CHAPTER 8

CHANGES IN THE ACTIVITY OF ACID AND ALKALINE PHOSPHATASES IN THE HYPOTHALAMO-HYPOPHYSIAL SYSTEM OF THE MIGRATORY STARLING, STURNUS ROSEUS (LINNAEUS) TOWARDS THE MIGRATORY PHASE

It is well known that enzymes are directly or indirectly influenced by hormones (Meyer and McShan, 1950; Gamzell and Samuels, 1950; Allen and Slater, 1956). The development of histochemical and cytochemical techniques for the localization of enzymes have considerably helped in the understanding of the physiological processes involved in the synthesis and secretion of hormones. Of the various enzymes studied with respect to hormonal secretion the acid and alkaline phosphatases have received considerable attention in the recent years particularly as a consequent of the discovery of the secretory activity in the hypothalamus and its influence on the endocrine system.

Abolins (1948) demonstrated the activity of alkaline phosphatase in the anterior pituitary of the guinea pig. Later Abolins and Abolins (1949) studied the acid phosphatase reaction in the anterior pituitary of the guinea pig at different pH. Eränkö (1951a)demonstrated phosphatase in the supraopticus and paraventricular nuclei. Cohn and Richster (1956) have shown an increase of phosphatase activity in the hypothalamus from the time the animal was born. Talanti et al.,(1958) reported acid phosphatase activity in the hypothalamus of the embryo of cow. Kobayashi and Kambara (1959a) have demonstrated in the rat greater

alkaline phosphatase activity in the posterior pituitary than in the anterior. Later they (1959b) studied the alkaline phosphatase activity in the hypothalamus and the pituitary body of the rat with special reference to neurosecretion. Kobayashi and Farner (1960) have shown the effect of increased daily photoperiod on the phosphatase activity in the hypothalamus and the pituitary of photosensitive and refractory birds. These studies have indicated the importance of these enzymes in preparing the neurohypophysis for its possible role in inducing migratory activity. It was therefore thought desirable to investigate the changes in the activity of these enzymes in the hypothalamus, median eminence, pars nervosa and anterior pituitary of the Rosy Pastor during the premigratory phase when the bird actively prepares itself for migration. The premigratory phase extends from February to the end of April. During this period, changes in body weight and fat deposition along with gonadial activity are rather gradual until the end of March or first week of April. From then on, rapid and phenomenal changes take place practically in the entire body of the bird preparing itself for the migratory phase which is observed to commence three or four days after the release of the neurosecretory material from the hypothalamus (Chapter 6). The present investigation was made during the period extending from the last week of March to the end of April.

Material and Methods

These birds arrive in Baroda (India) about September and leave by the end of April to return to their breeding grounds

in Europe. Birds of both sexes were collected by shooting them with an air rifle within the University Campus. The histochemical observations reported in this chapter were made in two sets: one in the last week of March and the other in April three or four days before migration. The brain was quickly dissected out along with the pituitary intact and fixed for 18 to 20 hours in cold 10% neutral formalin neutralized with NaOH, for the histochemical demonstration of alkaline and acid phosphatases. The material was washed in tap water followed by distilled water and embedded in 10% and 15% gelatin for 3 hours at 37°C. Gelatin blocks were prepared and preserved in cold 6% neutral formalin. Sections 12 u thick were cut on a freezing microtome, spread on albuminized slides and incubated at a temperature of 37°C for 5 to 6 hours in Gomori's modified media for alkaline phosphatase as described by Pearse (1960). Sections were also incubated in Gomori's modified medium for acid phosphatase for 3 to 4 hours at 37°C as described by Pearse (1960). The control slides for both enzymes were kept in the respective incubation medium after immersing in hot distilled water (80°C) for 10 minutes. The rest of the procedure was as described by Pearse (1960). The slides were mounted in glycerine jelly.

Results

Acid phosphatase:

During the last week of March acid phosphatase in the neurosecretory cells of the supraopticus and paraventricularis nuclei showed high enzyme activity in the nuclei of the neurosecretory cells but slightly less in the cytoplasm. A few days

prior to migration the activity increased tremendously in the nucleus as well as in the cytoplasm. The tract from the supra-opticus and paraventricularis nuclei which pass behind the optic chiasma showed the localization of the enzyme as fine granules in the axon fibres(Fig.1). Three or four days prior to migration the enzyme activity was found to increase in this region enormously (Fig. 2). The median eminence maintained steady high enzyme activity in March but shot up prior to migration (Fig. 3). At the junction of the median eminence and the anterior pituitary a very high concentration of the enzyme was observed(Figs. 4, 5). The enzyme activity in the anterior pituitary during the last week of March was found to be very high particularly more in the nucleus than the cytoplasm. On the whole, due to the very high enzyme concentration in the anterior pituitary,it was difficult to assess the difference in enzyme activity between acidophils and basophils (Fig.4). A few days prior to migration the enzyme activity increased enormously, so much so, that it was not possible to photograph this region. The posterior pituitary also showed a very high enzyme activity during the last week of March (Fig. 4). A few days prior to migration an increase in the enzyme activity was observed in this region also.

Alkaline phosphatase:

The alkaline phosphatase in the neurosecretory cells of the supraopticus and paraventricular nuclei showed greater activity of the enzyme than the ordinary nerve cells. The reaction was found more in the cytoplasm than the nuclei. A few days prior

Fig. 1 300μ

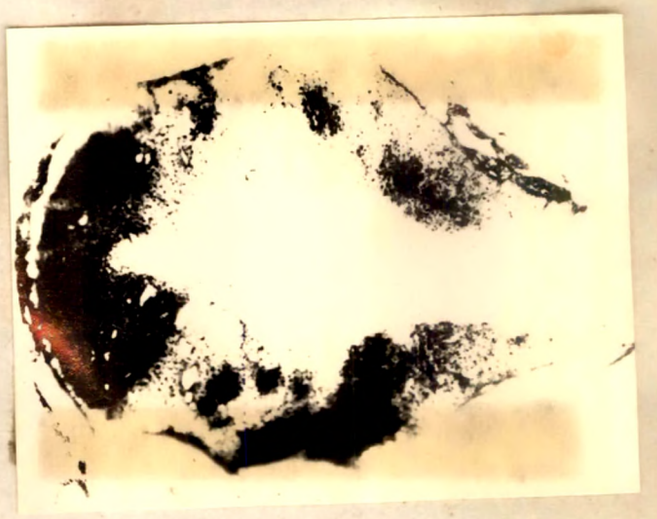

Fig. 2 100

Fig. 1. Photomicrograph showing acid phosphatase in the axon fibres of the hypothalamo-hypophysial tract in the Rosy Pastor in the last week of March.

Fig. 2. The same as above showing increased activity about the time of migration (last week of April).

300μ

Fig. 3. Acid phosphatase in the median eminence a few days prior to migration(last week of April). TThe blank portion [illegible] seen is due to a fold in the section which is out of focus)

Fig. 4 300 μ

Fig. 5 100 μ

Fig. 4. Tremendous increase in acid phosphatase, seen at the junction of the median eminence and the anterior pituitary a few days prior to migration.

Fig. 5. A portion at the junction of median eminence and anterior pituitary seen in fig. 4 magnified.

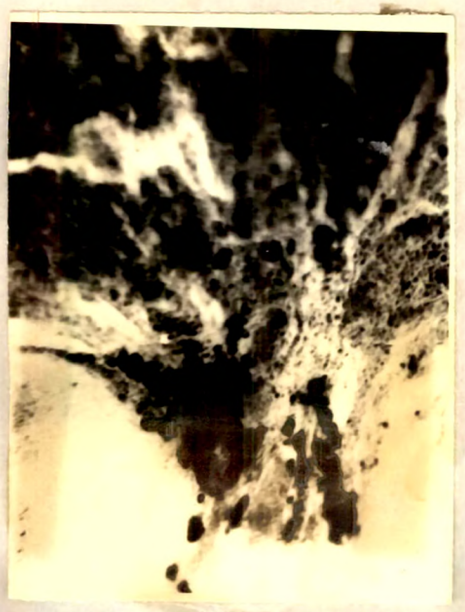

Fig. 6 300 μ

Fig. 7

Fig. 6. Photomicrograph showing the localization of alkaline phosphatase in the median eminence, and the anterior and posterior pituitary(last week of March).

Fig. 7. Increased alkaline phosphatase activity in the hypothalamo-hypophysial tract and in the anterior pituitary (last week of April).

to migration considerable increase in the enzyme activity was observed in the cytoplasm as well as in the nucleus.

The enzyme activity in the last week of March was however, not much in the anterior part of the optic tract but was considerably more in the median eminence, especially in the posterior part. In the anterior part of the median eminence the enzyme activity was observed in the cells of the neuroglea as well as in the fibrous tracts (Fig. 6). Due to the greater enzyme activity in the axon fibres passing through the median eminence towards the adenohypophysis, it was possible to trace the entire course of these axons. At the junction of the median eminence and the adenohypophysis, owing to the presence of considerably more connective tissue, the alkaline phosphatase activity was more at that region. The portal veins associated with the median eminence as well as those entering the adenohypophysis were also very rich in the enzyme activity.

A few days (three or four) prior to migration there was an increase of alkaline phosphatase in the neurosecretory cells of the supraoptic and paraventricular nuclei. The median eminence and the neurohypophysis showed the highest enzyme concentration, especially in the fibrous tract and the blood capillaries where it was the maximum (Fig. 7).

Alkaline phosphatase activity in the anterior pituitary during the last week of March was fairly high in almost all the cells, in the cytoplasm as well as in the nuclei (Fig. 6). But

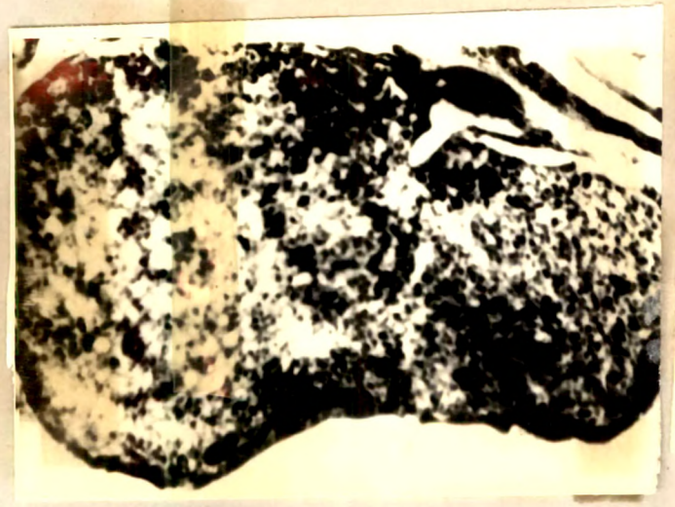

Fig. 8 300 μ

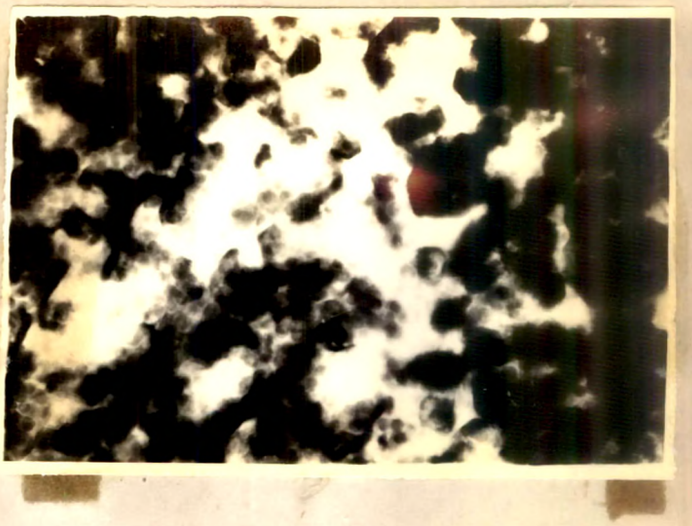

Fig. 9 50 μ

Fig. 8. The localization of alkaline phosphatase in the anterior pituitary (last week of April). Greater enzyme activity is seen in the acidophils than in the basophils.

Fig. 9. A portion of the peripheral region of fig. 8 magnified.

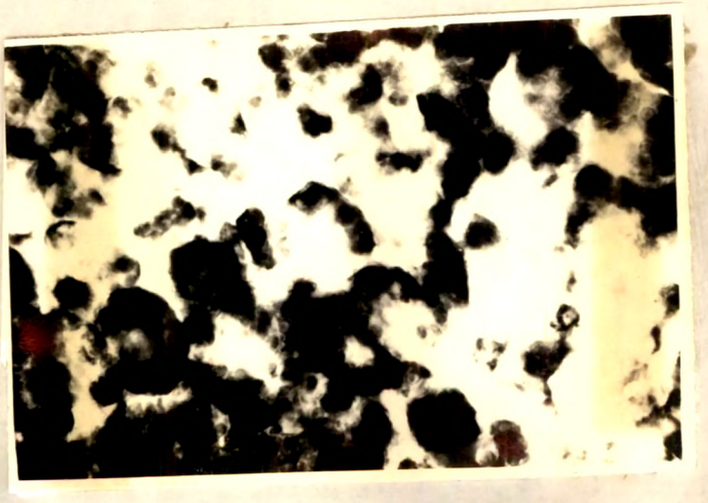

Fig. 10 50 μ

Fig. 10. A portion of the central region of fig. 8 magnified. The basophils are more in number and showing negligible amount of the enzyme.

no significant difference between acidophils and basophils for the enzyme activity was seen. The peripheral cells as well as the central cells of the gland showed more or less the same amount of enzyme activity.

Prior to migration the alkaline phosphatase in the acidophils of the anterior pituitary increased enormously, so much so, that it was difficult to see clearly the cell structure (Figs.8,9,10). The peripheral region of the anterior pituitary showed greater enzyme activity than the central (Fig.8), where there were more basophils which showed considerably less activity (Figs. 9,10). On the other hand, the central region showed higher enzyme activity at the end of March. Some of these basophils which lost most of the enzyme activity were identified as thyrotrope cells (Chapter, 7). Three or four days prior to migration these thyrotrope cells lost their cytoplasmic granules.

In pars nervosa, enzyme activity increased slightly prior to migration in the blood capillaries and nerve fibres. The concentration of the enzyme during this phase was comparatively less than that in the median eminence.

Discussion

In Rosy Pastor the neurosecretory cells of the supraopticus and paraventricular nuclei showed increased staining for paraldehyde fuchsin and Gomori's CHP a few days before migration (Chapter, 6). The activity of acid and alkaline phosphatases also increased prior to migration. Alkaline phosphatase activity in the hypo-

thalamus was histochemically demonstrated by Sloper (1955) and his observations were similar to that of Gomori's (1941) using the CHP staining reaction for neurosecretory cells. An increase in the acid phosphatase activity was observed by Kobayashi and Farner (1960) in the neurosecretory cells of the hypothalamus in the photoperiodically treated white crowned sparrows and they correlated this increased enzyme activity to the increased secretory activity of the neurosecretory cells. Talanti et al., (1958) found in the hypothalamus of three month old cow embryo, paraldehyde fuchsin-positive material in the neurosecretory cells. Eränkö (1951a) demonstrated intensive phosphatase activity in the supraoptic and paraventricular nuclei of the hypothalamus of the rat, indicating the existence of protein synthesis mechanism in these sites. Cohn and Ritcher (1956) using both histochemical and biochemical methods, observed concentrations of acid and alkaline phosphatases increasing in the hypothalamus of the rat as the animal grows from the time of birth. Methyl green pyronin Y - staining in the hypothalamus of the Rosy Pastor, showed an increase in the intensity of staining in the neurosecretory cells a few days prior to migration (Chapter, 6). This shows that RNA in the neurosecretory cells increases during this period and thereby denotes increased protein synthesis. It has also been suggested that the increase in acid phosphatase activity might be influencing the active synthesis of paraldehyde fuchsin-positive material (Talanti, et al., 1958; Kobayashi and Farner, 1960). The acid phosphatase activity in the optic tract and the median eminence of the Rosy Pastor also shows that the

increased enzyme activity may well be due to the increased formation of the paraldehyde fuchsin-positive material in these sites. In the median eminence and in the region between the median eminence and adenohypophysis, considerable acid phosphatase activity was observed (Figs. 4,5). This increased enzyme activity at these sites may be attributed to the high synthesis of the neurosecretory material taking place in the pituicytes of this region, and also due to the high rate of protein synthesis there. Kobayashi and Farner (1960) also observed a very high concentration of acid phosphatase in this region in the white crowned sparrows subjected to long daily photoperiod under artificial lighting. However, there was not much increase in the pars nervosa which shows that in this region there is more of storage of the neurosecretory material and less of active synthesis. The anterior pituitary showed very high acid phosphatase activity in all its cell types. In the nuclei of these cells,however, the enzyme activity was more than in the cytoplasm. This increase in acid phosphatase activity in the nuclei a few days prior to migration may be due to increased nucleic acid metabolism. Such a role for acid phosphatase has also been shown in cells of other material by Kalckar (1947). Therefore the increase in the enzyme activity in the cytoplasm may be due to the increased synthesis of the paraldehyde fuchsin-positive material. Kobayashi and Farner (1960) found that with the histochemical staining reaction for alkaline phosphatase, the axons of the neurosecretory cells could not be traced. On the other hand, in the present work it was possible to

trace the axon fibre from the median eminence to the adenohypophysis (Fig. 7). This tract was also traced with aldehyde fuchsin staining (Chapter, 6). It is a well established fact that the neurosecretory material is transported to the median eminence as well as the neurohypophysis from the hypothalamus. Similarly, the localization of this enzyme also tallied with the staining obtained with paraldehyde fuchsin. The intensity of this staining reaction also increased in the supraoptic tract, median eminence, the junction between the median eminence and adenohypophysis and the neurohypophysis a few days earlier to migration (Chapter, 6). It was also observed that alkaline phosphatase increases in all the above sites, side by side with the increase in the neurosecretory material. It is believed that this enzyme is responsible for the transport of material in the blood capillaries as well as in the axon fibres. For the transport of neurosecretory material in all the above mentioned tracts, a higher activity of alkaline phosphatase is to be expected. Kobayashi and Farner (1960) have demonstrated alkaline phosphatase in the anterior pituitary of the white crowned sparrow. They observed considerably greater activity of the enzyme in the nuclei than in the cytoplasm where it was very little. In the present work it was observed that the enzyme activity was very high in both the cytoplasm as well as the nuclei of acidophils and basophils during the last week of March. Towards the migratory phase the enzyme concentration increased enormously in the acidophils whereas it decreased in the basophils (Figs. 9, 10). The nuclei as well as the cytoplasm of the acidophils was completely loaded with the enzyme so much so

that it was difficult to identify the nuclei in the histochemical preparations. ~~(Fig. ——)~~. Abolins (1948) showed in the anterior pituitary of the guinea pig high alkaline phosphatase activity in the acidophil cells, whereas in the basophil cells and chromophobe cells, very little. In the Rosy Pastor three or four days prior to migration the thyrotropic cells were seen to undergo degranulation and the cytoplasm of the basophil cells became empty (Chapter, 7) and similarly the gonadotropic cells also showed slightly less stain with PAS (Chapter, 7). The low enzyme activity seen in the thyrotropes might have been due to the degranulation of the thyrotrope cells. The increase in enzyme concentration in the acidophils may be due to the increased activity of the hormonal secretion in these cells. It was already observed (Chapter, 7) that a few days prior to migration the acidophils increased in number and also showed higher intensity in the various staining reactions e.g. PAS, Alcian blue, Orange G (Chapter, 7). Three or four days before migration the chromophobe cells increased in number as a result of the degranulation of some of the basophils as well as acidophils, thus accounting for the low enzyme activity.

Kobayashi and Kambara (1959a; 1959b) observed that alkaline phosphatase activity in the pituitary of the rat was more in the posterior pituitary than in the anterior. Kobayashi and Farner (1960) observed in the white crowned sparrow more alkaline phosphatase in the posterior pituitary, the highest concentration being in the capillary wall of the lobe. In the present work also the same results were obtained. The increase

in enzyme activity a few days prior to migration may be enhanced by the release and active transport of the neurosecretory material supported by an increased circulation in this region.

Summary

1. The intensity and localization of the acid and alkaline phosphatases activity was studied in the hypothalamus, hypothalamo-hypophysial tract, median eminence, anterior and posterior pituitary in the last week of March and at the end of April three or four days prior to migration, in the Rosy Pastor.

2. A few days prior to migration a greater increase in the activity of acid phosphatase was observed in the nuclei of neurosecretory cells and the anterior pituitary than in their cytoplasm. The increased enzyme activity in the nuclei is correlated with the increased nucleic acid metabolism while that in the hypothalamo-hypophysial tract, median eminence, the cytoplasm of the neurosecretory cells and pars nervosa is correlated with the increased activity of the paraldehyde fuchsin-positive material in these sites.

3. The increased activity of alkaline phosphatase prior to migration in the neurosecretory cells, the hypothalamo-hypophysial tract, median eminence and the posterior pituitary is correlated with the released and active transport of the neurosecretory material and the increased ciculation of blood in those sites.

4. A few days prior to migration the activity of alkaline phosphatase reached its peak in the acidophil cells which more or

less coincided with the time of increased synthesis of hormones. The low activity of this enzyme noted in the basophils at this time is explained as due to the degranulation of thyrotrope cells and the decreasing activity of the gonadotrope cells. It is also suggested that the increase in the number of chromophobe cells might also contribute to the lowering of the enzyme activity.