Chapter IV

CIRCANNUAL VARIATIONS IN TISSUE ASCORBIC ACID CONTENTS IN RELATION TO TESTICULAR CYCLICITY AND THE INFLUENCE OF PINEALECTOMY OR ALTERED ADRENOCORTICAL ACTIVITY.

The annual breeding cycle of sub-tropical feral pigeons can be divided into pre-recrudescent preparatory (Dec.-Jan.), recrudescent (January - February), breeding (February - April), regression (May-June), and quiescent (July to November) phases. These phases of gonadal activity are associated with concomitant changes in the activity of thyroid and adrenal glands. In keeping with a parallel-adrenal-testes relationship and an inverse thyroid-testes relationship (Patel et al.,1985; Ramachandran and Patel, 1986; Ramachandran et al.,1987; Ramachandran and Patel, 1988),circulating levels of thyroxine (T_4) and corticosterone (CORT) have been recorded to show season specific alterations (Patel, 1993). Significant alterations in carbohydrate metabolism marked by tissue glycogen depletion and hyperglycemia in the recrudescent and breeding phases and vice-versa in the quiescent phase have also been noted to occur (Ramachandran and Patel, 1987; Patel et al.,1988). Besides the many implicated functions of ascorbic acid (AA), the levels of tissue AA content are known to reflect the metabolic potential as well as steroidogenic

status of the concerned organs (Biswas and Deb, 1970; Chinoy, 1972). In this context, an evaluation of circannual variations in the AA content of liver, adrenal and testis was considered meaningful. Since both PX as well as adrenocortical suppression in the breeding phase have been shown to bring about testicular regression and, administration of CORT in the regression and quiescent phases have been shown to have some favourable influences (Ramachandran et al., 1987; Ramachandran and Patel, 1988; Chapter I and II) with concomitant changes in carbohydrate metabolism, an evaluation of tissue contents of AA under these experimental conditions was also deemed pertinent.

MATERIALS AND METHODS.

As outlined in chapter III.

Parameters and Methodology of evaluation.

Ascorbic acid (AA) in liver, adrenal and testis.

Total AA content in the tissues was estimated by the method of Roe, (1954). The colour intensity was read colorimetrically at 540 nm. Ascorbic acid content was expressed in terms of mg/100g tissue weight.

Experimental setups.

As outlined in chapter III.

RESULTS.

A **Changes in tissue AA contents during annual gonadal cyclicity.**

The variations in tissue AA contents in relation to testicular cyclicity are shown in Fig. 4A. In the months of February-April (breeding season), the AA content of the testis was minimum. From mid-April onwards the testicular AA content increased and reached a maximum level in July (Regression) and, thereafter decreased again during August - September (Quiescence) and then increased steadily to reach a higher level in December. The adrenal and hepatic AA contents depicted parallel change with a maximum level in the month of February - April and a minimum level in month of August - September. (Fig 4 A)

B **Changes in tissue AA content during experimental manipulation of adrenocortical function in intact and PX pigeons.** (Tables IV a to IV c)

The hepatic and adrenal AA contents decreased under all

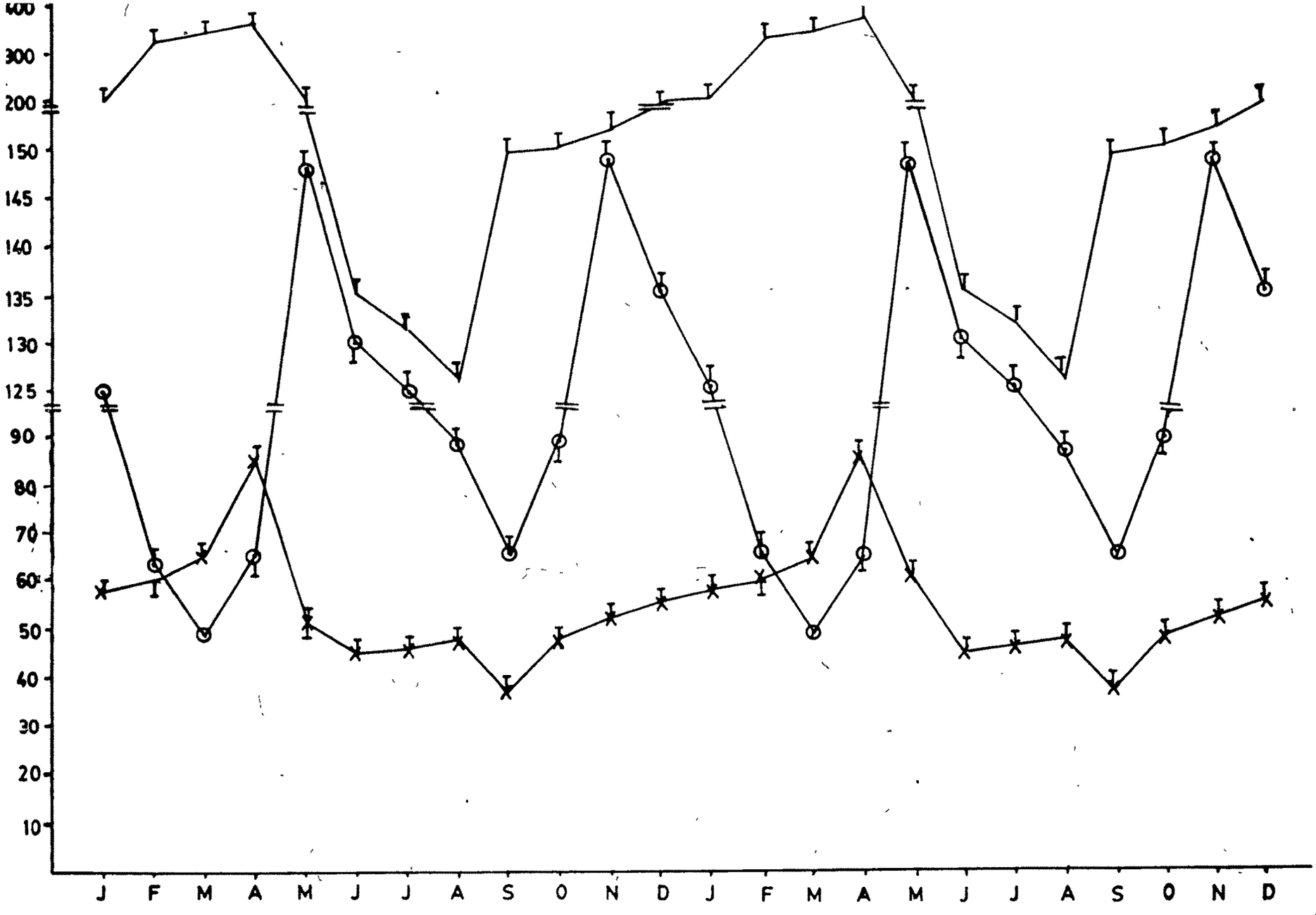

Fig 4A Cireannual variations (X ± SD) in tissue ascorbic acid contents of pigeons exposed to natural day-length.

(×——×) Liver ascorbic acid ; (——) Adrenal ascorbic acid ; (⊙——⊙) Testis ascorbic acid

Tissue Ascorbic Acid
(mg/100 g tissue)

Treatment	Testes	Adrenals	Thyroid
Control	86.42 ± 1.34	366.63 ± 13.41	29.81 ± 1.95
DXM	74.54* ± 4.47	143.11* ± 5.01	51.64* ± 1.86
PX	54.01 ± 1.79	105.30 ± 1.70	47.69 ± 1.69
PX+CORT	34.83* ± 2.68	126.33* ± 2.89	38.80* ± 1.77

Table IVa :Alterations in tissue ascorbic acid content of intact and PX pigeons subjected to functional manipulations of adrenals in the breeding phase.

(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

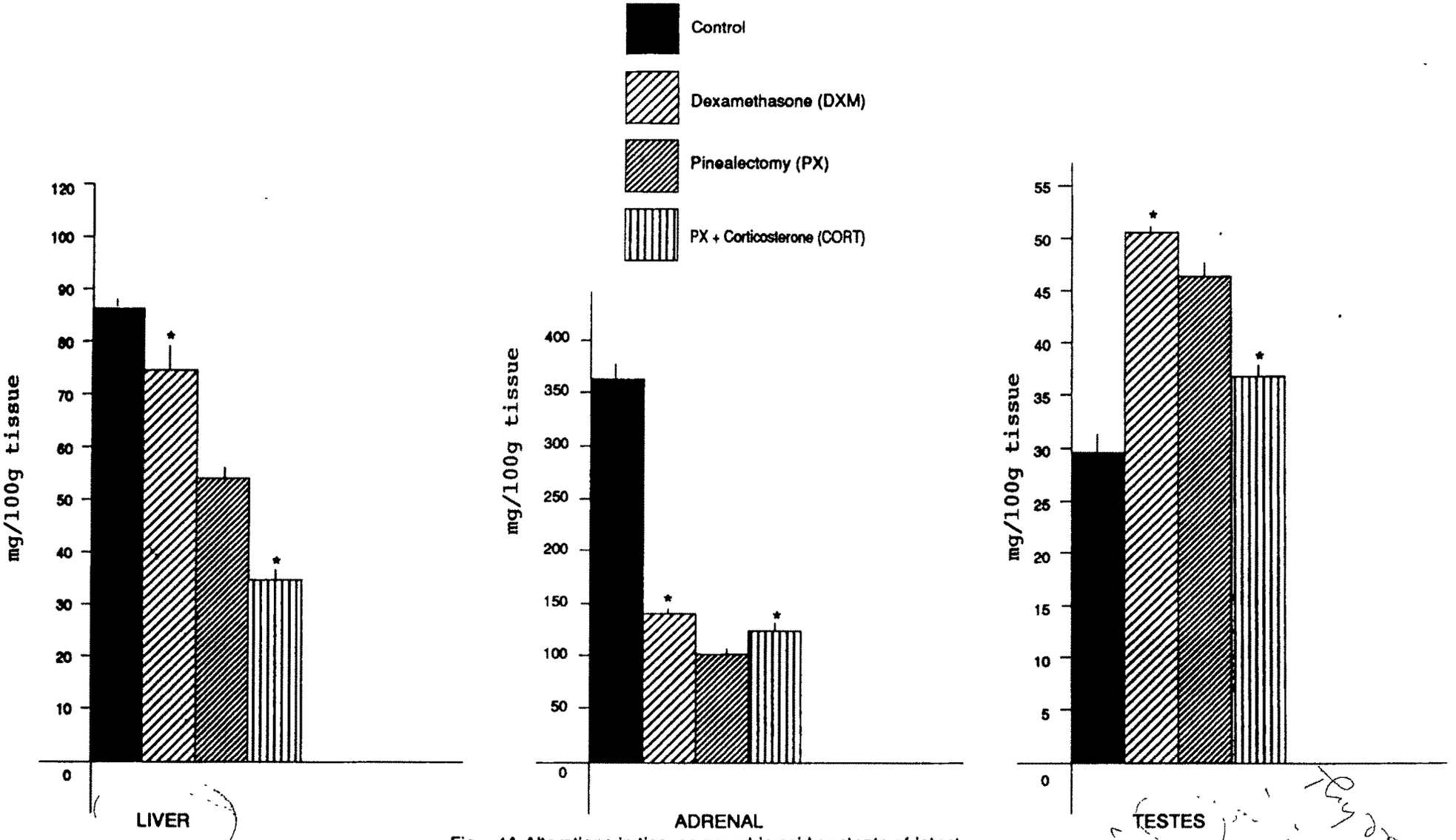

Fig 4A Alterations in tissues ascorbic acid contents of intact and PX pigeons subjected to functional manipulations of adrenal in the breeding phase (* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM).

Tissue Ascorbic Acid
(mg/100 g tissue)

Treatment	Testes	Adrenals	Thyroid
Control	54.20 ± 1.89	223.91 ± 4.47	46.01 ± 1.47
CORT	60.90* ± 2.32	160.47* ± 3.69	108.50 ± 1.79
PX	55.90 ± 1.47	200.26 ± 4.47	84.53 ± 1.94
PX+CORT	49.32* ± 1.89	164.72* ± 1.13	± 73.32 ± 4.24

Table IVb :Alterations in tissue ascorbic acid content of normal and PX pigeons treated with corticosterone (CORT) in the regression phase.

(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

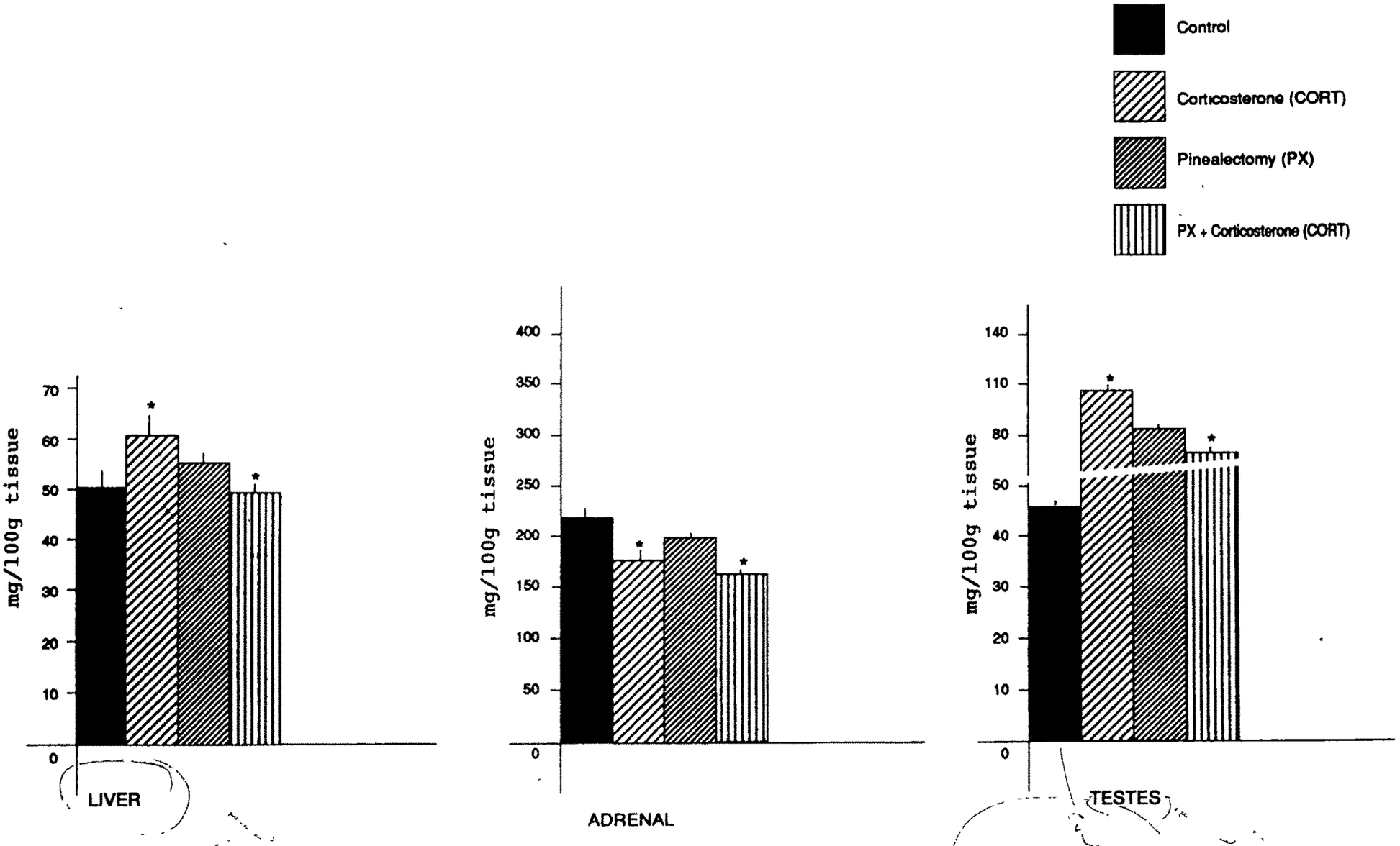

Fig : 4B Alterations in tissue ascorbic acid contents of intact and PX Pigeons treated with corticosterone (CORT) in the regression phase
(* - Significant at $p < 0.05$; values are $\bar{x} \pm$ SEM).

Treatment	Tissue Ascorbic Acid (mg/100 g tissue) Liver	Adrenal	Testis
Control	48.03 ± 2.23	150.72 ± 4.67	89.91 ± 2.70
CORT	58.11* ± 1.16	269.81* ± 11.62	172.72* ± 2.68

Table IVc. Alterations in tissue ascorbic acid content of pigeons subjected to hypercorticalism in the quiescent phase.

(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

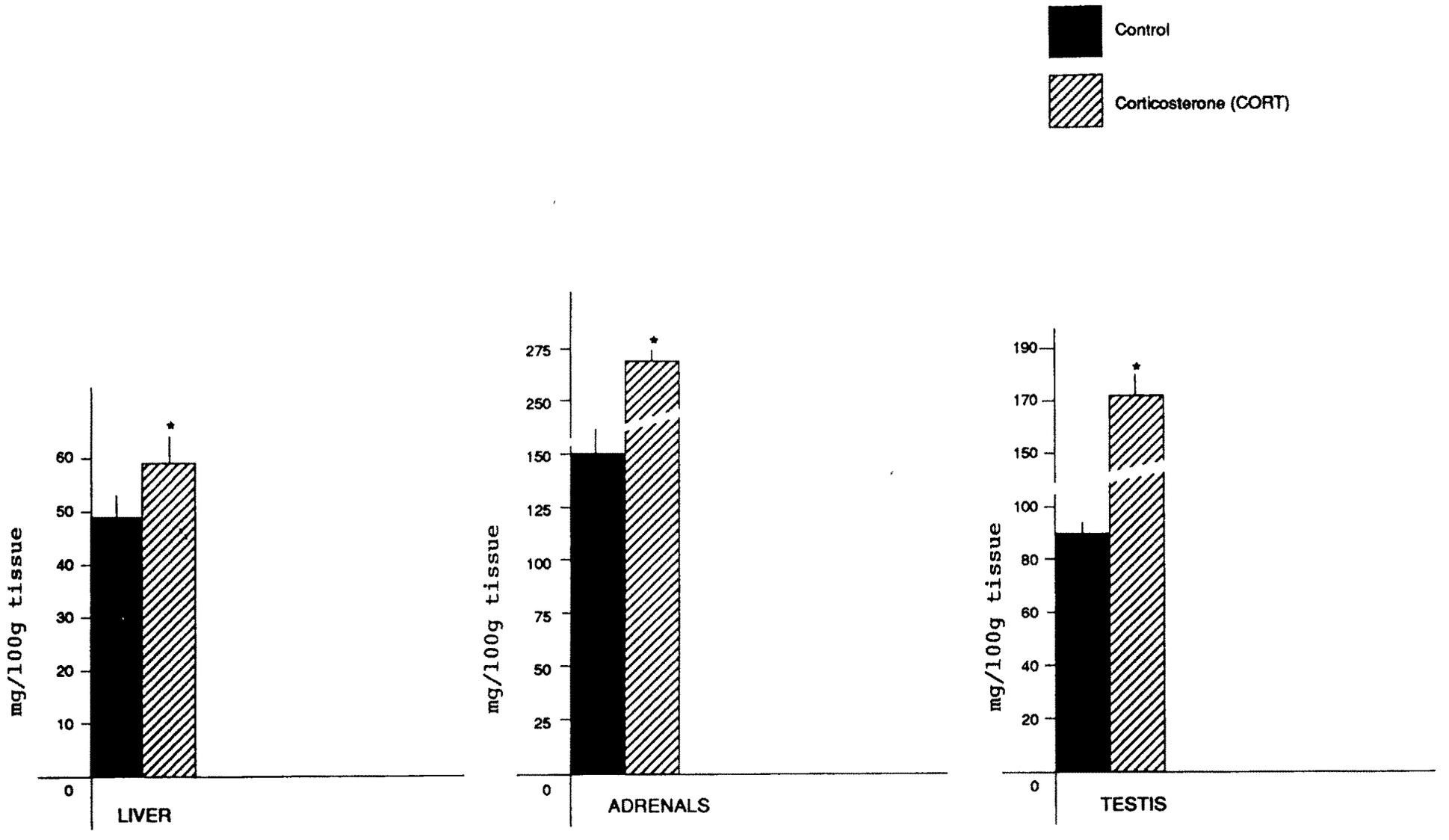

Fig 4 C Alterations in tissue ascorbic acid contents of pigeons subjected to hypercorticalism in the quiescent phase
(* Significant at $P < 0.05$, values are $\bar{x} \pm$ SEM)

experimental manipulations in the breeding season. Hepatic AA content increased and adrenal AA content decreased under various experimental schedules in the regression phase. In the quiescent phase, tissue AA contents increased with CORT treatment. Ascorbic acid content of testes increased under all treatment schedules irrespective of the season.

DISCUSSION.

The pattern of circannual variations in the AA content of liver (storage organ) and, adrenal and testis (organs of utilization) seen in the present study tend to suggest a parallelism between all the three organs. It would appear that the body as a whole enters into either a positive or a negative AA balance on a seasonal basis and that the changes can be correlated with adrenocortical activity and gonadal functions. A cursory glance of figure.4A reveals gradually increasing adrenal AA content from November onwards till a highest level is reached in April. Obviously, adrenal AA content starts increasing from the pre-recrudescent phase and continues through the recrudescent phase and attains a peak in the breeding phase, which corresponds well with the increasing adrenocortical activity and plasma CORT level recorded in the past studies (chapter I and II). Unlike in the case of mammals, the adrenal AA content in birds seems to run parallel with steroidogenic capacity as has been

inferred from the many studies on birds from this laboratory (Patel, 1982; Ayyar,1987; Joseph and Ramachandran, 1991). The increasing plasma CORT level induces a positive AA balance in the body as reflected in the concomitantly increasing hepatic and testicular AA content which dropped significantly between February and April, which is well correlatable with the increased spermatogenic and steroidogenic activities of the organ during this period. Ample evidences are available in this connection, linking AA utilization for steroidogenic and spermatogenic activities (Biswas, 1969; Biswas and Deb, 1970; Chinoy, 1972a; 1972b; Kitabachi and West, 1975). Though the post-breeding months extending from May to October were marked by decreasing adrenal and hepatic AA contents,the sudden elevation of testicular AA content in May could be attributed to a stock-piling of AA in the wake of a sudden cessation of steroidogenic and spermatogenic activities in the regressing gonads. The increased hepatic AA content in the recrudescent and the breeding months suggests increased metabolic activity of the organ which is supported by the previous observations showing increased glycogenolysis and hyperglycemia during these periods (chapter III). Overall, the circannual variations in tissue AA content in the pigeon are positively related with the alterations in adrenocortical activity and plasma CORT level and, is in keeping with the purported parallel testes-adrenal axis in

this bird (Patel et al.,1985; Ramachandran and Patel, 1986).

In the breeding season, both hypocorticalism as well as PX were shown to induce testicular regression and further, PX was also noted to have suppressive influence on adrenocortical activity (Patel et al.,1985; Ayyar, 1987; Ramachandran et al.,1987; Ramachandran and Patel, 1988). These observations have been confirmed by the decreased serum CORT level in PX birds as well as those rendered hypocorticalic. An evaluation of tissue AA content carried out in this study under the above condition reveals decreased hepatic and adrenal AA contents and increased testicular AA content. Apparently, hypocorticalism induces negative AA balance as has also been inferred earlier by Joseph and Ramachandran (1991) based on their studies in chicks. The decreased adrenal AA content noted here and the reduced serum CORT level reported earlier (chapter I), again allude to a parallel relationship between adrenal AA content and CORT output. The increased testicular AA content in both PX as well as DXM treated pigeons represents stock-piling of AA occurring due to the sudden testicular involution. These changes in tissue AA content under the experimental conditions in the breeding season mimic the changes occurring during the phase of normal gonadal regression. The inability of CORT to reverse the changes induced by PX suggests a positive interaction between pineal melatonin and CORT. This

has been justified by the earlier observations of the inability of CORT either to prevent testicular regression or the drop in serum CORT level induced by PX. The negative influence of PX on adrenal and testicular functions is further confirmed by the decreased adrenal AA content and increased testicular AA content in the regression phase as well. Administration of CORT to PX pigeons did not alter the changes in adrenal and testicular AA content while, that of the liver decreased, suggesting the insensitivity of PX pigeons to CORT or even an altered sensitivity in terms of hepatic AA content.

Corticosterone treatment either in the regression phase or the quiescent phase induced a positive AA balance in the body as a whole, as denoted by the increased hepatic and testis AA contents. Such an influence of CORT. was also inferred in chicks (Joseph and Ramachandran, 1991). Support to this concept comes from the observations of increased AA content in liver and blood in response to ACTH (Stewart et al., 1953; Sinha and Lahiri, 1964) and decreased AA content of liver in adrenalectomised rats (Giovanni et al., 1957; Cuzzocrea et al., 1959). The report of decreased activity of AA synthesizing enzymes, increased activity of AA catabolic enzymes and decreased AA content of liver, blood and urine in adrenalectomised rats (Nathani et al.,1971) provides further compelling evidences towards this. Interestingly,

adrenal AA content depicted a diametrically opposite trend in response to CORT in the regression and quiescent phases; while the adrenal AA content increased in the quiescent phase, it was decreased in the regression phase. A careful scrutiny of the circannual variations in adrenal AA content and the previously reported seasonal variations in serum CORT level (Patel, 1993; chapter I and III), reveals that the pre-recrudescent (Nov.-Jan.), recrudescent (Jan.-Feb.) and breeding phases (Feb.-April) are marked by a parallel increase in adrenal AA content and serum CORT level, while the regression phase (May-July) is marked by a parallel decrease in the two parameters. The increased adrenal AA content in response to CORT administration in the quiescent phase is in agreement with this and suggests a probable positive feedback action of CORT on ACTH secretion. Looking at the continuous increase in the adrenal AA content and serum CORT level throughout the recrudescent and breeding phases, it appears that the positive feedback action continues to function probably by way of decreasing the sensitivity of the negative feedback centre till, a high level of adrenocortical activity attained in the breeding phase sensitizes the negative feedback centre. This leads to a sudden decrease in the adrenal AA content and serum CORT level, heralding testicular regression. This contention is supported by the presently observed positive influence of CORT on adrenal AA in the quiescent phase and the negative

influence in the regression phase. Moreover, unpublished observations accruing from recent studies in progress, reveal a negative influence of CORT on adrenal AA content even in the breeding season. Obviously, exogenous CORT in the breeding season seems to increase its plasma level to the maximum threshold level, at which the negative feed back action comes into effect, leading to decreased adrenal AA content and serum CORT level, coupled with testicular regression (unpublished observations). Subsequent to this, the sensitivity of the feedback centre seems to be lowered as can be presumed from the observed influence of exogenous CORT in lowering adrenal AA content and serum CORT level in the regression phase, despite the fact that the adrenal AA content and serum CORT level are in the declining phase.

Overall, the present study suggests a parallel relationship between adrenal activity and AA content and an inverse relationship between testicular activity and AA content. Besides, it also gives evidence for the ability of CORT to induce a positive AA balance as well as for a differential season specific sensitivity of the hypothalamic feedback centre to CORT.