

CHAPTER 7

SEASONAL VARIATIONS IN THE ASCORBIC ACID
CONTENT OF LIVER, MUSCLE, KIDNEY AND GONADS OF NORMAL
AND PINEALECTOMIZED FERAL BLUE ROCK PIGEON,
COLUMBA LIVIA.

Ability to store and/or synthesize ascorbic acid is directly related to its metabolic significance in the body of any animal. Most invertebrates, the fishes and man lack the ability to synthesize ascorbic acid while the amphibian and reptilian kidney is capable of synthesizing ascorbic acid (Chatterjee, 1973). Hepatic tissue is known to be the site ^{of} ascorbic acid production in mammals except in primates and guinea pigs. Avian species reveal most varied and interesting facet in this regard. Non-passerine (primitive) birds like fowl and pigeon synthesize ascorbic acid in their kidney whereas in highly evolved passerines, both kidney and liver possess this capacity (Ray Chaudhri and Chatterjee, 1969; Chatterjee, 1973). However, the

red-vented bulbul totally lacks the ability to synthesize ascorbic acid (Roy and Guha, 1958). Variations in ascorbic acid levels in different tissues of insects, birds and mammals have been reported to be associated with reproductive activities and its participation in the metabolic turnover (Chinoy, 1969; 1970; 1972a; Chinoy et al., 1973). The role played by ascorbic acid in the biogenesis of hormones by gonads and other tissues has also been investigated (Chinoy and Parmar, 1975; Chinoy et al., 1978). Ascorbic acid is known to play an important role in various metabolic activities, and deficiency of this vitamin leads to disturbances in carbohydrate, lipid, protein and iron metabolisms (Rusch and Kline, 1941; Levine et al., 1941; Banerjee and Ghosh, 1947; Mazur et al., 1961; Banerjee and Ganguli, 1962). Ascorbic acid is also reported to assist in processes like haematopoiesis, immunity and metabolism of mucopolysaccharides. Due to the high redox potential, its participation in oxido-reduction processes in tissues was also suggested (Meiklejohn, 1953). Processes like connective tissue formation and enzyme stabilization too require ascorbic acid. The above cited manifold functions of vitamin C in several physiological processes and the reported influence of pineal on reproductive function in birds have prompted the present study on the ascorbic acid content in liver, muscle, kidney and gonads of normal,

sham-operated and pinealectomized wild pigeons, Columba livia. Since the pineal is suspected to show seasonal periodicity in relation to the annual cyclic reproductive activities, the study was extended to recrudescence, breeding as well as regressive phases of gonadal cycle. The present chapter thus attempts to correlate the possible alterations in ascorbic acid content post-pinealectomy with reproductive functioning as well as general metabolism of other tissues and organs of the body.

MATERIALS AND METHODS

Normal healthy wild pigeons of both sexes were obtained from the local animal dealer and were then acclimated to the laboratory conditions for a fortnight. Surgical removal of pineal was done according to the procedure described in chapter 1 and so operated birds were housed in an aviary of 12' x 12' x 12' size with cereals as food and water ad libitum. Experimental pigeons and controls (numbers and sexes as specified in chapter 1) were brought from the aviary at the end of each experimental period i.e. 30, 45 and 60 days post-pinealectomy, between 9.00 and 10.00 a.m. Body weights were noted and the birds sacrificed by decapitation. A piece of pectoral muscle, a part of liver lobe, the lower lobe of kidney and the gonad were taken out, blotted and used for biochemical estimation of ascorbic acid.

Estimation of ascorbic acid content in all the above tissues was carried out employing the method of Ree (1954). The ascorbic acid (AA) was first extracted with 6 % trichloroacetic acid (TCA), which reduces pH, stabilizes the vitamin and also prevents its catalytic oxidation. AA was then oxidized to dehydroascorbic acid (DHA) by shaking the extract with activated charcoal for 15 minutes. From the filtrate, 4 ml of aliquot was incubated with 2-4-dinitrophenyl hydrazine for 3 hrs. at 37°C. which yielded an osazone. This was allowed to react with 85 % sulphuric acid forming a reddish brown colour, which was read colorimetrically at 540 μ .

RESULTS

The levels of ascorbic acid (AA) in the liver, muscle, kidney and gonads of pinealectomized, sham-operated and normal intact pigeons in different seasons and for the three experimental time periods are recorded in table 1 and figures 1 to 4. It is evident that hepatic and gonadal AA contents were higher than that of kidney and muscle in general.

SEASONAL VARIATIONS IN ; NORMAL BIRDS:

Hepatic AA showed an increased trend during the recrudescence period reaching a maximal level by

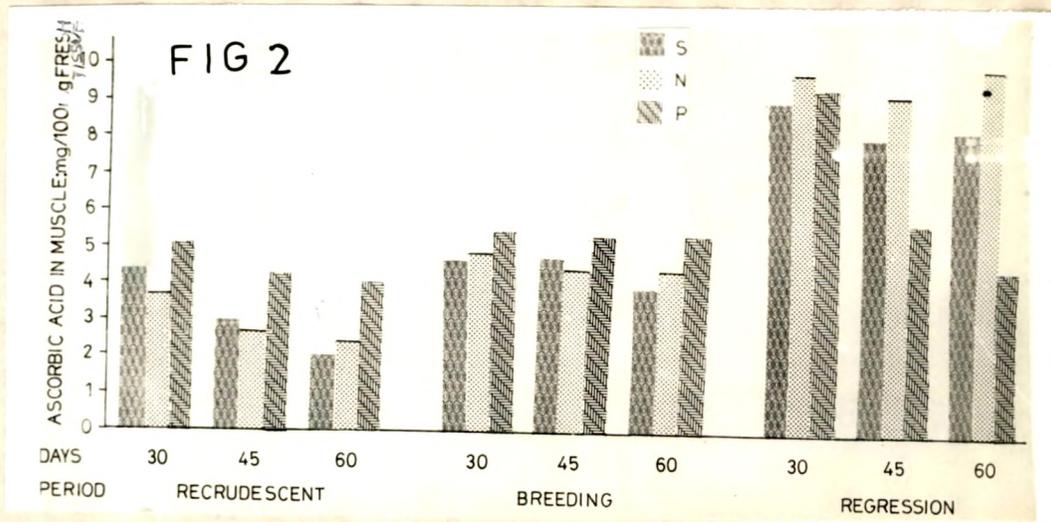
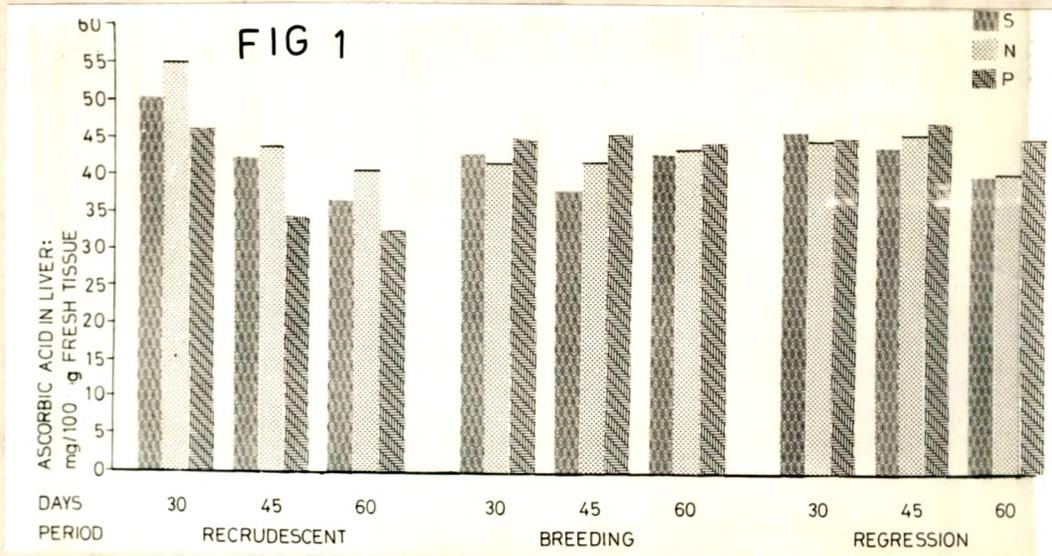
EXPLANATIONS FOR FIGURES

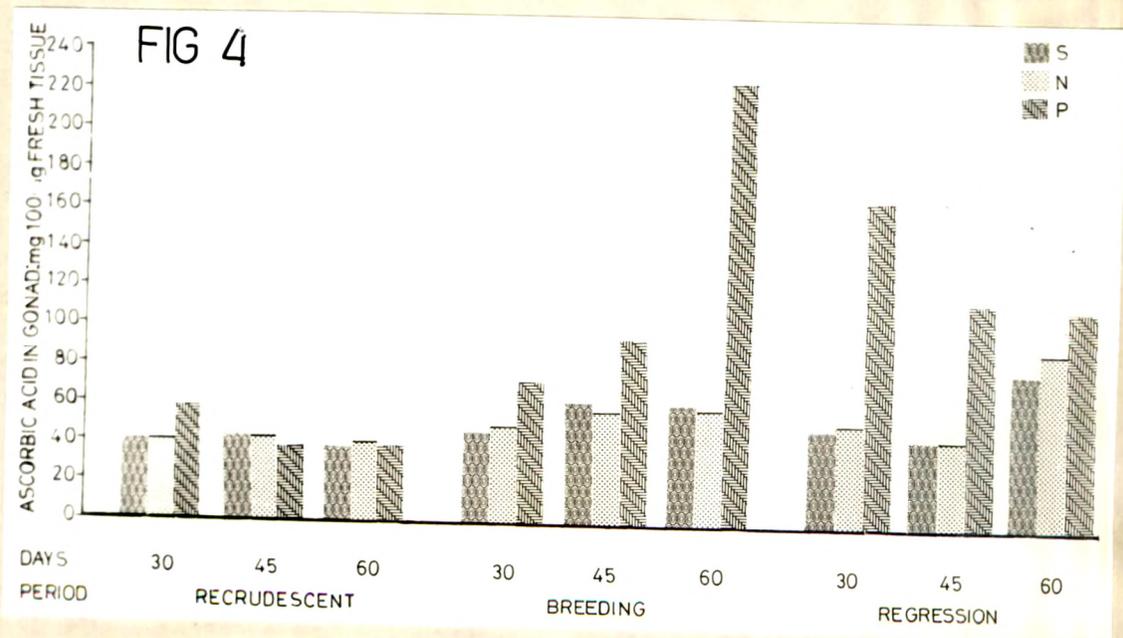
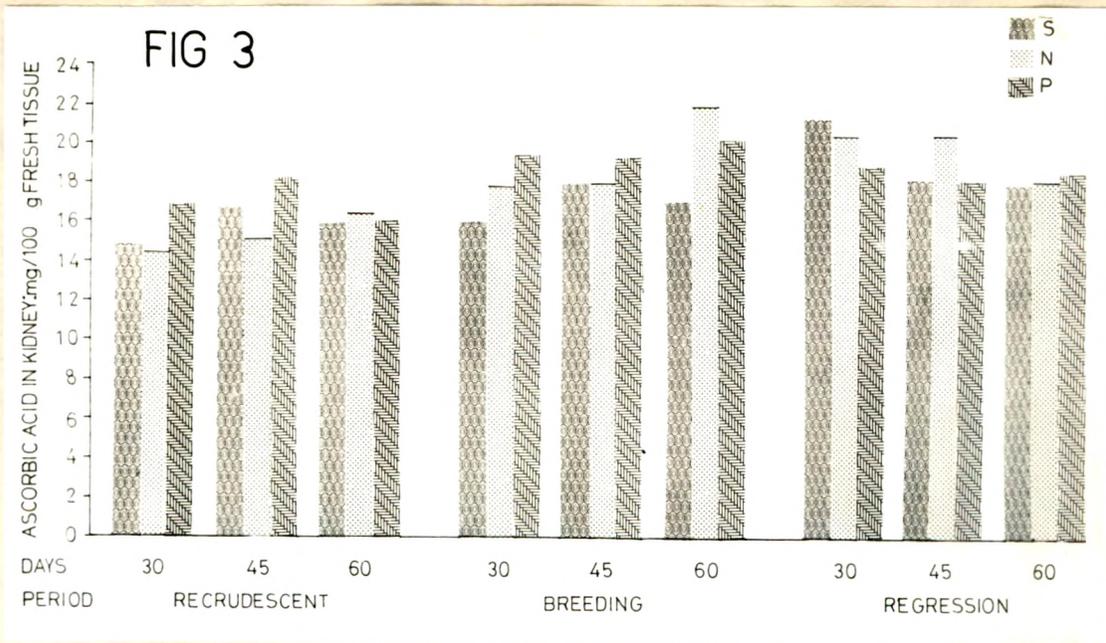
Figs. 1 to 4 : Histogrames showing alterations in ascorbic acid levels in three different intervals post-pinealectomy.

- Fig. 1 - Ascorbic acid in liver
- Fig. 2 - Ascorbic acid in muscle
- Fig. 3 - Ascorbic acid in kidney
- Fig. 4 - Ascorbic acid in gonad

Abbreviations :

- S - Sham operated.
- N - Normal unoperated.
- P - Pinealectomized.





late recrudescence period. Since then there was a fall in AA content during the breeding period which again started increasing by late breeding period and remained at a slightly higher level during the regression period. However, during the late regression / early recrudescence period there was again a fall in hepatic AA content. Renal AA content in contrast showed a depletion during the recrudescence period to reach the lowest level during the late recrudescence period. Since then the AA content remained high in this organ during breeding as well as regression periods. Muscle AA content was noted to be very high during the regression months. The commencement of recrudescence was marked by a 75 % drop in muscle AA content. Since then the AA content showed a slight increase during the recrudescence period and remained at this level during the entire breeding period which was still 50 % below the regression level. Gonadal AA content was found to show a decrease during the recrudescence period, however, during the breeding period it increased. During the late regression period gonadal AA content was found to be 100 % more than the recrudescence levels.

CHANGES DUE TO PINEALECTOMY:

In general, variations in AA content appear to be more significant during the recrudescence and breeding

periods in that order for all the tissues under study. During the regression period the changes in all these tissues except gonads were nonsignificant. Whereas pinealectomy increased the AA content in kidney, muscle and gonads during recedescence, it had a decreasing effect in the case of liver. However, during the breeding period it had a consistently increasing effect in all the organs with a pronounced increase being shown by the gonads. In the regression period whereas the AA contents of liver and kidney of pinealectomized birds were more or less in the normal range, in the muscle it was significantly low and in the gonads once again very high.

DISCUSSION

Disturbances in AA metabolism are known to occur under various abnormal conditions including hypophysectomy and adrenalectomy. Hypophysectomy is reported to lower AA synthesis in rats (Salmon and Stubbs, 1961), while Nathani et al. (1971) observed increased AA utilization from liver and kidney of adrenalectomized rats. Depletion in hepatic and adrenal AA concentration after castration in rats was also observed by Chimoy and Rao (1979). Evidently, levels of AA in various organs are controlled by subtle interaction of various physiological and endocrine factors. The present study has recorded seasonal alteration (in terms

of reproductive cycle) in AA content in gonads, kidney, muscle and liver of wild pigeons. Though the liver AA content has shown only marginal fluctuations, those of gonads, muscle and kidney have shown a sharp drop and depletion during the recrudescence period from the high levels characteristic of the regression period. Apparently AA is involved in metabolic and other activation processes at work in these organs associated with gonadal recrudescence. Slightly higher levels observable during the breeding period indicate the elevated metabolic turnover of the vitamin which may be linked with the prevailing high titres of gonadotropins and gonadal steroids. Elevated higher levels of AA recorded during the regression period probably indicate the reduced utilization and the resultant stock piling. However, the maximal contents attained by the late regression period represent the building up of a reserve vitamin source for utilization during gonadal activation and the resultant recrudescence associated biochemical events. In this pattern of alterations, liver also involves itself by showing two periods of increase and storage once during the late breeding / regression and the other during the progressive recrudescence period, and two phases of depletion - once during the late regression / early recrudescence and the other during the early breeding period.

A cursory glance at the data obtained in the present study does indicate definite alterations in ascorbic acid turnover in pinealectomized birds as compared to the intact controls. In general pinealectomized birds of all three experimental regimes show an increased AA content in all the tissues except for liver in the recrudescence period, and muscle in the regression phase, whence below control levels were recorded. High levels of AA in the gonads of pinealectomized pigeons with pronounced increase during breeding - early regression periods, together with the observed regression of gonads in pinealectomized condition (chapter 1) clearly indicate the accumulation of AA in the gonads due to its non-utilization. Literature on pineal in relation to AA metabolism and turnover is almost non-existent. A couple of reports available (Damian et al., 1979a,b) indicate increased AA content in the gonads after pineal removal and decrease after injection of pineal extracts in rats. Though an apparent similarity exists between these reports and the present study, an inherent point of contradiction lies in the antigonadal and progonadal influences of pineal in rat and wild pigeon respectively. In the wake of earlier observation of gonadal regression in pinealectomized birds (chapter 1), the currently obtained increased content of AA in the gonads can be considered logical under the antigonadotrophic influence and the resultant drop in

steroid^dogenic activity. This effect is as recorded more pronounced during the breeding period and early regression phase with the length of post-pineal ablation period having a definite positive influence. Apparently it is the utilization and not the mobilization which is affected by pineal ablation. Significant increase in renal AA content observable at 30 and 45 days post-pinelectomy during the recrudescence period might indicate the reduced AA release which in the controls is a noticeable feature. Similarly, the constant increase observable in the muscle of pinealectomized birds too during the recrudescence and breeding periods signifies less efficient utilization of AA and probably reduced metabolic efficiency of the organ. However, the diametrically opposite pattern of a decreased muscle AA content in the experimental birds during the regression phase might also denote the decreased ability to mobilize and store the vitamin required for the ensuing recrudescence activation. In contrast, the hepatic AA content has shown an opposite pattern from that of the muscle with pronounced decrease during recrudescence phase and increase during breeding and regression phases. The decrease may in the light of the reduced renal release and accumulation in muscle and gonads indicate inability of liver to bring about the normal AA mobilization. Nevertheless, the increase occurring during breeding and post-breeding periods agrees well with the reported

(early)
increase in hepatic AA content post-castration and its decrease on testosterone administration in rats (Ambadkar and Gangaramani, 1981).

Finally it may be concluded that pineal in wild pigeons is progonadal in nature and its extirpation does cause profound changes in AA turnover in tissues in general and gonads in particular, specifically during breeding and regression phases.