CHAPTER 9

CERTAIN CYCLIC CHANGES IN THE HISTOLOGY AND HISTOCHEMISTRY OF THE ADRENAL IN THE MIGRATORY STARLING, STURNUS ROSEUS (LINNAEUS)

Recent histophysiological studies on the mammalian adrenal have thrown much light on the physiology of this gland (Nicander, 1952; Greep, 1954; Hillarp and Hokfelt, 1954, 1955; Logan et al., 1955; Arvy, 1960, 1962; Smit-Vis, 1962). Comparatively little work has been done along these lines on the avian adrenal. Utila (1939), Nalbandov and Card (1943), Kar (1947a, 1947b) studied the histology of the chicken adrenal; Miller and Riddle (1942), the normal adrenal and of the adrenal cortex under experimentally induced atrophy and hypertrophy in the pigeon; and Hartman and Brownell (1949) of the adrenal of several wild species of birds. Hartman and Albertin (1951) examined the adrenals of over 400 species of wild birds and pointed out the species variation in the amount and pattern of the distribution of the chromaffin tissue in the gland. Knouff and Hartman (1951) showed that adrenal of the brown pelican (Pelicanus occidentalis) consists of the cortex and medulla as in the mammalian adrenal. Sinha and Ghosh (1961) observed zonal differentiation cytochemically in the adrenal cortex of the pigeon. Ray, [illegible] and Ghosh (1961) reported that the medulla of the pigeon adrenal is particularly rich in acid phosphatase, plasmogen and metachromatic substances. In a histochemical study of the adrenal of various birds, Ghosh (1962) found varying proportions of adrenalin and noradrenalin in the medullary tissue.

In the light of the studies mentioned above it was thought desirable to conduct morphological and histological studies on the changes in the adrenal gland of the migratory bird Sturnus roseus during different periods. Observations were made from the month of October soon after these birds arrived in Baroda to the end of April prior to migration to their breeding grounds abroad.

Material and Methods

Birds of both sexes were collected every month from October to April. The adrenals were fixed in Zenker formol, formol saline and Bouin's, and paraffin sections were taken. Some sections were stained with acid fuchsin and methyl green, some with Mallory's triple stain(Gurr, 1956) using fast green as counter stain and others with PAS according to Pearse (1960). For the demonstration of Golgi body Aoyama's method (1929) was adopted as described by Baker (1950). The Fettrot 7B was used according to Pearse (1960) for the demonstration of neutral fat as well as Golgi body. For the staining of RNA Zenker formol fixed tissues were used and stained with methyl green pyronin Y according to the method of Kurnick (1955a) as described by Pearse (1960). For adrenalin, noradrenalin and lipids in the same section certain modifications in fixation were introduced. In the modifications, certain steps in technique have been incorporated partly from the methods of Hillarp and Hokfelt (1955) for adrenalin and noradrenalin and partly from that of Baker (1946) for lipids by Sudan Black B.

Small pieces of the adrenal were first treated with a

mixture of 10 volumes of 5% potassium dichromate ($K_2Cr_2O_7$) and one volume of 5% potassium chromate ($K_2Cr_2O_4$) at pH 5.6 for 16 to 18 hours at room temperature as for adrenalin and noradrenalin (Hillarp and Hokfelt, 1955). The tissue was then transferred to calcium formol as fixative for lipids (Baker, 1946) for 24 hours, washed thoroughly in running water, passed through 10% and 15% gelatin for 2 hours at 37°C and embedded in gelatin. The blocks were preserved in cold 6% formalin. Sections were cut at 12 μ on a freezing microtome and were washed thoroughly in tap water and finally in distilled water. Sections were mounted in water and photomicrographs taken. The same sections were later stained with Sudan Black B and mounted in glycerine jelly and photomicrographed. According to the method of Hillarp and Hokfelt (1955) adrenalin containing cells stained dark brown and the noradrenalin containing ones yellow brown. The same result was obtained here without any loss of activity or any change in colour. Sudan Black B stained the cortical cells denoting the presence of fat.

Observations

In the adrenal of the Rosy Pastor as in other birds, the cortex and medulla are mixed and unlike that of mammals. With Mallory's triple stain and acid fuchsin methyl green stain, the cortex can be very well distinguished from the medulla (Figs. 1 & 3). Generally the cortical cells are in paired cords and spread throughout the adrenal. The cortical cells in the peripheral region in most cases, are small and thickly packed without the

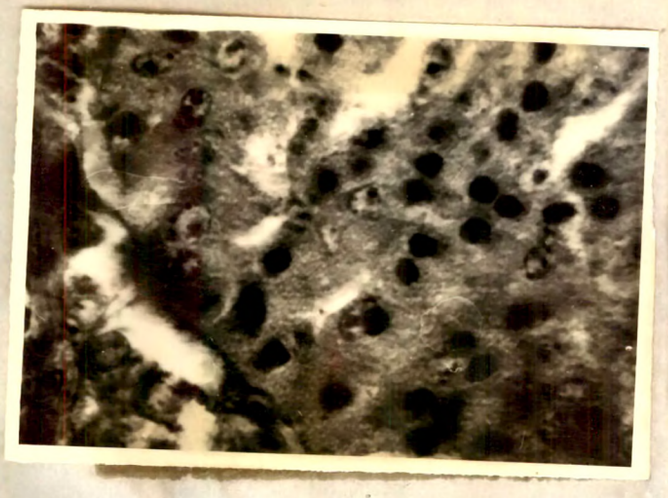


Fig. 1

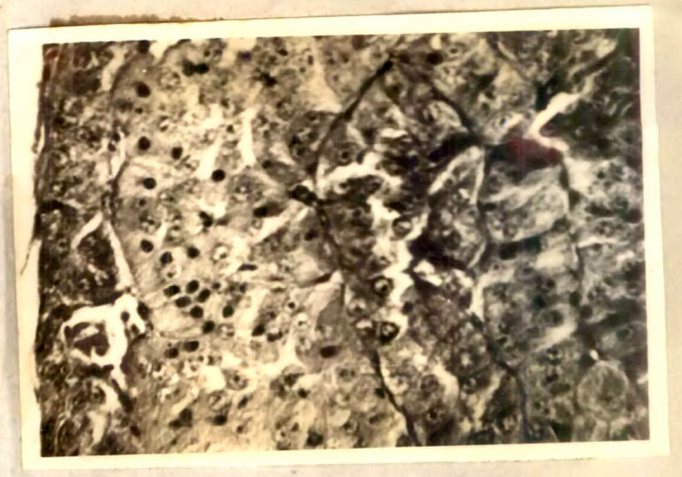


Fig. 2

50 μ

Fig. 1. T.S. of the adrenal of Rosy Pastor (October) showing the cords of cortex and medullary cells. The peripheral region is seen to contain more of the dividing and young cortical cells having darkly stained dense nuclei.(Mallory's triple stain)

Fig. 2. Photomicrograph of T.S. of the adrenal showing the hypertrophied cortical cells(third week of April) with dense cytoplasm and darkly stained nuclei.(Acid fuchsin methyl green).

100 μ

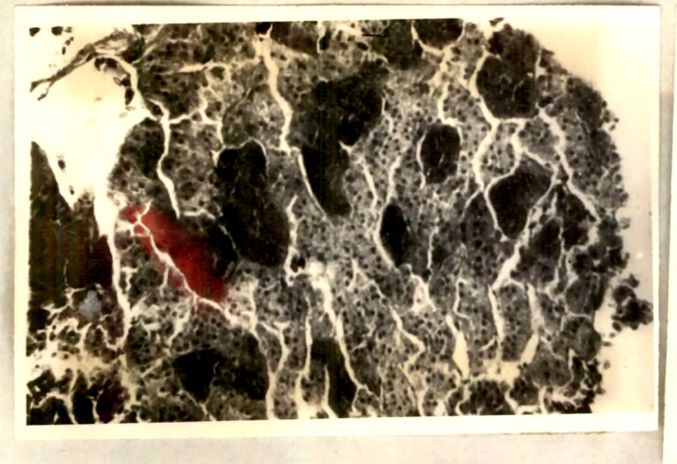


Fig. 3

Fig. 4

50 μ

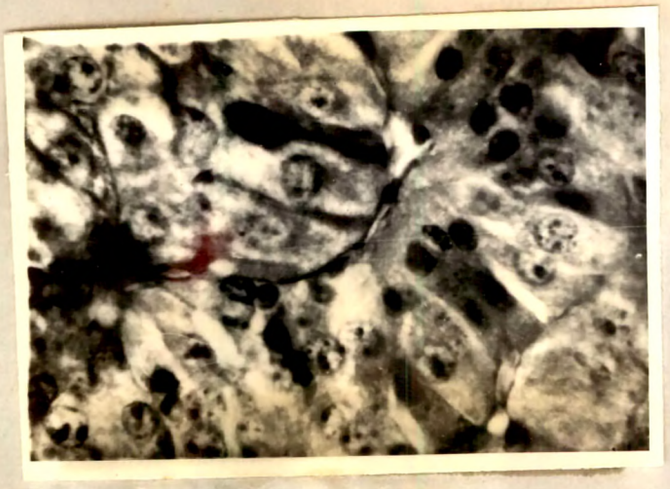
Fig. 3. Photomicrograph showing the adrenal cortex and medulla (first week of March). All cortical cells are seen more or less to be alike with less number of young cells(Mallory's triple stain)

Fig. 4. A peripheral portion of fig. 1 magnified, showing the numerous young cells in the cortex.

paired arrangement. These peripheral cells are smaller than the inner ones and possess dense nuclei and cytoplasm (Figs. 1 & 4), whereas the inner cells are bigger with less cytoplasmic material and large nucleus with scattered chromatin (Figs. 5 & 7). However, some of the inner cortical cells which are columnar in shape, have a dense nucleus and are very rich in cytoplasm (Figs. 5 & 7).

Staining with acid fuchsin and methyl green, mitochondria can be seen in the cortical cells as definite cytoplasmic granules, stained deep red. Mitochondria are numerous in the young peripheral as well as the columnar cortical cells and less in the large cells of the cortex. Similar granules are also found in the medullary cells but few in number.

Aoyama's silver nitrate impregnation for Golgi body, practically did not show much difference between the peripheral and the inner cortical cells due to the dense reaction in the cytoplasm (Fig. 6). PAS-haematoxylin staining showed a difference between the Golgi bodies of the peripheral and the inner cortical cells. The peripheral young cells and the inner columnar cortical cells showed a large deeply stained pink Golgi body whereas the big inner cortical cells showed a small light pink Golgi body (Figs. 5 & 7). The cytoplasmic PAS reaction in the cortical cells might be due to the corticoids (Chapter, 10). The Fettrot 7B also revealed very clearly the Golgi body (Fig. 8). This stain can therefore be used also to differentiate the Golgi body of the young as well as old cells as mentioned above with the PAS reaction.



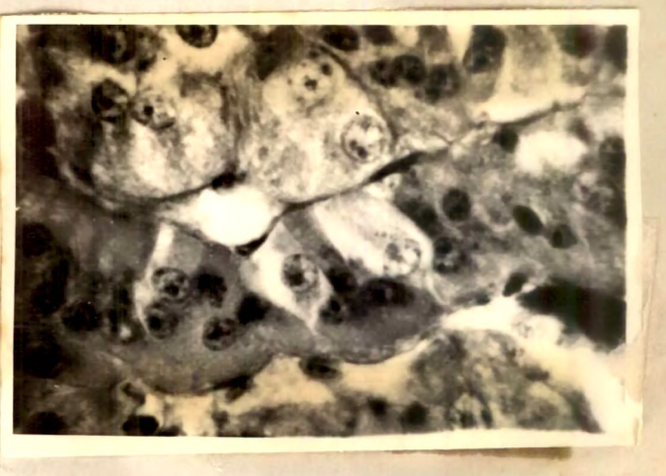
50 μ

Fig. 5 Fig. 6

Fig. 5. Central portion of the adrenal cortex showing the cells of different sizes, the columnar cells, and the large cells some rich in cytoplasm and others having less cytoplasm. The Golgi bodies of the different types of cells are clearly visible near the nuclei (PAS, haematoxylin)

Fig. 6. Photomicrograph of the adrenal showing darkly stained region of cortical cells and the Golgi bodies in the medullary cells (Aoyama's silver nitrate method).

50 μ



fo

Fig. 7 Fig. 8 100 μ

Fig. 7. Same as fig. 5

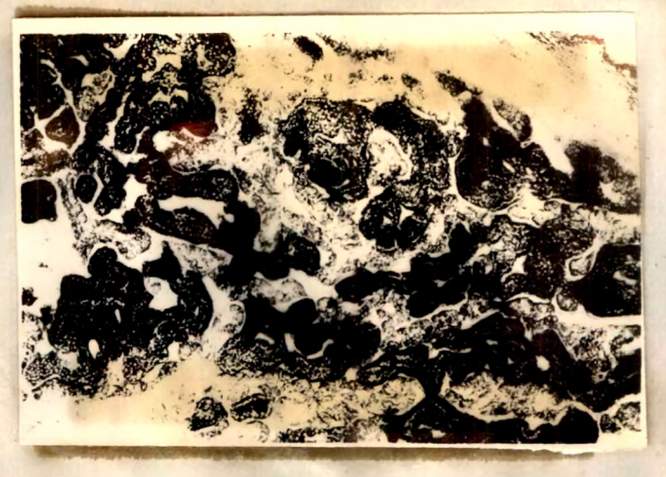
Fig. 8. Photomicrograph of the adrenal showing the distribution of neutral fat along side the Golgi bodies (Fettrot 7 B)

Medullary Cells:

These cells which are bigger than the cortical cells are found in small groups could be identified distinctly with Mallory's triple stain as well as acid fuchsin and methyl green staining (Figs. 1, 2, 3). They were usually present near blood vessels in the centre but few were found at the peripheral region of the gland below the capsule. This region consists of generally one or two layers of small cells (Figs. 9 & 10). The central cells are very large some possessing the normal cytoplasm with a large nucleus, while others have pycnotic nuclei with disrupted cytoplasm indicative of old cells.

The PAS staining gave a strong positive reaction in the cytoplasm of the medullary cells including the Golgi body (Fig. 5). Lillie (1948) also observed the same in the medullary cells.

It was possible to distinguish the cortex from the medulla with the adoption of a modification in the fixing and staining procedure mentioned earlier. The cytoplasm of the cortical cells was clearly stained as deep blue granules with Sudan Black B (Figs. 11, 12 & 13). The majority of medullary cells showed granules coloured deep brown (Figs. 12, 13, 14, 15), in the cytoplasm while very few cells were stained brownish yellow. The former are the adrenalin cells and the latter noradrenalin ones. The adrenalin granules were more distinct and numerous in the bigger medullary cells than in the smaller peripheral cells. The cells near the blood vessels were found to be very rich in adrenalin



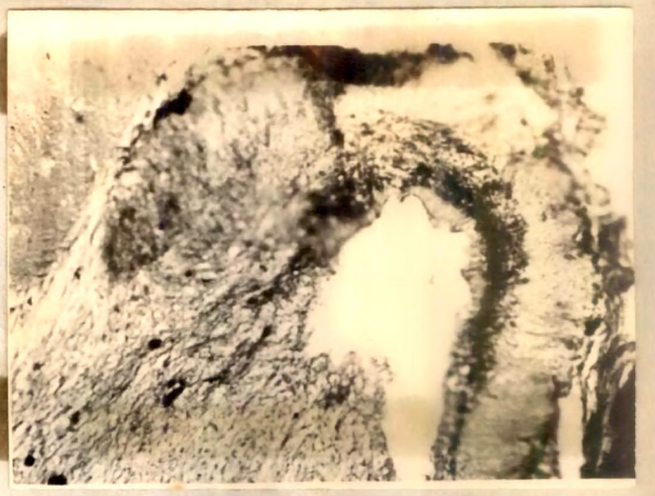
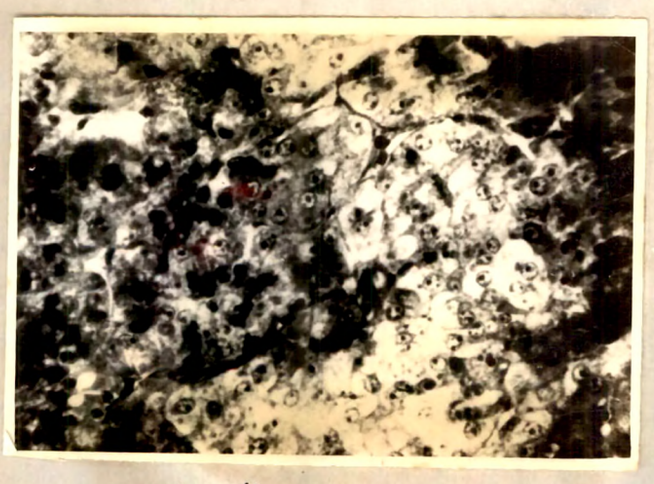
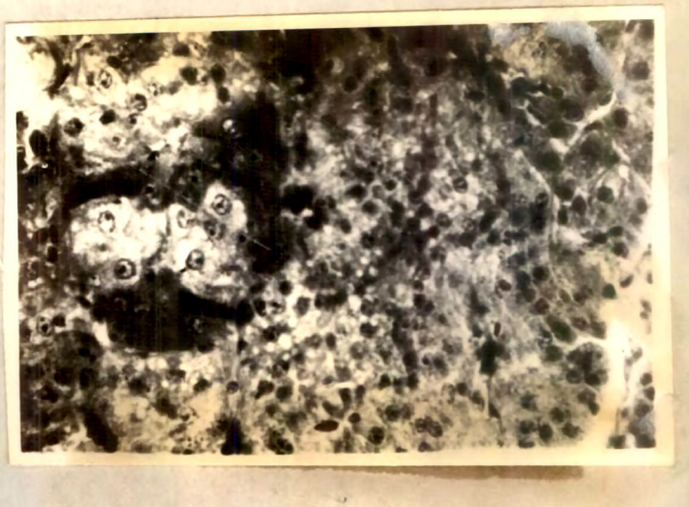


Fig. 9 50 μ Fig. 10

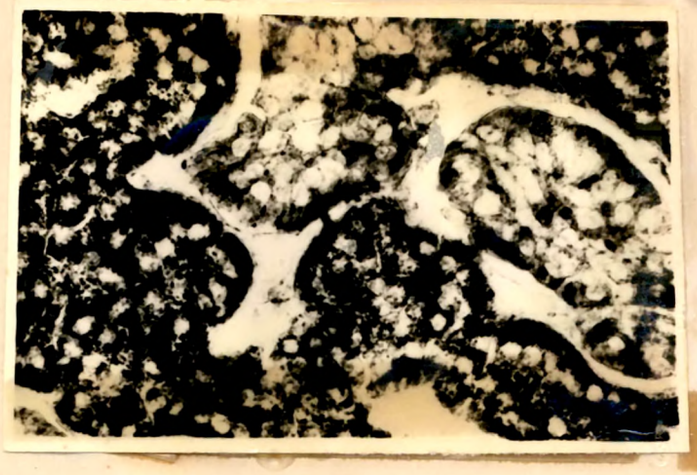
Fig. 9. Photomicrograph showing the cortical and medullary cells (acid fuchsin methyle green). Some medullary cells are very old and pycnotic and some vacuolar(last week of March).
Fig. 10. Same as fig. 9.





400 μ Fig. 11 100 μ Fig. 12

Fig. 11. Photomicrograph of the adrenal (Sudan Black B). The cortex is much more deeply stained than the medulla(last week of March).
Fig. 12. Photomicrograph of the adrenal showing the distribution of adrenalin, noradrenalin and fat in the first week of April (Potassium dichromate and chromate + Sudan Black B)



400 μ

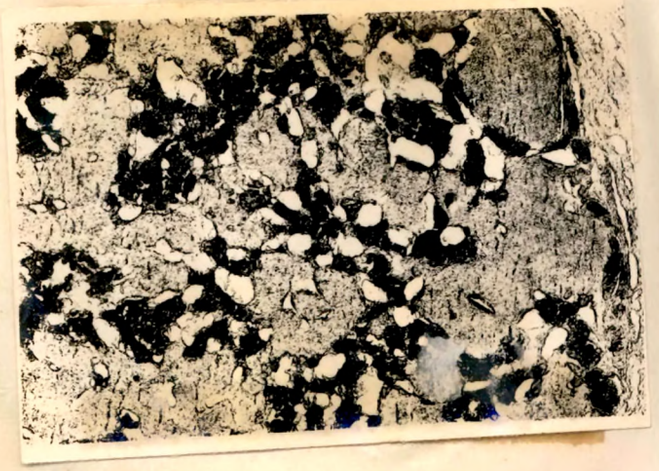
Fig. 13

Fig. 14

Fig. 13. Photomicrograph showing the greater concentration of adrenalin, noradrenalin and fat in the last week of April a few days prior to migration(stained as fig. 12).

Fig. 14. Portion of fig. 13 magnified. The cytoplasm of the cortical cells fully loaded with fat. The medullary cells contain more of adrenalin and noradrenalin.

50 μ



100 μ

Fig. 15

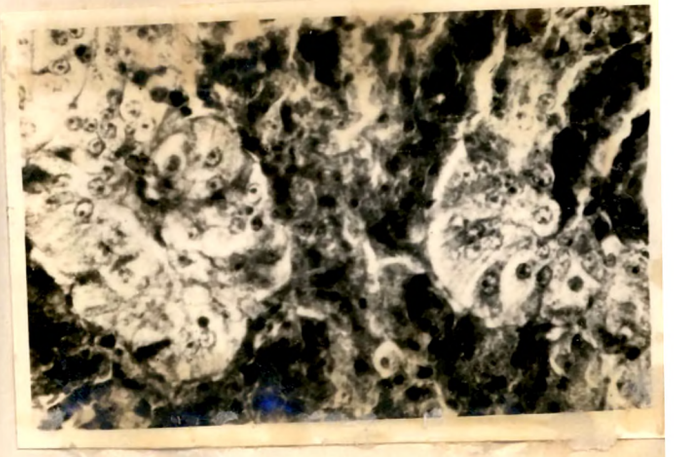


Fig. 16

Fig. 15. Photomicrograph showing the localization of adrenalin, noradrenalin and fat in the hypertrophied adrenal gland 3-4 days prior to migration. The clear patches are the empty blood vessels

Fig. 16. Hypertrophied adrenal cortex and medullary cells 3-4 days prior to migration. The cortex cells do not show the normal cell structure and the medullary cells and their nuclei are increased in size (Mallory's triple stain).

granules. The pycnotic cells showed very poor reaction for adrenalin.

Cyclic changes in the Adrenal Gland:

In terms of the histological and histochemical changes observed in the cortical region of the adrenal, three phases (from October to April) in the activity of the gland could be visualized.

1. October to January:- Period of moderate activity.
2. February to March:- " " low activity.
3. April:- " " highest activity.

During the first phase, the peripheral cortical cells were found to divide mitotically, giving rise to numerous young cells (Figs. 1 & 4). This phase continued until December and thereafter gradually slowed down in activity to reach the low level attained in the second phase. The interior columnar cells as well as the large cortical cells, however, increased in cytoplasm storing more fat. The peripheral young cells as well as the columnar cortical cells showed a greater intensity of PAS reaction in the Golgi body than those of the large cortical cells. The number of mitochondria increased in almost all the cortical cells. The RNA reaction also increased considerably and much of the cells generally showed hypertrophy.

In the second phase the young peripheral cells were very few in number (Fig. 3) and amongst these cells only a few showed mitotic activity. Most of the peripheral cells were larger

with less cytoplasm and the columnar cells were also few with less cytoplasm. The Golgi body gave a weak reaction with PAS. The nuclei, though bigger in size appeared more as the vesicular type with little nucleoplasm, the staining of RNA was seen comparatively light and the mitochondria were few in number.

In the third phase beginning from early April, the activity of the cortical cells showed rapid increase giving rise to numerous young cells. These new young cells as well as the old ones showed a tremendous increase in lipids, RNA, mitochondria and PAS-positive material in the Golgi bodies resulting in an overall hypertrophy of the cortical cells (Figs. 2 & 16).

Regarding the medullary cells no distinct histological and histochemical changes could be observed except at the time just prior to migration. At this time, however, a definite increase in cell size, RNA, mitochondria and adrenalin and noradrenalin reaction was noted(Figs. 15 & 16). Blood supply towards the medullary cells was also found to increase towards migration.

Discussion

It has already been mentioned that by fixation with calcium formol for lipids after treatment with potassium dichromate and potassium chromate, it was possible to histochemically demonstrate adrenalin and noradrenalin and on further staining with Sudan Black B, lipids also in the same section. On staining fresh section with Fettrot 7B which is considered to be more or less specific for neutral fats, it was confirmed that the regions stained with Sudan

Black B by and large consisted of neutral fat. The treatment with potassium dichromate and potassium chromate, therefore offered fixation for neutral fat at the same time stained adrenalin and noradrenalin in the tissue. It should also be mentioned that transferring the tissue from the dichromate solution having a pH 5.6 to the neutral calcium formol, produced no adverse effect on the staining reaction. On the other hand, when the tissue slices were treated with a mixture of the dichromate solution and neutral calcium formol the staining offered for adrenalin and noradrenalin was very poor. The inhibition of the staining reaction for adrenalin and noradrenalin by previous treatment with formalin, has already been pointed out by Lillie (1948).

It is well known that in the mammalian adrenal, the cortical cells arise mitotically on the periphery of the cortex and these young cells later migrate towards the interior of the gland. The same proceds was observed in the pigeon also by Miller and Riddle (1942). It is generally believed that the newly formed young cells are very slow in synthesizing the cortical hormones. But in the Rosy Pastor on the other hand a number of new cells were found to be formed mitotically which were highly active as far as could be judged cytologically and cytochemically. These young cells in the Rosy Pastor showed a dense nucleus with a higher concentration of cytoplasm than that of the large cortical cells. The Golgi body too showed a higher concentration of the PAS-positive as well as Fettrot 7B positive materials. The higher activity of the Golgi body is to be regarded as indirectly related to the greater synthesis of the

hormones, since it is well established that the Golgi body in the glandular cells has an important role in the synthesis of the secretion (Junqueira and Hirsch, 1956; Naik, 1959). In the light of these observations it can be concluded that the young cortical cells are more actively engaged in the synthesis of the hormonal material than the old cells. When the cortical cells contain more of young cells in the peripheral region, such a gland is to be considered as in an active state. In the Rosy Pastor the cortex at the time of migration increased in bulk as a result of rapid multiplication of the peripheral cells as well as of the hypertrophy of these cells due to greater hormone synthesis. In the period just prior to migration, the cortex showed higher concentrations of fat and RNA. It is therefore clear that the increased activity in the adrenal cortex is not only morphological but also biochemical. The increase in fat in the cortex is in some way connected with increased corticoid synthesis which has already been demonstrated (Chapter, 10).

The increase of RNA and fat in the cortex and the overall hypertrophy of the cortex just prior to migration may be due to the increased influence of ACTH on the adrenal cortex (Chapter, 7). Logan et al., (1956) demonstrated that ACTH increased in the incorporation of P^{32} labelled phosphate into the ribonucleotides of rat adrenal. Hartman et al., (1954) observed the increase in the amount of fat in pelican adrenal cortex by treating with ACTH. Höhn (1961) and Zarrow et al., (1962) found adrenal enlargement in ACTH injected birds. An increase in fat in the cortical cells

as a result of cold stress has been shown (Wendler et al., 1954; Knigge, 1957; Raths and Schulze, 1957; Zimny, 1959). Cold stress is also known to stimulate thyroid function (Knigge, 1957; Söderberg, 1958; Hoffman and Zarrow, 1958). The increase of cortical activity in the first phase may be due to the pre-winter hyperphagia and the higher activity of the thyroid towards the winter months (November to January). In the Rosy Pastor, from October to January, the thyroid was found to be active (Chapter, 4). It is now well established that the thyroid influences the cortical activity in the adrenal (Brown-Grant et al., 1954; Levin and Daugnaday, 1955; Money, 1955; Brown-Grant, 1956; Evans, et al., 1956; Smit-Vis, 1962). It is also experimentally shown that if the thyroid activity increases, the cortical activity in the adrenal also increases and vice versa (Deane and ~~Roy~~ Greep, 1947; Feldman, 1951; Berkheiser, 1955 and Peterson, 1958). Similarly in the Rosy Pastor, the thyroid activity from late January to March is rather low (Chapter, 4)and correspondingly the adrenal cortex is also less active. Towards migration there was a sudden rise in the adrenal cortical activity and a corresponding rise in thyroid activity (Chapter, 4).

During the period just prior to migration the change in the medullary area could not be clearly differentiated. However, from the histochemical demonstration of adrenalin and noradrenalin, it was possible to conclude that the intensity of the dichromate reaction obtained increased in the medullary cells a few days prior to migration and that the blood supply near the medullary cells also increased.

In the Rosy Pastor the noradrenalin cells were very few in number. Ghosh (1962) analyzed the proportion of adrenalin to noradrenalin secreting cells in the adrenal glands of various birds. Certain birds of the family Sturnidae to which the Rosy Pastor also belongs were found to possess only a few noradrenalin cells in the adrenal medulla.

Summary

1. Histological and histochemical changes in the adrenal cortex and medulla of a migratory bird, Sturnus roseus, were studied in different months from October to April.

2. From the changes observed in the cortical region three distinct phases in the activity of the adrenal could be visualized: (1) period of moderate activity (October to January) (2) of low activity (February to March) and (3) of highest activity (April).

3. During the first and the third phases, considerable increase in the amount of RNA, fat and the number of mitochondria was observed in the cortical cells. An increase in the activity of the Golgi bodies (as shown by PAS-positive reaction) was also observed.

4. The seasonal changes observed have been discussed in relation to the corresponding changes in the thyroid as well as the influence of ACTH release towards the migratory phase.

5. No direct seasonal changes in the medulla could be observed except an increase in adrenalin and noradrenalin, a few days prior to migration.