

CHAPTER 11

HISTOCHEMICAL CHANGES IN THE ACTIVITY OF ALKALINE AND
ACID PHOSPHATASES, IN THE ADRENAL OF THE MIGRATORY STARLING;
STURNUS ROSEUS (LINNAEUS) TOWARDS THE MIGRATORY PHASE

It has been shown that one of the major influences of hormonal action is on enzyme systems (Meyer and McShan, 1950; Miner, 1951; Roberts and Szego, 1953). The influence of hormonal moieties on the structure and physiology of the adrenal cortex is well known (Burrows, 1949; Yoffey, 1952). Gemzell and Samuels (1950) have shown that the rate of incorporation of P^{32} into the intracellular inorganic phosphate component of rat adrenal, was increased by adrenocorticotrophic hormone (ACTH). Allen and Slater (1956) found that in castrated male ^{mice} ~~rats~~ alkaline phosphatase activity in the adrenal cortex was considerably low. On treating with testosterone propionate or ACTH the enzyme activity was raised to its normal level. They also observed that on treating the castrates with estradiol benzoate a strikingly higher elevation of the enzyme activity resulted in the outer fasciculata of the adrenal. In the adrenal medulla of mouse (Allen, 1956) under conditions of insulin shock, inanition or cold stress, increase in alkaline phosphatase activity has been demonstrated. With regard to acid phosphatase activity Eränkö (1951^b, 1952) observed regions of positive and negative islets of the enzyme activity in the adrenal medulla.

In migratory birds, some observations have been made on

the changes in alkaline and acid phosphatases activity in the hypothalamus and neurohypophysis (Kobayashi and Farner, 1960). These studies have indicated the importance of these enzymes in preparing the neurohypophysis for its possible role in inducing the migratory activity. In the light of these findings it was thought desirable to investigate the changes in the activity of these enzymes in the adrenal during the premigratory phase when the bird is actively preparing for migration. In the Rosy Pastor, the bird under investigation, this period was found to extend from the last week of March to the end of April.

Material and Methods

Birds of both sexes were shot in the morning with an air rifle. One set of observation was made in the last week of March and another towards the end of April. The adrenals were immediately removed and fixed for 12 to 16 hours in cold 10% neutral formalin neutralized with NaOH, for the histochemical demonstration of alkaline and acid phosphatases. After fixing, the material was washed thoroughly in running tap water. Gelatin blocks were prepared and 12 μ sections were cut on freezing microtome. The sections were placed on albuminized slides and incubated at a temperature of 37°C for 4 to 6 hours in Gomori's modified medium for alkaline phosphatase as given by Pearse (1960). Sections were also incubated in Gomori's modified medium for acid phosphatase for 3 hours at 37°C as given by Pearse (1960). The control slides for both enzymes were kept in the respective incubating medium after immersing in

hot distilled water (80°C) for 10 minutes. The rest of the procedure was as described by Pearce (1960). The slides were mounted in glycerine jelly.

Results

Alkaline phosphatase:

The localization of alkaline phosphatase in the adrenal cortex showed considerable seasonal variation. About 3 weeks prior to migration the cortex showed abundant alkaline phosphatase activity in the cytoplasm and little in the nucleus (Figs. 1 & 2). The adrenal cortex in males were found to be more active for this enzyme than in the females. In the medulla, however, there was very little enzyme activity. At the time of migration the activity of the enzyme in the cortex increased enormously as was denoted by the extremely dense staining in the whole of the cortex. In the nucleus too, considerable increase in the enzyme activity was noticed (Figs. 3&4). It should also be mentioned that there was an increase in area in the peripheral region of the cortex, but without any zonal differentiation with respect to the enzyme as was observed in the pigeon by Ghosh (1962).

Acid phosphatase:

Seasonal variation in the level of acid phosphatase was also observed. This enzyme activity however, was little in the cytoplasm of the medullary cells about 3 weeks prior to migration (Figs. 5 & 6). But in the nucleus as well as in the Golgi bodies of the medullary cells the enzyme activity was a little more.

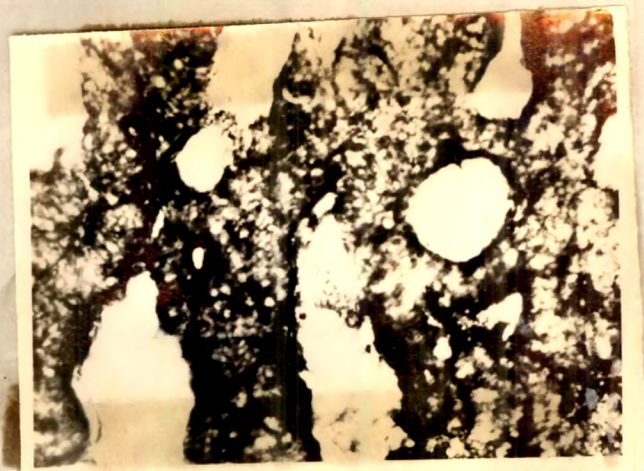


Fig. 1

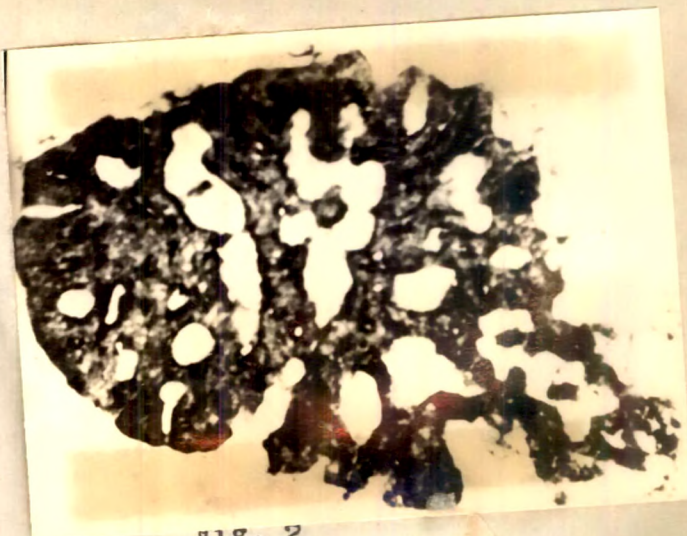


Fig. 2

Fig. 1. Photomicrograph of the T.S. of adrenal of Rosy Pastor in the last week of March showing higher alkaline phosphatase activity in the adrenal cortex than in the medulla.

Fig. 2. A portion of Fig. 1 magnified.



Fig. 3

300 μ



Fig. 4

50 μ

Fig. 3. Increased alkaline phosphatase activity in the adrenal cortex a few days prior to migration (last week of April).

Fig. 4. A portion of Fig. 3 magnified.



Fig. 5

Fig. 6

Fig. 5. Photomicrograph of the T.S. of the adrenal of Rosy Pastor in the last week of March showing the localization of Acid phosphatase in the cortex and medulla. The enzyme activity in the cytoplasm of the medullary cells are seen to be more than that in the cortex cells. The nuclei also show greater activity of the enzyme.

Fig. 6. A portion of Fig. 5. magnified.

50 μ

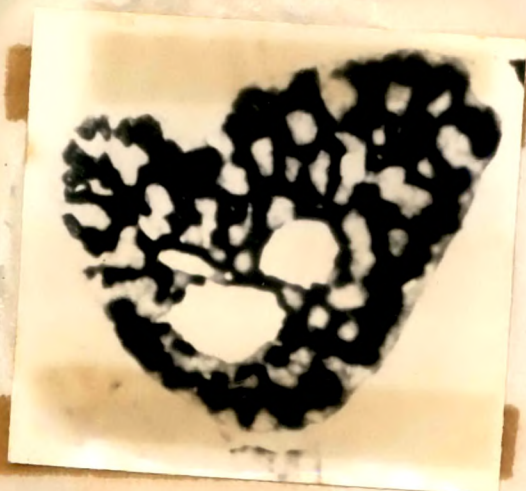


Fig. 7

50 μ

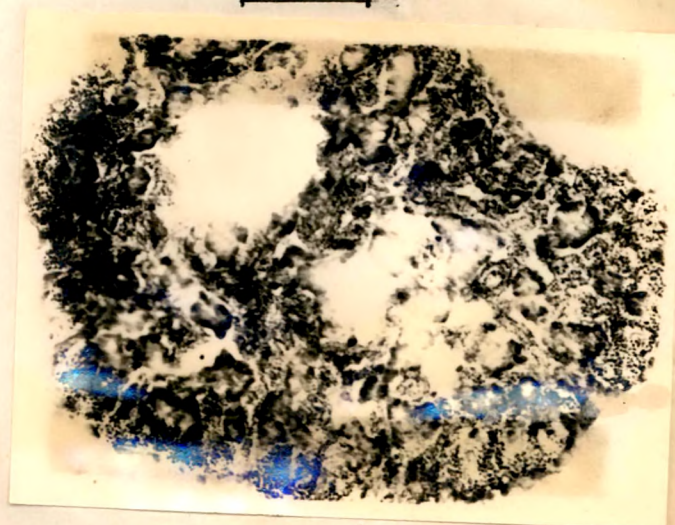


Fig. 8

Fig. 7. The increased activity of acid phosphatase in the medullary cells just prior to migration (last week of April).

Fig. 8. A portion of fig. 7. magnified.

In the cytoplasm of the cortical cells also the enzyme activity was very meagre while the nuclei and Golgi body showed slightly higher activity. At the time of migration the peripheral region of the cells of the medulla showed very high increase in the acid phosphatase activity (Figs. 7 & 8), whereas there was only little increase in the nuclei of these cells. In the cytoplasm of the cortical cells there was an increase but in the nuclei considerable increase in the enzyme activity was noticed.

Discussion

It is known in mammals that there is a higher concentration of alkaline phosphatase in the adrenal cortex of males (Elftman, 1947; Dempsey *et al.*, 1949; Nicander, 1952). Similar sex difference with regard to the enzyme activity in the adrenal cortex was also seen in the Rosy Pastor. In this bird, however, the difference could not be ascertained histochemically when compared to those females during the month of April, since in both males and females the activity of the enzyme in the adrenal cortex increased considerably. It has been shown in male mice that alkaline phosphatase activity ceased completely in the adrenal cortex after castration (Allen and Slater, 1956). Testosterone propionate administration in castrated males however, restored the enzyme activity to normal (Allen and Slater, 1956). Estradiol benzoate treatment in castrates also resulted in the development of strong activity of the enzyme in the outer fasciculata of the adrenal cortex (Allen and Slater, 1956). Elftman (1947) observed that alkaline phosphatase is absent or

present only in traces, in the normal female mice, the castrated male and the immature male. Treatment with testosterone propionate has also been shown to result in the appearance of alkaline phosphatase in the adrenal cortex of the castrated male, the ovariectomized female and in immature ones of both sexes (Elftman, 1947). So it is clear that there is a relationship between a greater supply of gonadial hormone and increased alkaline phosphatase activity in the adrenal cortex. In male Rosy Pastor after the last week of March when the testes develop at a very fast rate and while in the female the gonads correspondingly show hardly any development, the enzyme activity was considerably higher in the male adrenal cortex than that in the female. The higher enzyme activity obviously was influenced by male hormones. But it has been reported by Kar (1950) that in pigeons treated with sex hormones the cortical phosphatase concentration is reduced. However, the degree of this reduction was found to be more pronounced in birds receiving estrogen than in those receiving androgens. These observations of Kar which are contrary to the findings of several others, need confirmation.

ACTH administration has been found to increase the metabolic activity in the adrenal cortex during steroidogenesis (Carpenter et al., 1946; Ganong and Forsham, 1960) and since the role of alkaline phosphatase in glucose transport (Rosenberg and Wilbrandt, 1952), is known, it is logical to expect an increase of this enzyme with ACTH stimulation. According to Allen and Slater (1956) ACTH does not have any appreciable effect upon cytoplasmic alkaline phosphatase but does increase the alkaline phosphatase activity

associated with the capillary sinus. Symington et al., (1958) and Burns and Hale (1959) found after stimulation with ACTH the adrenal cortex increased in RNA and alkaline phosphatase. Logan et al., (1955) demonstrated increased incorporation of P^{32} labelled phosphate into the ribonucleotides of rat adrenal as a result of ACTH administration. Chèveremont and Firket (1953) suggested that the increased alkaline phosphatase activity is ^{concerned} ~~considered~~ with the increased nuclear metabolism involving increased nucleic acid synthesis. In the case of the Rosy Pastor an increase of the corticoids in the adrenal cortex has been histochemically demonstrated (Naik and George, 1963). Increase in lipids, RNA and the cortex cells themselves with a corresponding increase of ACTH has been noted a few days prior to migration. The increase in alkaline phosphatase activity in the cortex should be expected to increase the glucose transport and the intracellular phosphatase turnover thereby bring about an increase in cortical cells resulting in the general hypertrophy of the gland. The increase in general corticoid synthesis towards migration noticed in this bird has been ^tattributed to a possible increase in ACTH level (Chapter 10).

According to Allen (1956) an increase in alkaline phosphatase activity in the medulla under conditions of stress, is accompanied by a differentiatial release of adrenalin or noradrenalin. An elevation in alkaline phosphatase activity under conditions of cold stress, particularly in cells which appear to be depleted of noradrenalin, offers further evidence in support of the thesis put forth by Hillarp and Hokfelt (1954) that cells identifiable by

potassium iodate oxidation represented specific secretory elements and not secretory phases of a common cell type. But from the observations made in the present investigation it became clear that alkaline phosphatase activity in the medulla does not show any appreciable change during the period under investigation.

Rapid increase in the acid phosphatase level of the medulla during the period of about 3 weeks prior to migration is a striking change, especially the greater activity of acid phosphatase on the peripheral region of the medullary cells 3 weeks prior to migration and the full distribution of this enzyme in the entire cell of the medulla just before migration indicates the role of these cells for the synthesis of adrenalin and noradrenalin. It is already shown that a few days prior to migration the histochemical localization of adrenalin and noradrenalin increases in the medullary cells (Chapter 9). Eränkö (1951, 1952) has investigated the acid phosphatase of rat adrenal medulla and has found that isolated clusters of medullary cells are rich in this enzyme. He finds that the distribution and the concentration of acid phosphatase are not changed by insulin shock or treatment with thyroxin.

The increase of acid phosphatase in the nuclei of medullary as well as cortical cells indicates the higher activity in the nucleus prior to migration. The increase of the size of the nucleus as well as the increase in nucleoplasm of the cortical and the medullary cells towards the migratory phase has already been mentioned (Chapter 9). The increase of acid phosphatase in the nuclei of the cortical and medullary cells may be due to the higher synthesis of

nucleic acids in the nuclei as Kalckar suggested (1947).

Summary

1. The activity of alkaline and acid phosphatases was studied in the adrenal cortex and medulla in the two periods 3 weeks prior to and just a few days before actual migration in the Rosy Pastor.
2. The alkaline phosphatase in the cortex and acid phosphatase in the medulla showed very high increase towards actual migration.
3. The significance of the increase in alkaline phosphatase activity is discussed in relation with the increase in ACTH, RNA, corticoids and fat in the adrenal cortex.
4. The increase in acid phosphatase in adrenal medulla is also discussed in relation to the increase in adrenalin and noradrenalin.
5. The increase in acid phosphatase in the nuclei of the cortical and medullary cells is correlated with increase of nucleic acid metabolism.