CHAPTER 7

ASCORBIC ACID CONTENT IN THE LIVER OF ROSY PASTOR DURING THE POST- AND PRE- MIGRATORY PERIODS

Ascorbic acid (Vitamin C) is now known to be a non-essential dietary inclusion since majority of the animals can synthesize it. But, man, other primates and guinea pigs are unable to do so (Wiss and Weber, 1964). Among the birds, only the red vented bulbul has been reported so far, to be incapable of synthesizing it (Roy and Guha, 1958). The liver is found to be the site of ascorbic acid (AA) production in mammals whereas, it is the kidney in reptiles and birds (Grollman and Lehninger, 1957; Roy and Guha, 1958). This vitamin is found in a greater concentration in tissues rather than in the plasma, denoting its existence in a bound state which takes an active part in various metabolic processes in the cells (Burns *et al.*, 1951). The bound form was reported to be a protective measure against the degrading action of enzymes, especially those of the liver (Hassan and Lehninger, 1956). A certain amount is always stored in tissues such as adrenals, liver, spleen and bone marrow, in varied concentrations (Burns *et al.*,1951). The normal rat liver (Hassan and Lehninger, 1956) and that of both normal and vitamin C deficient guinea pigs (Dayton *et al.*,1956) were found to store good amounts of ascorbic acid.

Although, a large number of studies have been carried out on AA and its effect on various biological processes, yet a teleological concept is far from known. The majority of information is obtained from experiments on the scorbutic animals or on those maintained on a deficient diet. A deficiency in this vitamin however, was known to disturb carbohydrate, fat and protein metabolisms. Recently, it was realized that AA exerts a profound influence on iron metabolism too (Mazur et al.,1961). Due to the ease with which it undergoes oxidation and reduction, it was suggested that it participates in oxidation- reduction processes in the tissues (Cf. Meiklejohn, 1953).

Since it has been observed that vitamin C affects the metabolism of many nutrients and assists in a number of other physiological processes, a study on its content in the liver of a migratory starling, Sturnus roseus, was thought useful. Moreover, since these birds undergo many physiological and biochemical changes in connection with their migration, such an investigation would be greatly informative in elucidating the role of vitamin C upon their migratory activities.

MATERIALS AND METHODS

The changes of the ascorbic acid content in the liver of Rosy Pastor were determined just when they arrived here and regularly in different months during the premigratory phase. The birds were shot in the early morning hours and brought to the laboratory within 15 minutes. Thin slices of the liver were then quickly removed, blotted and weighed. These weighed slices were homogenized in 6% Trichloroacetic acid (TCA) and diluted with thw same solution to a suitable volume. The estimation of the ascorbic acid was carried out by the colorimetric method of Roe and Kuether (1943).

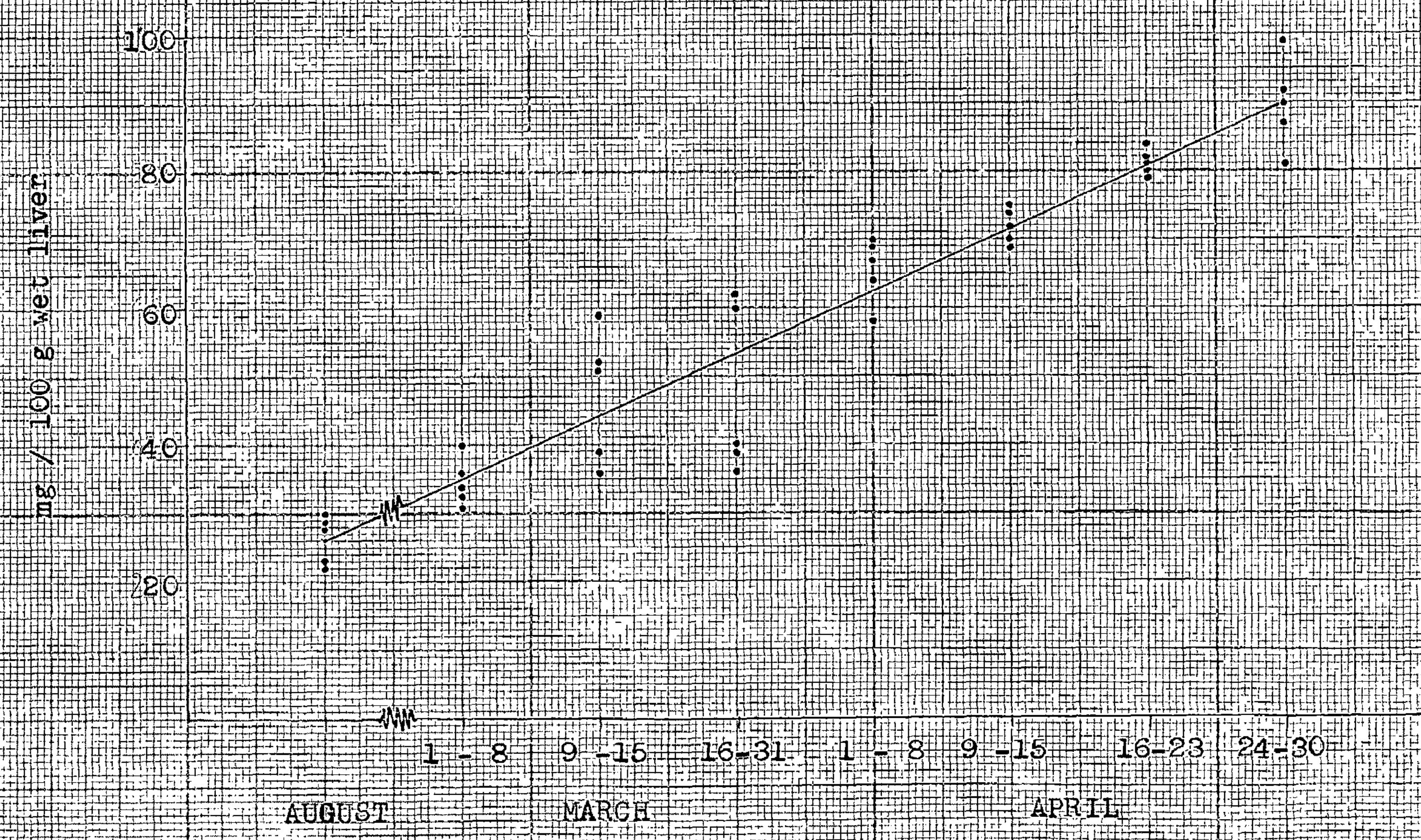
To the TCA extract, activated charcoal (0.3g/20 ml) was added. After vigorous shaking, the mixture was allowed to stand for 10 minutes at room temperature and then filtered. Out of this filtrate, 4 ml aliquotes were transferred to test tubes to which a drop of 10% thiourea and 1 ml of 2% 2,4-dinitrophenyl hydrazine was added. The test tubes were then kept in a water bath at 37°C for 3 hours inorder to incubate the solution. After the incubation period, the tubes were placed on ice and 5 ml of 85% sulphuric acid was added drop by drop with constant shaking. They were removed from ice, kept at room temperature for 30 minutes and the readings were taken at 540 mμ on a Klett-Summerson photoelectric colorimeter. The amount of ascorbic acid in the tissue is expressed as mg/100g fresh liver.

The standard and blank were prepared according to the specification given by Roe (1954).

RESULTS

The amount of ascorbic acid in the liver during various periods are presented in Fig. 1. During postmigratory period the amount of ascorbic acid was found to be low (26.6 mg per 100 g) compared to that of premigratory period. In the first week of March the average value was 34.5 mg, which was slightly higher than in August. Thereafter a constant increase

Fig.1. Ascorbic acid content of the liver of Rosy Pastor during the post- and pre- migratory periods.



in its amount was observed, reaching the maximum (90 mg/100 g) by the end of April.

DISCUSSION

Since, the level of AA was found to increase together with the fat content (Chapter 1), a possible relationship of AA with the metabolism of fat could be suggested. Though the evidences on the mechanism by which AA affects the fat metabolism are meagre, a reduction in the fat content was reported in scorbutic animals (Murray and Morgan, 1946). The importance of AA in normal fatty acid metabolism and during catabolic processes of phospholipid oxidation in the liver were suggested (Rush and Kline, 1941). Abramson (1949) found no change in the phospholipid content of the liver in scorbutic guinea pigs. It could be stated then, that AA might assist in the process of phospholipid breakdown and not the synthesis. It could be recalled here that the phospholipid content in the liver of Rosy Pastors was found to decrease towards the time of migration (Chapter 3) and at the same time AA was present in high concentrations. These facts may suggest that the decreased amount of phospholipids observed in the liver was due to the influence of AA on the phospholipolysis.

There are a number of reports which show a similar relationship between AA and fat. AA is known to be associated with the lipid bodies and golgi apparatus in the silkworm Bombyx mori (Aggarwal, 1962). In this connection it is interesting to note that AA was not only found to influence the size of golgi bodies and affect the excretion of bile pigments by the liver (Murphy and Johns, 1953), but also enhanced the formation of bile acids from cholesterol (Guchait et al., 1963; Read, 1954). John (1967) reported that in the Rosy Pastor liver the total cholesterol increases significantly towards migration. Since both AA and cholesterol were found in high concentrations, the production of bile acids could be at its highest during the premigratory period. In the intrascapular fat pads AA was found in appreciably high amounts (Gero, 1962). It mobilizes the fat that is deposited on the aortic walls due to a high cholesterol diet (Volkova, 1961). Feeding rats with high unsaturated fatty acids leads to a higher concentration of AA in the liver (Merezhinski and Nikitina, 1962). Taking all these observations into account, it could be tentatively concluded that its presence might greatly influence the mobilization of lipids from tissues like adipose and liver. This suggestion is exemplified by the fact that a fatty infiltration takes place in the liver of scorbutic animals (Read, 1954).

It is known that the migratory birds require a high energy supply, so the oxidative metabolism using fat is preferred by them during such long distant flights (George and Berger, 1966). If fat is the chief fuel then the metabolic reactions are bound to proceed through the citric acid cycle thereby producing more energy in the form of ATP. The AA was found to be essential for the functioning of citric acid cycle, since in scorbutic guinea pigs, a decreased SDH activity

(Banerjee et al., 1959), an increased excretion of pyruvate and citric acid (Banerjee and Singh, 1960), the appearance of oxaloacetic acid in the urine and depressed α- ketoglutaric acid excretion (Banerjee and Biswas, 1959) were observed. Therefore, the increased concentration of AA in the liver towards the time of migration could be to maintain an active metabolic state via the citric acid cycle. Thus, ascorbic acid assisting in the breakdown of fats and facilitating their course via citric acid cycle, might play an immensely significant role in the migratory bird, where the fat forms the cheif fuel during migration.

Recently, it was found that the presence of AA was essential for the utilization of iron by animals. Changes in the iron content of the liver of Rosy Pastor were observed in different migratory phases (Chapter 4) and hence a possibility of some influence of AA in this respect can not be ruled out. Ascorbic acid increases the absorption of ferric salts from the diet (Moore et al.,1940) and large doses of AA were found necessary to overcome the effects of some blocking agents in the intestinal mucosa, thereby enhancing the absorption of iron (Pirzio- Biroli et al.,1958). As a reducing agent, it was also found to play an important role in the process whereby the iron is taken up by the tissues, to be incorporated into ferritin (Loewus and Fineberg, 1957; Fineberg et al., 1959). Biellig and Bayer (1955) reported that iron in the ferrous gets directly incorporated into apoferritin, while the ferric iron fails to do so (Mazur *et al.*,1955). The fact that iron is transported to tissues in the ferric state and seldom in the ferrous, suggests, that some biological process mediated via certain agents is necessary for reducing the ferric form of iron to the ferrous for its incorporation into the tissues iron binding proteins. Since it was observed that the incorporation of transferrin bound iron to hepatic ferritin was depended on the energy derived from oxidative metabolism (Mazur *et al.*, 1960; 1961) in which both ATP and ascorbic acid participate, it was suggested that ATP activates the oxidation of AA in the presence of iron bound to transferrin. It is this reaction which helps to reduce the ferric form to ferrous state (Bothwell and Finch, 1962). Ascorbic acid not only facilitates the absorption of iron by the intestinal mucosa or its incorporation into storage protein in the tissues, but also it's release from the storage organs (Bielig and Bayer, 1965; Mazur *et al.*,1955). Lockhead and Goldenberg (1956) also suggested that vitamin C greatly assists the release of iron from liver ferritin and haemosiderin. An active mobilization of iron from the liver (Chapter 4) and increased erythropoiesis (Chapter 5) were observed in Rosy Pastor during the premigratory period. Therefore, an increased AA content in the liver towards the end of April might also enhance the release of more iron from ferritn or haemosiderin for the synthesis of haemoglobin.

A possible relation of AA to the carbohydrate metabolism should also be considered, since, in scorbutic guinea pigs a lowered absorption of glucose and a reduction in the amount of glycogen from the liver and muscle was reported (Murray and Morgan, 1946; Banerjee and Ghosh, 1947). This was suggested to be due to the depressed hexokinase activity (Banerjee and Ghosh, 1947) which in turn lowered the glucose-6- phosphate (G-6-P) concentration (Banerjee and Ganguli, 1962). G-6-P acts as a regulating factor for the rate of glycogen synthetase activity (Banerjee and Ganguli, 1962). Thus , ascorbic acid is shown to be an essential participant in the reactions which lead to the synthesis of glycogen. The deposition of more glycogen by the last week of April (Chapter 1) could then be due to the glucogenic effect (influencing the hexokinase activity) of the ascorbic add which was present in high concentrations at the corresponding period.

Ascorbic acid is known to be capable of being reversibly oxidized and reduced, a fact which prompted many workers to suggest the role of AA as an hydrogen aceptor in the oxidative processes in the cells. But it is mainly present in the reduced form (dehydro-ascorbic acid) in tissues, thus Meiklejohn (1953) stated that AA acts more likely as an antioxidant " by providing a supply of readily available hydrogen". This property is well utilized in the bone marrow and erythrocytes where AA maintains the iron in the ferrous state, as well as that of certain iron containing hepatic enzymes (Takeda and Hara, 1955).

It could be stated in conclusion that the increased mobilization of iron from the liver, the enhanced fat and glycogen synthesis and over all increase of oxidative metaboilsm observed in the Rosy Pastors during the premigratory period, might have been largely influenced or assisted by the high amounts of AA that was recorded in the liver. Whether the increased hepatic AA content was a result of an increased synthesis or absorption is not explainable on the basis of this preliminary study.