General Summary:

The present work deals with certain aspects of colour-change mechanism in some freshwater fishes, Garra gotyla (Gray) and Mystus bleekeri (Day). The salient features of this study are summarised as follows:

I. Normal colour pattern in the fishes, G. gotyla and M. bleekeri

The pigmentary system of G. gotyla comprised of melanophores, xanthophores, guanophores-leucophores and iridophores. Extensive study was carried out on melanophores which were identified to be of 4 different types. On the basis of their size and nature of branching pattern they were classified into macro-melanophores (types 'A', 'B' and 'C') and micromelanophores (type 'D'). Under normal conditions the fish has a dark dorsal and a light ventral surface and the shade of the head region was lighter than the body region. A study on the topographical distribution of the different types of melanophores in the head and body regions of the fish was undertaken by dividing the head region into 3 areas and the body region into 9 areas (Fig. 3; p. 10). The head skin had only type 'A' and 'D' melanophores. Body scales had all the four types of melanophores, with type 'B' melanophores restricted to the ventral area scales. Melanophores were more densely populated in the body scales than in the head skin thus justifying the lighter shade of the head region. In the dorsal and lateral areas of the head region type 'D' melanophores formed characteristic ring-like structures which were absent in the body scales. Such ring-like structures (Fig. 25 p. 22) have not been reported in any other teleost fish worked out so far.
The dark dorsal surface of the body region was due to the presence of greater number of melanophores with greater melanin content. The lighter shade of the ventral area was due to the presence of mostly type 'B' melanophores and xanthophores. Xanthophores were more abundant than melanophores which gave the fish, a steel-grey colour in normal condition. Iridophores were absent in the head skin but present in the scales; mostly in the ventral area scales.

The pigmentary system of *M. bleekeri* comprised of melanophores, xanthophores and leucophores. Melanophores were of 3 types (types 'A' and 'B' - macromelanophores and type 'C' - micromelanophores). To study the characteristic colour pattern of the fish, the body region was divided into 9 areas (Fig. 4, p. 10). A study on the topographical distribution of melanophores revealed that all the 3 types of melanophores were present in the dorsal and lateral band areas, whereas in the general body surface type 'A' melanophores were completely absent, which gave a light shade to the general body surface. The steel-grey colour of the fish was due to the presence of xanthophores which were more numerous than melanophores.

A gradual reduction in the number of melanophores was observed both from before backwards and from the dorsal to the ventral area of both the fishes.

II. Quantitative colour-change mechanism in the fish, *Garra gotyla* (Gray)

Effect of long term background adaptation on the structural characteristics of melanophores and their population without and
with pituitary extract administration was studied. A quantitative study on the melanophore population of the fish after its adaptation to normal, white and black background for a period of 80 days showed marked difference in their number and melanin content.

On a normal background, in the absence of a perfect black and white background stimulus, the fish was deprived of an active role of either neurohumor(s) or the melanophore aggregating hormone(s). Hence, not much variation in the size of melanophores and their melanin content was observed. Numerically there was a slight increase in the number of type 'A' melanophores of the dorsal area of the head and body regions and type 'B' melanophores of the ventral area scales, while all the other melanophore types showed a decline in both head and body region in all the 12 sites of the non-injected fish. In the injected fish a pronounced decline in the population of all the four melanophore types was seen in the head and body regions, which was most likely accentuated due to the presence of an active melanophore aggregating hormone in addition to neurohumors which were responsible for causing aggregation and loss of melanophores and at the same time inhibiting melanophore dispersion and thus preventing an increase in their number.

On a white background, the reflected light from the background stimulates the dorsal part of the retina and the impulses thus generated, stimulate the aggregating nerve centre of the autonomic nervous system resulting in the secretion of neuro-
humoral transmitter at the nerve terminals to bring about quick aggregation of melanophores. The white background stimulus received by the eye (retina) evokes the secretion of melanophore aggregating hormone (MAH/MCH) and its release through the pituitary gland into systemic circulation thus causing melanin aggregation. Hence, a reduction in the number of melanophores and melanin content of all the four melanophore types of both the head and body regions took place. With additional stimulation, i.e., by the administration of pituitary extract, a marked percentage decrease in the number of melanophores with greater number of depleted melanophores in the injected fish than the non-injected fish was found. The rapid loss of melanophores occurred in the fishes after 20 days of their adaptation i.e., in the first phase of the experiment, later on the process of degradation of melanophores became slow. In the non-injected white-adapted fish percentage loss was maximum in case of type 'D' melanophores and least percentage fluctuation was seen in case of type 'B' melanophores. Out of the four types of melanophores the type 'A' and type 'B' melanophores showed greater percentage reduction in the injected fish as compared to that of the non-injected fish than in type 'C' and type 'D' melanophores of which the percentage reduction between the injected and non-injected fish did not vary much.

On a black background the dorsal part of the retina of the eye is deprived of any stimulation from the reflected light, as such the melanophores attain their passive state exhibiting maximal dispersion. Continued maximal dispersion of the melanin
for prolonged period potentiated melanin synthesis and an increase in the melanophore population of all the four melanophore types. The presence of melanophore concentrating hormone in the pituitary extract was further confirmed by the action of the pituitary extract on melanophores of the black-adapted fish in which a reduction in the melanophore population and melanin content was clearly seen.

On the four melanophore types, type 'B' melanophores had a comparatively smaller role to play in the adaptation of the fish in response to changing backgrounds in their natural habitat.

The results clearly pointed that the intensity of stimulation on the dorsal part of the retina, in the manifestation of release of neurotransmitters from the chromatic fibres and hormone from the pituitary gland was greatest in the fish placed on a white background, less on the normal background and least on a black background.

The experimental results showed a definite relationship between transitory and quantitative colour change mechanisms. On a long-term adaptation of the fish on a white background the physiological and behavioural factors tended to aggregate the pigment granules (transitory colour change) and thereby causing a reduction in the melanophore population (quantitative colour change); whereas the passive state of long-term black background adaptation of the fish caused melanin dispersion (transitory colour change) and an increase in the number of melanophores (quantitative colour change).
III. Transitory colour-change mechanism:

(A) Methods used for the measurement of chromatic responses

Melanophores of a fish differed in their response to different backgrounds i.e., becoming dispersed on a black background (black-adapted) and becoming pale on a white background (white-adapted). Methods adopted by various researchers in this field have been described in Part III-A.

The derived oswald index (D.O.I.) method proposed by Healey (1967) has been adopted for the 'in vivo' studies of the chromatic responses of the fishes, *Garra goltula* (Gray) and *Mystus bleekeri* (Day), keeping in mind the chromatic behaviour of the fishes due to handling. The melanophore index (M.I.) method devised by Hogben and Slome (1931) was used for 'in vitro' studies i.e., studies utilizing the isolated scales of *G.goltula* to see the effect of external agents. The M.I. was prepared for both types 'A' and 'B' melanophores by aggregating the fully dispersed melanophores with a sympathomimetic drug and selecting 3 intermediate stages between the fully dispersed (stage 5) and fully aggregated (stage 1) states of the melanophore.

(B) Studies on the rate of colour-change mechanism as a result of background response

In both the fishes, *G.goltula* and *M.bleekeri* the rate of darkening was faster than the rate of paling. The white-adapted fishes, when placed on a black background attained darkening i.e., 5.5 and 6.5 grades of the D.O.I. in the head and body regions of *G.goltula* and 6.5, 4.5 and 5.5 grades of the D.O.I.in the dorsal band, general body surface and lateral band areas
respectively of *M. bleekeri* in 40 minutes. The black-adapted fishes when placed on a white background, attained a paling i.e., 1.5 and 2.0 grades of the D.O.I. in the head and body regions of *G. gotyla* in 60 minutes and 2.0, 1.0 and 1.5 grades of the D.O.I. in the three areas in 50 minutes. The rate of colour-change mechanism was rapid during the initial stages as a response to background which indicated the predominant role of the nervous system. The hormonal control was slow and took time to set in its effect. The results with spinal-sectioned fishes, when left on a white background for longer period, indicated that the hormonal control played its role in maintaining the pallor attained through the neural control. A greater darkening attained in the blinded and spinal-sectioned fishes (6.0 and 7.0 grades of the D.O.I. in the two regions of *G. gotyla* and 7.0, 5.0 and 6.0 grades of the D.O.I. in the three areas of *M. bleekeri*) than in the normal intact black-adapted fish was due to the total non-stimulation of the paling centre (as in the blinded fish) or complete cessation of the release of neurotransmitter(s) from the nerve terminals by severance of autonomic chromatic nerve fibres (as in the spinal-sectioned fish despite the intact paling centre in the brain).

The changes observed in the blinded *G. gotyla*, when kept on an illuminated white background for long period, was probably due to the photosensitive nature of the pineal organ in the absence of eyes.

The long-term adaptation of the spinal-sectioned *G. gotyla*
and *M. bleekeri* on a white background clearly pointed towards the presence of a melanophore concentrating/aggregating hormone in the pituitary gland which helped in maintaining the pallor attained by the fish through the neural control mechanism.

(C) *In vivo* and *in vitro* effects of pituitary extracts on melanophores

The *in vivo* effect of pituitary extracts of *G. gotyla* and *M. bleekeri* in *Garra gotyla* and an *in vitro* study of the effects of pituitary extracts of these two fishes on type 'A' and type 'B' melanophores of *G. gotyla* has been undertaken to find the nature and role of the possible hormone(s) released from the pituitary gland of fishes influencing transitory colour change mechanism in *G. gotyla*.

On administration of the pituitary extract, it was found that in any of the condition (white-adapted, black-adapted, blinded and spinal-sectioned) the response of the fish was always paling. These results suggested the presence of a hormone (melanophore aggregating/concentrating hormone, MAH/MCH) in the pituitary gland of both the fishes. The paling nature of the pituitary-borne hormone was further evident from the experiments in which the pituitary extracts evoked paling in the fully black-adapted fishes placed on a black background; blinded and spinal-sectioned fishes placed on either backgrounds. There was no indication in the pituitary of both the fishes of a hormone with melanophore dispersing tendency (MSH or MDH). The data obtained from *in vivo* and *in vitro* studies on the effect of pituitary extracts of *G. gotyla* and *M. bleekeri* very clearly
indicated that the pituitary extract of *G. gotyla* was more potent and faster in action than that of *M. bleekeri* which was slower in eliciting its paling response which was, however, longer lasting.

(D) **Studies on the pathway of chromatic nerve fibres, the vertebral level at which they leave the spinal cord, and the nature of neural colour change mechanism in Garra gotyla (Gray) and in two species of Mystus i.e., M. bleekeri (Day) M. vittatus (Bloch) has been described**

The chromatic nerve fibres responsible for neural control of melanophores in the fish were interrupted by sectioning of the spinal cord at different vertebral and intervertebral levels in a large number of fishes. The findings as a result of spinal-ectomy clearly showed that the chromatic nerve fibres left the spinal cord through the spinal nerves issuing out between seventh and eighth vertebrae in *G. gotyla*, between the fourteenth and fifteenth vertebrae in *M. bleekeri* and between the thirteenth and fourteenth vertebrae in *M. vittatus* behind the anterior complex vertebra.

The detailed chromatic nerve fibre pathway, as worked out in *Phoxinus* (von Frisch, 1911 and Healey, 1940, 1948, 1951, 1954), running from the spinal cord through the spinal nerves into the sympathetic chain to make synaptic relation with the peripheral neurons and then the pathway of postganglionic fibres into the spinal nerves of the body region and trigeminal nerves of the head region has not been carried out in the present study, but the indirect evidence that spinalectomy in these fishes behind
the level of spinal outflow of chromatic fibres evoked paling as in the normal intact fishes; and the darkening caused in the head and body regions of these fishes as a result of spinal-ectomy anterior to the level of spinal outflow of the fibres clearly pointed out to the presence of a similar pathway as in Phoxinus.

IV. **Effect of pharmacological drugs and hormones**:

The effects of various pharmacological drugs and hormones known for their effects in mammals were examined both in 'in vivo' and 'in vitro' studies in _G. gottleb_ and only in 'in vivo' studies in _M. bleekeri_ to determine the nature of innervation of melanophores, the nature of neuro-melanophore transmission and to characterise the receptors involved in the neural control of colour change mechanism in both the fishes.

1. The results clearly indicated that the melanophores of both the fishes were innervated by sympathetic pigment-aggregating fibres only (supporting the mono-neuronic hypothesis-Hogben, 1924; Waring, 1963) and that these fibres were adrenergic in nature.

2. All the five sympathomimetic drugs, catecholamines (adrenaline tartrate, epinephrine, arternol, dopamine) and non-catecholamine (ephedrine), tested in the two fishes, resulted in paling in all the eight different experimental conditions. The most potent pigment aggregating agent in the present study were the catecholamines i.e., adrenaline tartrate, epinephrine and arternol than other drugs. The pharma-
colological observations with regard to these catecholamines suggested that either of them served as a chemical transmitter in an adrenergic system controlling the melanophores, just as noradrenaline served in the adrenergic system in higher vertebrates. The aggregating activity of all these sympathomimetic drugs supported the existence of sympathetic-pigment aggregating fibres of adrenergic character, controlling the active rapid aggregation of melanophores in the two fishes which was mediated through the $\alpha$-adrenoceptors. Blinded and spinal-sectioned fishes restoring to the pre-experimental shade after the effect of the drug is over, provided a supporting evidence for the presence of mono-neuronic control of melanophores mediated through adrenergic nerves.

3. Of the different $\alpha$-adrenergic antagonists (yohimbine, tolazoline, ergotamine and prazosin), yohimbine was found to be the most potent drug in blocking the paling responses of the $\alpha$-adrenoceptor. Yohimbine has been known as a selective $\alpha_2$-adrenoceptor antagonist. A greater darkening observed after yohimbine administration in all the different experimental conditions than other $\alpha$-adrenergic antagonists and the normal black-adapted fish was due to the complete blockade of $\alpha$-adrenoceptors, especially $\alpha_2$-subtype. Tolazoline and ergotamine were only moderately effective in bringing about dispersion (or blockade of $\alpha$-adrenoceptors) of the melanophores. Prazosin has been known to be a specific $\alpha_1$-adrenergic antagonist. It produced only weak or no inhibition of the nerve induced or noradrenaline induced pigment
aggregation. Hence, the role of $\alpha_1$-adrenoceptors in the mediation of pigment aggregation could be ruled out in the two fishes. $\beta$-adrenoceptors mediating pigment dispersion has been observed in some fishes. In the present study $\beta$-adrenergic antagonist, propranolol did not induce any aggregation of melanophores thus indicating the absence of role of $\beta$-adrenoceptors in the mediation of pigment dispersion.

The catecholamine depleting action of the anti-adrenergic drug reserpine was seen in the two fishes as it elicited greater darkening by complete depletion of catecholamine stores. The slow and long-lasting effect of reserpine was observed because it reduced the overall uptake of catecholamine by adrenergic neurons.

The results with various sympatholytic drugs confirmed the mechanism of aggregation of melanophores in C.gotyla and M. bleekeri through the post-ganglionic fibres and mediated through $\alpha_2$-adrenoceptors of $\alpha_2$-subtype.

4. Parasymathomimetic drugs like cholinergic (acetylcholine chloride, acetyl $\beta$-methyl choline chloride and carbamyl $\beta$-methyl choline chloride); cholinomimetic (arecoline and pilocarpine) and anticholinesterase (eserine) were tested in both the fishes in all the eight experimental conditions. The results obtained with these drugs provided no evidence for the existence of cholinergic fibres controlling pigment dispersion.

5. A parasymatholytic drug atropine sulphate provided no evidence to prove the melanophore dispersing activity through a
possible parasympathetic nervous system in the two fishes. The dispersing effect of atropine sulphate was probably the result of its direct action on melanophores rather than mediated through the nerves.

6. Melatonin, a pineal hormone known for its extremely potent aggregating action on melanophores, was found effective in bringing about aggregation in both the fishes in all the different experimental conditions. The paling action of melatonin was not as potent as that of the sympathomimetic drugs. α-melanophore stimulating hormone (α-MSH), known for its dispersing action on the melanophores, was found eliciting dispersion of melanophores and hence darkening in both the fishes.

7. Studies with various pharmalcological agents 'in vitro' on type 'A' melanophores of G. gotyla were found in confirmation with the 'in vivo' studies. All the sympathomimetic drugs brought about full aggregation of melanophores (time differing for different drugs). Epinephrine was found to be the most potent aggregating drug. Yohimbine, tolazoline and ergotamine were effective in bringing about dispersion of aggregated melanophores, yohimbine being the most potent one; while prazosin, propranolol and reserpine were ineffective in bringing about any chromatic change. All the parasympathomimetic drugs tested could not produce any change either in the aggregated melanophores or melanophores dispersed in Young's Ringer solution. Parasympatholytic drug atropine sulphate was also ineffective in bringing about any change. Melatonin and α-MSH brought about
aggregation and dispersion respectively in the isolated scale melanophores.

The results with pharmacological drugs suggested that as in almost all other teleost fishes, in *Garra gotyla* and *Mystus bleekeri* also, the aggregation of melanophores was elicited through sympathetic post-ganglionic adrenergic fibres of the autonomic nervous system and mediated through $\alpha$-adrenoceptors of the $\alpha_2$ subtype.