CHAPTER X

SEASONAL ALTERATIONS IN TRANSAMINASES AND PROTEIN CONTENT OF LIVER AND GONADS IN NORMAL AND ADRENAL MANIPULATED PIGEONS, COLUMBA LIVIA

Transminases or aminotransferases (the term aminopherase also has been suggested) are a group of enzymes known to occur in various tissues of animals and have also been demonstrated to occur in higher plants and in many micro-organisms. These enzymes are known to bring about transfer of amino groups to L-keto acids generating the corresponding amino acids. The first definite evidence for the presence in animal tissues of enzymes that catalyse such reactions was provided by Braunstein and Kritzmann (1937). Though there are many specific transaminases bringing about transfer of amino groups from specific amino acids, two of the most commonly studied ones are the glutamate - pyruvate transaminase also known as alanine aminotransferase and abbreviated as GPT and glutamate - oxaloacetate transaminase also known as aspartate amino transferase and abbreviated as GOT. Since these two enzymes are reportedly capable of acting on almost all amino acids, they are considered to be the major transaminases of animal tissues. Because of the pivotal role in linking the metabolism of carbohydrates and lipids with that of proteins, GPT and GOT have gained more importance in the studies of tissue metabolism. Though these enzymes do play an important role in tissue metabolism, most of the studies on GOT and GPT in general have been done with a pathological or clinical bias (Wroblewski and La Due, 1955, 1956; De Ritis _et al._, 1956; Waldman and Borman, 1959; Sacks and Lan Chantin, 1960). Barring a few studies (Felig, 1975; Muralimohan and Sasirababu, 1976; Whitting and Wiggs, 1977), no attempt has been made in the direction of corrrelating the activity of these enzymes with that of normal protein metabolism and tissue functioning.

Alterations in the protein content of gonads and other organs such as liver and muscle of seasonally breeding subtropical Indian birds has been apparent from the present study as well as from the reports of Patel (1982) and Patel (1984). Seasonal alterations with respect to testicular amino acid uptake in turtles have also been reported (Silva and Guillermo, 1982). Although various studies in the recent years deal with the effect of glucocorticoid action on transaminase activity, the possible influence of season as a factor in such type of studies has not been considered. Moreover, the studies of late pertain to tyrosine amino transaminase in several organs (see reviews by Laborit and Thuret, 1977; Myshuna _et al._, 1978; Mishunia, 1978). Previous studies carried out in this laboratory on pigeons have indicated alterations in lipid and carbohydrate metabolisms with respect to the annual breeding cycle (Patel, 1982; Patel, 1984). Since there is lack of literature regarding the inter-relationship between transaminase activity and the role of these enzymes in tissue metabolism, an attempt has been made to study the alterations in the activity levels of GOT and GPT along with the total protein content in liver, gonads and muscle of normal and experimental pigeons on a seasonal basis.

MATERIALS AND METHODS

As outlined in Chapter I

RESULTS

Changes in the total protein content and activity levels of transaminases are depicted in tables (1-7) and figures (1 - 7).

The values represented are the cumulative values of the tissues of birds of both sexes as no remarkable sex differences in either liver, muscle or the gonads was noted.

Changes in normal birds

Protein content : The protein content of all the tissues studied have exhibited seasonal alterations. The total protein content of muscle and liver was nearly the same, and that of gonads was half as that of liver and muscle. All the three tissues exhibited almost parallel seasonal changes in the total protein content with a slightly higher content during the non-breeding than during the breeding season. Liver exhibited a decrease of about 12% from regression to recrudescence and a further decrease of about 5% from recrudescence to breeding. Muscle protein content too exhibited a decrease of about 3.4% and 16% from regression to recrudescence and from recrudescence to breeding respectively. Though the gonadal protein content too depicted a similar change, the decrease from regression to recrudescence was more pronounced and amounted to about 23%. There was just a marginal increase of 5% in the protein content from recrudescence to breeding.

GOT and GPT activity.

Both the transaminases exhibited season specific changes in the liver as well as the gonads. Whereas the GOT activity was higher than that of GPT at all phases, the seasonal changes were of greater magnitude for GPT than that for GOT in both the tissues. Hepatic GOT activity was found to be least during the non-breeding phase and higher during recrudescence and breeding in that order.

Gonadal GOT activity too exhibited similar pattern of changes with a greater magnitude.

The season specific alterations pertaining to GPT activity in the liver and gonads not only showed parallel changes but also exhibited similar pattern of changes as that of GOT. Increased enzyme activity was the feature during the breeding phase in the gonads while reduced activity was observed during the non-breeding phase. Maximal enzyme activity was recorded during the recrudescent phase in both liver and gonads.

Changes in Experimental Birds

Protein Content - Protein content of adrenal suppressed birds exhibited increase under all the three doses of dxm used and in all tissues studied viz., liver, muscle and gonads. ACTH administration elicited a significant increase in the total protein content of liver, a decrease in the muscle and an insignificant increase in the gonads. LCM and LCE failed to bring about any significant alterations in the total protein content of either hepatic or gonadal tissues while the muscle protein content was decreased. High doses of corticosterone exhibited time specific influence on the total protein content in the sense that only HCM resulted in increased protein content of liver

TABLE-1 : SEASONAL CHANGES OF HEPATIC GOT ACTIVITY ("KARPMAN" UNITS/mg PROTEIN/ 60 MINUTES±SD) IN NORMAL AND EXPERIMENTAL PIGEONS *C. LIVIA*

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80μg	120μg	160μg	0.5 I.U.	1μgM	1μgE	3μgM	3μgE
RECRUDESCENT	269.12 ±79.12	191.31* ±43.54	132.89+ ±8.62	72.27*** ±16.14	-	-	-	-	-
BREEDING	257.84 ±26.87	226.56 ±52.72	239.51 ±30.56	257.28 ±26.10	-	-	-	-	-
REGRESSION	202.14 ±43.66	-	-	-	302.07** ±48.09	220.87 ±41.01	142.04* ±30.20	139.70+ ±17.38	109.74*** ±27.89

+ $P < 0.01$ * $P < 0.05$ ** $P < 0.005$ *** $P < 0.0005$

M - MORNING   E - EVENING

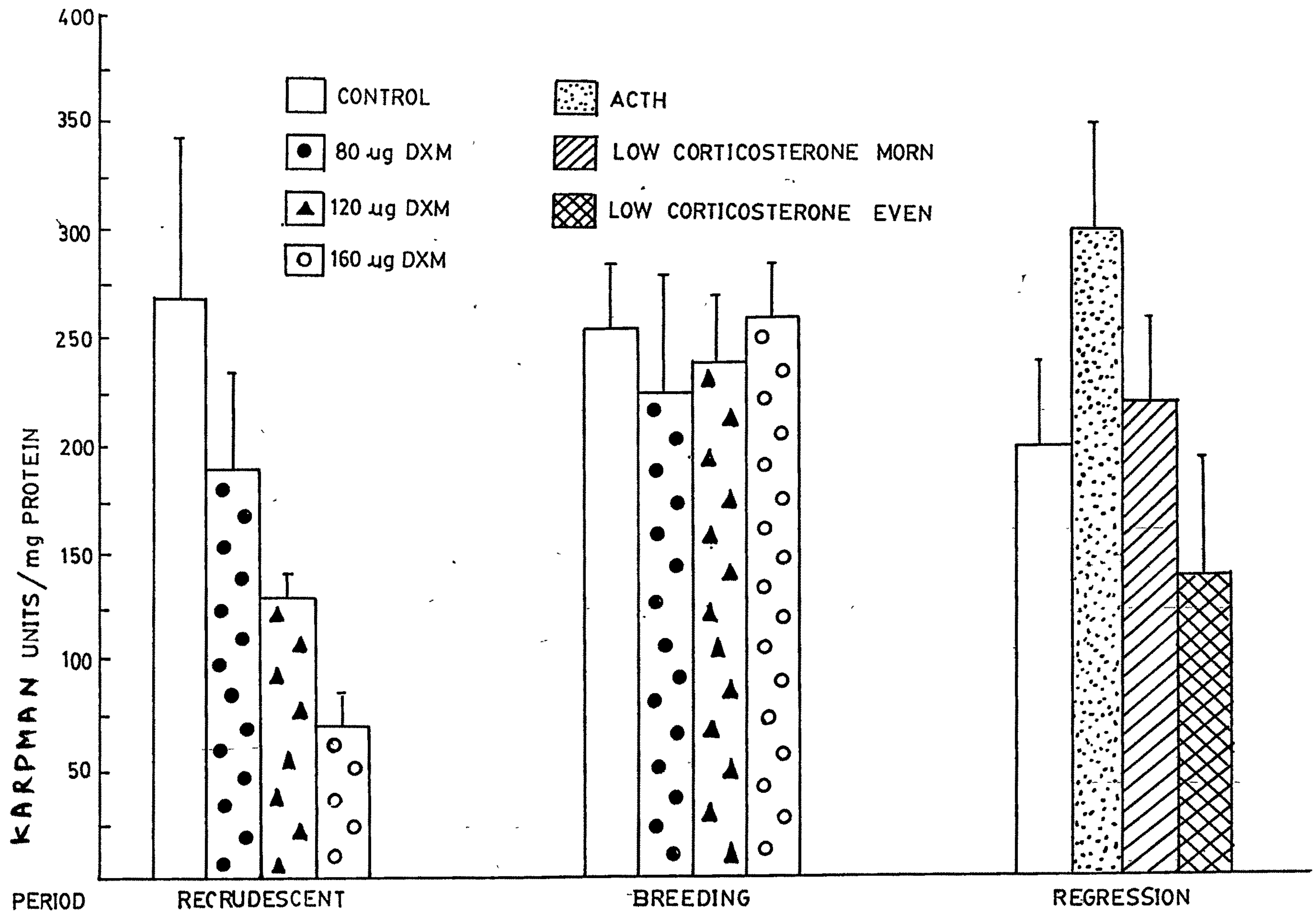

FIG.1. CHANGES IN HEPATIC GOT ACTIVITY

TABLE-2 : SEASONAL CHANGES OF GONADAL GOT ACTIVITY ("KARPMAN" UNITS/Mg PROTEIN/ 60 MINUTES) IN NORMAL AND EXPERIMENTAL BIRDS (± S D)

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	379.64	146.26***	118.70***	254.81+	-	-	-	-	-
	±73.37	±31.04	±18.94	±50.25					
BREEDING	226.50	132.66***	154.22***	175.94***	-	-	-	-	-
	±33.32	±24.72	±33.95	±20.56					
REGRESSION	169.36	-	-	-	325.30***	221.43++	80.03***	146.84	128.72*
	±32.19	-	-	-	±81.16	±39.91	±22.61	±21.34	±11.96

+ $P < 0.01$ ++ $P < 0.001$ * $P < 0.05$ *** $p < 0.0005$

M - MORNING E - EVENING

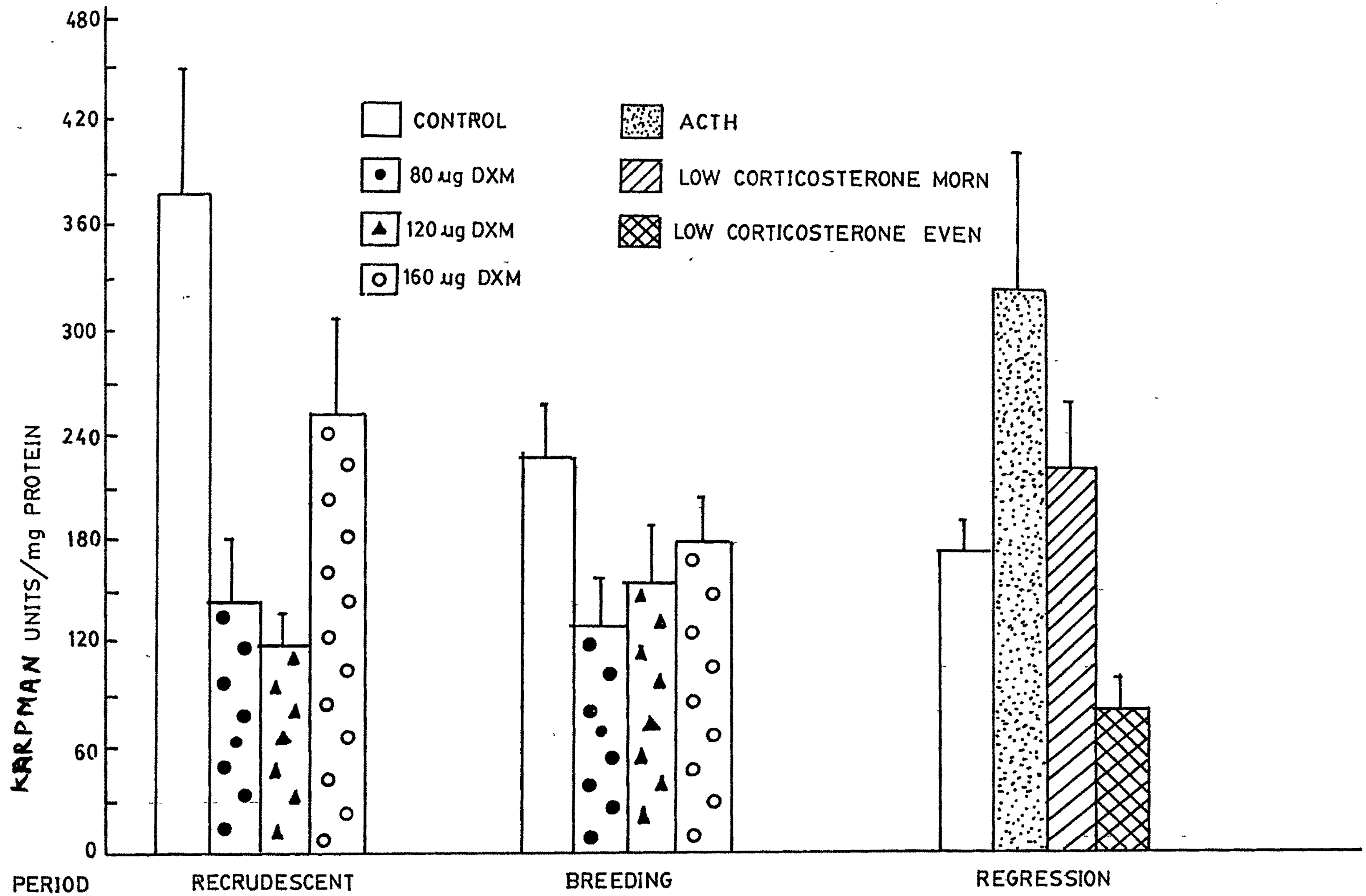

FIG.2. CHANGES IN GONAD GOT ACTIVITY

TABLE-3 : SEASONAL CHANGES OF HEPATIC GPT ACTIVITY ("KARPMAN" UNITS/mg PROTEIN/30 MINUTES) IN NORMAL AND EXPERIMENTAL BIRDS, *C. LIVIA*. (± S D)

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	135.97 ±46.41	148.36 ±55.91	138.17 ±25.44	167.59* ±12.67	-	-	-	-	-
BREEDING	30.21 ±9.27	71.24*** ±26.74	82.73*** ±4.35	53.84*** ±14.78	-	-	-	-	-
REGRESSION	52.43 ±16.12	-	-	-	38.27* ±7.14	37.64* ±7.06	26.22** ±4.96	32.40*** ±8.63	19.86++ ±4.33

++ $P < 0.001$ * $P < 0.05$ ** $P < 0.005$ *** $P < 0.0005$

M - MORNING   E - EVENING

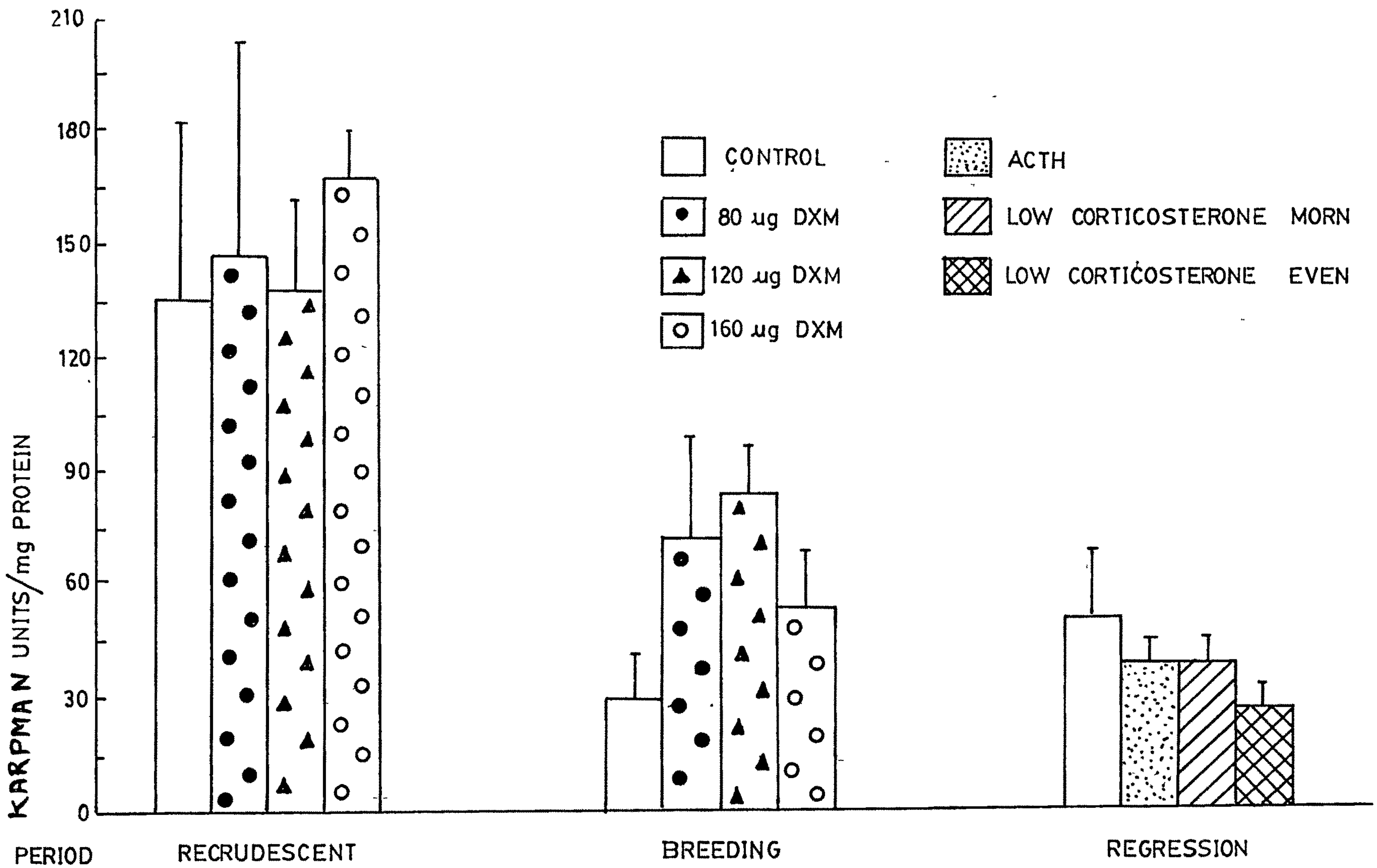

FIG.3. CHANGES IN HEPATIC GPT ACTIVITY

TABLE-4 : SEASONAL CHANGES OF GONADAL GPT ACTIVITY ("KARPMAN" UNITS/mg PROTEIN/30 MINUTES) 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	339.10	47.73**	35.36**	33.40**	-	-	-	-	-
	±115.35	±15.87	±7.09	±7.36					
BREEDING	142.49	18.79***	35.26***	22.50***	-	-	-	-	-
	±31.40	±2.32	±7.76	±7.33					
REGRESSION	19.43	-	-	-	26.81*	35.73**	14.64*	8.54***	11.86+
	±5.04				±3.91	±9.06	±4.16	±1.77	±4.53

+ $P < 0.01$ * $P < 0.05$ ** $P < 0.005$ *** $P < 0.0005$

M - MORNING E - EVENING

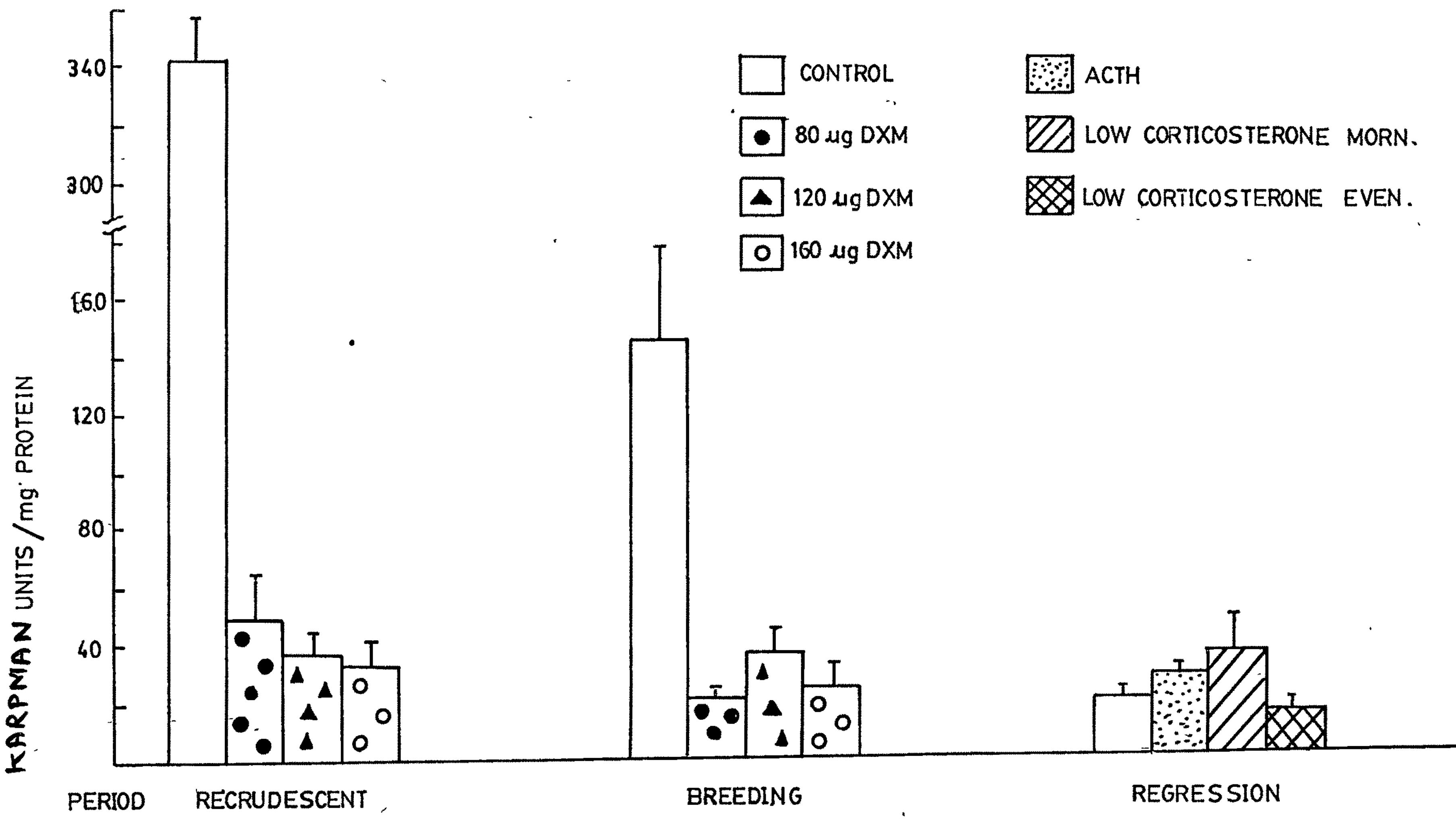

FIG. 4. CHANGES IN GONADAL GPT ACTIVITY

TABLE-5 : SEASONAL CHANGES IN HEPATIC PROTEIN CONTENT (mg/100 mg 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	8.00	9.77*	8.33	9.24*	-	-	-	-	-
	±1.48	±1.33	±1.20	±1.12					
BREEDING	7.59	9.68***	7.19	9.29++	-	-	-	-	-
	±0.59	±0.87	±0.77	±1.14					
REGRESSION	9.09	-	-	-	10.35*	9.95	8.10	11.83*	9.09
	±1.65				±0.78	±1.17	±0.74	±3.3	±1.39

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

M - MORNING E - EVENING

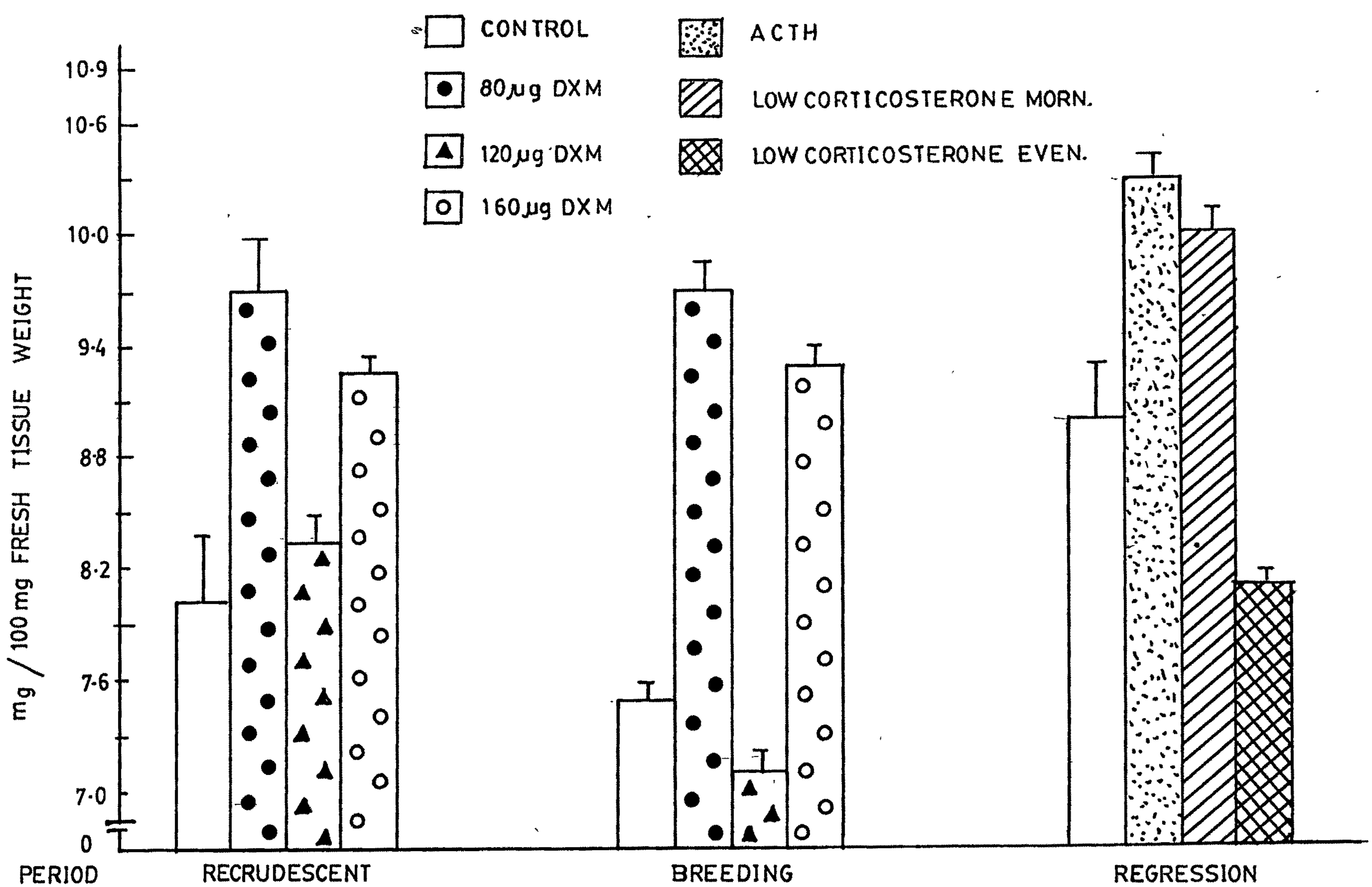

FIG. 5. CHANGES IN HEPATIC PROTEIN CONTENT

TABLE-6 : SEASONAL CHANGES IN MUSCLE PROTEIN CONTENT (mg/100 mg 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
RECRUDESCENT	8.67 ±1.21	10.99^{++} ±1.00	10.29^{+} ±2.26	9.74* ±1.19	-	-	-
BREEDING	7.27 ±0.96	10.14*** ±1.19	8.59** ±1.31	7.69 ±0.86	-	-	-
REGRESSION	8.97 ±1.09	-	-	-	7.42* ±0.48	7.45** ±0.87	6.62** ±1.19

+ $P < 0.01$ ++ $P < 0.001$ * $P < 0.05$ ** $P < 0.005$ *** $P < 0.0005$

M - MORNING E - EVENING

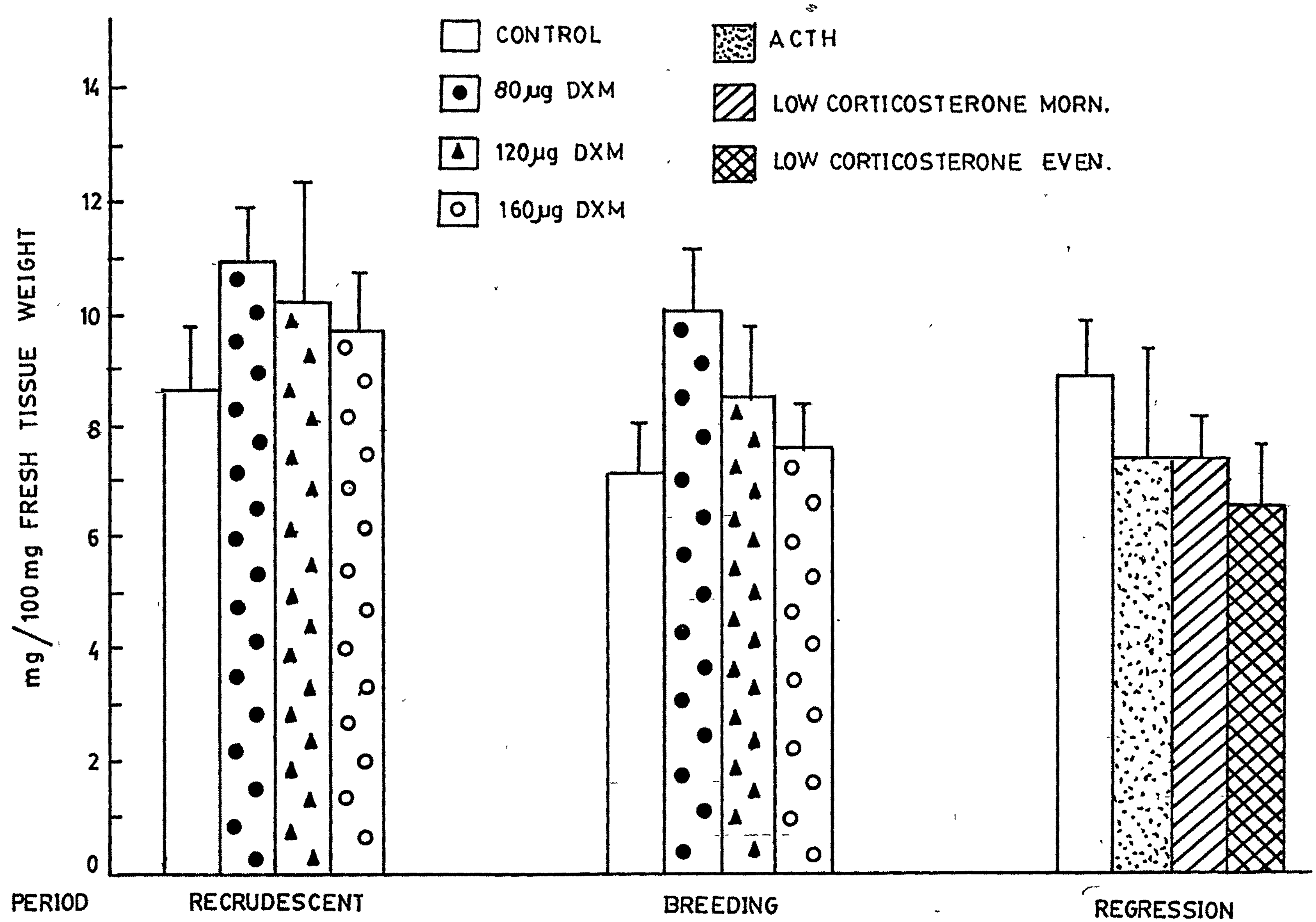

FIG.-6. CHANGES IN MUSCLE PROTEIN CONTENT

TABLE-7 : SEASONAL CHANGES IN GONADAL PROTEIN CONTENT (mg/100 mg TISSUE WEIGHT) IN NORMAL AND EXPERIMENTAL PIGEONS, *C. LIVIA* (±SD)

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80μg	120μg	160μg	0.5 I.U.	1μgM	1μgE	3μgM	3μgE
RECRUDESCENT	4.37	7.82***	8.03***	10.10***	-	-	-	-	-
	±0.82	±1.97	±1.03	±2.82					
BREEDING	4.58	6.66***	5.08	6.30***	-	-	-	-	-
	±0.87	±0.75	±0.89	±0.73					
REGRESSION	5.71	-	-	-	6.44	5.32	5.07	11.31***	7.44*
	±0.48				±1.27	±0.80	±1.04	±2.31	±1.32

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

M - MORNING   E - EVENING

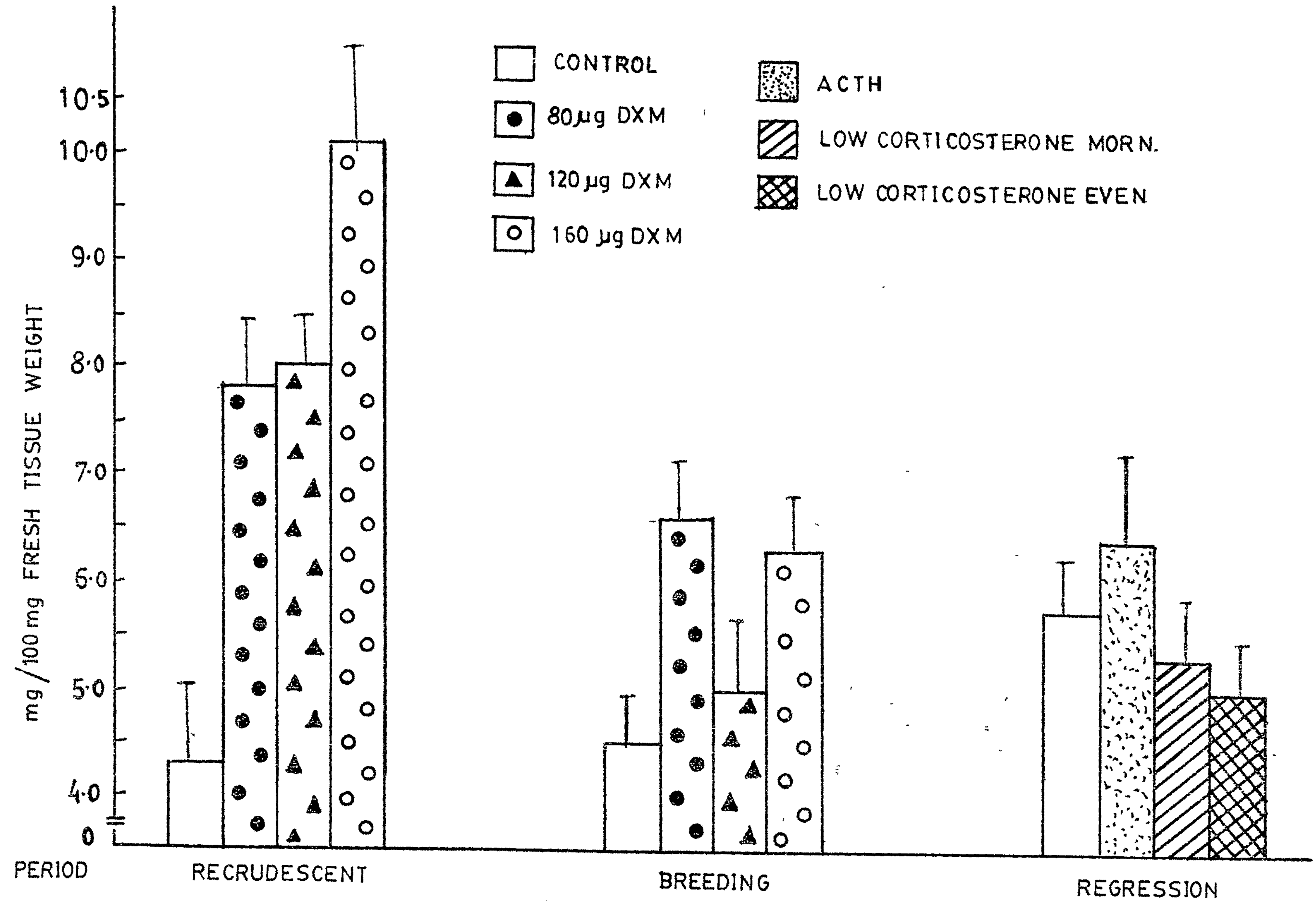

FIG.7. CHANGES IN GONADAL PROTEIN CONTENT

and gonads. However, HCE revealed significant decrease in muscle protein content.

GOT and GPT activity -

Tremendous alterations in the activity levels of these two enzymes have been recorded in the present study. Another characteristic feature worth mentioning is that whereas the changes in GOT activity were identical in liver and gonads under adrenal suppression, GPT activity exhibited differential alterations. An increase in liver GPT activity was exhibited in adrenal suppressed birds while decreased GPT activity was recorded in gonads. In contrast, the GOT activity showed a similar pattern of decrease in both liver and gonads of dxm treated birds. Administration of ACTH and LCM brought about favourable increase in GOT activity levels, a picture comparable to the pattern characteristic of control birds during recrudescence and breeding. However, LCE, HCM and HCE resulted in significant decrease in the activity levels of the enzyme in liver and gonads. Administration of ACTH and all doses of corticosterone elicited a positive response in the form of decreased GPT activity in hepatic tissue; the levels corresponding to those recorded for normal controls in the breeding phase. In the case of gonads, although injection of ACTH and LCM exhibited increased activity, the levels were still very much subnormal to those of the control levels during the breeding phase. LCE, HCM and HCE elicited a reverse effect and decreased the levels of enzyme activity further.

DISCUSSION

Organs undergoing seasonal functional alterations could be expected to show some enzymological variations and changes in total protein content. In the present study, significant alterations in GOT and GPT activities and subtle changes in protein content could be noticed on a seasonal basis. Accordingly higher protein content coupled with very low GOT and GPT levels during the non-breeding season and reduced protein content with significantly elevated GOT and GPT activity during the breeding have been recorded. Similar inverse relationship between gonadal protein content and breeding activities has been reported by Patel (1984). Further, Patel (1982) has observed decreased protein content not only in the gonads but also in liver, muscle and kidney of normal birds during the breeding season. Obviously, increased functional attributes of organs are marked by lowered protein content and such a change could be controlled by the subtle adaptive variations in the circulating levels of various endocrine principles. The observed increased adrenal activity coupled with decreased thyroid functioning during these periods (Chapters II, III; Patel, 1976) may probably constitute a hormonal balance favouring such changes in tissue protein content. Conversely, the increased protein content recorded in the non-breeding months was accompanied by decreased adrenal functioning and increased thyroid activity. This becomes obvious from the observable increase in tissue protein content and thyroid activity (Chapters II, III) in adrenal suppressed birds during the recrudescence and breeding phases. Administration of either ACTH, LCM or LCE in the non-breeding phase failed to elicit any significant alteration either in hepatic or gonadal protein content. However, muscle protein content was found to be decreased significantly. HCM or HCE administration brought about increased protein content in liver and gonads.

The importance of certain amino acids in testicular function has been known for quite sometime. However, this particular aspect has received very scant attention especially in species such as birds which show seasonal changes in gonadal activity. Levintow (1954) showed that glutamine synthetase was very active in rat and mouse testis. Later, Roussel and Stallcup (1967) and Joel and Herzberg (1965) demonstrated high levels of GOT activity coupled with lesser GPT activity in the male reproductive system of bull and man respectively. Similar results have been obtained in the present study in not only gonads but also in the liver of pigeons during the reproductively active phases. Thus the production of amino acids and the amides seems to be important for functional gonads. The concomitant increase in the activity levels of LDH and SDH (Chapter VII) could easily provide sufficient pyruvate and oxaloacetate necessary for the transmination reactions. It has been reported that of the specific amino acids formed from labelled glucose or glucose intermediates, those resulting from simple transmination reactions with glycolytic or citric acid cycle intermediates contained the most label in rat, rabbit and cod testes _in vitro_ (Hollinger and Davis, 1968; Mounib, 1967) and in the testicular fluid collected from conscious ram (Setchell _et al._, 1967). In the above studies aspartate was found to incorporate most label in rat, and glutamate in ram. Setchell _et al._ (1967) reported high concentration of glutamate, glutamine, glycine, alanine and aspartate in the testicular fluid of ram and suggested that such a presence may represent an especially favourable environment for nucleic acid synthesis within the seminiferous tubules as these amino acids are involved in purine and pyrimidine biosynthesis in one way or other. The increased GOT and GPT activities recorded in the present study during the reproductively active phases in this context could signify similar dependance of avian gonads too on aspartate and alamine family of amino acids for successful gametogenic activity. The parallel increase in enzymes' activity in the hepatic tissue apart from ensuring a supply of amino acids to the active gonads could also be purported to provide amino acids for gluconeogenesis as an increased hepatic G6-Pase activity has been observed in correspondence (Chapter IV).

Manipulation of adrenal activity during both breeding and non-breeding phases did bring about alterations in GOT and GPT activities in liver and gonads. Accordingly, dxm induced adrenal suppression brought about decrease in hepatic GOT, and gonadal GOT and GPT activities. A discordant note in this context was the increased hepatic GPT activity. Although literature dealing with correlation of adrenal steroids and transminase activity and amino acid content is scanty, a few reports available do indicate a possible correlation. Silva and Guillermo (1982) have opined that the seasonal variation in amino acid uptake could be due to testicular and hormonal cycles and also due to changes in membrane functions induced by acclimatization. Dunn et al. (1971) reported that while adrenalectomy does not diminish the incorporation of aspartate or glutamate into glucose or lower the aspartate trasaminase activity *in vivo*, it does however diminish the incorporation of alanine into glucose, alanine permeability as well as alanine transaminase activity. The present findings on an avian system depicts a slight difference in the sense that adrenal insufficiency suppressed both GOT and GPT in gonads. The resultant effect on transamination activities as well as amino acid metabolism in the gonads could be a significant contributory factor in the observed gonadal regression (Chapters II, III). Moreover, the reduced hepatic GOT activity and increased GPT activity apart from suggesting a differential influence of the adrenal steroids on hepatic and gonadal transaminases could also be responsible for disturbing the existing favourable metabolic equations leading to the observed gonadal regression during recrudescent and breeding seasons in adrenal suppressed pigeons.

The influence of adrenal steroids in regulating transaminase activity gains further credence from the currently observed increased gonadal GOT and GPT activities and hepatic GOT activity by ACTH or LCM administration during the non-breeding phase. Whereas the increased hepatic and gonadal GOT activity was very much comparable to the breeding levels, the increase in gonadal GPT activity though significant was very much less relative to the breeding level. The present findings on transaminase activity with respect to ACTH or corticosterone administration and dxm induced adrenal suppression indicate the definite influence of adrenal steroids in modulating hepatic and gonadal transaminases in relation to seasonal breeding activities in birds. Worth mentioning in this context is the report of Knox and Greengard (1965) of the stimulatory influence of cortisone on transaminases, especially hepatic GPT. However, the present observations of hepatic GPT activity is at variance and might suggest a negative influence of corticosteroids at least in birds. Another interesting result is the negative influence of LCE, HCM and HCE on both GOT and GPT of liver and gonads. Obviously, this once again underscores the dose and time specificity involved in corticosterone action as well as tissue sensitivity with respect to the seasonal physiological modulations characteristic of birds.

Though the metabolic effect of adrenal steroids in regulating protein metabolism is still far from clear, the few reports available do indicate a generalised influence of corticosteroids on transaminase activity and more studies are warranted to clarify the relationship between corticosteroids and protein metabolism. Many studies have indicated a stimulatary influence of corticosteroids on tissue tyrosine amino transferase activity, (Myshunina et al., 1978; Myshunina, 1978, 1979) and only one report of Roussel and Stallcup (1967) deals with GOT activity with respect to hormonal changes during oestrous cycle in rats. The present study in this context apart from filling the existing lacuna has also underscored the relative importance of adrenal steroids in shifting protein metabolism towards catabolism during the reproductively active phases. The report of Sadasivudu *et al.* (1978) of the influence of hydrocortisone in shifting nitrogen metabolism towards catabolism in the brain tissue coupled with increased glutamate utilization [illegible] is quite relevant.

S U M M A R Y

Total protein content and activity levels of transaminases (GOT & GPT) have been quatitatively assayed in the liver and gonads of pigeons during the three reproductive phases. Moreover, the effect of adrenal manipulation has also been assessed. The tissue protein content and GOT and GPT activities revealed a reciprocal relationship. Low protein content and increased levels of enzyme activity characterised the recrudescent and breeding phases while reverse was found to be true for the regression phase. Adrenal suppression in the active phases led to a reduction in transaminase activity in the gonads while its activation by either ACTH or corticosterone in the non-breeding phase increased the activity of the both the enzymes. These observations indicate that transamination reactions are involved in amino acid metabolism in the gonads during their active phases and that the adrenal steroids are capable of modulating these normal seasonal changes in the gonads of the pigeon.