CHAPTER VII

SEASONAL ALTERATIONS IN SUCCINATE DEHYDROGENASE, LACTATE DEHYDROGENASE AND ADENOSINE TRIPHOSPHATASE IN THE TISSUES OF NORMAL AND ADRENAL MANIPULATED PIGEONS, COLUMBA LIVIA

Seasonal breeders attune their breeding activities to a specific period of the year when the conditions are most favourable to bring about success in "reproduction" and also in the rearing of young ones. The entrained seasonal breeding pattern proceeds by means of established internal rhythms aided by environmental cues. Characteristically such annual rhythms of reproductive activity involve cyclic changes in reproductive organs and endrocrine glands. Along with the waxing and waning of gonads and endocrine glands (especially adrenals and thyroid) certain organs like kidney and liver undergo specific alterations with reference to enzymological and biochemical set-up.

Changes in certain enzymes in relation to breeding cycles in liver as well as in kidney of pigeon have been reported by Patel (1982). Histophysiological alterations with reference to breeding cycles have been documented in many seasonally breeding birds (Chalana and Guraya, 1974; 1978; Ambadkar and Chauhan, 1976; Mori and George, 1978). Garnier et al. (1973) have reported hormonal changes (testicular hormones) during testicular cycles of the pekin duck. In this wake, the possible role of adrenal

hormones in energy metabolism associated with reproductive cyclicity would be of interest and has received attention in the recent past (Chan and Norman, 1978; Petroiu et al., 1977; Cherkasova, et al., 1978; Valivullah et al., 1983a,b). Garstka et al. (1983) have reported metabolic changes in male snakes during the breeding period and have correlated the changes in oxygen consumption, nutrient distribution and alterations in lipid content and serum glucose with that of changing energy demands associated with the recrudescence of gonads.

Changing energy demands associated with seasonal reproductive cyclicity should involve qualitative and quantitative alterations in the operations of glycolytic and oxidative pathways of metabolism. Hence in the present study relative participation of glycolytic and oxidative pathways coupled with energy metabolism has been assessed by evaluating the activity levels of lactate dehydrogenase (LDH), succinate dehydrogenases (SDH), and Na^{+}- K^{+}- and Mg^{++} activated adenosine triphosphatases (ATPases) in liver and gonads of normal and adrenal manipulated pigeons on a seasonal basis so as to reveal the role played by adrenocortical hormones in the metabolic physiology associated with gonadal cyclicity in birds.

MATERIALS AND METHODS

As outlined in Chapter I

RESULTS

Results of the present findings have been depicted in tables 1-8 and figs. 1-8.

Changes in the Normal Birds

All the enzymes studied viz., LDH, SDH and ATPases have exhibited season specific alterations in both liver and gonads. LDH of both liver and gonads exhibited tremendous increase in activity during recrudescence from the lowest levels during regression; the increase being to the tune of 218% and 213% respectively. The enzyme activity in both the tissues registered a decrease during the breeding phase. SDH activity too, like LDH, showed lowest level of activity during regression from which it increased by 38% and 82% respectively in the liver and gonads during recrudescence. However, whereas the hepatic SDH activity exhibited a further increase from recrudescence to breeding, the gonadal enzyme activity decreased on the other hand.

The $Na^{+}K^{+}$ ATPase as well as Mg^{++} ATPase showed similar changes in both the tissues. Accordingly both the ATPases increased gradually through recrudescence to breeding from the lowest levels during regression.

TABLE-1 : SEASONAL CHANGES OF HEPATIC LDH ACTIVITY (μ MOLES PYRUVATE OXIDISED/ mg PROTEIN/15 MINUTES) IN NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.)

RECRUDESCENT PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80μg	120μg	160μg	0.5 I.U.	1μgM	1μgE
RECRUDESCENT	22.29 ±4.01	16.19++ ±1.43	18.77* ±1.65	14.76*** ±1.71	-	-	-
BREEDING	15.92 ±2.48	15.28 ±2.10	18.29 ±3.58	14.84 ±2.59	-	-	-
REGRESSION	7.00 ±1.92	-	-	-	13.26*** ±1.26	10.01*** ±1.01	10.74*** ±1.95

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

M - MORNING E - EVENING

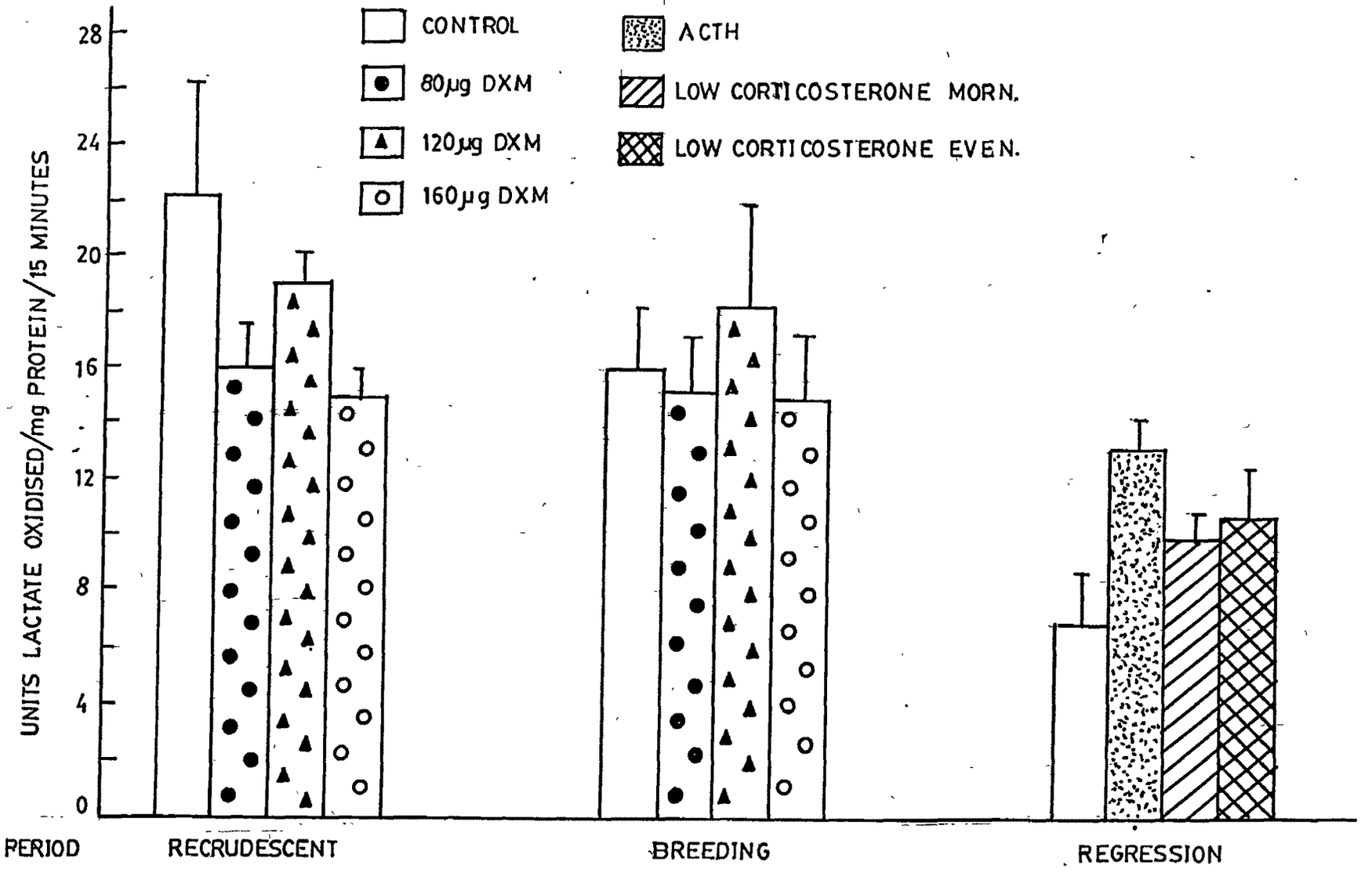

FIG. 1. CHANGES IN HEPATIC LACTATE DEHYDROGENASE ACTIVITY

TABLE-2 : SEASONAL CHANGES OF GONADAL LDH ACTIVITY ('µ' MOLES PYRUVATE OXIDISED/ mg PROTEIN/15 MINUTES) IN NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80µg	120µg	160ug	0.5 I.U.	1µgM	1µgE
RECRUDESCENT	44.34 ±6.40	38.75* ±4.8	32.46@@ ±6.10	27.73@ ±10.011	-	-	-
BREEDING	39.95 ±9.34	29.40@ ±5.91	30.83@ ±7.73	32.66* ±9.30	-	-	-
REGRESSION	14.17 ±3.2	-	-	-	34.02*** ±5.92	22.80** ±4.03	22.80** ±4.41

@ P<0.02 @@ P<0.002 * P<0.05 ** P<0.005 *** P<0.0005

M - MORNING E - EVENING

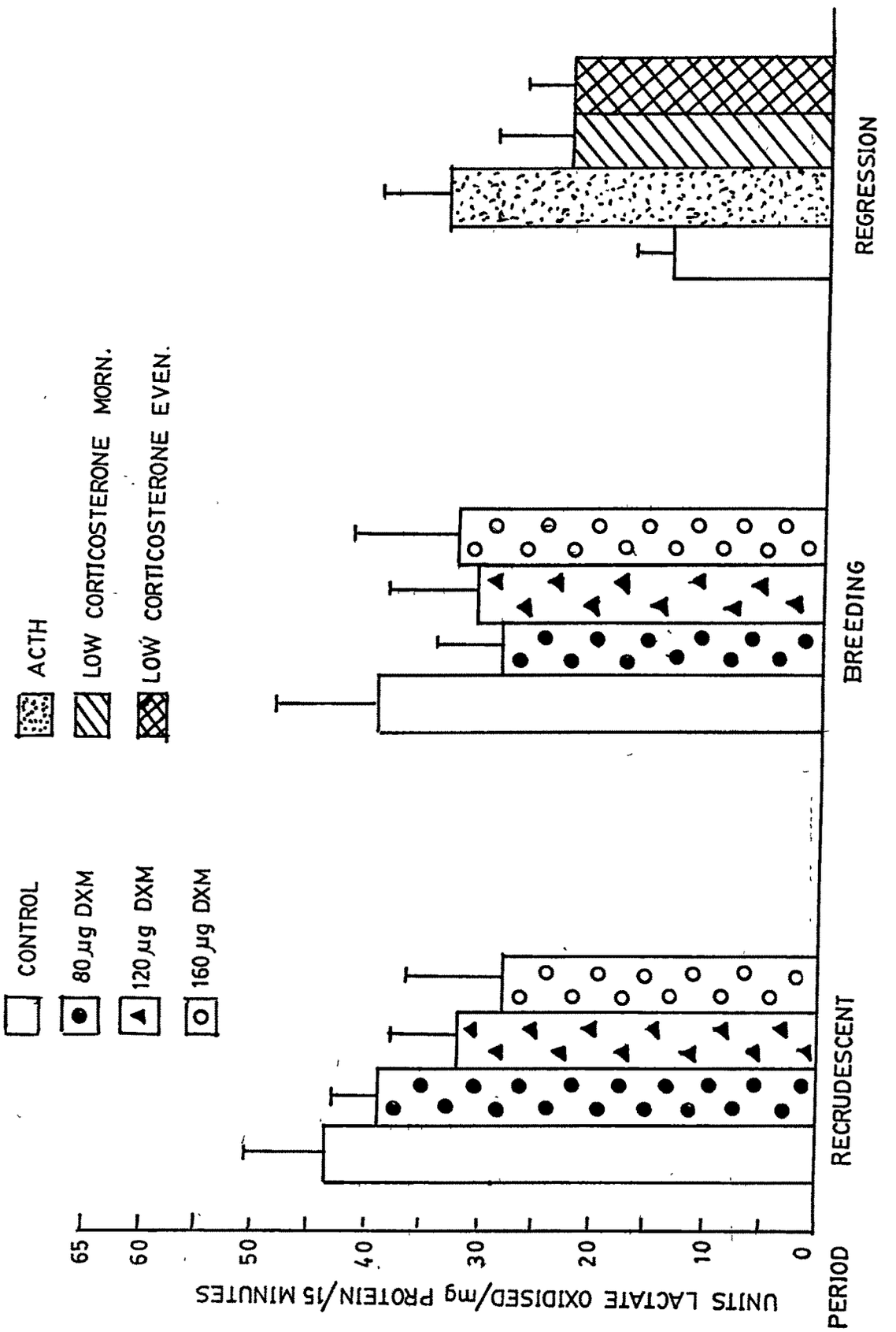

FIG. 2. CHANGES IN GONAD LACTATE DEHYDROGENASE ACTIVITY

TABLE-3 : SEASONAL CHANGES IN HEPATIC SDH ACTIVITY ('µ' MOLES INT REDUCED/mg PROTEIN/ 15 MINUTES) OF NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE
RECRUDESCENT	10.89 ±1.09	6.43*** 0.90	6.63*** ±0.30	7.00** ±0.41	-	-	-
BREEDING	14.99 ±0.70	8.29*** ±0.64	8.00*** ±0.80	8.00*** ±0.60	-	-	-
REGRESSION	7.89 ±0.15	-	-	-	9.98@@ 0.56	11.41*** ±1.00	8.20 ±1.13

@@ P< 0.002 ** P< 0.005 *** P< 0.0005

M - MORNING E - EVENING

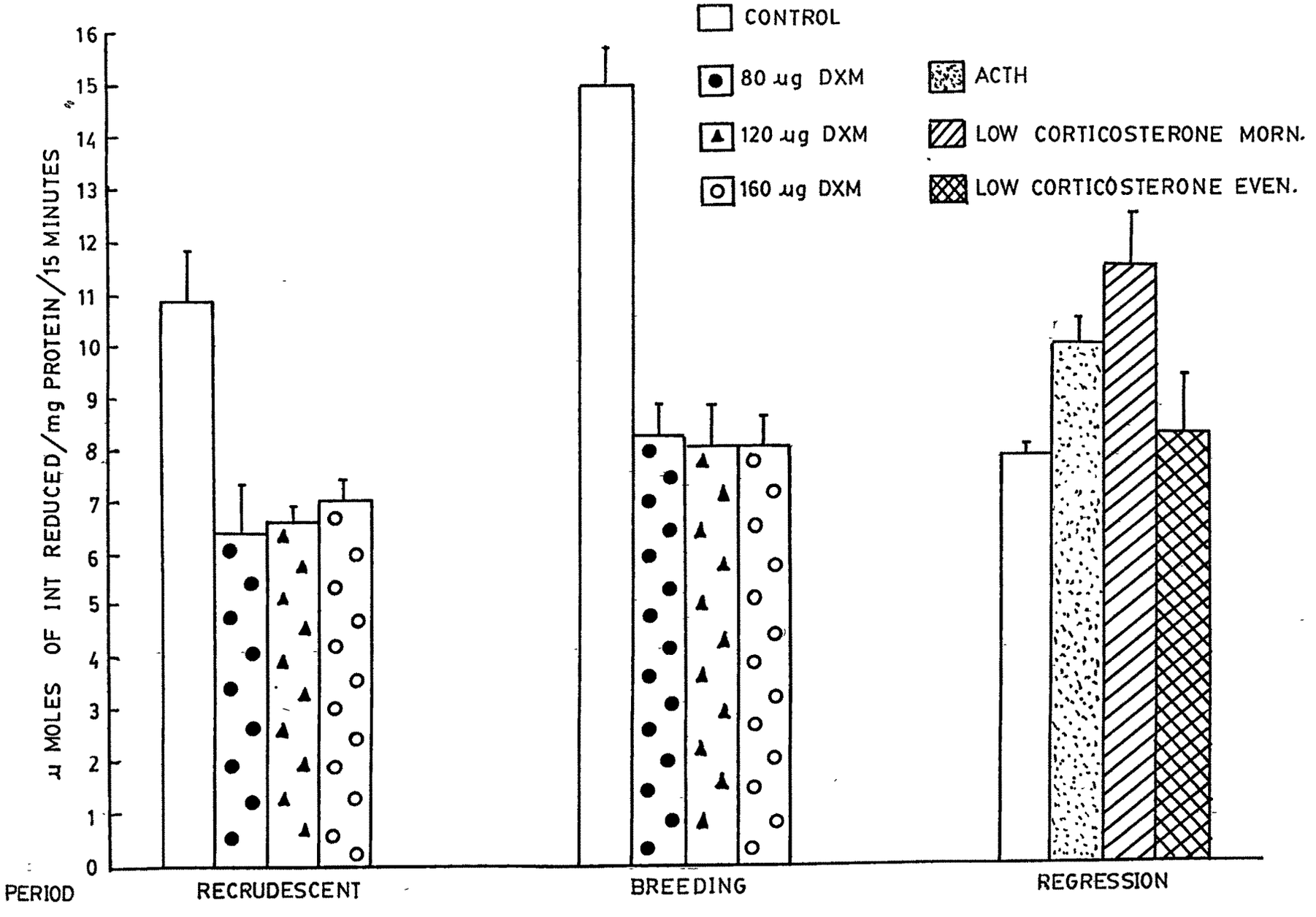

FIG.3. CHANGES IN HEPATIC SDH ACTIVITY

TABLE-4 : SEASONAL CHANGES IN GONADAL SDH ACTIVITY ('µ' MOLES INT REDUCED/ mg PROTEIN/15MINUTES) OF NORMAL AND EXPERIMENTAL PIGEONS, C.LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE
RECRUDESCENT	8.98 ±0.41	5.00*** ±0.09	4.68*** ±0.61	4.36*** ±0.43	-	-	-
BREEDING	6.01 ±0.61	4.68++ ±0.21	4.69++ ±0.40	4.93++ ±0.43	-	-	-
REGRESSION	4.92 ±1.00	-	-	-	7.01++ 0.61	7.23++ 0.64	5.39 0.52

++ P < 0.001 *** P < 0.0005

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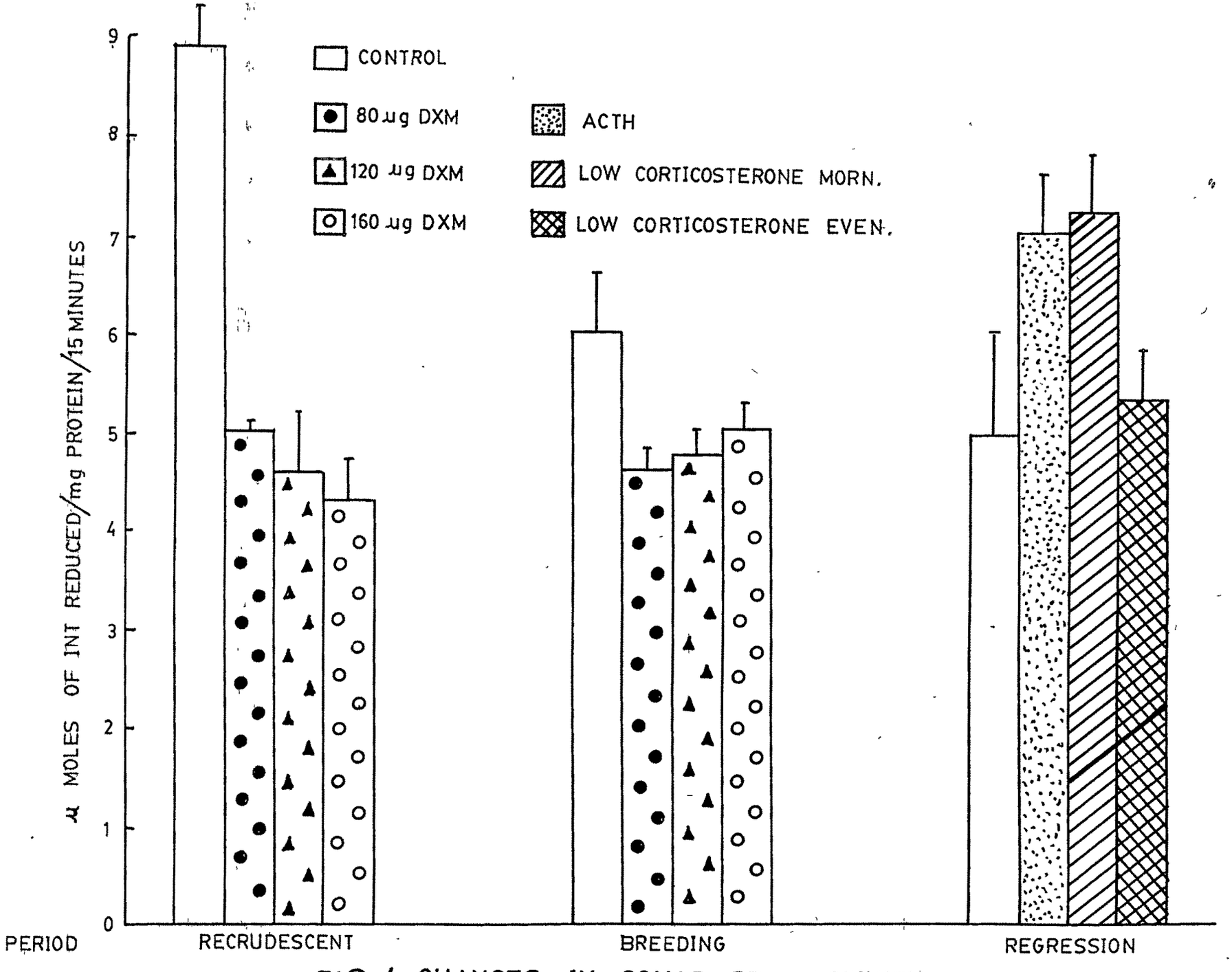

FIG. 4. CHANGES IN GONAD SDH ACTIVITY

TABLE-5 : SEASONAL CHANGES OF HEPATIC Na^+ K^+ ATPase ('µ' MOLES 'p' RELEASED/Mg PROTEIN/10 MINUTES) IN NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

PRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE
RECRUDESCENT	149.70 ±28.76	121.12* ±20.86	156.58 ±24.36	165.59 ±31.81	-	-	-
BREEDING	228.43 ±58.66	120.03*** ±22.0	128.03@@ ±23.14	133.41@ ±18.97	-	-	-
REGRESSION	67.93 ±16.89	-	-	-	83.03 ±9.89	88.11 ±5.30	80.31 ±13.97

@ P< 0.02 @@ P< 0.002 * P< 0.05 *** P< 0.0005

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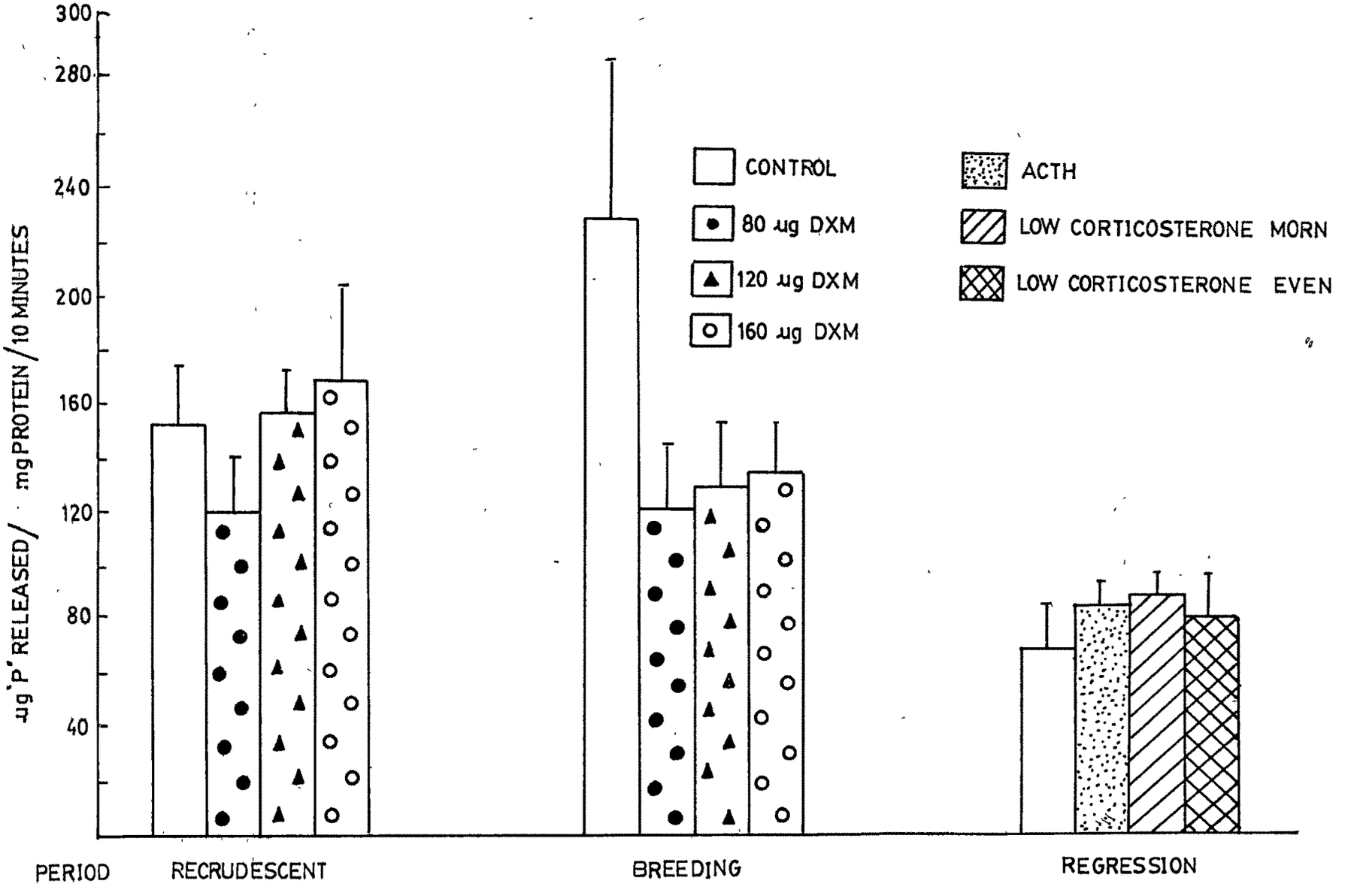

FIG. 5. CHANGES IN Na^+ K^+ ACTIVATED HEPATIC ATPase

TABLE-6 : SEASONAL CHANGES OF GONADAL Na^+ K^+ ATPase ('µ' MOLES 'p' RELEASED/mg PROTEIN/10 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
RECRUDESCENT	65.74 ±9.80	57.47* ±6.89	44.24** ±9.73	43.50** ±8.64	-	-	-
BREEDING	84.20 ±10.99	62.04@ ±18.77	61.43@@ ±10.12	60.44** ±11.86	-	-	-
REGRESSION	33.12 ±10.18	-	-	-	46.06* ±9.60	42.75* ±12.45	46.49* ±6.20

@ $P < 0.02$ @@ $P < 0.002$ * $P < 0.05$ ** $P < 0.005$

M - MORNING E - EVENING

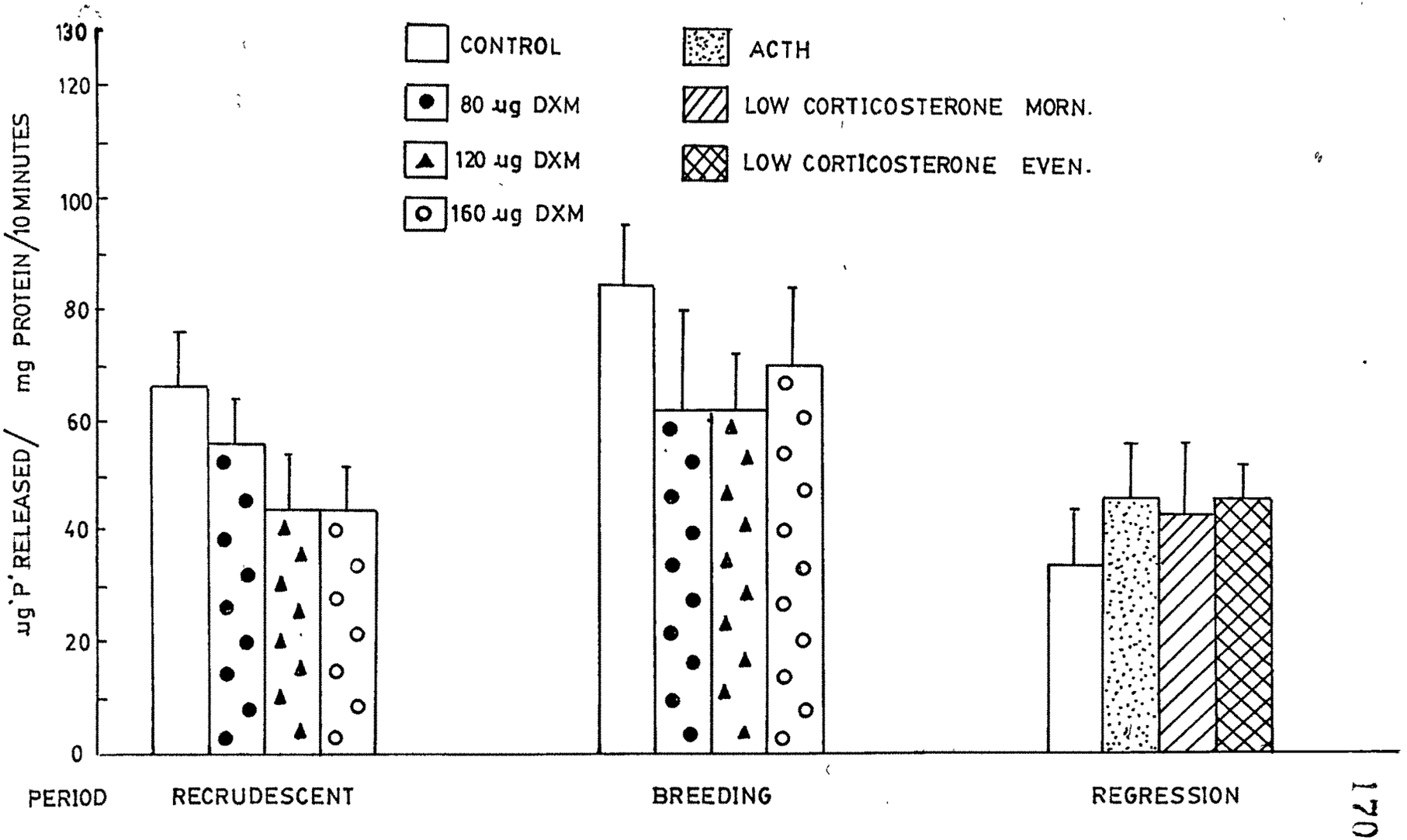

FIG. 6. CHANGES IN $Na^+ K^+$ ACTIVATED GONADAL ATPase

TABLE-7 : SEASONAL CHANGES IN HEPATIC Mg^{++} ATPase ('µ' MOLES 'p' RELEASED/mg PROTEIN/10 MINUTES) OF NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE
RECRUDESCENT	92.50 ±19.18	109.50 ±24.67	149.15*** ±27.25	134.97*** ±10.68	-	-	-
BREEDING	162.68 ±40.5	92.97** ±17.84	105.23@ ±18.11	111.50@ ±17.30	-	-	-
REGRESSION	57.01 ±14.02	-	-	-	79.14* ±9.69	81.48+ ±16.5	64.20 ±12.49

@ P<0.02 + P<0.01 * P<0.05 ** P<0.005 *** P<0.0005

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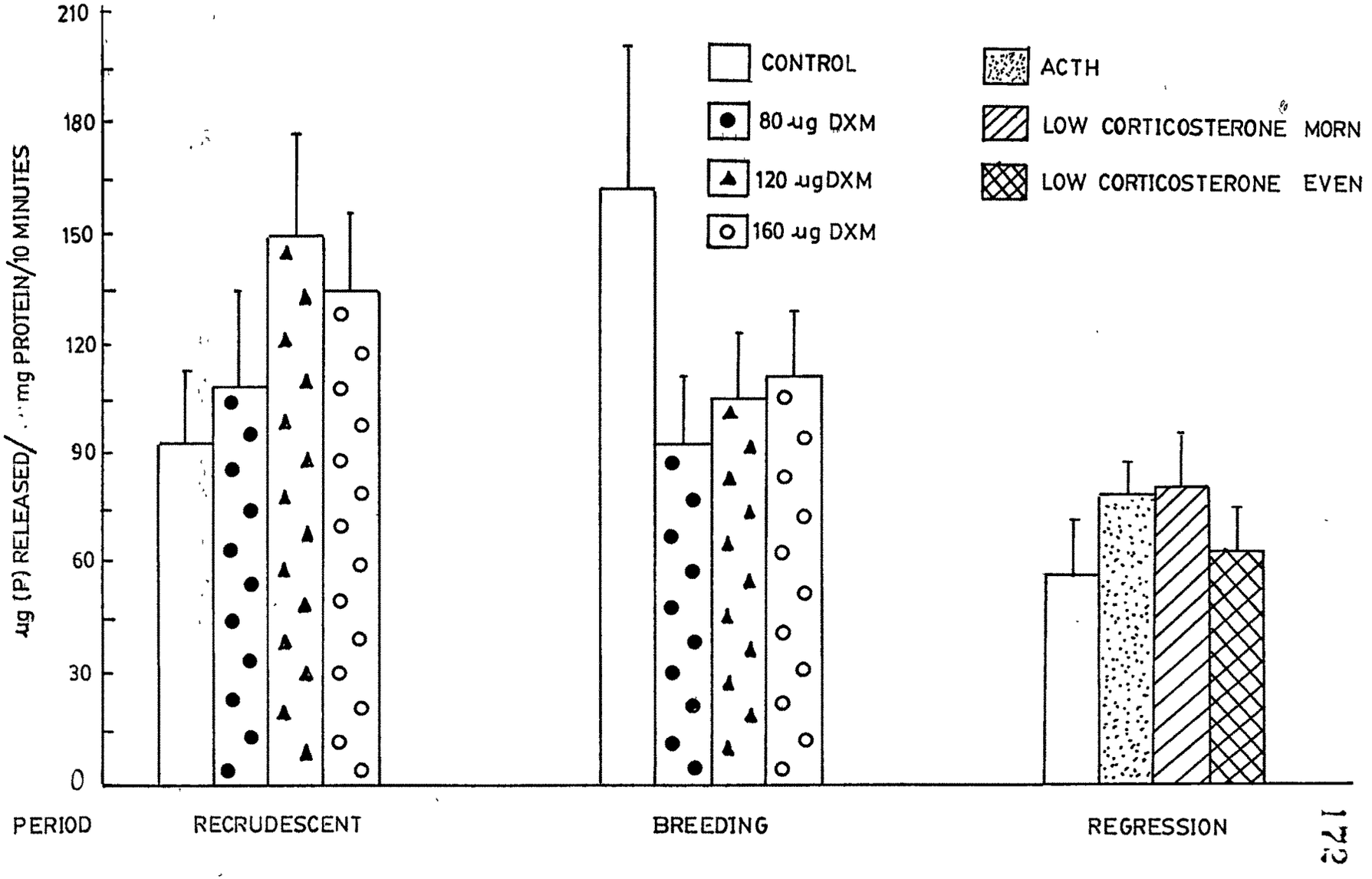

FIG. 7. CHANGES IN Mg^{++} ACTIVATED HEPATIC ATPase

TABLE-8 : SEASONAL CHANGES IN GONADAL Mg^{++} ATPase ('µ' MOLES 'p' RELEASED/mg PROTEIN/10 MINUTES) OF NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE
RECRUDESCENT	38.72 ±3.31	33.54* ±4.64	22.12*** ±6.72	27.65* ±8.8	-	-	-
BREEDING	63.19 ±19.9	31.91** ±9.96	34.25++ ±4.11	36.58* ±12.10	-	-	-
REGRESSION	15.66 ±3.28	-	-	-	26.40* ±8.12	31.98*** ±5.25	16.38 ±2.91

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

M - MORNING E - EVENING

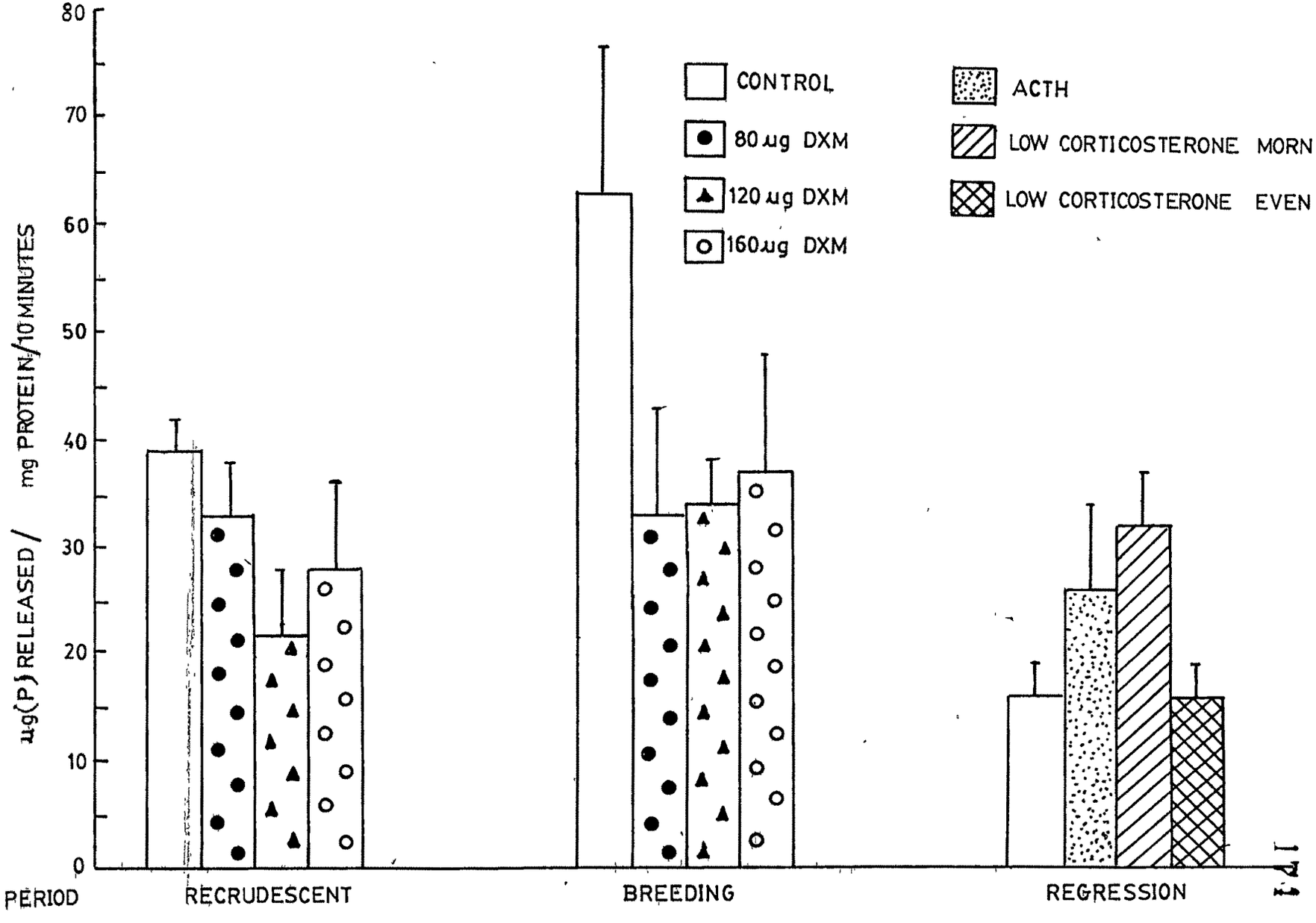

FIG. 8. CHANGES IN Mg^{++} ACTIVATED GONADAL ATPase

Changes Under Experimental Conditions

In general the activity levels of all the four enzymes in both liver and gonads were found to be decreased under dxm induced adrenal suppression during both recrudescence and breeding. The decrease was significant in all cases except for the hepatic LDH activity during the breeding season, and the Na^{+}- K^{+} ATPase activity during the recrudescent phase. Again, the administration of ACTH/corticosterone during the regression phase significantly elevated the activity of all the four enzymes in both the tissues. This increased activity in most of the cases was still subnormal in relation to the levels of activity observed in the control birds during the recrudescent and breeding phases.

DISCUSSION

Presence of high activity of SDH is indicative of the operation of TCA cycle oxidations while LDH activity points towards the operation of anaerobic glycolysis. In the present study, the activity of both the enzymes was found to be increased during the reproductively active phases of recrudescence and breeding. This would suggest the active participation of glycolytic pathway coupled with TCA cycle oxidations in both liver and gonads in order to meet the increased energy demands associated with gonadal

activation and functioning. As a corollary the decreased levels of enzyme activity during the regression phase is indicative of the ebb in enzyme activity related to reduced energy demands associated with gonadal quiescence. Except for some species of reptiles and amphibians the gonadal content of carbohydrate reserves is very low and unequally distributed (Cavazos and Melampy, 1957). Hence the gonads are probably dependent upon a constant supply of exogenous substrates. It is presumable that the process of gametogenesis entails an expenditure of energy for biosynthesis and the contribution of glucose, pyruvate, or the glycolytic products towards energy production in the gonads of many species has been reported by Mounib (1967) and Annison et al. (1963). Further, it has been reported that higher RQ in a tissue is indicative of anaerobic glycolysis and the testis has considerable ability for lactate formation from glucose anaerobically (Ewing and VanDemark, 1963; Ewing et al., 1966). Moreover, Dickens and Simer (1929) have shown increased anaerobic glycolysis in rat testis with increase in glucose concentration. Although similar observations have not been made with regard to avian gonads, such possibilities can be presumed from the increased LDH activity recorded herein during the recrudescent and breeding phases and the cocomitantly increased blood glucose levels (Chapter IV). Adrenal suppressed birds in the present

study depicted decreased LDH activity in both liver and gonads along with gonadal regression (Chapters II and III). Apparently, decreased glycolytic activity due to adrenocortical insufficiency may bear either a cause or effect relationship with the observed gonadal involution. Some supportive evidence in this context can be drawn from the report of Valivullah et al. (1983a) of reduced LDH activity and depleted glycogen content in the testis of prepubertal rats due to adrenalectomy. The role of corticosteroids in regulating LDH activity and carbohydrate metabolism is further emphasized by the herein observed increase in LDH activity due to ACTH/corticosterone administration during the regression phase. Further validity to this aspect is provided by the report of Takaishi et al. (1977) which shows elevated serum levels of LDH in patients with Cushing's syndrome and its normalisation by surgery.

SDH activity in testis has been measured by a number of workers (Blackshaw, 1963; Brown et al., 1966; Blackshaw and Samisoni, 1967), and SDH along with MDH and ICDH are considered to be the most active TCA cycle enzymes in the testis. Season specific alterations in the pattern of respiration in fertile adult testis were also evidenced in the cyclic regeneration of the fertile testis of the cotton rat, Sigmodon hispidus (Ewing et al., 1965). In the present study as well, alterations in the form of relatively high

SDH activity in the gonad and liver during recrudescence and breeding as compared to regression have been observed. It is known that TCA cycle intermediates are utilised for biosynthetic processes and the present observation emphasizes this aspect to be of utility value during gonadal recrudescence and active spermatogenesis in the pigeon. Increased SDH activity in the tissues of migratory avian species has also been reported to occur in the premigratory phase prior to migration to the breeding ground (Patel et al., 1976). Moreover, increased hepatic SDH activity in the liver of wild pigeons has also been documented by Patel (1982). Obviously the favourable disposition of the TCA cycle, as denoted by the SDH activity, in the biosynthetic activities during the active phases of reproduction is clearly evident. In this light the largest drain on TCA cycle intermediates could be for the synthesis of nucleic acids and proteins as can be inferred from the reports of the appearance of specific labelled amino acids in the testicular fluid after infusion of labelled glucose (Mounib, 1967; Setchel et al., 1967) as well as the currently recorded high GOT and GPT activities (Chapter IX). Similar high SDH activity in the liver concomitantly reflects the increased metabolic transformations occurring in the hepatic tissue associated with recrudescence and breeding. Marked elevation in the activity levels of many respiratory and pentose cycle enzymes has been reported to occur in the testis of infantile rats

following chronic gonadotrophin administration (Schor et al., 1963; Ostadalova et al., 1967).

However in the present study, decreased SDH activity subsequent to adrenal suppression during the active phases and increased activity after ACTH/corticosterone administration during the inactive phase have been documented. Similarly Sharma and Patnaik (1982) have shown decreased activity of MDH isoenzymes in the liver of adrenalectomized rats and its reversal by hydrocortisone treatment. Langdon et al.. (1984) have also observed increased SDH activity in a suspension of gill cells under the influence of ACTH and cortisol in juvenile Atlantic Salmon. From these it can be presumed that the adrenal corticosteroids do have a favourable influence on oxidative metabolism associated with active gonadal functioning during the seasonal breeding activity in Columba livia.

Activities of Na^{+}-K^{+} dependant and Mg^{++} dependant ATPases due to their membrane and mitochondrial localisations respectively are generally correlated with transport functions and energy transformations respectively. In this wake increased activity of both the enzymes in the present study in liver and gonads during the recrudescent and breeding phases portend their involvement in active transport and energy metabolism. Similar seasonal alterations in Mg^{++}

ATPase activity in the liver of wild pigeons and Ca^{++} ATPase in the testis of common frog during active spermatogenesis have also been reported (Patel, 1982; Juelich et al., 1982). Beyond this, the present study also highlights the role of adrenal corticosteroids in mediating these changes as seen by the decreased enzyme activity in adrenal suppressed birds and increased activity in ACTH/corticosterone treated birds which is in keeping with the inferred parallel adrenal-gonad axis in pigeons (Chapters II and III, Patel et al., 1986; Ramachandran and Patel, 1987). Though there are reports indicating the favourable influence of adrenal corticosteroids on Na^{+}- K^{+}-ATPase of intestinal mucosa (Gnanaprakasan and Srivatsava, 1978), liver (Miner et al., 1980), Red cell ghosts (Wambach et al., 1980) and sub-mandibular gland (Marisa et al., 1983), there is no report on the influence of corticosterone on ATPase activity of gonads and hence the present study becomes interesting in this respect and bespeaks of the involvement of the adrenal gland in modulating biochemical activities of the gonads in birds associated with seasonal cyclicity. The present findings also reveal the influence of corticosteroids in regulating mitochandrial functioning as denoted by the Mg^{++} dependent ATPase aetivity. This is in contrast to the observations of Wambach et al. (1980) who observed no change in Mg^{++} ATPase in the red cell ghosts of patients with

Cushing's syndrome. Marisa et al. (1983) too failed to observe any change in Mg^{++} ATPase in the submandibular gland after the administration of corticosterone. However, Patel (1982) had observed increased Mg^{++} ATPase in the liver of wild pigeons during the breeding season. Apparently, in pigeons the adrenal hormones do influence mitochondrial functioning in relation to breeding activities.

S U M M A R Y

Levels of activity of LDH and SDH, key enzymes of glycolytic pathway and TCA cycle, together with those of Na^{+} - K^{+} and Mg^{++} - activated ATPases have been evaluated in the liver and gonads of normal and adrenal manipulated pigeons on a seasonal basis. All the four enzymes were found to be maximally active during the breeding months in both the organs. However, ACTH/corticosterone administration in the non-breeding season could restore the levels of activity closer to those characteristic of the breeding phase. Similarly, adrenal suppression by dexamethasone in the breeding months lowered the [illegible] enzyme activity to the non-breeding levels. The results obtained indicate active metabolic interconversions, transport and energy transactions during the recrudescent and breeding seasons, and the probable involvement of corticosterone in modulating these processes in relation to gonadal cyclicity.