CHAPTER VI

SEASONAL ALTERATIONS IN THE ASCORBIC ACID CONTENT OF LIVER, ADRENAL AND GONADS IN NORMAL AND ADRENAL MANIPULATED PIGEONS, *COLUMBA LIVIA*

Ascorbic acid (AA) an important biologically active reductant as well as an important vitamin has been known to be widely distributed in animal and plant cells. AA is known to be involved in multiple tissue functions and metabolism. Importance of AA due to its high redox potential was reported by Meiklejohn (1953). Its participation in oxido-reduction processes in tissues has been widely recognised by several workers (Mapson 1953; Goodwin, 1960; Ramachandran *et al.*, 1975; Asnani *et al.*, 1976). Apart from this, several other roles have been ascribed to AA. Its importantance in developing and regenerating tissues, in drug metabolism and detoxification, in enzyme activation, in protein, carbohydrate & nucleic acid metabolism, and, in cancer therapy has also been well quoted by number of authors. (Rusch and Kline, 1941; Levine *et al.*, 1941; Banerjee and Ghosh, 1947; Mazur *et al.*, 1961; Banerjee and Ganguli, 1962). Yet another important function attributed to this vitamin is its role in reproductive functions and steroidogenesis. This particular aspect is being greately ascertained and explored in recent years. Synergistic action of AA with testosterone in increasing germ cell

maturation and enhancing the activity of a number androgen dependent enzymes has been reported by Kutsky (1973) and Chinoy and Seethalakshmi (1977). Adenyl cyclase a key enzyme for cAMP formation which in turn plays a crucial role in mediating hormone responses in reproductive tissues is known to be activated by AA (Lewin, 1975). Role of AA in steroidogenesis has been reported by many workers (Szent-Gyorgyii, 1957; Bacq and Alexander, 1961; Biswas and Deb, 1970; Chinoy, 1972; a,b; Chinoy et al., 1978). Ascorbic acid content is known to undergo season specific alterations associated with reproductive activities, and metabolic turnover in gonads as well as in other tissues (Chinoy, 1969, 1970; Patel, 1982,1984).

Adrenals and gonads are the main centers of steroidogenesis in vertebrates while liver is the storage organ in passerine birds, and hence all these organs are known to contain greater store of AA. Depletion of this vitamin during the active phase of tissue functioning is also well documented. Since the AA content is reported to show veriations with respect to gonadal activation and regression, and as adrenal activity seems to parallel gonadal functioning in tropical wild pigeons, it was thought worthwhile to study the AA content in the three tisses viz., liver, adrenal and gonads of normal and experimental (adrenal suppressed and adrenal activated) birds on a seasonal basis.

MATERIALS AND METHODS

As outlined in Chapter - I

RESULTS

The results are represented in tables 1-3 and in figure 1-3. The tables 1 and 2 give comparative levels of AA content in the adrenal and gonads of male and female birds. Though ovarian AA content is slightly higher than that of testicular AA contemt, the difference in the levels is however not significant. The adrenal of female birds showed comparatively less AA content than that of males and the differences are significant. The pattern of normal seasonal changes as well as under adrenal manipulation was identical in gonads and adrenals of both sexes. Hence the changes in the AA content of both these organs are discussed in general terms in text with no sex bias and the figures 1 and 2 represent the computed values of both the sexes put together.

Seasonal Variations in Normal Birds.

Of the three tissues studied, the ascorbic acid content of adrenals was found to be maximum followed by that of gonads and liver in that order. All the tissues depicted seasonal variations, with maximum content during

TABLE-1a : SEASONAL CHANGES OF ADRENAL ASCORBIC ACID (mg/100 gms TISSUE WEIGHT) OF MALE NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DAXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	337.0 ±29.7	162.9*** ±30.61	207.8*** ±5.8	182.73*** ±21.51	-	-	-	-	-
BREEDING	157.60 ±2.63	119.2** ±4.7	129.4** ±2.02	139.29@ ±13.99	-	-	-	-	-
REGRESSION	118.07 ±30.19	-	-	-	90.16* ±30.19	86.64* ±6.25	128.88 ±20.88	259.55++ ±60.11	187.29++ ±10.29

@ P< 0.02 ++ P<0.001 * P<0.05 ** P<0.005 *** P< 0.0005

M - MORNING E - EVENING

TABLE 1b : SEASONAL CHANGES OF ADRENAL ASCORBIC ACID (mg/100 gms TISSUE WEIGHT) OF FEMALE NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DAXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	262.3 ±21.8	112.5*** ±18.5	174.87++ ±30.4	169.8+ ±13.23	-	-	-	-	-
BREEDING	187.13 ± 9.74	52.63*** ±10.29	68.29*** ±6.12	48.29*** ±6.28	-	-	-	-	-
REGRESSION	135.59 ±7.39	-	-	-	78.96++ ±14.23	73.45++ ±10.16	110.25+ ±12.25	169.18 ±42.10	218.13+ ±58.34

+ $P < 0.01$ ++ $P < 0.001$ *** $P < 0.0005$

M - MORNING E - EVENING

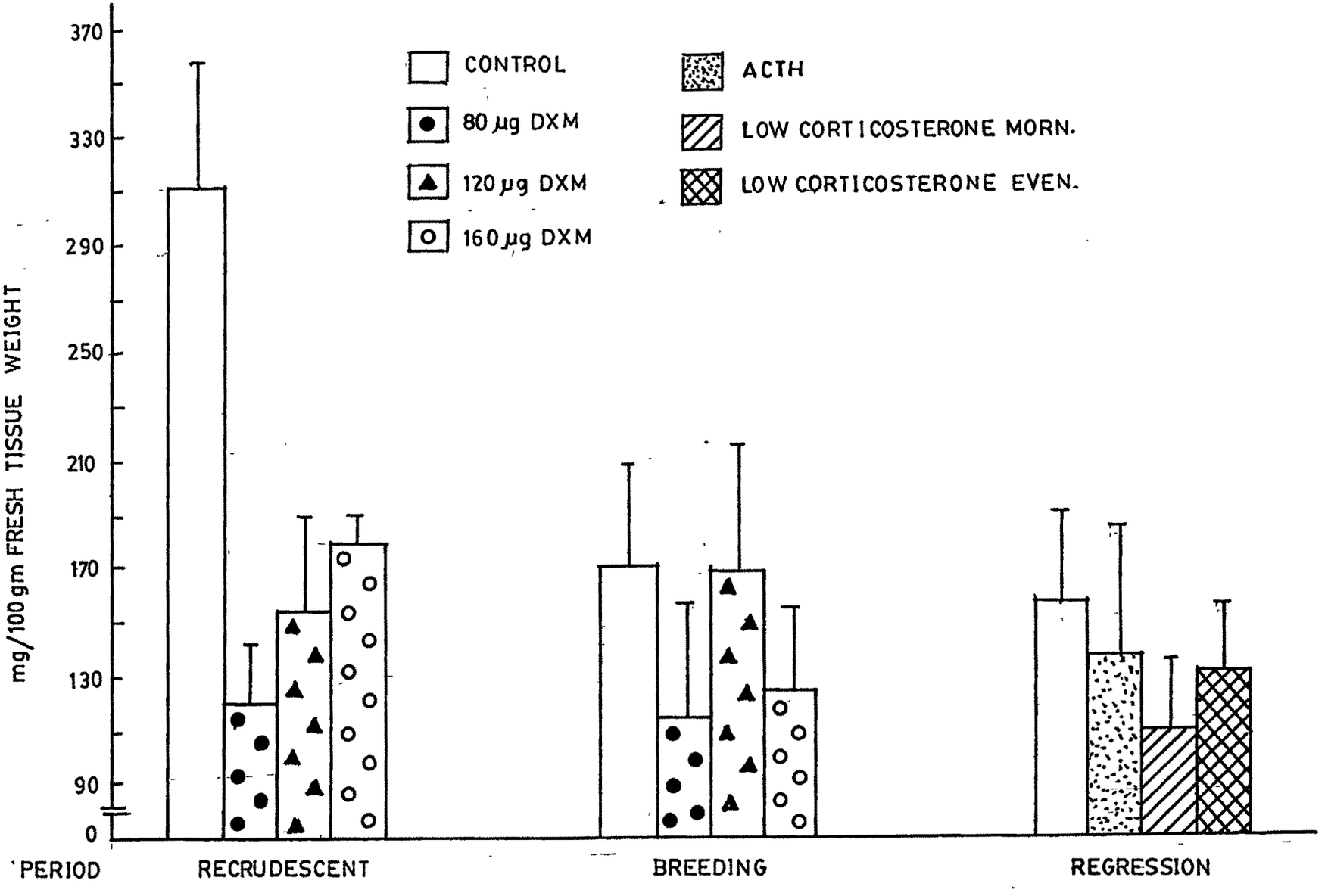

FIG. 1. CHANGES IN ADRENAL ASCORBIC ACID CONTENT

TABLE 2a : SEASONAL CHANGES OF GONADAL ASCORBIC ACID CONTENT (TESTIS; mg/100 gms TISSUE WEIGHT) IN NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DAXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	65.30 ±16.80	75.40 ±9.01	142.94^{++} ±15.9	128.7 $^{++}$ ±14.60	-	-	-	-	-
BREEDING	33.52 ±10.0	68.25*** ±12.74	71.37*** ±16.21	95.46*** ±14.77	-	-	-	-	-
REGRESSION	130.85 ±33.95	-	-	-	26.44*** ±7.03	28.72*** ±5.61	52.26*** ±10.24	114.26 ±12.45	82.77* ±23.79

++ $P < 0.001$ * $P < 0.05$ *** $p < 0.0005$

M - MORNING E - EVENING

TABLE-2b : SEASONAL CHANGES OF GONADAL ASCORBIC ACID CONTENT (OVARY mg/100 gms TISSUE WEIGHT) IN NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	86.27 ±9.31	96.59 ±24.49	106.94* ±12.0	162.64*** ±5.80	-	-	-	-	-
BREEDING	36.69 ±4.32	72.83*** ±10.24	74.89*** ±14.26	119.08*** ±14.26	-	-	-	-	-
REGRESSION	149.79 ±21.56	-	-	-	58.62*** ±15.52	53.27*** ±15.22	50.28*** ±8.24	65.29*** ±20.98	100.37* ±15.39

* $P < 0.05$ *** $P < 0.0005$

M - MORNING E - EVENING

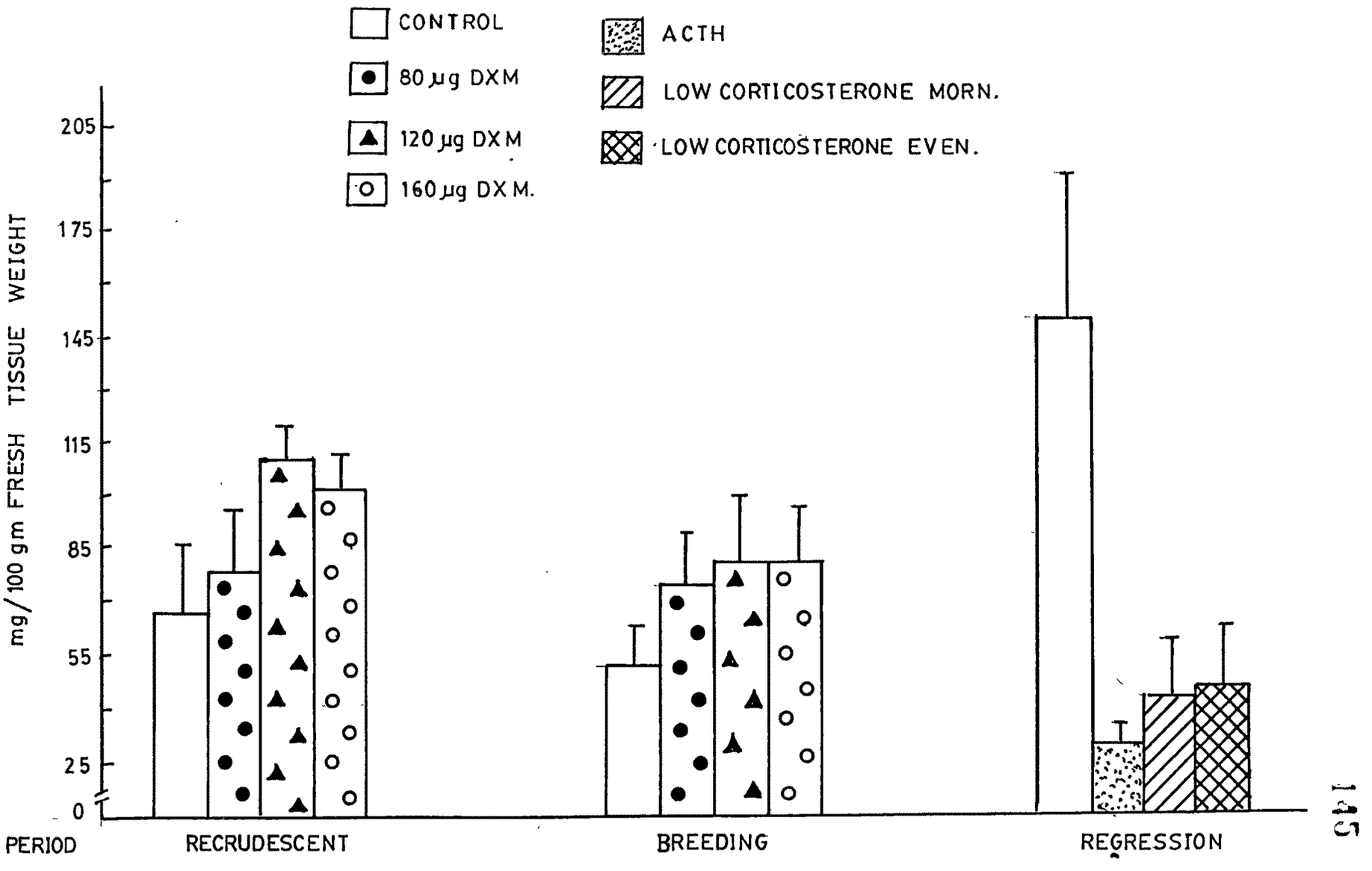

FIG. 2. CHANGES IN GONADAL ASCORBIC ACID CONTENT

TABLE- 3 : SEASONAL CHANGES IN HEPATIC ASCORBIC ACID CONTENT (Mg/100 gms TISSUE WEIGHT) IN NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	48.51 ±5.50	37.75 ±10.0	26.53++ ±4.13	24.72@ ±5.60	-	-	-	-	-
BREEDING	29.39 ±6.40	35.05 ±6.19	34.5 ±7.10	33.76 ±6.65	-	-	-	-	-
REGRESSION	45.69 ±11.5	-	-	-	33.62* ±5.99	33.23* ±5.90	28.07** ±4.8	40.63 ±8.57	42.11 ±10.41

++ $P < 0.001$ @ $P < 0.02$ * $P < 0.05$ ** $P < 0.005$

M - MORNING E - EVENING

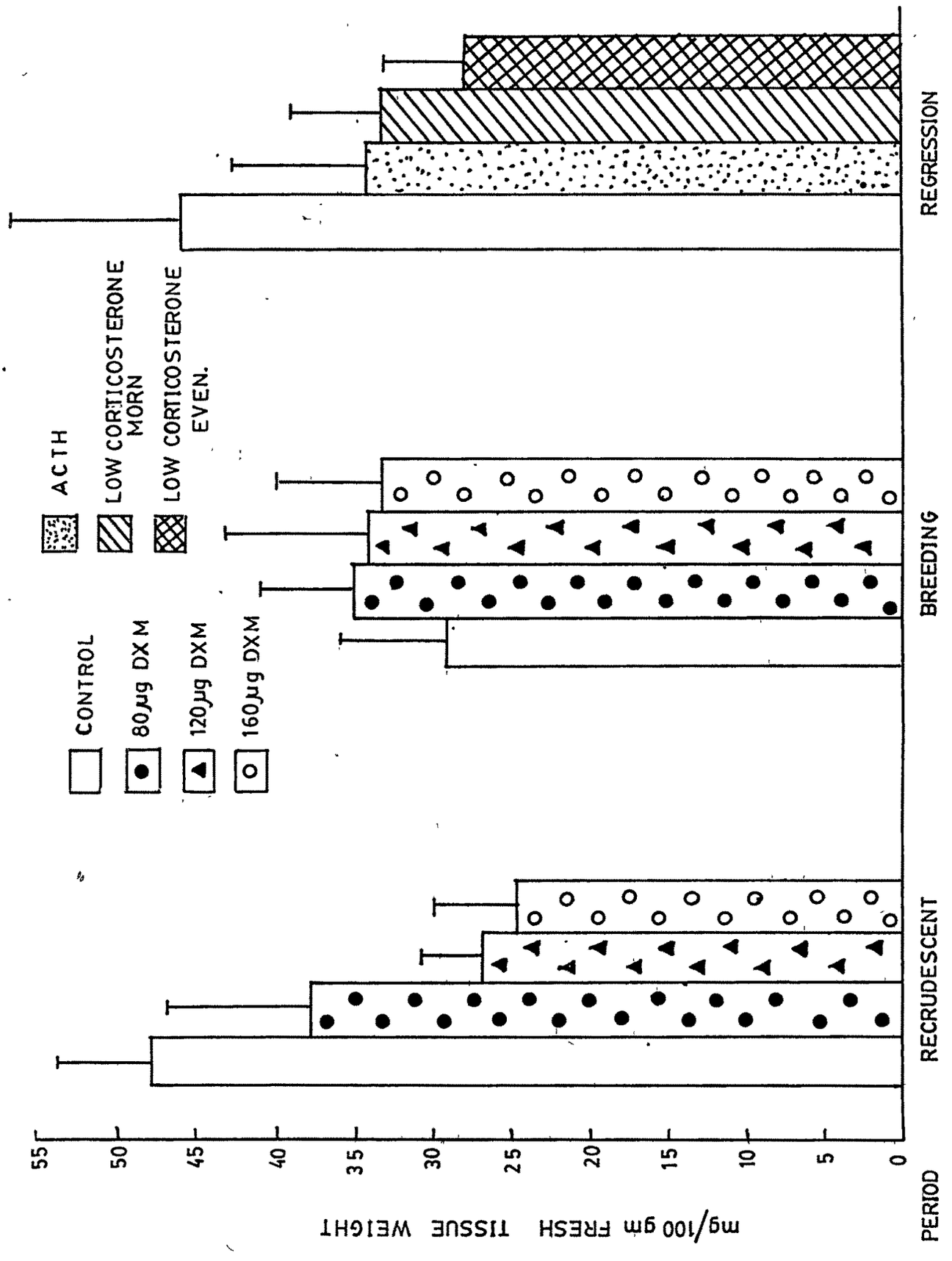

FIG. 3. CHANGES IN HEPATIC ASCORBIC ACID CONTENT

non-breeding and minimum during breeding except for adrenals which depicted least content during non-breeding and maximum during recrudescence. The adrenals recorded a 100% increase in ascorbic acid content from regression to recrudescence which later showed a fall of 46% from recrudescence to breeding. On the other hand, gonadal ascorbic acid depicted a tremendous decrease of 58.3% from the regression phase to recrudescent phase and a further fall between recrudescence and breeding which totalled to a maximum fall of 64.6% from the basal level during regression.

The hepatic ascorbic acid content was found to show significant depletion between recrudescence and breeding (35.6%) while the difference in the level of AA content between regression and recrudescence was insignificant.

Changes Under Experimental Conditions

Adrenal suppression and activation undoubtedly brought about notable alterations in the vitamin content of the three tissues. Adrenal suppression during the active phases as well as injection of either ACTH or corticosterone during the inactive phase brought about significant decrease in the content of liver and adrenals. Adrenal suppression resulted in increased content of this vitamin in gonads during recrudescence and breeding while injection of

corticosterone or ACTH brought about significant decrease. However high doses of corticosterone failed to evoke such a response in adrenals and significant increase in AA content was effected by HCM and HCE.

DISCUSSION

Tissue AA content has been known to undergo alterations under several experimental conditions and also on a seasonal basis in relation to the breeding cycle in several species of animals. Increase in hepatic ascorbic acid content post-castration (early effects) and its decrease on administration of testosterone propionate has been reported by Amabadkar and Gangaramani (1981). Hypophysectomy is reported to lower ascorbic acid synthesis in rats (Salmon and Stubbs, 1961). Depletion in hepatic and adrenal ascorbic acid contents after castration and its recovery on administration of testosterone propionate has also been reported by Chinoy and Rao (1979) and Muddeswar et al. (1984). Thus the prevailing levels of ascorbic acid in tissues could be modulated by subtle interactions of hormones and metabolic factors. Higher ascorbic acid content of liver and adrenals noted herein during the recrudescent phase probably represents a built up reserve for utilisation during the ensuing breeding season. This contention stands vindicated

by the observed depletion of hepatic and adrenal ascorbic acid stores between recrudescence and breeding. The depletion of adrenal ascorbic acid can reflect its (adrenal's) activity as AA is known to act as electron donor in hydroxylation reactions involved in the biosynthesis of corticosterone (Kerstein et al., 1958). Same could be considered to hold true for gonadal ascorbic acid, as steroidogenesis in gonads also necessarily involves hydroxylation reactions. Synergistic cumulative association between LH and AA in increasing the activity of Δ^5 - 3β hydroxysteroid dehydrogenase (Δ^5 - 3β HSDH) has been shown in normal toads (Biswas, 1969). The increased AA content in the liver during the regression phase could easily indicate reduced utilisation post-breeding and it could be mentioned in this connection that a reciprocal relationship between hepatic AA and gonadal hormones has been reported by Chaturvedi and Thaliyal (1978).

On the other hand, the low level of adrenal AA in the post-breeding months could indicate the depleted condition after a phase of increased adrenal activity and hence a low key functioning of the adrenals in the non-breeding phase. Decreasing content of gonadal AA during recrudescence and breeding coupled with the elevated level during regression could easily indicate greater utilisation of the vitamin

for steroidogenesis and gonadal functioning in the former periods and the stockpiling due to reduced utilisation and cessation of steroidogenesis in the latter period. It may be mentioned in this connection that high levels of ascorbic acid in the gonads of immature birds (with non-functional gonads) and low levels in the ovary of egg laying birds have been reported by Prabhakhar et al. (1975). Similarly, Chand et al. (1978) in their study on relation of testicular ascorbic acid with testicular development in Desi and white leghorn poultry, observed depletion of AA content alongwith gonadal maturation and increase in weight. Studies on the influence of adrenal steroids on tissue AA content being scanty, discussion on this line becomes difficult. However the few reports available suggest that adrenal hormones do play an important role in the turnover of vitamin C. Nathani et al. (1971) observed increased utilisation of ascorbic acid from liver and kidney of adrenalectamized rats. Involvement of adrenal corticosteroids in modulating tissue AA contemts can be inferred from the significant alterations observed under experimental conditions in the present study.

Adrenal suppression by dxm during the recrudescent and breeding periods induced organ specific differential alterations in ascorbic acid content . Whereas the hepatic AA content depicted a decrement in the recrudescent phase and no change in the breeding period, adrenals depicted

decrement and gonads an increment during both the phases. However, ACTH/corticosterone administration induced generalised AA depletion from all the three organs. In general it could be presumed that either dxm treatment or ACTH/corticosterone administration can bring about AA depletion from both liver and adrenal. Apparently, adrenal steroids can induce increased utilisation and/or release of AA from hepatic tissue. The depletion in hepatic AA content of dxm treated birds during the recrudescent phase may essentially be due to the inability of the hepatic tissue to distinguish between native corticosterone and the synthetic one. In this respect, however, the unresponsiveness of hepatic AA to dxm treatment during the breeding season could be attributed to the prevailing low level of AA content. This level of hepatic AA might then represent a low threshold level making it insensitive to corticosteroids. It is pertinent to quote here the report of Chinoy and Rao (1979) of castration induced reduced utilisation of hepatic AA and increased utilisation on administration of testosterone propionate, thereby suggesting the influence of gonadal steroids in decreasing the hepatic AA content. However in the present study, dxm induced depletion of hepatic AA was marked by gonadal involution (Chapter III) which over-rules the possible involvement of gonadal steroids and thereby emphasises the role of adrenal corticosteroids.

Adrenals have shown a uniform response of AA depletion under all experimental regimes i.e. dxm treatment as well as ACTH/corticostcrone administration. Though the ACTH induced adrenal AA depletion in the wake of increased adrenal weight and activity (Chapters II & III) is understandable, the dxm and corticosterone induced depletion is rather enigmatic. The corticosterone induced depletion appears tenable in the light of the related reports of increased testosterone production by the baboon testes following ACTH injection (Katsya and Goncherov, 1982), of cortisol and progesterone mediating the stimulatory effect of ACTH on testosterone production in porcine testes (Juniewiez and Johnson, 1984), and of the ability of testosterone to increase adrenal AA utilisation in rat (Chinoy and Rao, 1979) and the herein recorded increased gonadal weight and activity of adrenals and gonads and increased weight and activity of adrenals in corticosterone treated birds tend to disfavour the above possibility. The only plausible explanation that could be suggested in this wake is that of an autoregulatory role of corticosteroid in inducing adrenal AA depletion which may or may not be coupled to steroidogenesis; in which case the adrenals too like the liver appear to have no ability to distinguish between native and synthetic corticosteroids and thus seems to be endowed with less specific costicosteroid receptors.

The observed changes in gonadal AA content are well correlable with the functional status of the gonads under the two different experimental conditions. Accordingly, the increased vitamin content of the gonads under dxm induced adrenal suppression is in keeping with the reduced utilisation leading to stockpiling of the vitamin occurring due to gonadal regression (Chapters II & III). Similarly, the reduced gonadal AA content under ACTH and corticosterone injections indicates the utilisation of the vitamin in association with the noted gonadal enlargement and activity (Chapters II & III). Importance of AA in gonadal functioning is stressed not only by the reports of Parlow (1958, 1961) suggesting AA as an indicator of steroidogenesis in ovary for bioassay of LH but also by the observations of Chatterjee (1967) and Biswas (1967) of testicular atrophy and spermatogenic arrest respectively under Vitamin C deficiency. From the observations made in the present study, it could be surmised that seasonal reproductive cyclicity in subtropical Indian pigeons involves adaptive modulations in tissue AA content and that corticosteroid do play a key role either directly or indirectly in bringing about such alterations.

S U M M A R Y

Hepatic, adrenal and gonadal AA contents have been assayed during the three reproductive phases and under conditions of adrenal manipulation in the pigeon, Columba livia. Hepatic and gonadal AA contents were high in the non-breeding phase and low in the breeding phase while adrenal AA content recorded a reverse set of changes showing a progressive decrement from recrudescence to regression through breeding. Whereas the changes in liver and gonads are indicative of increased utilisation during the active phases and decreased utilization in the inactive phase, changes in the adrenals are suggestive of stockpiling during recrudescence and increasing utilization through breeding to regression. Adrenal suppression in the breeding phases led to increased gonadal AA content while adrenal activation in the non-breeding phase induced AA depletion from all the three tissues; changes suggestive of non-utilization in the former and increased utilization in the latter case. These aspects are discussed in detail in the text.