# CHAPTER V

# VARIATIONS OF ASCORBIC ACID CONCENTRATIONS IN THE LIVER, GONADS, KIDNEY AND BLOOD SERUM OF FERAL BLUE ROCK PIGEON COLUMBA LIVIA G. DURING THE BREEDING AND NON-BREEDING PHASES

With respect to the biosynthesis of ascorbic acid (AA) in different tissues, animal kingdom reveals interesting facets. Most invertebrates including insects and also the fishes lack the ability the synthesize AA, probably because of their negligible requirements (Chatterjee, 1973). Commenting on the phylogenetic trend, Chatterjee (1973) further added that, capacity to synthesize AA starts with the amphibian kidney, resides in the reptilian kidney and gets transferred to the liver in mammals. It is once again noteworthy to find the absence of AA synthesizing enzymes in some mammals like the guinea pig, bat, monkey and man and therefore they depend upon the dietary source for this vitamin.

Avian species perhaps show most varied examples in this regard. Biosynthesis of AA in the non-passerines (eg. chicken, pigeon) occurs in the kidney as in the case of amphibians and reptiles whereas in the higher Passeriformes, both the kidney as well as liver possess this capacity (eg. House crow: *Corvus splendens* and Common myna : *Acrydotherus tristis*) (Ray Chaudhri and Chatterjee, 1969; Chatterjee, 1973). Chatterjee *et al.* (1968) have even remarked that some birds like the

Red-vented bulbul (Picnonotus cafer) do not have the ability to synthesize AA. Recently, Chinoy (1972a) has studied the AA levels in different tissues of pigeon (Columba livia) by histochemical means together with cytophotoelectrometric measurements; commenting on the participation of AA in the metabolic turnover by donating its free radicle.

The role played by AA in the biogenesis of hormones by the brain, adrenals and testes have also been investigated (Szent Gyorgii, 1957; Biswas and Deb, 1970, Chinoy, 1970; 1972a and b). The interrelationship between metabolism of AA and testosterone levels has been investigated by many workers (Dieter, 1969; Majumdar and Chatterjee, 1974; Chinoy and Parmar, 1975; Chinoy et al., 1978). Dieter (1969) and Majumdar and Chatterjee (1974) have suggested that in the cockerel and rat respectively, testosterone influences the rate of biosynthesis of AA in the kidney and the liver, the relationship between the androgen and AA being directly proportional. Besides this, AA is also known to assist in resisting cold stress, haematopoiesis and is associated with intercellular substances (Reid, 1954) like in the synthesis and maturation of collagen, reticulin, dentine and bone matrix and metabolism of GAG (glycossiminoglycans). Asnani et al. (1976) while studying the AA levels in the developing pigeon liver and kidney have remarked that the hepatic concentrations touch the peak mark on the 20th day of development when the liver attains both its structural as well functional maturity.

The present investigation reports on the concentrations of AA in the liver, gonads, kidney and blood serum of the feral pigeons during the breeding and non-breeding phases of the annual reproductive cycle as have been noted earlier (Chapters I, II and III). A correlation has been attempted between the levels of AA and gonadal sex steroids.

## MATERIAL AND METHODS

Feral blue rock pigeons (Columba livia G.) were shot down with an air rifle in the University campus between 9.00 a.m. and 10.00 a.m. in the year 1977. Both the adult males and females were utilized for the study. Blood was collected either from the jugular vein or directly from the heart on the very site of killing. Birds were then immediately brought to the laboratory. A part of right liver lobe, first left kidney lobe and a part of left gonad were taken out, blotted and used for biochemical measurements of AA. Blood was centrifuged and serum was seperated.

AA content in all the above mentioned tissues was estimated employing the method of Roe (1954). The vitamin was first extracted with 6% trichloroacetic acid (TCA) which also reduces the pH, stabilized the vitamin and prevents its catalytic oxidation. AA was then oxidized to dehydroascorbic acid (DHA) by shaking the extract with activated charcoal. A 4ml. aliqout of the filtrate was incubated with 2, 4 dinitrophenyl hydrazine for 3 hrs. at 37°C yeilding an osazone. This was allowed to react with 85% sulphuric acid forming a reddish brown colour, which was read colorimetrically at 540 mµ.

## RESULTS

The levels of AA in different tissues of both the sexes are presented in the table and figure. It is clear that as far as the AA levels are concerned, the male and female gonads exhibit a significant sex difference. On the whole, testicular AA levels recorded higher values than those of ovaries. It is also noteworthy that overall AA concentration in the liver, gonads and serum were higher in males than in the corresponding tissues of females. The highest value at any time was that recorded for the testes during the month of June (98.35 mg/100g. wet weight). Among the three organs (liver, gonads and kidney), the kidney showed minimum concentrations of the vitamin in both the breeding and non-breeding phases. Serum AA levels varied between 3.00 to 6.50 mg/100 ml.

<u>SEASONAL VARIATIONS</u>: It is evident from the values given in the table and figure that barring the blood serum, all

TABLE I

ASCORBIC ACID LEVELS (MEAN ± S.D.)

| Months | LIVER | GONADS | KIDNEY | BLOOD SERUM |
|---|---|---|---|---|
| | milligrams/100g. wet weight | | | mg/100ml. |
| MALE BIRDS | | | | |
| MARCH | 44.09 ± 4.51 | 45.70 ± 4.12 | 19.07 ± 4.39 | 6.15 ± 0.55 |
| APRIL | 44.15 ± 3.40 | 36.30 ± 5.08 | 18.18 ± 2.14 | 3.60 ± 0.97 |
| JUNE | 55.29 ± 3.82 | 98.35 ± 10.31 | 18.93 ± 1.62 | 3.09 ± 1.18 |
| JULY | 58.51 ± 4.59 | 65.02 ± 6.86 | 34.43 ± 6.05 | 4.14 ± 0.52 |
| FEMALE BIRDS | | | | |
| MARCH | 35.83 ± 3.68 | 37.67 ± 4.68 | 16.40 ± 3.20 | 2.73 ± 0.84 |
| APRIL | 38.26 ± 3.97 | 57.66 ± 6.21 | 14.43 ± 2.37 | 3.45 ± 0.51 |
| JUNE | 63.08 ± 5.10 | 75.70 ± 4.31 | 19.30 ± 2.69 | 3.93 ± 0.53 |
| JULY | 57.23 ± 5.31 | 60.05 ± 2.98 | 30.44 ± 4.82 | 4.00 ± 0.65 |

## EXPLANATION TO FIGURE

Fig. 1. Graphic representation of variations in Ascorbic acid levels in liver, kidney gonads and blood serum during breeding (March-April) and non-breeding (June-July) phases, in both sexes of feral pigeons.

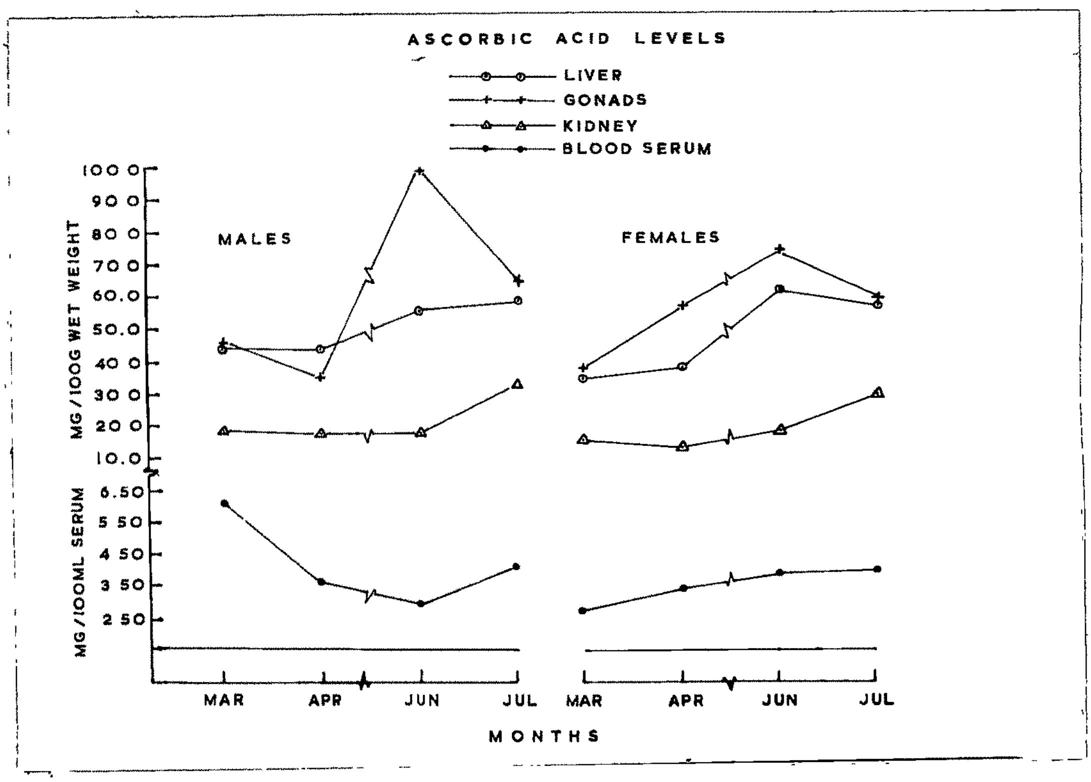
ASCORBIC ACID LEVELS
LIVER
GONADS
KIDNEY
BLOOD SERUM
MALES
FEMALES
MG/100G WET WEIGHT
MG/100ML SERUM
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
6.50
5.50
4.50
3.50
2.50
MAR
APR
JUN
JUL
MONTHS

FIG. 1

the three organs show low levels of AA in the breeding phase and higher levels during the non-breeding season. Liver AA levels of both sexes remained at more or less the same levels during March and April (breeding) and increased significantly in June and July (non-breeding). Testicular AA concentrations exhibited a drop from March to April (Fig. ) and then shot up drastically (three fold approximately) in the month of June, decreasing in July. Ovarian AA levels too, showed somewhat similar trend; rising significantly in the non-breeding phase. Highest values for the same were encountered in the month of June (75.70 mg/100g. wet weight).

Comparatively low levels of AA in the kidney of both sexes revealed no significant fluctuations upto the month of June (i.e. from March to June) but there was a marked increase in the month of July reaching highest concentrations.

Serum AA levels in males showed higher levels in March; thereafter the same declined and touched the lowest mark in June. There was a slight increase in July. No significant change was noticeable in the blood serum of female birds except for a mild rise in the month of June.

## DISCUSSION

Based on the ability to synthesize AA in different tissues of various avian species, Ray Chaudhri and

Chatterjee (1969) and Chatterjee (1973) have shown a phylogenetic trend in birds. As mentioned earlier, L.AA in higher passerines is synthesized in the liver and kidney whereas in the fowl and pigeon, kidney is the main site. (Roy and Guha, 1958; Isherwood *et al*., 1960; Chatterjee *et al*., 1961; Ray Chaudhri and Chatterjee, 1969; Chatterjee, 1973). Histochemical findings of Chinoy (1972a) reveal that among the different tissues of pigeon, brain contains highest levels of AA per cell and that the liver, ovary, pancreatic acini, kidney, adrenals and testes follow in that order. In the present investigation however, gonads exhibited maximum concentrations of AA among the three organs whereas kidney showed the lowest. Asnani *et al*. (1976) too, have obtained similar low values of renal AA concentrations in the developing and adult pigeons. It is plausible to assume that low levels of AA in the kidney (site of synthesis) could be due to its prompt release into the general circulation for the supply to various tissues and this could perhaps result in an apparantly low levels of the same.

Dieter (1969) comments that, as in the case of guinea pigs (Stubbs and McKernan, 1967), the synthesis and distribution of AA in cockerels is under hormonal control and that the synthesis and distribution of AA in pigeons is similar to that of fowl. According to

Chinoy (1972a), in all probability therefore, both the kidney as well as the liver may contribute to the AA pool in the pigeon. This assumption however, demands further investigations.

Higher AA concentrations in the testis than those in the ovary and overall higher AA levels in all the tissues under investigation in case of male birds obviously exemplified the sex difference. This could perhaps be due to the influence of androgens. It has been shown in the case of rat that males have higher hepatic enzyme activities (enzymes associated with AA synthesis) and the AA content in 6 out of 8 tissues studied were higher in males than in females (Stubbs and McKernan, 1967). It has also been demonstrated in hypophysectomized and castrated rats that there was a significant reduction in the enzymic activities that are involved in the AA synthesis (Stubbs et al., 1967) and the concentrations of AA dropped down to that characteristic of the females. It was suggested by these workers that the high enzymic activities in intact males were androgen dependent and the decline after hypophysectomy was due to reduced androgen secretion. It is possible that androgens (as compared to estrogens) may have greater effect over AA turnover rates.

Adrenal cortex and interstitial Leydig cells have high AA content in mammals and birds and ascorbic acid (AA), monodehydroascorbic acid (MDHA) and dehydroascorbic acid (DHA) are all involved in steroidogenesis in these sites (Szent Gyorgii, 1957; Bacq and Alexzander, 1961; Biswas and Deb, 1970; Chinoy, 1972 a & b; Chinoy et al., 1978). In normal toads, AA administration in conjugation with leutinizing hormone (LH) increases the testicular $\Delta^5$-3β-hydroxysteroid dehydrogenase activity whereas hypophysectomy reduces the same (Biswas, 1969) which suggests that AA acts in conjugation with LH augmenting testicular $\Delta^5$-3β-HSDH. In another study (Biswas and Deb, 1970), it has been pointed out that DHA elevates the enzyme activity whereas same doze of AA proved to be ineffective. During the course of the present investigation, high concentrations of hepatic, renal and gonadal AA in the months of June and July (non-breeding) were apparantly evident when $\Delta^5$-3β-HSDH activity in the gonads was at its minimum intensity (Chapters II & III). It appears therefore that higher AA concentrations doesn't enhance the enzyme activity in the gonads of pigeon and thereby increase the steroidogenic rate. This perhaps, could be due to unavailability of leutinizing hormone during the non-breeding phase which might otherwise facilitate the elevation of steroid synthesizing key enzyme by augmenting with ascorbic acid.

Dieter (1969) and Majumdar & Chatterjee (1974) have demonstrated that in the cockerels and rats, tissue localization and synthesis of AA is under the control of testosterone. It is interesting to note that a treatment of testosterone propionate to intact 10 week old cockerels (Gallus domesticus) significantly decreases gulonate-NADP-oxidoreductase activity in the kidney, however, subsequent to which hepatic and renal vitamin levels get elevated. It must be made clear that in the pigeon and chick (Grollman and Lehninger, 1957) and also in the turtle, only kidney contains all the 3 enzymes necessary for the synthesis of L.AA from D. glucoronic acid. During the current investigation, low levels of AA in the liver, gonads and kidney during March and April (breeding) could indicate increased rate of utilization, probably for steroidogenesis. Blood serum of male birds reflected the higher turnover rates in March when the levels of AA were the highest (6.10 mg/100ml). Gonadal 3β-HSDH activity was at one its peak intensities and total cholesterol reveled depleting levels during these breeding months (Chapters I, II & III). Taking the above mentioned facts into consideration, AA (coupled probably with LH) could increase the $\Delta^5$-3β-hydroxysteroid dehydrogenase activity which would ultimately be associated with an increased biosynthetic rate of steroid sex hormones. The key role played by this enzyme in gonadal steroidogenesis of various vertebrates has amply been proved.

On the other hand, higher AA contents in the liver, kidney and the gonads during the non-breeding phases would possibly be due to decreased rate of utilization and a subsequent accumulation of the vitamin leading to storage of the same in bound form in these organs. Many birds (including the pigeon and chicken) and mammals show occurrence of bound AA in their livers and kidneys (Needham, 1963; Malakar, 1963; Chinoy, 1972b).

On the whole, it seems more likely that the low levels of AA in the breeding months were possibly due to the increased uptake or the vitamin which may be required for gonadal steroidogenesis. The circulating sex steroid hormones, particularly androgens may influence the AA levels, perhaps by affecting the AA synthesizing enzyme system. Higher concentrations of AA in June and July were probably suggestive of unaltered synthetic rate coupled with the post-nuptial non-utilization of AA resulting in the accumulation of AA in the tissues. High levels of AA in the immature birds and significantly low values of the same in the layers have also been reported in the case of White Leghorn hens (Prabhakar *et al*., 1975).