CHAPTER 2

CHANGES IN THE SODIUM, POTASSIUM AND WATER CONTENTS OF THE LIVER, MUSCLE AND KIDNEY OF THE ROSY PASTOR DURING DIFFERENT MIGRATORY PHASES

Sodium forms the major component of the cations of the extracellular fluid while potassium is the principal cation in the intracellular fluid compartment. The osmotic processes of the cells are controlled by the concentrations of these two ions. Since water is freely diffusible across the cell membrane, its movement is effected by the changes in the concentrations of these two electrolytes across the cell membrane. Sodium and potassium also keep the normal state of excitability of the muscle Moreover, a large number of enzymes are activated by either Na^+ or K^+ or by both together. As, potassium is present mostly intracellularly, its outflux from the cells, draws out a certain concentration of the intracellular water also. Thus, it has the function of retaining the cellular water too.

It is known that water and H^+ should be optimal for production of fat. If water is more in the cell, the reaction that leads to the formation of fat is reversed, or in other words, a higher level of water than the optimum concentration enhances lipolysis. Bleicher et al. (1966) suggested that the Na^+/K^+ flux may influence the basal metabolic state of adipose tissue by regulating its capacity to respond to any stimuli that may activate lipolysis. This property of the electrolytes could then be decisive in orienting the tissue in either the catabolic of anabolic directions. That, potassium is increasingly deposited when there is a~~n~~ high amount of glycogen in the cells, is well known (0.34 mM potassium is retained with each g of glycogen. Harper,1965). Together with potassium a sufficient amount of water is also taken into the cell inorder to maintain osmotic equilibrium. Similarly, potassium followed by water also moves out of the cell when glycogenolysis takes place.

Thus, it is evident that the ionic concentration, and thereby the amount of water also, are influenced great~~e~~ly by the metabolic changes and adaptations in the cells. In many cases it was observed that the water content decreased with an increase in the amount of fat, such as in the liver (Naik,1963) and muscle (Vallyathan,1963) of the Rosy Pastor where heavy deposition of fat occurs during the premigratory period. Such depletion of water, either could bring about ionic movement or could be the result of changes in the ionic concentration. The present investigation was undertaken to find out if such movement of ions occurred along with the reduction of water concomit~~t~~ant with an increased fat deposition in different tissues like liver, muscle and kidney of the Rosy Pastor.

MATERIAL AND METHODS

All the determinations were carried out on birds which were shot in the evening. The tissues were removed, cut into small pieces and blotted thoroughly to remove traces of blood. These were then transfered to tared weighing bottles and dried.

The difference in the wieght after complete drying was taken as the weight of water. The values are expressed as g/100g fresh tissue. The fat content in the liver was determined by the method described in Chapter 1.

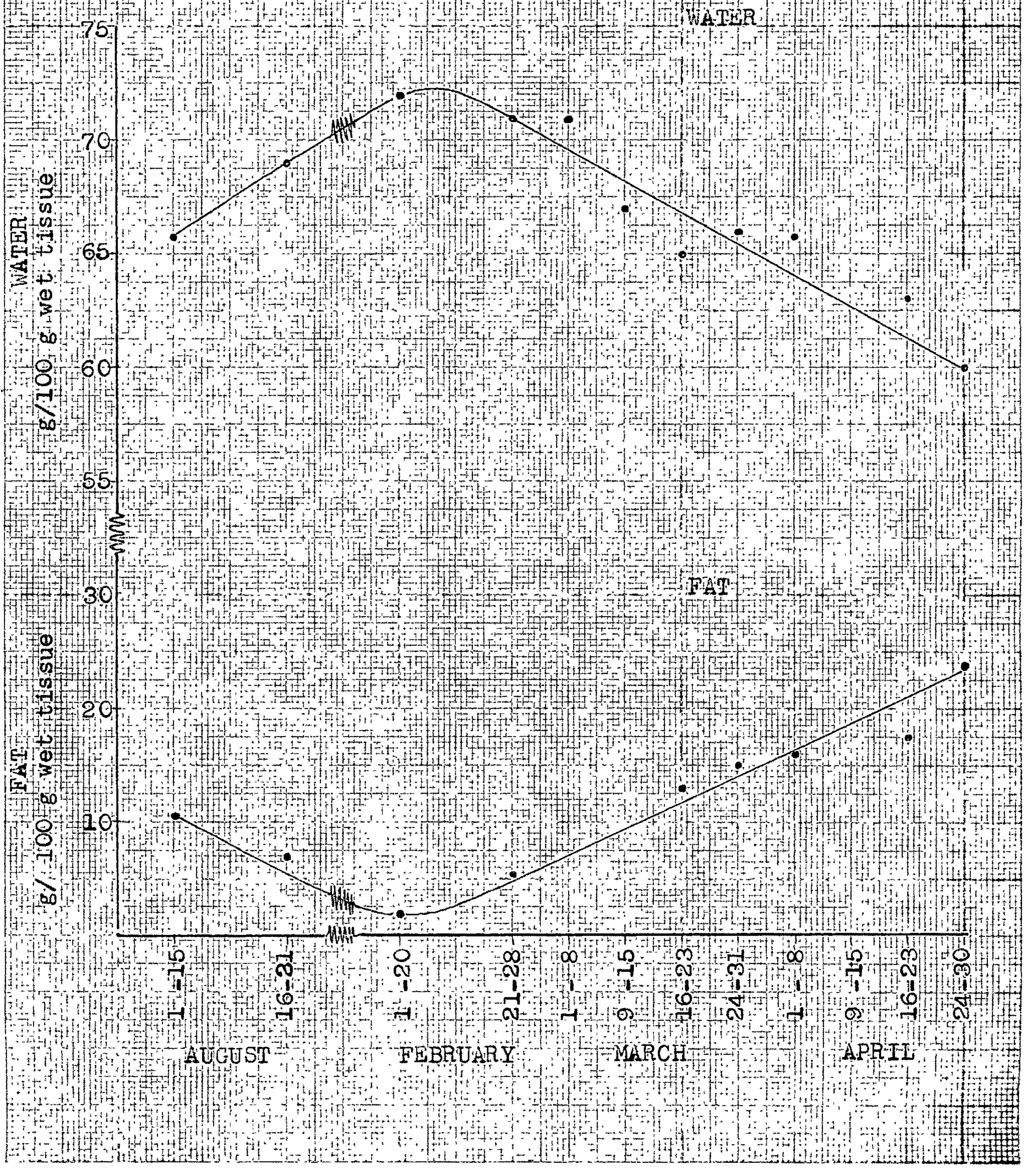
Suitable amount of dried tissues were subjected to dry ashing at 600-700°C. The ashes were then dissolved in 1N HCl and the determination of sodium and potassium were carried out by the flame photometric method using 'EEL' flame photometer.

RESULTS

The results are presented graphically in Figs. 1,2,3 and 4. It was observed that in the liver, the water content was inversely proportional to the fat level (Fig.1). But, the values for the fat free dry tissue remained more or less constant. In the post migratory period, 66% of water was present in the liver during the third week of August and by the end of this month it increased to 69 %, while the fat content declined from 10.5 g/100g fresh liver to 7g. In the premigratory period however, the water content was very high (72%) in February, while the fat content was at a lower concentration (2%). Thereafter a gradual fall in water and corresponding rise in the fat content were noticed (Fig. 1).

The changes in the concentration of potassium and sodium in the liver were equally striking. The potassium level was low (200mg/100g wet tissue) in the postmigratory period, whereas in the premigratory period the level rose to 343mg/100g wet liver in March. Thereafter it decreased gradually to 243 mg (Fig.2). Sodium showed a more or less balanced picture with a

Fig.1. The amounts of fat and water in the liver of Rosy Pastor during post- and pre- migratory periods.



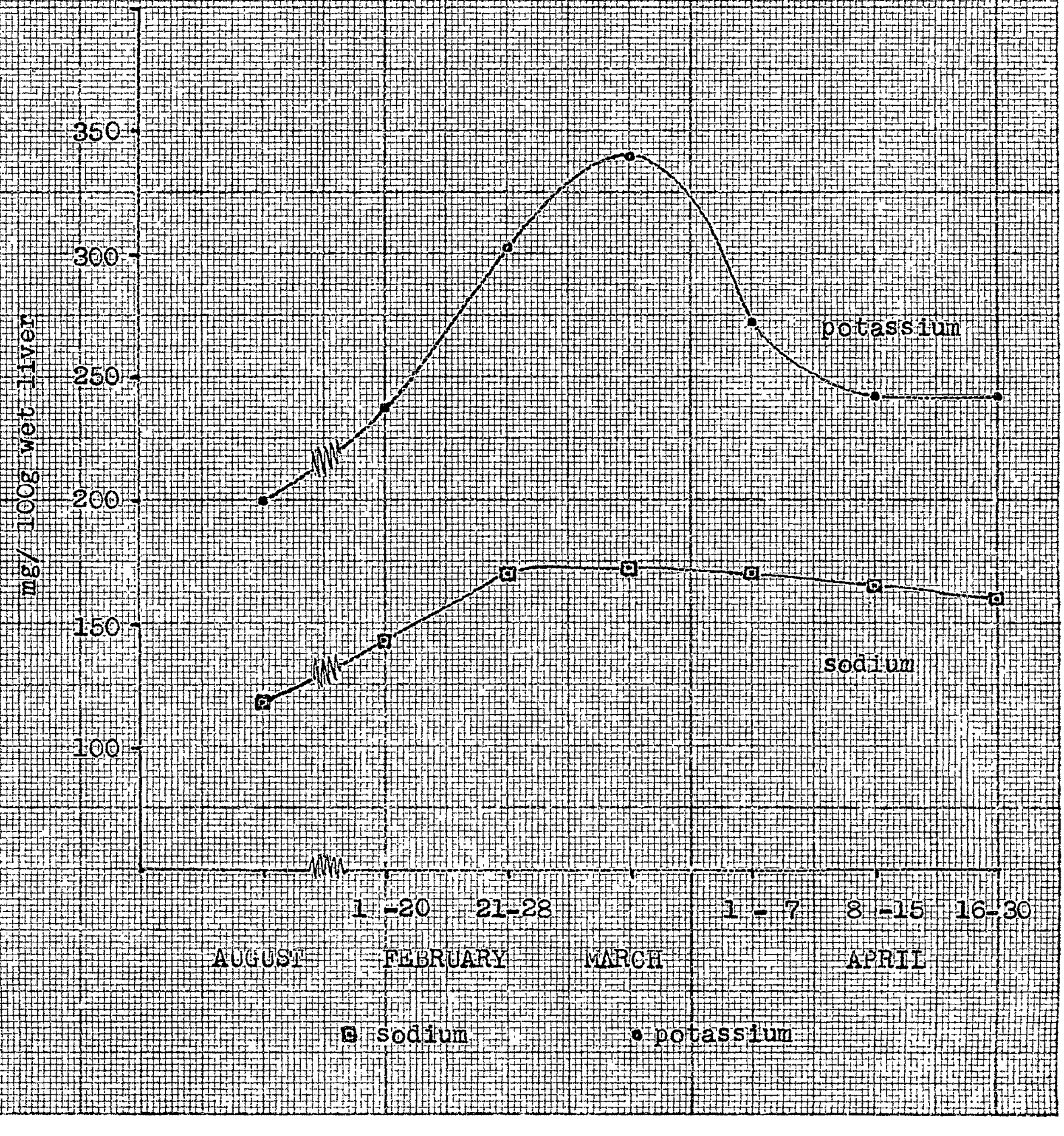


Fig.2. Potassium and sodium contents of the liver of Rosy Pastor during the post- and pre- migratory periods.

Fig.3. Sodium, potassium and water contents of the muscle of Rosy Pastor during the premigratory period.

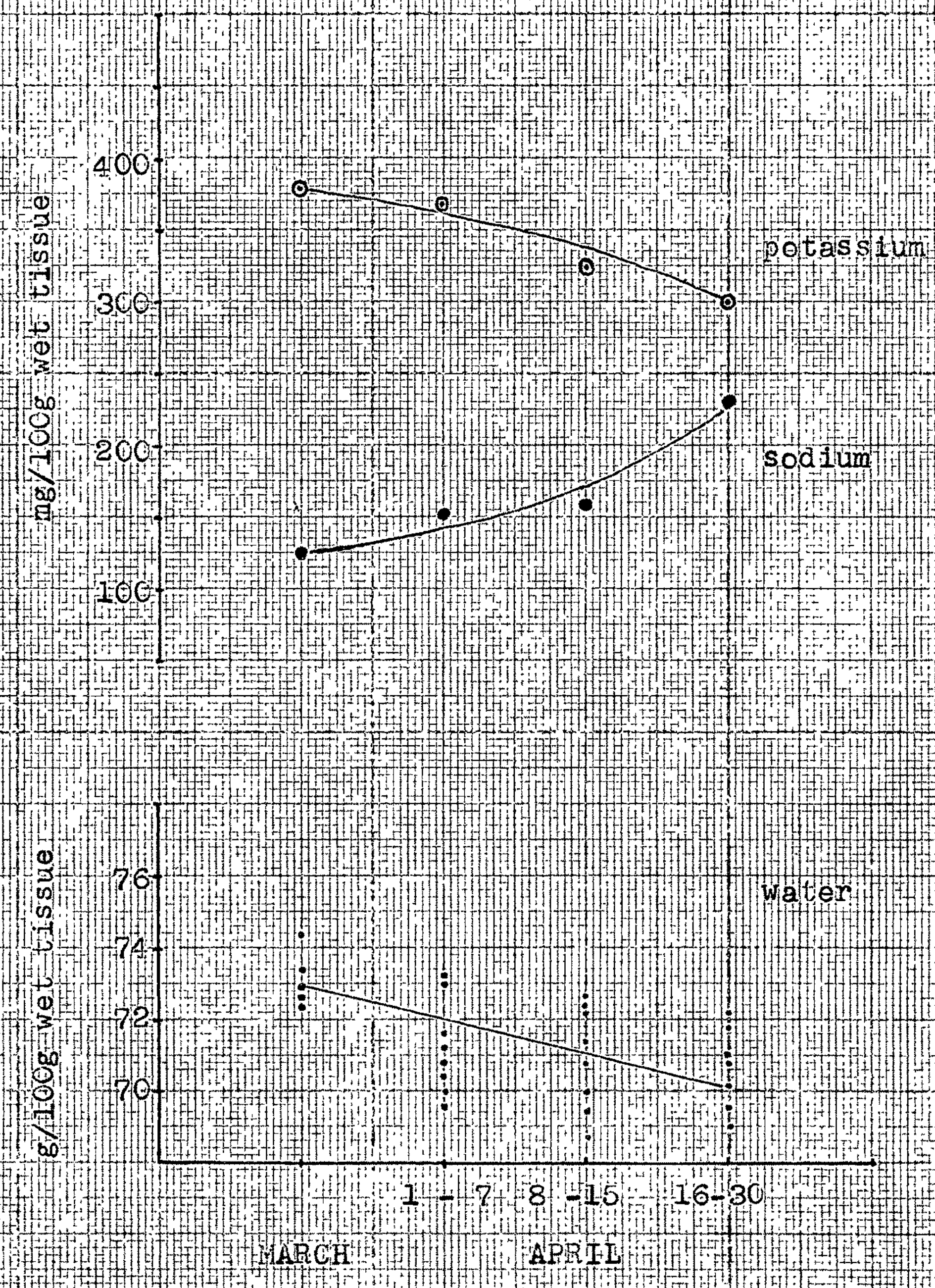
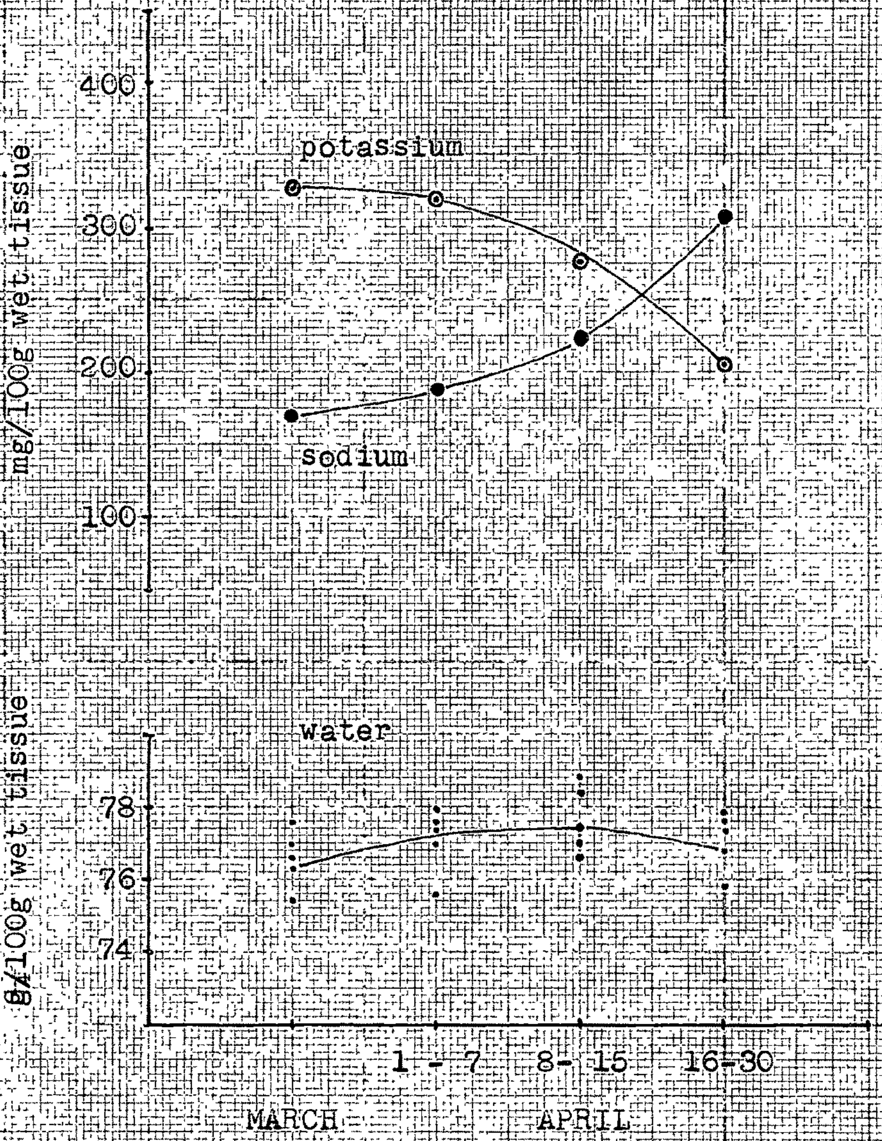


Fig.4. Sodium, potassium and water contents of the kidney of Rosy Pastor during premigratory period.



slight initial increase in the premigratory period than in the postmigratory period followed by a negligible decrease towards the end of April (Fig.2).

The determination of Na and K contents in the muscle as well as kidney were done only in the months of March and April. A decrease in the content of potassium was observed in the muscle while Na increased. However, the concentration of the former was more than that of the latter in all the stages. The watermcontent tended to decrease slightly in the muscle (Fig.3).

The potassium level in the kidney was found to decrease gradually during premigratory period and sodium increased reciprocally to potassium at the same time. However, no significant change could be noticed in the water content of the kidney (Fig.4)

DISCUSSION

A gradual decrease in the water content was evident in the liver during premigratory period beginning in February. But the decline of K was seen only by the first week of April. The electrolyte levels were found to increase in February when the water content was at the maximum. Thereafter, the Na level was kept constant and K reduced. It is thus reasoned out that the amount of water in the hepatic cells depended on the total ionic concentrations. When one (K^+) was sent out, while the other (Na^+) was prevented from increasing its concentration, the ionic equilibrium was upset and the water moved out to compensate this.

The decrease in the concentration of K and water, while Na level remained constant in the liver in the premigratory period, suggests that K^+ may have a positive role in bringing down the water content of the liver. The movement of ions out of the cells depends on two conditions: either the concentration of ions outside the cells should increase so that the water may come out of the cells to dilute the extracellular fluid or a reduction of cellular ions should occur so that the water would be forced out to maintain the osmotic equilibrium. If the outflux of water took place in response to a high ionic concentration in the extracellular fluid (ECF) then the K^+ movement would not occur. Hence, the reduction of water level was due to the shift of K^+ out of the cells and not because of an increase of ions in ECF. The effective sodium pump may prevent the free movement of Na^+ out of the cells and hence the sodium level was more or less maintained constant during March and April. Explaining the principles underlying the movement of Na and K ions across the cell membrane, Conway (1960) suggested that the shift of K^+ was an unrestricted one while the Na^+ was under the control of 'sodium pump' so that the energy consumption was minimized while keeping a balanced ionic state. Once the K^+ was extruded due to some factor, the water would passively cross the cell membrane into the ECF, and the place thus vacated might get filled up with fat. It is known that when water content is experimentally altered, replacement by tissue materials such as fat often takes place (Adolf,1947). This replacement of water by fat might be a secondary event since the fat itself cannot induce such changes (Flear et al.,1965). Then the questian arises as to why the water and potassium moved out of the cells ?. Only a cursory attempt could be made to explain such a phenomena. One explanation may be that the water is forced to go outside so as to accommodate more fat. However, in the adipose tissue the increased deposition of neutral fat was not found to be followed by a reduction in the water content (Pandazi <u>et</u> <u>al</u>., 1960). The fat in this case need not displace water, since the adipose tissue cells themselves enlarge to accommodate more fat. But, wherever the size of the cell is more or less fixed, as in the case of hepatic cells, the water may have to decrease inorder to facilitate the deposition of fat which is continuously synthesized at a high rat. If this is so, the prior evacuation of certain amount of water, which could be brought about by the extrusion of K$^+$ out of the cells, would be only for increasing the fat storing capacity of the liver cells. But a more plausible explanation could be taken from the observations of Bleicher <u>et</u> <u>al</u>. (1966) who by extrapolating their experimental data suggested that Na$^+$/K$^+$ flux might control the synthesis or breakdown of fat in the adipose tissue. The high K concentration in a Ca^{++}-free medium enhanced lipolysis. So that one could mention that a high K concentration in the absence of Ca^{++} could elevate lipolysis. In Rosy Pastor, such a possibility was eliminated by depleting K$^+$ and taking up Ca^{++} into the cells. An increased Ca content in the liver was seen at a period when active lipogenesis occurred (Chapter 3). Thus, the displacement of K^+ and thereby water also might be a prerequisite for a high rate of fat synthesis and perhaps the same factors that activate lipogenesis might also effect these too. It is obvious that the movement of $K^+$and water brings down the osmotic pressure of liver cells. The osmotic pressure of the liver is twice greater than that of blood and is often reduced by toxic agents such as carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$) (Popper and Schaffner,1957). Kume (1962) reported that CCl_4, when injected, decreased the K content but increased the sodium content in the rat liver. That, CCl_4 causes heavy deposition of fat in the liver, due to the failure of hepatic cells to remove or break-down fat, is well known. If we exclude the pathological conditions and injury that accompany CCl_4 administration, all other factors such as high fat levels, reduced K and water concentrations etc., were seen in the liver of Rosy Pastor during the premigratory period. Although some factors are similar in both the cases, yet many differ greatly. In the liver of Rosy Pastor there was no increase of Na content, the fat was removed as effectively as it was synthesized (Chapter 1) and there was no inhibition of lipogenesis. But greater amount of fat was found to be stored in the liver during this period. Thus, as in the case of CCl_4 affected liver, lipolysis was considerably inhibited in the liver of Rosy Pastor also during the premigratory period, This fact could be attributed to the lesser K and water contents.

Another factor that could influence the K and water levels is the changes in the glycogen metabolism. Whenever there is an increased glycogen formation the K concentration was also found to be high. Deane <u>et</u> <u>al</u>. (1947) suggested that K^+ must be in optimal concentration for the synthesis of glycogen. The report of Elliot and Bilodeau (1962) is of interest in this connection. They found that a low K level increases glycolysis. There are other evidences also which show that it is K^+ that determines the formation or breakdown of glycogen (vide infra). It goes without saying that the influx of K^+ during glycogen synthesis is followed by water also. About 3.6 to 4.4 g of water is retained with each gram of glycogen (Mac Kay and Bergman,1934). During the secretory phase of the liver the glycogen as well as water gets depleted. Thus, it could be reasoned out that the reduction of K and water contents from the liver of Rosy Pastor during the premigratory period might be influencing the prevention of the conversion of glucose to glycogen, in which case, the glucose load increases and the adaptive hyperlipogenesis sets in . The slightly higher level of glycogen in the last two weeks of April (Chapter 1) occurred at a period when a further reduction of K content was not seen.

In the muscle also, water showed a tendency to decrease. Depletion of water in the muscle of Rosy Pastor was also reported by Vallyathan (1963). Here, obviously, a reduction of K concentration was evident. However, the reciprocal increase of Na content rules out the possibility of an ionically induced diminition of intracellular water. Since the fat deposition in muscle usually occurs in the connective tissue in between fibres (Joseph,1967), which could reduce the size of the intercellular fluid compartment, the decrease in the water content may be simply due to its displacement by fat.

Another reason for the decrease of K in the muscle may be the muscular activity itself. Potassium is liberated from the muscles during contraction (Fenn,1939). This loss of K^+ is balanced by the gain of Na^+ (Fenn _et_ _al_.,1938). The extrusion of K^+ is also compensated by the movement of water into the blood (Fenn _et_ _al_.,1939). During the last weeks of April the Rosy Pastors were in a state of restlessness and the muscles were in an active condition being highly sensitive to stimulation. In this period the birds take longer time to calm down, circling over the trees before they perch, fighting and moving from one tree top to another. This hyperactivity termed as the ' premigratory excercises or restlessness' could be due to hormonal or nervous stimulations and this state may be one of the factors causing the ionic movements in the muscle.

In the kidney also, as in the muscle, the decreasing K level was accompanied by a reciprocal increase of Na level. But no change in the water content was observed. The lower K concentration could be due to the increased excretion of this ion, while higher sodium level might be because of an elevated Na^+-reabsorption. It is a well established fact that the kidney could excrete more K^+ only by an exchange of Na^+. This exchange mechanism is suggested to be an aldosterone mediated one (White _et_ _al_.,1964). Aldosterone raises the extracellular concentration of Na and this consequently draws out the K ions from the cells in exchange for Na$^+$. The K$^+$ that enters the plasma is eventually excreted by the kidney. Perhaps this is how adrenocortical hormones could bring about a decreased K$^+$ in the serum. In Rosy Pastor only in the liver the Na was not found to increase whereas in the muscle and kidney the outflux of K$^+$ was followed by the influx of Na$^+$. In the liver there may be some factors that prevent the influx of Na$^+$ thereby the lipogenesis was allowed to procede without any hind~~e~~rance. Niedermeir and Carmichael (1960) found that the injection of deoxycorticosterone acetate (DOCA) brings a depletion of K$^+$ from the serum and liver followed by an inhibition of glycogen formation. But when DOCA was injected with 1% KCl there was no such effect~~s~~ on K concentration or on glycogen synthesis ~~were obtained~~. They suggested that the inhibition of glycogen formation is secondary to the K$^+$ depletion in the DOCA treatement.

Since, all the tissues like liver, muscle and kidney showed a reduction of K$^+$, it could be taken as due to the stimulation ~~of~~ by adrenocortical hormones. This suggestion is well supported, since hyperactivity of adrenal cortex was observed in the Rosy Pastor during premigratory period (Naik and George,1965). In this connection it should be mentioned that the hormones of adrenal cortex influence so many physiological changes which were also found in the Rosy Pastor during the premigratory period. These hormones were found to decrease the amount of glycogen in the chick liver (Clawson and Domm,1964) and reduce the hepatic glucose and inhibit glucose utilization by peripheral tissues of rats (Lecocq *et al*.,1964).Administration of ACTH, which activate the adrenal glands, indirectly increase fat content (Li *et al*.,1949) and enhances lipogenesis (Nejad and Chaikoff,1964) in the rat liver. It could be thus reasonably suggested that the adrenal cortical hormones by affecting the ionic concentrations might have induced necessary changes for increased lipogenesis as well as the capacity of the liver for storing a good amount of fat. However, one could only say, that an interplay of a number of hormones and not of any one particular might have influenced the manifestation of all these changes in the migratory birds.