CHAPTER 3

CYCLIC HISTOLOGICAL AND HISTOCHEMICAL CHANGES IN THE PANCREAS IN RELATION TO BLOOD GLUCOSE LEVELS IN THE MIGRATORY STARLING, STURNUS ROSEUS (LINNAEUS)

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The distribution of the alpha and beta islets in chickens of various ages, has been studied by Oakberg (1949) and found that the number of alpha cells remains fairly constant upto three hundred days but the beta cells increase. Epple (1961) has studied the pancreas of the black bird in different seasons and found variations in the different types of cells in the post nuptial and moulting periods and in Spring and Winter.

In mammals the production of new alpha and beta cells by the conversion of acini cells or from the duct cells has been described by several authors (Hughes, 1947; Johnson, 1950; House, 1958).

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Seasonal variations in the blood glucose level in different species of animals have been studied (Lee, 1936a, 1936b; Smith, 1950; Herandez and Coulson, 1951; Dessauer, 1952). Miller and Wurster (1959) reviewed the earlier literature on the seasonal variations in the blood glucose level and the morphology and the physiology of the pancreatic islets in different animals.

The role of insulin which is produced by the beta cells of the islets in maintaining the glucose level of blood is well known. In migratory birds there are several metabolic changes taking place in the body during the pre-migratory, migratory and post-migratory phases. One of the important changes occurring is the storage of fat in large amounts during the pre-migratory phase. In the process of diverting carbohydrates for fat production and storage, insulin should play a significant role. It was therefore thought desirable to study the seasonal changes in the islets of the pancreas in relation to the changes in the glucose levels in the blood of the migratory starling, Sturnus roseus.

Material and Methods

These starlings arrive in Baroda (India) about the month of September and leave towards the end of April. Birds of both sexes were collected every month from October to April by shooting them with an air rifle, the pancreas was quickly removed, cut into small pieces and fixed in Bouin, Zenker formol, Helly's and neutral formol for 24 hours. Paraffin blocks were prepared and 5 to 7 µ thick sections were cut and stained with Gomori's (1941) Chrome-alum haematoxylin for pancreas as described by Gray (1954), Heidenhain azan(Gurr, 1956) methyl green pyronin Y for RNA by Kurnick (1955a) as described by Pearse(1960).

For the estimation of blood glucose the birds were trapped by means of Japanese mist nets at 4 O' clock in the morning and blood samples were immediately taken. Blood glucose content was determined by the copper iodometric method of Somogyi et al. (1945).

Observations

In the Rosy Pastor also three types of cells were seen in the islets of Langerhans as was observed by Miller (1942) in pigeons. The alpha, beta and delta cells were found to be mixed up in the islets and not separated out into distinct islets for certain types of cells containing either alpha and delta cells or beta and delta cells together as in the pigeon. The beta cells were generally the largest cells and prepondering over all the other cell types. The cytoplasmic granules of these cells were small and stained yellow with Heidenhain azan and blue with Gomori's chrome-alum haematoxylin stain. The nuclei of these cells were spherical with little chromatin and having only one nucleolus (Fig. 1). The alpha cells were fewer than the beta cells and also smaller in size having an oval or round nucleus with generally two prominent nucleoli or rarely one(Fig. 1). The cytoplasmic granules were stained deep red with Heidenhain's azan as well as Gomori's chrome-alum haematoxylin. The delta cells were very few in number and smallest in size having different shapes but generally more elongated. The cytoplasmic granules stain deep blue with Heidenhain's azan and pink with

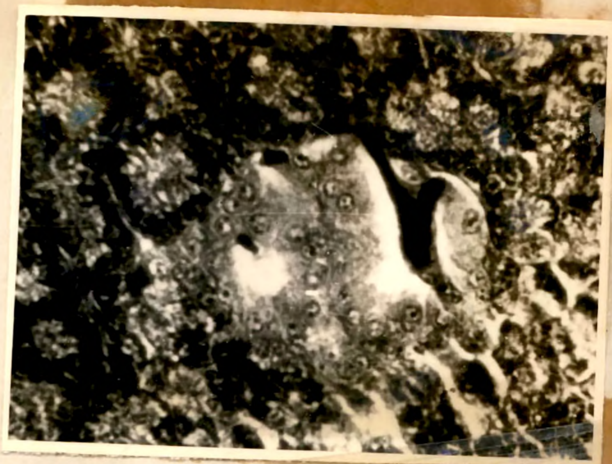


Fig. 1

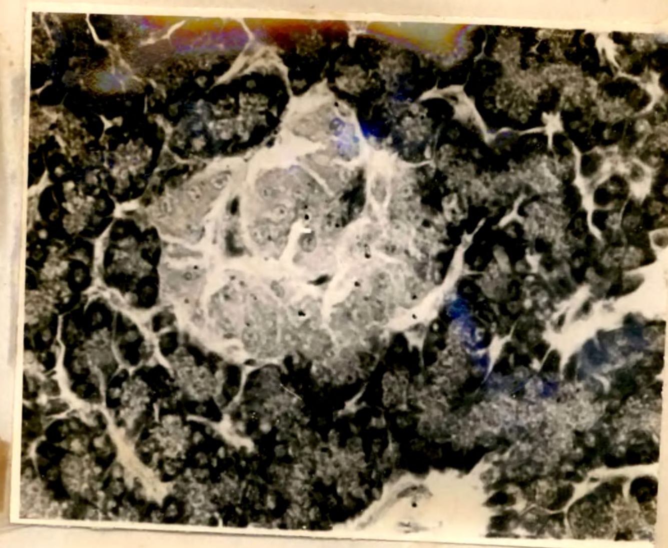


Fig. 2

Fig. 1. Photomicrograph of T.S. of pancreas of Rosy Pastor showing the acini and the islet cells (alpha, beta and delta), stained with Heidenhain azan.

Fig. 2. Photomicrograph showing the formation of new islets on the wall of the blood vessel. (Methyl green pyronin Y)

Fig. 3

50 µ

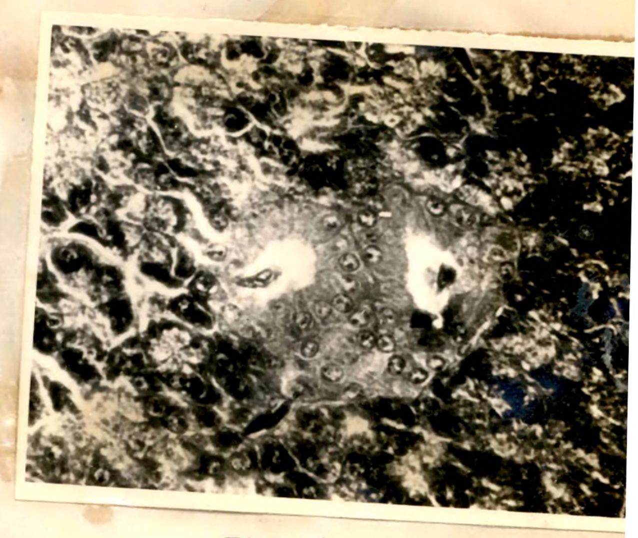


Fig. 4

Fig. 3. Photomicrograph showing the formation of new islets on the pancreatic duct. (Methyl green pyronin Y)

Fig. 4. Acini cells converted into islet cells. (Methyl green pyronin Y)

chrome-alum haematoxylin. The nuclei were small and dense.

The big islets were found generally on the anterior end of the pancreas. The beta cells were scattered throughout islets but the alpha cells were restricted mostly on the posterior ends of the pancreas. There was very little mitotic activity in these islet cells. In most cases the new islets were observed on the peripheral region of the blood vessel (Fig. 2). The epithelial cells on the wall of the blood vessel gave rise to these islet cells. These newly transformed islet cells may be either alpha or beta cells. The wall of the longitudinally or obliquely cut blood vessel showed the formation of these new islets very clearly. In few cases it was also found that the epithelial cells of the pancreatic duct gave rise to islet cells just like those of the blood vessel (Fig. 3). In many cases it was observed that the acini cells were transformed into islet cells. This production of islet cells from acini cells in Rosy Pastor formed the main type of cell conversion. When the acini cells were converted to islet cells, in the beginning they shrink and become smaller in size with considerable reduction in the number of zymogen granules. The staining for RNA confirmed the conversion of the acini cells to islet cells. The amount of RNA decreased considerably when the acini cells were converted into islet cells. The gradual decrease of RNA could be correlated with the degranulation of zymogen granules. The successive stages in the reduction in the intensity of staining for RNA could be studied only with the methyl green pyronin Y stain. The newly forming islets contain about 5 to 6 cells in one group and gave exactly the appearance of the round acini group of cells having a central empty space (Figs. 4 & 5). Later the islets arranged themselves so that more and more new cells were accommodated within the space resulting in the complete disappearance of the central lumen. In most cases when the islet was freshly formed from an acini group the lumen was probably occupied with blood cells. When three or four acini groups were converted to individual islets they ultimately united to form a single islet. In such islets some times the blood capillaries which remained in between the acini groups took up a position at the centre of the newly formed large islet. Those acini cells were converted either into alpha or beta cells. In most cases it was found that the delta cells were also present at the peripheral region of the islet.

Cyclic changes:

On the basis of the activity of the islet cells as assessed from the increase in their numbers and in the formation of the secretory substances under investigation it was possible to distinguish four phases in the pancreas of the Rosy Pastor during the period October to April.

(1).	October	:	Period of moderate activity.
(2).	November to mid-December	:	" " high activity.
(3).	Mid-December to mid-March	:	" " low activity.
(4).	Mid-March to late April (till migration)	:	" " high activity.

During the first phase the newly formed small islets were numerous whereas the big islets were very few. The acini cells were moderately active and contained a good amount of RNA. The islets contained most of the beta cells and only few alpha cells.

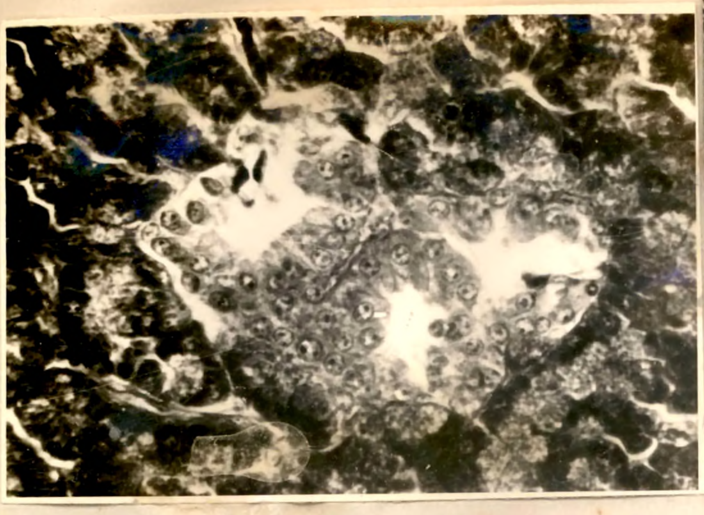


Fig. 5 50 µ

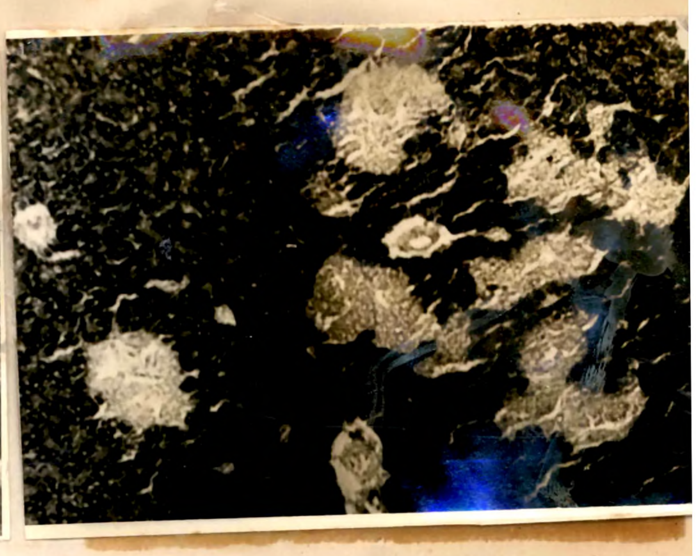


Fig. 6 200 µ

Fig. 5. Photomicrograph of the islets newly formed from acini cells. (Stained with methyl green pyronin Y).

Fig. 6. The degeneration of the islets (December) showing in some places invasion of the islets by the acini cells.

Fig. 7 50 µ

Fig. 8

Fig. 7. The hypertrophy of acini cells towards the migratory phase. (Methyl green pyronin Y stain)

Fig. 8. Disintegration of acini cells just 3-4 days prior to migration. (Methyl green pyronin Y)

In the second phase most of the islets were very large and the few others very small (Fig. 6). The former had an irregular outline and did not have the typical round shape. In several cases the acini cells were found invading the islet tissue (Fig. 6). Towards the end of the second phase the islet cells were found to be degenerating and most of the nuclei of these cells became pycnotic.

The first part of the third phase which marked the advent of winter may be considered as the most inactive phase. All the large islets were completely degenerated leaving a few small ones. By the end of March which is also the end of the third phase regenerative activity in several new islets was observed.

The last or the fourth phase was again marked by increased activity in the islets rapidly forming numerous new islets so as to present a state of hypertrophy. As for the acini cells in the beginning of the phase they were very active but towards the end, a few days prior to migration almost all the acini cells were found losing their content of RNA and zymogen granules. Most cells were vacuolated and completely inactive (Figs. 7 & 8).

Three or four days prior to migration the stomach and the intestine were found empty without any food material.

The blood glucose values in the Rosy Pastor are presented in Table I. The highest value 360.84 mg./100 ml of blood was obtained in the month of February. The lowest value 212.17 mg./100 ml of blood was obtained in the last week of April a few days prior to migration.

Microphotographs of T.S. of alloxan treated pigeons pancreas stained with Heidenhain azan.

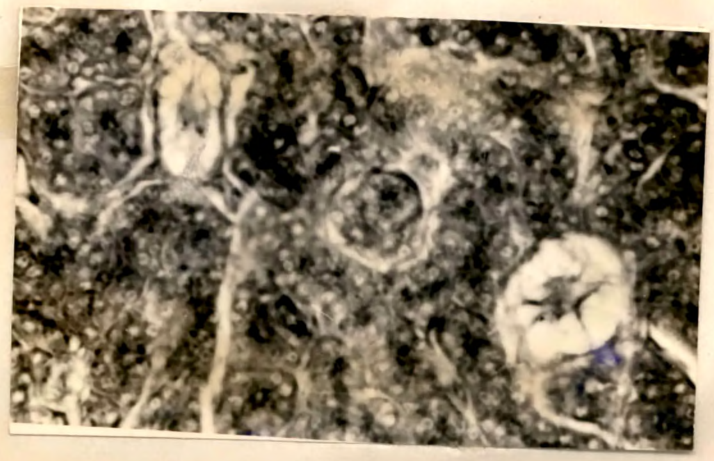
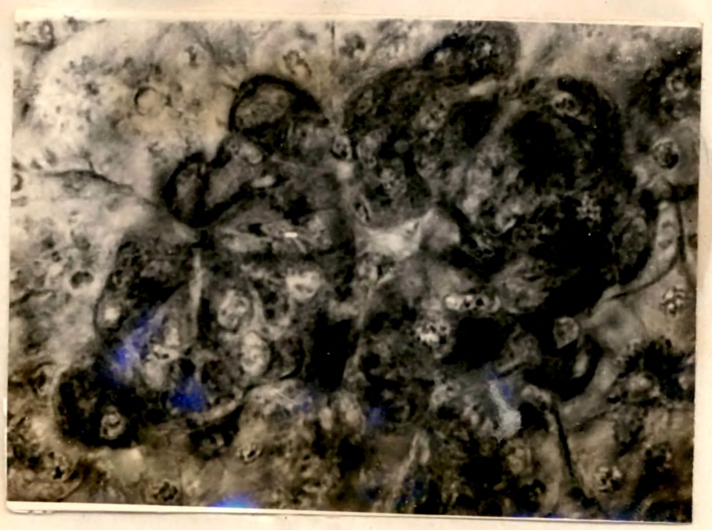


Fig. 9 50 µ

Fig. 9. The newly formed islets seen on the wall of the blood vessel(extreme left) and the pancreatic duct(extreme right) and a newly formed islet derived from the acini cells (in between).



50 µ

Fig. 10

Fig. 10. Fully formed normal islet after the sixth injection of alloxan.

Table I

Showing the changes in the glucose content of the blood of Rosy Pastor during the period February to April.

Number of birds studied	Months	Glucose content, mg/100ml of blood
5	February (first week)	360.84 ± 7.05
9	April (First week)	286.79 ± 9.92
10	April (last week)	212.17 ± 13.92

For comparison the following observations were made on pigeons.

1. Effect of alloxan treatment in pigeons:

The pigeons were given a daily intraveneous injection of alloxan (120 mg for 1000 gm body weight, dissolved in 3 ml of distilled water). The pancreas was then studied histologically. After the third injection the beta cells of the islets were found to be degranulated but thre was no complete destruction of the cells. After the fourth or fifth injection, there was an increase of new small islets containing beta cells (Figs. 9 & 10). These new cells were the result of the acini cells being converted into beta cells as was observed normally in the pancreas of the Rosy Pastor. Similarly some islets were derived from the walls of the blood capillaries as well as from that of the pancreatic duct. After the sixth injection

Pancreas of cobalt chloride treated pigeon(heidenhain azan)stained).

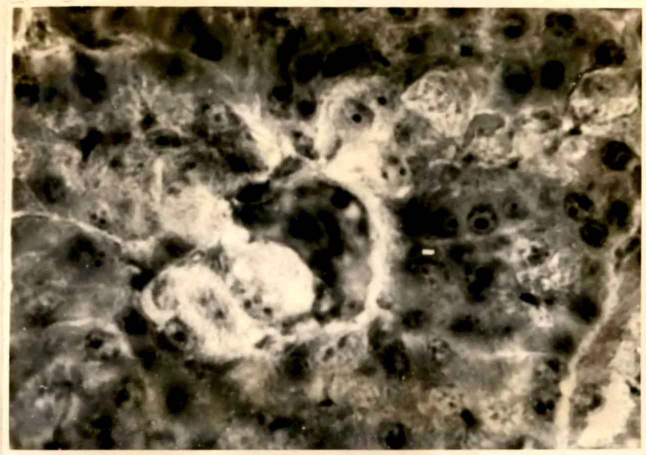


Fig. 11 50 µ

Fig. 11. Photomicrograph showing the region of dead alpha cells in the islets near the blood vessel and numerous degranulated new alpha cells.

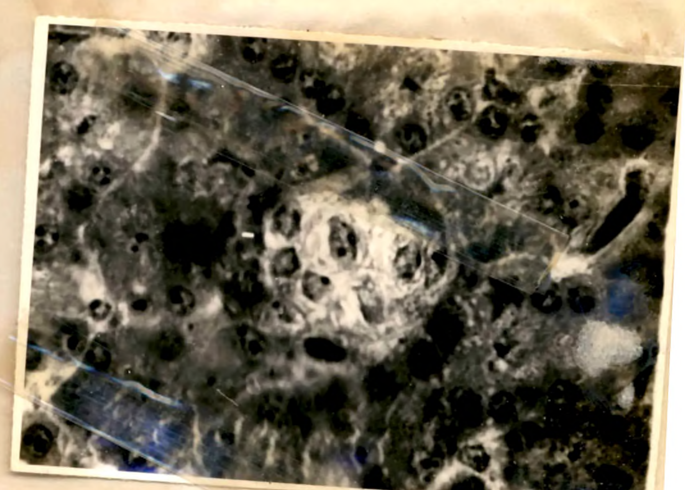


Fig. 12 300 µ 50 µ Fig. 13

Fig. 12. Photomicrograph showing several newly formed islets of alpha cells.

Fig. 13. Region of one islet magnified.

onwards there was not much preceptible change in the islet cells.

2. Effect of cobalt chloride treatment in pigeons:

The pigeons were daily given an intravenous injection of cobalt chloride (30 mg in 1000 gm body weight dissolved in 2 ml of distilled water).

With two or three injections many alpha cells were completely destroyed and many cells appeared degranulated and vacuolated (Fig. 11). After the fifth injection numerous new islets were formed and were increasing in size (Figs. 12 & 13). These islets were formed through the conversion of acini cells into alpha cells. In some cases these new alpha islets got transformed from the epithelial cells of the walls of the blood capillaries and pancreatic ducts.

Discussion

In comparison to other species of birds studied (Miller, 1942; Oakberg, 1949) the islets in the Rosy Pastor, were small and few in number. The conversion of acini cells into islet cells has been shown in most mammals (Hughes, 1947; Johnson, 1950; House, 1958), but has not been reported in birds. In the Rosy Pastor however, many acini cells were converted into islet cells and by studying the RNA content histochemically it was possible to observe the various successive stages in the conversion of these cells. It was also observed that in many cases the epithelial cells of blood vessels gave rise to the islet cells. This has also been observed in the Rosy Pastor. In certain cases however, it was observed, as was seen in mammals (House, 1958) and a bird (Epple, 1961), that the epithelial cells of the pancreatic duct also gave rise to islet cells.

In the first phase a slow increase of new islet cells from the acini cells as well as from the other sources as mentioned above, was seen. This process was further accelerated in the second phase. Meanwhile the slight hyperphagia that developed continued giving rise to greater food intake resulting in the accumulation of fat in the body (Chapter 1). In this context it should also be mentioned that the thyroid activity is lowered in November thus promoting the accumulation of fat (Chapter, 1). Miller (1942) demonstrated that the beta cells could be stimulated by over feeding normal pigeons. Starvation is known to decrease the number of beta cells and forced feeding to increase it (Sturkie, 1954). In the Rosy Pastor also during the second phase as well as towards the migratory phase (March and April) the accumulation of fat could be regarded as a result of over feeding and the stimulation of beta cells. It is therefore possible that the increase in islet cells brought about increased production of insulin in these phases as a result of which more and more glucose was converted to fat and stored in the fat depots. According to Houssay (1959) insulin increases the uptake of glucose by cells resulting in the conversion of excess glucose to fat, protein or glycogen. Excess of insulin is known to increase fat synthesis and its deficiency to reduce the capacity for fatty acid synthesis. Thus insulin acts as an anabolic hormone producing materials for storage and for growth (Houssay, 1959;

Menon, 1961). With regard to the glucose level in blood, there was a distinct decrease from February to the end of April. This reduction in glucose indicates a possible increase in insulin or decrease in glycogen or both. The histological observations support the possibility that at least insulin production was increased towards migration.

Degranulation in the islet cells during the winter months was observed in the European black bird by Epple (1961). The decreased activity of the islet cells in this species might have been caused by the low food intake in winter. In order to meet the special energy requirements of the bird during the winter months, the winter fat store should prove handy.

The observations made on alloxan and cobalt treated pigeons have shown that the newly formed beta and alpha cells respectively, are derived from the acini cells, the cells of the pancreatic ducts as well as from those of the blood capillaries. These findings also support the conclusion that the production of new islet cells from those precursor cells mentioned above takes place normally in the Rosy Pastor during the pre-migratory phase.

Summary

1. Histological changes in the pancreas in relation to blood glucose level in the migratory starling, Sturnus roseus was studied during the period October to April.

2. From the changes observed in the islets of Langerhans, four distinct phases in the activity of the pancreas could be visualized.

3. The increase in number of islet cells was mainly through the conversion of acini cells into islet cells. Production of islet cells were also from the cells of the epithelial lining of blood vessels and pancreatic ducts. These observations were confined in the pancreas of alloxan and cobalt treated pigeons.

4. The increased fat accumulation in the body towards winter as well as migration is attributed to the increased activity of the islet cells during the periods November to mid-December and later mid-March to April end.

5. The lower blood glucose level towards migration is also attributed to the increase of insulin.

6. In view of the observations that the acini cells undergo degeneration a few days prior to migration and that at the same time the alimentary tract of these birds contains no food, it is suggested that there is a loss of appetite during this period.