CHAPTER 3

LEVELS OF CALCIUM AND PHOSPHORUS IN SOME TISSUES OF THE ROSY PASTOR DURING VARIOUS MIGRATORY PHASES

The metabolism of calcium and phosphorus are mostly interrelated, when either of them is in excess the other one is usually less in plasma or in the tissues. Phosphorus is essential for innumerable functions in both the cytoplasm and nucleus of cells. Phosphorylation of intermediate substances in the metabolism of carbohydrate, fat or protein is of utmost importance to the normal functioning of any living cell. Calcium, on the other hand, does not take part in the intermediary metabolism of any nutrients but is an essential factor for blood coagulation, in the normal functioning of muscle and nerves, in the activation of certain enzymes and reactions and in the permeability of membranes.

The major role played by phosphorus is in the formation of energy rich phosphate bonds (ATP).Other important compounds wherein the phosphorus forms an integrated part, are the phospholipids. The liver is the only organ which is greatly concerned with the synthesis and removal of phospholipids. Moreover, the lipid phosphorus may also indirectly indicate the amount of phospholipids.

It is well known that a lesser fat absorption via the intestine is accompanied by a lower Ca transport through the membrane. The concentration of Ca in hepatic cells could

also have some influence on fat or vice versa. Bliecher <u>et al</u>., (1966) suggested that Ca⁺⁺ may prevent lipolysis, While Kaye and Mommaerts (1960) found that it stimulated glycolysis.

In view of all these facts, the concentrations of Ca and phosphorus were estimated in the liver, muscle and kidney of Rosy Pastors during the different migratory phases in order to obtain more insight into the various physiological processes in which they participate.

MATERIALS AND METHODS

The birds were shot in the evening and brought to the laboratory. The required tissues were removed, cut into small pieces, blotted and dried in an oven at 60- 70° C. Suitable amounts were subjected to dry ashing at 600 to 700°C. The ashed materials were dissolved in 1 N HCl and the Ca content was determined using 'EEL' flame photometer. The value is expressed as mg/100g fresh tissue.

For the estimation of phosphorus, a known amount of the dry tissue was subjectd to extraction with a chloroform : methanol (2:1) mixture. The lipids were thus extracted and the residual tissues were taken seperately for the determination of phosphorus. They were digested in $5N H_2SO_4$ with a few drops of 30% hydrogen peroxide (oxidizing agent). After digestion, the solution was diluted to a suitable volume. The estimation of phosphorus was then carried out by the Fiske and Subbarow (1925) method, in 1 ml aliquots. The values are presented as mg/100g fresh tissue.

RESULTS

The Ca content in the liver was 6 mg/100g fresh tissue in the post migratory period. In March (premigratory period) the value increased to 7.2 mg and remained more or less steady till the first week of April. By the middle of April the value again rose to 8.6 mg, but by the end of this month the level came down to 6.8 mg which corresponds with that of the postmigratory period (Fig.1). In the muscle, the Ca content was found to increase towards the end of April (Fig. 3) and in kidney it was higher in April than in March (Fig. 4).

The hepatic lipid phosphorus concentration was high (500 to 600 mg) in August (Fig. 2) but in March (premigratory period), however, it was reduced to 200 mg/100 g wet tissue. By the first week of April, the value decreased further to 100 mg. By the middle of this month, a slight increase was observed, which declined below 100mg again by the last week of April. The non-lipid phosphorus or the tissue phosphorus was found to increase gradually in the premigratory period.

DISCUSSION

It is well established that Ca^{++} has profound influence on the permeability of Na⁺ and K⁺ (Bronner, 1964). Since the concentration of the latter two ions were found to change in the liver, muscle and kidney during the premigratory period [Chapter 2), the role of Ca⁺⁺ in their movements could be suggested. Frankenhaeuser and Hodgkin (1957) observed that an increased Ca concentration reduces the membrane permeability to K⁺ and Na⁺

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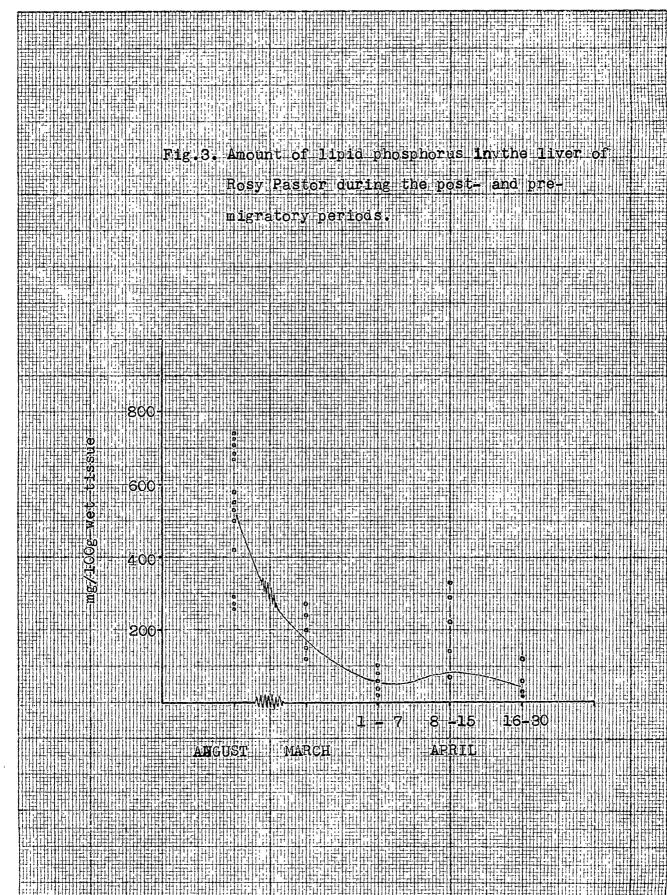
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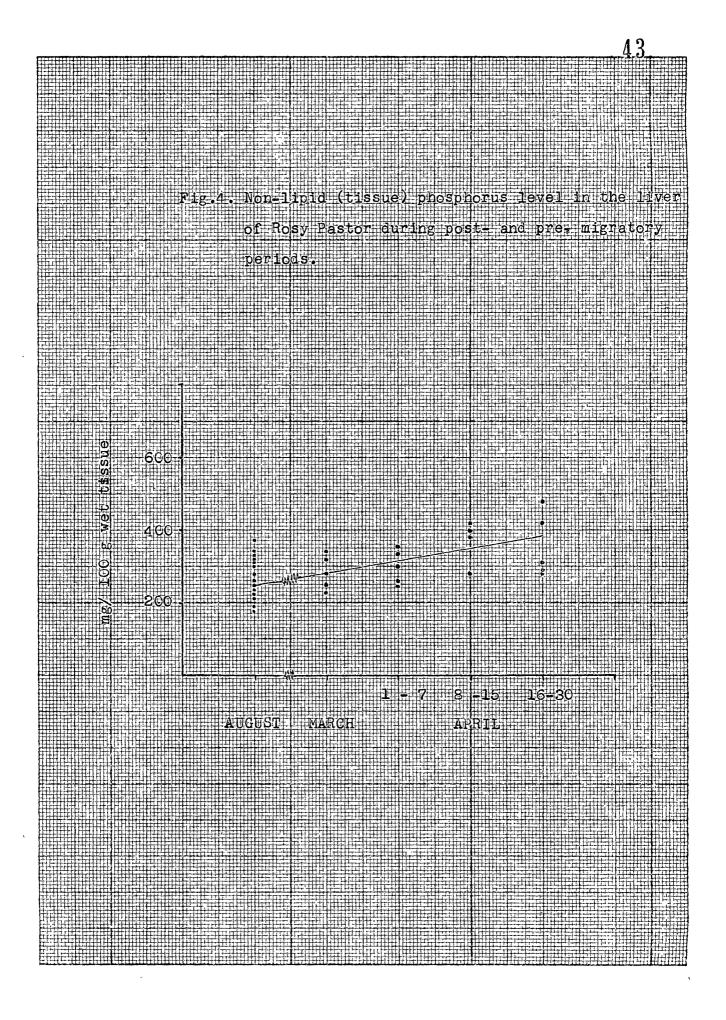
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Fig.2. Calcium content of the muscle and kidney of the Ros Bosy Pastor Ouring premigratory period. <u>T</u> निर्मान n. - FEG Ŧ ATDNEY MARCH APRIL 画明





and vice versa in high concentration. That the increased K extrusion from the liver of Rsoy Pastor during premigratory period might have been greatly influenced by the presence of Ca⁺⁺, is plausible, since Gardos (1960), has suggested such a role for Ca⁺⁺. It was also observed that as Ca⁺⁺ increased in the liver of Rsoy Pastor (in the first week of April)., the K content which was very high in March (Chapter 2), decreased. There was however no further reduction in K value from that present in the middle of April (Chapter 2), when Ca decreased by the end of April. Similarly, when an increased outflux of K⁺ and influx of Na⁺ into the muscle and kidney cells were found (Chapter 2), the Ca⁺⁺ in both the tissues increased (though not significantly in the kidney).

The increase of Ca content in the liver of Resy Pastor by the first week of April could be very effective in preventing the breakdown of fat. Bleicher <u>et al</u>. (1966) demonstrated a spontaneous lipolysis in a Ca-free medium containing a high concentration of K⁺. If a high K concentration influences lipolysis, then such a possiblity is eliminated from the liver since there is a depletion of K out of the cells. Calcium could also assist in fat synthesis by making available more ATP since Ca⁺⁺ was found to increase the turnover and activity of ATP (Judah and Ahmed, 1963). These workers also suggested that the metabolism of ATP in the liver cells was greatly influenced by the balance between the activities of Na⁺, Ca⁺⁺ and K^+ , wherein a diminished K concentration and increased Ca⁺⁺ activate ATP production.

It was also observed that high concentration of Ca increases the glycolysis (Simon <u>et al.</u>,1962). If such is the case, the decreased glycogen value in the liver (Chapter 1) when Ca level was high (by the middle of April) together with its reduction (last week of April), when an increased glycogen deposition was noted, could be explained.

Calcium was found to be influenced by thyroxine. Hypothyroidism increased its level in the serum by drawing it out of intracellular spaces (Royer and Mathieu, 1962). A slightly higher thyroxine in the blood might have caused its increase in the liver of RSoy Pastor while a high colloidal release from the thyroid gland in the last week of April (Chapter 8) could probably result in the reduction of Ca content during this period.

In muscle, the calcium is an important entity of the contractile processes. ^By facilitating the movements of K and Na ions (Brink, 1954), changing the membrane potential (Spere-lakis, 1962), and by depolarizing the membrane (Bianchi,1961), Ca^{++} improves the response of the muscle to stimulation. Cosmos (1958) observed that muscle Ca^{++} increased in stimulation. Cosmos (1958) observed that muscle Ca^{++} increased in stimulation. The restlessness of Rsoy Pastor (Chapter 1) before migration might be due to the excited condition of muscles at this period aided by an incressed Ca^{++} , sodium ions (Chapter 2) and thyroxine (Chapter 8) but a reduced K^{+} (Chapter 2).

As has been already mentioned, the major part of the

phosphorus exists in combination with various intermediary substances. The increase in the non-lipid (tissue) phosphorus could then denote higher concentrations of these compounds as well as nucleic acids, phosphorylated nucleotides, ATP etc. It also may show an increase in free phosphorus.

Since the amounts of phospholipids could be deduced from the level of lipid- phosphorus, the decreased level pf the latter therefore could be due to reduced phospholipid concentration in the liver. That phospholipid sythesis was greater in the postmigratory than in the premigratory period could also be deduced from the fact that the lipid-phosphorus level was very high in the former period, But Odum and Perkinson (1956) observed a constant phospholipid (PL) value in the liver of the white throated sparrow (\underline{Z} . <u>1. gambelii</u>). Bloor (1943) also pointed out that the PL content of most organs were more of less constant. This discrepancy seen in the liver of Rosy Pastor points to some other mechanism probably at variance from other migratory birds which resulted in the increased PL during **post**migratory period.

Among the hormones, thyroxine has influence over phospholipids. In hyperthyroidism or after administration of TSH or thyroxine, a decrease in the serum PL was observed (Deuel, 1955; Myant, 1964). This diminished PL level could be due to a depressed production in the liver, since it is the chelif source of serum PL. The effect of thyroxine has to be considered because a thyroid hyperactivity was noticed in Rosy Pastors during the premigratory period (Chapter 8). Perhaps this may be the reason why Odum and Perkinson had not seen any fluctuation in the PL content in the liver of \underline{Z} . <u>L. gambelii</u>, in which thyroid hyperplasia before migration was not reported (Oakeson and Lilley,1957). Ascorbic acid is also found to reduce Pl by accelerating their breakdown, and the increased ascorbic acid content in the liver towards the end of April (Chapter 7) might also have influenced the reduction of PL.Another factor pperating in this direction, is the low dietary supply of choline and similar substances due to a poor protein diet. Moreover, the tissue phosphorus was found to increase, indicating a nondiversion of these phosphorus for PL synthesis.

The slight increase of lipid phosphorus during the third week of April could be due to the fact that the birds captured at that time might have belonged to an entirely new flock whose premigratory activities might be belatedly set in.