CHAPTER III

MORPHOLOGICAL COLOUR CHANGES

A. GENERAL INTRODUCTION
A number of stimuli cause increase or decrease in either net content of pigmentary substances or the number of the pigment cells. These changes are known as morphological colour changes.

There is conclusive evidence that the teleosts, when subjected to prolonged sojourn on illuminated, white and black backgrounds, exhibit absolute melanin loss or gain, respectively (Sumner, 1934, 1939, 1940 and 1943; Sumner and Fox, 1933; Sumner and Doudoroff, 1939 and 1943; Odiorne, 1937; Fries, 1933 and many others).

Among the other stimuli, which induce the morphological changes in the teleost fishes, pituitary intermedin is most widely recognised substance. Pickford and Atz (1957) have divided teleost fishes into two groups, according to their chromatophore reactions to pituitary preparations. Group I, includes, those fishes that always react to active pituitary preparations by the dispersion of the pigment granules within the melanophores. Although unresponsive to purified intermedin, group II has further been divided into three categories: IIa is characterised by a high sensitivity to the melanophore concentrating hormone and little or no response to intermedin, IIb includes the fishes, which respond to intermedin by
either melanophore dispersion or aggregation, while fishes belonging to group IIc, show the dispersion of pigment granules in 'lipophores' only.

According to Odiorne (1943), prolonged dispersion of melanophores in response to MSH provides a favourite condition for the synthesis of melanin in the melanophores. Pickford and Atz (1957) detected an increase in skin melanogenesis in Fundulus, by injecting MSH. On the other hand Reidinger (1952) using Phoxinus, found that the yellow background adaptation resulting from xanthophore activity, is controlled by both epinephrine and MSH. He further observed that epinephrine actually increases the number of xanthophores without causing dispersion of pigment inside the cells. He, therefore, concluded that morphological colour changes are not initiated by the physiological colour changes and are directly regulated by hormonal substances.

Kosto et al (1959) found that MSH stimulates the proliferation of melanocytes in Fundulus. Hu and Chavin (1960) stated that MSH is inactive in promoting melanogenesis in vitro. However, Tchen et al. (1964) demonstrated that both and B MSH stimulate the formation of melanophores from premelanophores. Baker and Ball (1975)
demonstrated that the presence of two opposing hormones inducing either melanin dispersion or melanin aggregation.

Hu and Chavin (1960) demonstrated in goldfish that, among the pituitary hormones, other than MSH, only ACTH is capable of inducing melanogenesis. This observation was confirmed by many workers (Chavin, 1956; Kosto et al., 1959; Kim et al., 1961; Loud and Mishima, 1963 and Matsumoto, 1965). Kosto et al. (1959) demonstrated that besides ACTH, prolactin, promotes the melanin synthesis in Fundulus.

Egami et al., (1962) demonstrated that X-ray radiation induces melanogenesis, a process to form new melanocytes and ultimately new melanophores in the goldfish. Thyroxine treated larvae of brown trout Salmo trutta show significant increase in the number of melanophores, whereas in thiourea treated group, the number of melanophores decreases. Thyroxine treated fish contains less melanin than the control and thiourea treated fish contains more melanin (Woodhead, 1966).

Epinephrine and yohimbine are able to induce nuptial colouration in certain fishes (Marath, 1959). Arai and Egami (1961) reported that adrennergic steroids
are effective in increasing the number of leucophores in adult male *Oryzias latipes*, while cholesterol, progesterone and estrogens are not effective. Chavin (1963) found that hydroquinone is effective in destroying melanocytes and melanophores of black moor goldfish. A variety of substances have been categorised as depigmentising agents (Chavin and Schlesinger, 1967).
CHAPTER III

MORPHOLOGICAL COLOUR CHANGES

B. INFLUENCE OF PROLONGED BACKGROUND ADAPTATION ON THE INTEGUMENTARY MELANOPHORES OF L. THERMALIS
Introduction

Teleosts have the ability to become pale or dark depending upon the colour of the surroundings, where they live. The change of tint from pale to dark and vice-versa, may be rapid, requiring a few seconds or slower taking several days. The rapid colour changes among fishes, brought about by the movement of melanin granules to the centre (i.e. aggregating) or into the extended branching processes of the melanophores (i.e. dispersing), are called as physiological colour changes. Contrary to these rapid but temporary colour changes, fishes exhibit a second type of colour change of a more permanent nature, which is, however, slower and requires greater time than that is required for physiological colour changes. This involves the actual alteration (decrease or increase) in the net pigimentary content, as well as, the number of pigimentary cells in the integument. These colour changes are usually evoked by maintaining the animals on a specific background, for a few days, to several weeks or even months and are termed as morphological colour changes.

Secerov (1909) was the first to coin the terms, physiological and morphological colour changes, to these
activities of chromatophores. This nomenclature remained in vague for a long time in the literature, until Sumner and his co-workers (Sumner and Wells, 1933; Sumner and Fox, 1933; Sumner and Doudoroff, 1937, 1939, 1943 and Sumner, 1939, 1940 and 1943), amended the term 'Physiological' as transitory and 'Morphological' as quantitative colour change, on the ground that both the processes referred above are basically physiological. In the present studies, I have used the old nomenclature, as the later findings of many workers, have shown that morphological and physiological colour changes are not initiated simultaneously, but are independent from each other (Reidinger, 1952).

Keeble and Gamble (1904) were the first to observe the increase or decrease of chromatophore number, in an adult shrimp, Hippolyte varians. Among the fishes, Franz (1910), while working on flatfish, Pluronectus platessa, paid attention to this type of colour change. Thereafter this problem was extensively studied by various workers (Von Frisch, 1911, 1920; Kuntz, 1915, 1917; Murisier, 1920-1; Kudo, 1922; Sumner and Fox, 1933, 1935; Odorone, 1933, 1936, 1937, 1943 and 1957; Sumner and Doudoroff, 1937, 1938 and 1943; Osborn, 1940a and b and 1941a, b, c and d; Parker, 1948; Reidinger, 1952;

In the present investigation the rate of decrease or increase in the melanophore number, from various sites of the fishes, adapted to illuminated normal, black and white backgrounds for 40 days, has been determined. The rate of recovery in the number of melanophores from the fishes of 'white history' and the rate of disappearance of melanophores from the fishes of 'black history' has also been observed. The present study helps in understanding the nature of control of colour change mechanism in L. thermalis.

Materials and Methods

L. thermalis were obtained from Kham river near Aurangabad and were kept in glass aquarium (90 x 37 x 37 cm), provided with aerator. Dichlorinated water was provided to them, every alternate day. They were fed with commercial fish food, every third day. The fishes were kept in this condition at least for two weeks to acclimatize to laboratory conditions.

The tanks used to provide normal, black and white backgrounds, to the experimental fishes, were the aquaria
with clear walls and those of black and white painted, from the outside walls; respectively. Each tank measured 45 x 27 x 27 cm. A thin bottom layer of sand was provided to produce a normal background, in clear walled aquarium.

In all 90 fishes were selected (showing normal background responses) from the stock aquarium, for the present experimental work. Thirty fishes were transferred to clear walled tank and thirty were transferred to each one of the black and white painted tanks.

Dichlorinated water was provided to the experimental fishes, every alternate day. To prevent the aquarium water from becoming foul, due to the excreta and other wastes of food material, they were removed daily, by making the use of wash-bottle. Electrically operated aerators were provided to oxygenate water in the aquaria. Fishes behaving abnormally or dying during the course of experiment, were immediately removed and discarded. A 40 W bulb hanging at a distance of about 1 metre from the surface of the water in aquaria, was used as a source of constant light to each of the three aquaria. Care was taken to maintain the aquaria clean during the period of experimental work.

In the earlier studies, concerning the normal colour pattern of the fish (Chapter I), the whole fish
Fig. 1 Diagrammatic representation of the fish, showing the sites in the dorsal region of the body, considered for recording the quantitative data from three major parts of the body i.e.,

1 - cephalic,
2 - the trunk and
3 - the caudal of *L. thermalis*. 
body was divided into four regions i.e. the dorsal, the dorso-lateral, the latero-ventral and the ventral regions. While studying the rate of colour change mechanism (Chapter IIe), it has been noticed that the dorsal region of the body of the fish, showed quicker and uniform responses to the background changes. As the object of the present study was to determine the quantitative changes in the melanophore number, the dorsal region of the cephalic and trunk and the dorsal lobe of the caudal fin, were considered appropriate for the observations in the present investigation.

The present experiment lasting for 40 days, included 16 groups of experimental fishes with each group consisting of 5 animals. The quantitative data regarding the epidermal melanophores, was recorded from the various but fixed sites in the dorsal region of the three major parts of the fish body i.e. the cephalic, the trunk and the dorsal caudal part (Fig. 1). The sites for recording the quantitative changes in the melanophore numbers, were fixed at posterior part of operculum, anterior end of the dorsal fin and anterior part of the dorsal caudal lobe, in the cephalic, the trunk and the caudal regions, respectively (Fig. 1). Before the commencement of the experiments, the melanophore numbers from various sites were recorded. Further observations were made on the experimental fishes, in a group of
five, at a definite time interval, i.e. 10, 20, 30 and 40 days, during the course of prolonged adaptations to normal, white and black backgrounds.

To study the rate of decrease or increase of the melanophores in the fishes, the fishes (10, in number) adapted to black background for 40 days were transferred to white background for a period of 20 days and similarly, the fishes (10 in number) adapted to white background for 40 days were transferred to black background, for a period of 20 days. The changes in the melanophore populations from the various sites were recorded at an interval of 10 and 20 days, during the course of these experiments.

The counting of the epidermal melanophores from the various sites in the dorsal region of the cephalic, the trunk and the dorsal part of the caudal fin was made, by using the graticulum.

Observations and Results

The results of the present experimental work, of the prolonged adaptation to the normal, white and black backgrounds are graphically shown in Figs. 2, 3 and 6, respectively. Further, the results obtained by the
Fig. 2  Quantitative changes in the epidermal melanophores of the fishes, kept on illuminated normal background for a period of 40 days at various sites in the dorsal regions of cephalic [], trunk [] and caudal [] part. A significant change ($P < 0.01$) was observed in the number of epidermal melanophores, following 10, 20, 30 and 40 days. Pre = Pre-experimental.
background reversals of 'white history' fishes and 'black history' fishes, for 20 days, are graphically represented in Figs. 3a and 3b, respectively.

1. Quantitative changes in the epidermal melanophores of the fishes, kept on normal background for a period of 40 days:

Fig. 2, clearly illustrates the gradual increase in the total number of epidermal melanophores in all the sites of the fishes, placed on illuminated normal background for a period of 40 days. A significant \( P < 0.01 \) change was observed in the numbers of epidermal melanophores, following 10, 20, 30 and 40 days. However, it was observed that the increase in the epidermal melanophage numbers in the first 20 days was much faster (approximately 10%) than that was observed in the later part of the experiment (approximately, 5%). The total increase in the epidermal melanophores at various sites in the dorsal regions of cephalic, trunk and caudal, was observed to be approximately 15% at the end of the experiment.

2. Quantitative changes in the epidermal melanophores of the fishes, kept on white background for a period of 40 days:

Fig. 3, indicates that there is a marked decrease in the total number of the epidermal melanophores, in all
Fig. 3 Quantitative changes in the epidermal melanophores of the fishes, kept on illuminated white background for a period of 40 days, at various sites in the dorsal regions of cephalic □ , trunk □ and caudal □ parts. The decrease in the number of epidermal melanophores was found to be statistically significant (P < 0.01), following 10, 20, 30 and 40 days.

Pre = Pre-experimental.
Fig. 4 *Lepidocephalichthys thermalis*

(a) after the adaptation to the illuminated white background for 40 days,

(b) after the adaptation to the illuminated black background for 40 days.
Fig. 5(a) Melanophores with aggregated melanin, from the antero-dorsal region of the caudal fin, after the adaptation of the fish to a illuminated white background for 20 days.

M = melanophores.

100 x.

(b) Melanophores with disappearing melanin granules, from the antero-dorsal region of the caudal fin, after the adaptation of the fish to a illuminated white background for 40 days.

M = melanophores.

100 x.
the sites of the fishes, placed on illuminated white background, for a period of 40 days. This decrease in the number of epidermal melanophores, was found to be statistically significant (P \( \leq 0.01 \)), following, 10, 20, 30 and 40 days. Further, it was observed that the rate of decrease in the epidermal melanophore number, in the first 20 days was faster (approximately, 30%) than that was observed in the later part of the experiment (approximately, 11%). The total decrease in the epidermal melanophore number at various sites, was found to be approximately 41% (Fig. 3), at the end of the experiment. Visual and microscopic examination lend an additional support to the above mentioned observations. After 40 days of adaptation to the illuminated white background, the fishes blanched to D.M.I., 1.5 ± 0.23 (Fig. 4(a)).

Microscopic observations, after 20 and 40 days of white background adaptation, revealed that the melanophores loose their melanin granules and appear to be fragmented (Fig. 5a and 5b).

3. **Quantitative changes in the epidermal melanophores of the fishes, kept on black background, for 40 days:**

   It was observed that, there was a gradual increase in the total number of epidermal melanophores, at the
Fig. 6 Quantitative changes in the epidermal melanophores of the fishes, kept on illuminated black background for a period of 40 days, at various sites in the dorsal regions of cephalic [I], trunk [II] and caudal [III] parts. The increase in the epidermal melanophore number was found to be significant ($P < 0.01$), following 10, 20, 30 and 40 days.

Pre - Pre-experimental.
Fig. 7(a) Melanophores with increased content of melanin granules, from the antero-dorsal part of the caudal fin, after the adaptation of the fish to the illuminated black background for 20 days.

M = melanophores.

100 X

(b) Melanophores with increased content of melanin granules, from the antero-dorsal part of the caudal fin, after the adaptation of the fish to the illuminated black background for 40 days.

M = melanophores.

100 X
various sites of the fishes, kept on illuminated black background, for a period of 40 days. The increase in the epidermal melanophore numbers was found to be statistically significant ($P \leq 0.01$), following 10, 20, 30 and 40 days. Further, it was noticed that the rate of increase in the epidermal melanophore number, in the first part (first, 20 days) of the experiment, was comparatively faster (approximately, 13%), than the one observed in the later part of the experiment (approximately, 7%). The total increase in the epidermal melanophore number at various sites, by the end of the experiment (40 days), was observed to be approximately 25% (Fig. 6).

Microscopic and visual examination gave an additional evidence to the above observations. After 40 days of adaptation to the illuminated black background, the fishes darkened to D.M.I., 7.2 ± 0.25 (Fig. 4(b)). Under the microscope, it was observed that after 20 and 40 days of black background adaptation, the melanophores contained increased number of melanin granules and became fully dispersed (Fig. 7a and 7b).

4. **Quantitative changes in the epidermal melanophores by transferring the fishes of white history to black background and the fishes of black history to white background for a period of 20 days.**

It was observed that, when the white history fishes were transferred to illuminated black background for a
Fig. 3(a) Increase in the epidermal melanophore number of white history fishes, on black background for a period of 20 days, at the various sites in the dorsal regions of cephalic □, trunk □, and the caudal □□ parts. The increase in the epidermal melanophore number was found to be significant ($P < 0.01$), following 10 and 20 days.

Ini - Initial experimental.

(b) Decrease in the epidermal melanophore number of black history fish, on white background for a period of 20 days, at the various sites in the dorsal regions of cephalic □, trunk □, and the caudal □□ parts. The decrease in the epidermal melanophore number was found to be significant ($P < 0.01$), following 10 and 20 days.

Ini - Initial experimental.
period of 20 days, there was an increase in the total number of epidermal melanophores at various sites in the dorsal region of cephalic, trunk and caudal parts of the fishes (Fig. 3a). It was found that the increase in the number of epidermal melanophores, was statistically significant (\( p < 0.01 \)). The total increase in the epidermal melanophores at various sites, by the end of the experiment was approximately 12% (Fig. 3a).

When the fishes of the black-history, were transferred to illuminated white background for a period of 20 days, it was noticed that there was a marked decrease (approximately, 20%) in the number of the epidermal melanophores, at various sites. The decrease in the number of epidermal melanophores was found to be statistically significant (\( p < 0.01 \)). Further, it was noticed that the rate of decrease in the epidermal melanophore number was faster, than the rate of increase of epidermal melanophore number at various sites in the major parts of the fish body (Figs. 3a and 3b).

Discussion

The chromatophoral system of an adult animal experiences, from time to time, the increase or decrease
in the pigment granule content of the pigmented cells. Kuntz (1915), Murisier (1920-1), Sumner and Wells (1933), Odiorne (1937), Osborn (1940a and b; 1941a, b, c and d), Reidinger (1952), Brantner (1956), Umrath (1957), Ahmad (1972) and Jain (1976) have shown in various teleost species, that the absolute increase in both, the amount of melanin and number of melanophores, can be achieved by keeping the animals to long term adaptation, on an illuminated black background. Conversely, a longterm adaptation to an illuminated white background, reverses the effect, with a decrease in the quantity of melanin granules and the number of melanophores.

The results of the present experiments on quantitative changes in the epidermal melanophores of the fish, L. teresalis, due to prolonged black or white background adaptation, are in accordance with the findings of above mentioned workers.

Many earlier workers (Odiorne, 1936, 1937; Reidinger, 1952; Brantner, 1956; Umrath, 1957 and Jain, 1976) observed the changes in the total number of melanophores by counting them at the beginning of the experiment and finally at the end of the experiment and describing the net changes which had taken place. The same method has been followed in the present study.
Keeble and Gamble (1904) suggested that the state of chromatophores (dispersed or aggregated), is the deciding factor for melanogenesis. Later workers (Franz, 1910; Babak, 1910, 1912 and 1913, Von Frisch, 1911; Odiome, 1933, 1937 and 1943; Osborn, 1941a, b and c) revealed that, the long-term dispersion of the pigments leads to an increase in the net quantity of the pigment granules and the number of the pigmentary cells. Whereas, the long-term aggregation of the pigmentary cells leads to a decrease in the net amount of the pigment granules and the number of the pigmentary cells. The long-term dispersion or aggregation is based primarily on the principles of transitory (i.e. Physiological) colour changes. The pigmentary changes in the teleost fishes are controlled either neurally, hormonally or by both. In the present study, the slower and time consuming nature of morphological colour changes, demonstrates the possibility of hormonal control in L. thermalis, after the faster predominant nervous control in the initial stages of the experiments.

That the nervous control is superimposed by the hormonal control is already shown in the fish, L. thermalis, as mentioned in Chapter IIa. The nervous control in the colour change mechanism is effected through the pigment aggregating nerve fibres (Chapter, IIa and IIb).
The results obtained in the present work are interpreted in the light of the above mentioned facts.

White background:

The results obtained in respect of the quantitative changes in the epidermal melanophores, are consistent with the events occurring, during the long-term adaptation to the illuminated white background.

On the white background the retina of the fish, possibly evokes the impulses in the optic nerves and then in the aggregating- autonomic nerve centre, which through its paling fibres brings about the rapid aggregation of the melanin in the melanophores (Chapter, IIe). It is believed that pigment- aggregating fibres exert their action by secreting a neurohumoral transmitter of an aggregating nature at the nerve terminals, innervating the melanophores. At the same time the white- background stimulus received by eye (retina) evokes the secretion of melanophore aggregating hormone in the cells of parsintermedia of the pituitary and its release into the blood, which also causes the melanin aggregation.

By maintaining, the white background stimulus, by retaining the fishes for a long period, lasting several days, results in an increased concentration of aggregating
neurohumoral transmitter and an increased concentration of aggregating hormone in the vicinity of melanophores. Under these longterm biochemical and physiological conditions, the formation of melanin and melanophores is retarded, checked and finally the fishes suffer a gradual loss in its melanophage population. The most rapid loss of the melano- phores was observed in the animal sacrificed, in first phase of the experiment (first 20 days), but afterwards the process of disappearance of melanophores becomes considerably slower.

The same mechanism described above, seems responsible for the reduction in the amount of melanin in the melanophores as estimated visually [Fig. 4(a)]i, disappearing melanophores and the fragments of melanin granules are also observed microscopically (Figs. 5a and 5b).

**Black background:**

The results with regard to the quantitative changes in the epidermal melanophores, fit well with the mechanism involved— during the longterm adaptation to an illuminated black background.

On the black background (illuminated), the fishes become dark rapidly, due to the dispersion of the pigment
granules in the melanophores. It is believed that the black background stimulus works as a negative factor to the functioning of the agencies, inhibiting pigment aggregation. Consequently it appears that the production of neurohumoral transmitter of an aggregating nature is either stopped or slowed at the nerve terminals, innervating the melanophores. Simultaneously the stimulus received by retina and in turn reaching the pars-intermedia via the optic nerve, either stops or reduces the production and release of the melanophore aggregating hormone, thus, leading to the darkening of the fish.

By maintaining the fishes, on an illuminated black background for a prolonged period, the pigment aggregating agencies are greatly hampered and this results in maximal dispersion of melanophores. Under this physiological condition, there would be a minimum antagonism to melanogenesis and as such, fish experiences an increase in its pigmentation. The most rapid increase in the melanophore number, is seen in the first phase (first, 20 days) of the experiment, after which the formation of melanophores is slow. The increase in the amount of melanin, in the melanophores as estimated visually [Fig. 4(b)], is due to the mechanism described above.
The experiments with prolonged, white and black background adaptations, clearly indicate that, there is a definite relationship between physiological and morphological colour change, in respect of their control mechanisms. The stimulus and biochemical agents which tend to aggregate the pigment granules (Physiological colour change), also cause a decrease in the melanophore population (morphological colour change). The stimuli and biochemical agents which bring about the dispersion of the melanin granules (physiological colour change) are also responsible for an increase in the number of melanophores (morphological colour change). The results of the present experimental work, on teleost, L. thermalis, are in conformity with the observations of earlier workers (Odiorne, 1949; Lerner and Case, 1959; Abbott, 1973; and Jain, 1976).

By assuming, the involvement of an active pigment-dispersing mechanism, on an illuminated black background, working antagonistically to the active aggregating mechanism, stimulated by illuminated white background, one would expect, an approximate equal average percentage loss of melanophores in a fish on the illuminated white background, to average percentage increase in number of melanophores on illuminated black background, for a equal
time period. The present study reveals that the decrease in number of epidermal melanophores is always greater (approximately 41%), than that of reappearing melanophores (approximately 25%), on an illuminated white and black background (Figs. 3 and 6), respectively.

The data, thus, indicates that the increase in the epidermal melanophore population, on prolonged illuminated black background is the result of an inhibition of active pigment aggregating mechanism, rather than the functioning of any sort of dispersing mechanism.

Similar relationship holds true in case of the fishes of white history and fishes of black-history. The disappearance of melanophores in the fishes of black history is approximately twice than that of the formation of the new melanophores in the fishes of white history, for a equal period of 20 days (Figs. 8a and 8b).

These observations, thus support the existence of pigment aggregating nerve fibres and melanophore aggregating hormone, which control the quantitative (morphological) colour change mechanism in the fish, I. thermals.
Normal background

Before interpreting the results obtained from the prolonged adaptation of the fishes to the normal background, it is necessary to consider a few important facts.

On the normal background, the shade of the dorsal, latero-ventral spots and that of the dorso-lateral and ventral regions, ranges from 3.5 to 4.5, D.M.I. and 2.0 to 2.5, D.M.I., respectively. The general brownish black colour of the fish, is due to the dispersed melanophores and xanthophores. The fishes normally dwell at bottom of the aquarium and in this case (on the normal background), the bottom of the aquarium was covered with a thin layer of sand, making the background greyish in colour.

The average percentage increase in the epidermal melanophore population in the fishes, kept on illuminated normal background (14%, Fig. 2) is nearly half than that of the increase in the number of epidermal melanophores in the fishes, kept on illuminated black background (Approximately 25%, Fig. 6) and about 1/3 of the decrease in the epidermal melanophore population of the fishes, kept on illuminated white background (41%, Fig. 2), for the equal time period of 40 days. It is, thus, observed
that the quantitative changes in the fishes adapted to normal background are comparatively less marked, than the rest of the two backgrounds.

The fishes on an illuminated normal background receive neither a perfectly black background, nor a true white background stimulus. Instead, a grey background stimulus (due to the sandy bottom) helps the fishes in maintaining the melanophores in semidispersed condition (a condition favourable for the melanogenesis) by slightly reducing the amount of aggregating neurohumoral transmitter at the nerve endings and the concentration of melanophore aggregating hormone in the blood. These two conditions seems to favour a slight increase in the melanophore population, during the longterm adaptation to the normal background.

The results of the present study, are in agreement with results of earlier workers (Hadley and Quevodo, 1967; Bagnara and Farris, 1971 and Jain, 1976). The results obtained in the present experimental work, suggest that, there exists a mono-neuronic (pigment- aggregating nerve fibres) nervous control and a single hormonal control (melanophore- aggregating hormone), in the co-ordinating mechanisms of the chromatic system of L. thermalis.
GENERAL SUMMARY AND CONCLUSIONS
General Summary and Conclusions

The fresh-water teleost, L. <i>thermialis</i> was chosen for the present work, because of its availability throughout the year. Its maintenance in the laboratory and size is suitable to carry the research work on physiology of colour changes.

The integumentary system of the fish, possesses four kinds of chromatophores viz: melanophores, xanthophores, guanophores and iridophores. The quantitative estimation of the chromatophore number and their distribution has led the author to conclude that:

(1) the dark spotted regions in the body of the fish are produced due to, the greater number of melanophores and xanthophores per unit area, greater melanin and orange pigment granule content, lesser interspacing between the chromatophores and greater number of guanophores and iridophores in this regions of the body,

(2) the dull dotted regions in the body of the fish are produced due to the exactly reverse conditions, as mentioned above,
(3) the melanophores of the dorsal and latero-ventral regions contain higher amount of melanin pigment granules, whereas the ones, in dorso-lateral and ventral regions have lesser amount of these pigment granules. This helps in maintaining the separate identity of these regions. Further, this produces suitable colour pattern to match with the natural background of the animal.

(4) the gradual increase in guanophore and iridophore number, from the dorsal to ventral regions, helps in producing iridescent shining on the ventral side of the body of the fish, and

(5) the spots and dots, produced due to the differential distribution of melanophores and xanthophores are helpful to the animal, for concealing against the sandy background.

The pigmentary responses of the melanophores are measured, throughout the present work, with the help of (a) melanophore index method (Hogben and Slone, 1931) or (b) derived munsell index method, modified by the author following the procedure in principle, adapted by Healey (1967).

The application of electrical stimulation to melanophores, through central and peripheral nerves, has
led to conclude that melanophores in *L. thermalis* are under the control of melanin-aggregating nerves only.

The effects of all the pharmacological agents which stimulate or block the sympathetic nervous system, confirmed that the post-ganglionic fibres, innervating the melanophores of *L. thermalis* are $\alpha$-adrenergic and induce pigment aggregation only. The evidence for cholinergic dispersing mechanism, based upon the drug's effects is strongly negative.

Cytochalasin B inhibited pigment granule dispersion and induced pigment granule aggregation significantly. Colchicine inhibited pigment granule aggregation and induced the pigment granule dispersion significantly. This has led to presume that microtubules assist in the dispersion of the melanin granules. The effect of cytochalasin B does not give use any clear idea about the involvement of microfilaments in the pigment migration.

Potassium and sodium ions had a pigment granule aggregating and dispersing effect respectively. Under the special experimental conditions, isotonic KCl solution had a pigment dispersing effect. Potassium and sodium ions act directly on the melanophore, which is evident from the their action on innervated and denervated melanophores.
Pulsatory activity due to BaCl₂ was observed in the innervated and denervated melanophores in split tail preparations of L. thermalis. This has led to presume that the pulsating activity, caused by Ba ions, may be due to the contraction and relaxation of cell body as suggested by Obika (1976).

The rate of colour change mechanism, as a background response in L. thermalis, led the author to conclude that, the co-ordination of colour change process is done not only by nerves but also by hormone. It seems likely that the initial rapid colour change is the result of nervous co-ordination, while the later phases are controlled by hormone.

The influence of prolonged adaptation to the normal, white and black backgrounds, indicated the presence of mono-neuronic nervous control i.e. by pigment aggregating nerve fibres and a single hormonal control i.e. by melanophore aggregating hormone, in the co-ordinating mechanism of the chromatic system of L. thermalis.