CHAPTER IV

SEASONAL ALTERATIONS IN BLOOD GLUCOSE LEVEL, TISSUE GLYCOGEN CONTENT AND HEPATIC GLUCOSE-6-PHOSPHATASE ACTIVITY IN NORMAL AND ADRENAL MANIPULATED PIGEONS, COLUMBA LIVIA

Carbohydrates, the major source of energy in the body are known to be widely distributed in almost all tissues. The most important carbohydrate reserve of animal tissues is glycogen which is also the most preferred stored energy material because of its gigantic molecular size, limited solubility and less osmotic effect. Glycogen is also known to contribute effectively to the maintanance of blood glucose level. Glycogen is reported to be present in almost all the tissues, the liver and muscle being the major storage sites of the metabolite.

Several hormones are known to affect carbohydrate metabolism. Apart from insulin and glucagon, avian pancreatic polypeptide, corticosteroids, catecholamines and pineal principles are also known to modulate carbohydrate metabolism. However, insulin and glucagon are the known major hormones that are involved in modulations of carbohydrate metabolism. Birds are known to be primarily glucagon dependent with insulin playing only a secondary role (Hazelwood, 1973). Seasonal breeders are known to exhibit cyclic variations in their metabolic pattern thereby inducing modulations in

energy equilibrium to ensure the success of breeding activities. Concomitantly one can expect definite alterations in the quantitative levels of metabolites, enzymes etc.

Seasonal alterations in tissues glycogen content have been reported by several workers in varied species of animals. (Castineiras et al., 1977; Ramachandran et al., 1986; Ramachandran and Patel, 1987). Blood glucose has also been noted to undergo variations with respect to the breeding cycle. This has been observed in lizards, Iguana iguana and Hemidactylus flaviviridis by Acuna et al. (1977) and Ramachandran and Chacko (1987) as well as in pigeons, Columba livia by Patel et al. (1983) and Ramachandran and Patel (1987). Corticosteroids are known to be glucogeogenic in nature. Further proof regarding the glucogeogenic nature of corticosteroids comes from the reports of Abraham and Istran (1978) who observed that hydrocortisone inhibited glucose uptake, intensified glucogeogenic pathway; the pool of acetate and glycine formed as a result of the same being converted to glycogen. Further, the activity level of the gluconeogenic enzyme G-6-Pase has been observed to undergo season specific alterations (Patel, 1982). Since carbohydrate metabolism has been noted to undergo seasonal changes with respect to breeding cycle and as corticosteroids are capable of influencing the same, the present study on blood glucose levels and the quantitative content of glycogen in tissues, viz -

Liver, muscle and gonads was undertaken in feral blue rock pigeons, Columba livia under adrenal suppression during the breeding season and ACTH/Corticosterone administration in the non-breeding season along with that of hepatic G-6-Pase activity.

MATERIALS AND METHODS

As outlined in Chapter I

RESULTS

The various changes are depicted in tables 1-3 and figs. 1-3. Gonadal glycogen content represented are values of testis and ovary put together as no remarkable sex difference was discernible.

Seasonal Changes in Normal Birds

Whereas the glycogen content of muscle and liver was maximum during the regression period, the glycogen content of gonads was found to be maximum during the recrudescent months. Liver depicted a progressive reduction in the glycogen content from regression to breeding through recrudescence, an overall depletion of 61%. Muscle glycogen too showed a depletion from regression to recrudescence (55%). However, there was an increment in the glycogen content between recrudescence and breeding. Gonadal glycogen content showed a gradual increase from a minimum during regression to a maximum during breeding through recrudescence, an increase of about 165%.

Blood glucose was maximum during the recrudescent months which gradually decreased through the breeding months to a minimal level during the regression months.

Glucose-6-Phosphatase activity (G-6-Pase) was greatest during the breeding months. The activity level of this enzyme was found to be least during the non-breeding months. There was a significant increase in the activity level of G-6-Pase from regression to recrudescence with a further increase from recrudescent to breeding thereby attaining peak activity in the breeding season. On a percentage basis the increase in the enzyme activity from the lowest level during regression to its maximum level during breeding was to the tune of 198%.

Changes Under Experimental Conditions

Adrenal suppression by dxm and activation by injection of corticosteriods brought about tissue and season specific alterations in the glycogen content. Adrenal suppressed birds depicted significant deposition of glycogen in liver both during recrudescence and breeding while there was depletion under ACTH and corticosterone administrations during regression. Muscle and gonads exhibited differential response under hypoadrenalism in the two seasons studied viz., recrudescent and breeding. Whereas muscle glycogen content was found to be increased during breeding, the gonad glycogen content recorded significant

TABLE-1 : SEASONAL CHANGES OF BLOOD GLUCOSE LIVER (mg/100 ml; ±.S.D.) 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	210.17 ±15.29	172.51*** ±16.60	165.83*** ±11.48	148.78*** ±7.57	-	-	-	-	-
BREEDING	194.87 ±18.88	163.36*** ±20.44	142.75*** ±18.31	153.69** ±16.01	-	-	-	-	-
REGRESSION	180.06 ±23.35	-	-	-	197.47 ±6.32	230.28*** ±16.07	184.80 ±29.90	171.69 ±31.47	171.90 ±20.0

*** P< 0.0005 ** P< 0.005

M - MORNING E - EVENING

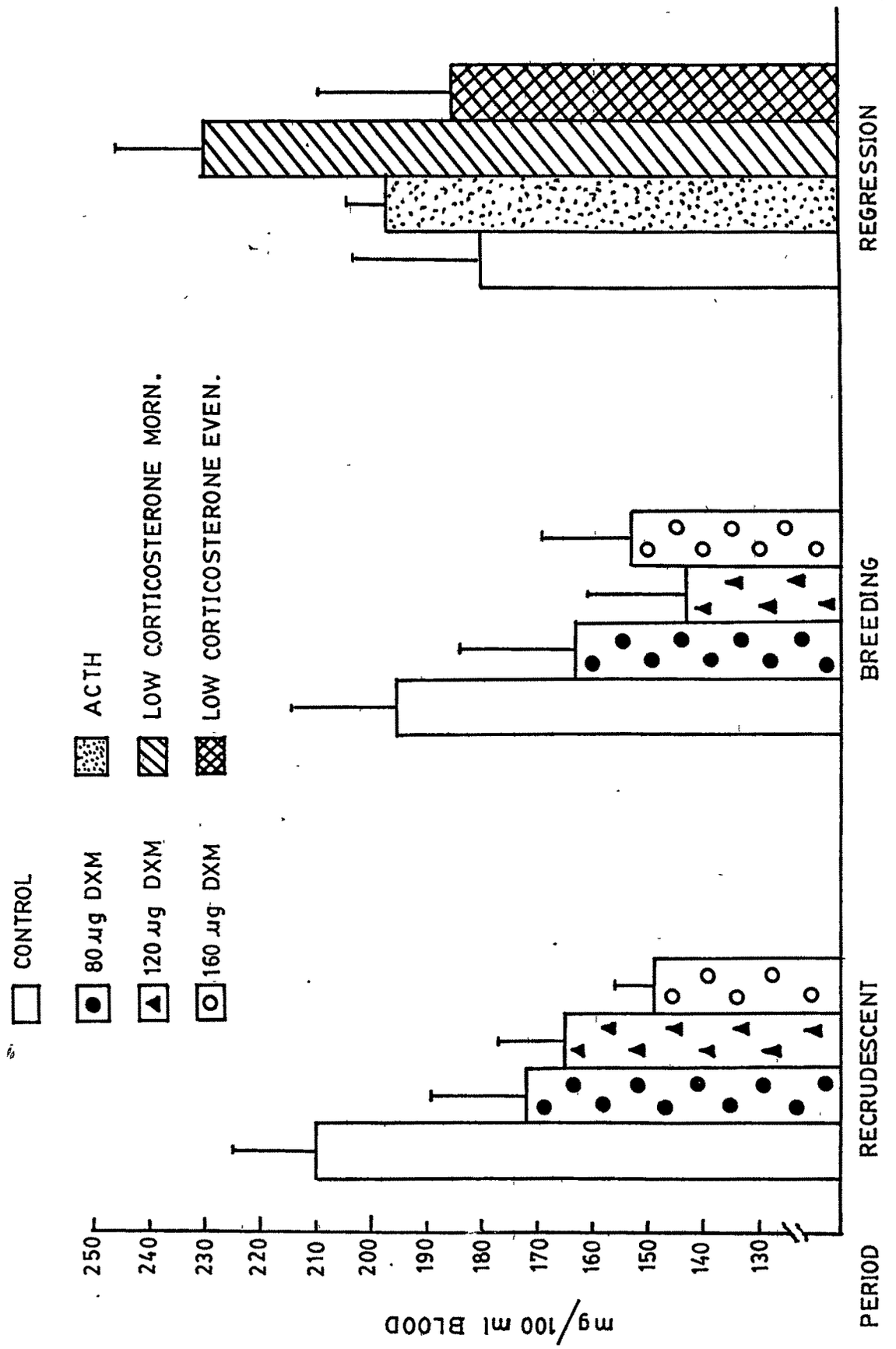

FIG. 1. CHANGES IN BLOOD GLUCOSE LEVEL

TABLE-2a : SEASONAL CHANGES OF HEPATIC GLYCOGEN CONTENT (mg/100mg FRESH TISSUE WEIGHT) ± S.D.) IN NORMAL AND EXPERIMENTAL BIRDS, C. LIVIA

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	1.63 ±0.40	2.93** ±0.72	2.08 ±0.59	1.41 ±0.43	-	-	-	-	-
BREEDING	1.15 ±0.31	1.60* ±0.50	2.14+ ±0.66	2.50** ±0.73	-	-	-	-	-
REGRESSION	3.02 ±0.05	-	-	-	1.19*** ±0.32	2.24* ±0.86	1.76+ ±0.73	1.99** ±0.53	2.08** ±0.52

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

M - MORNING E - EVENING

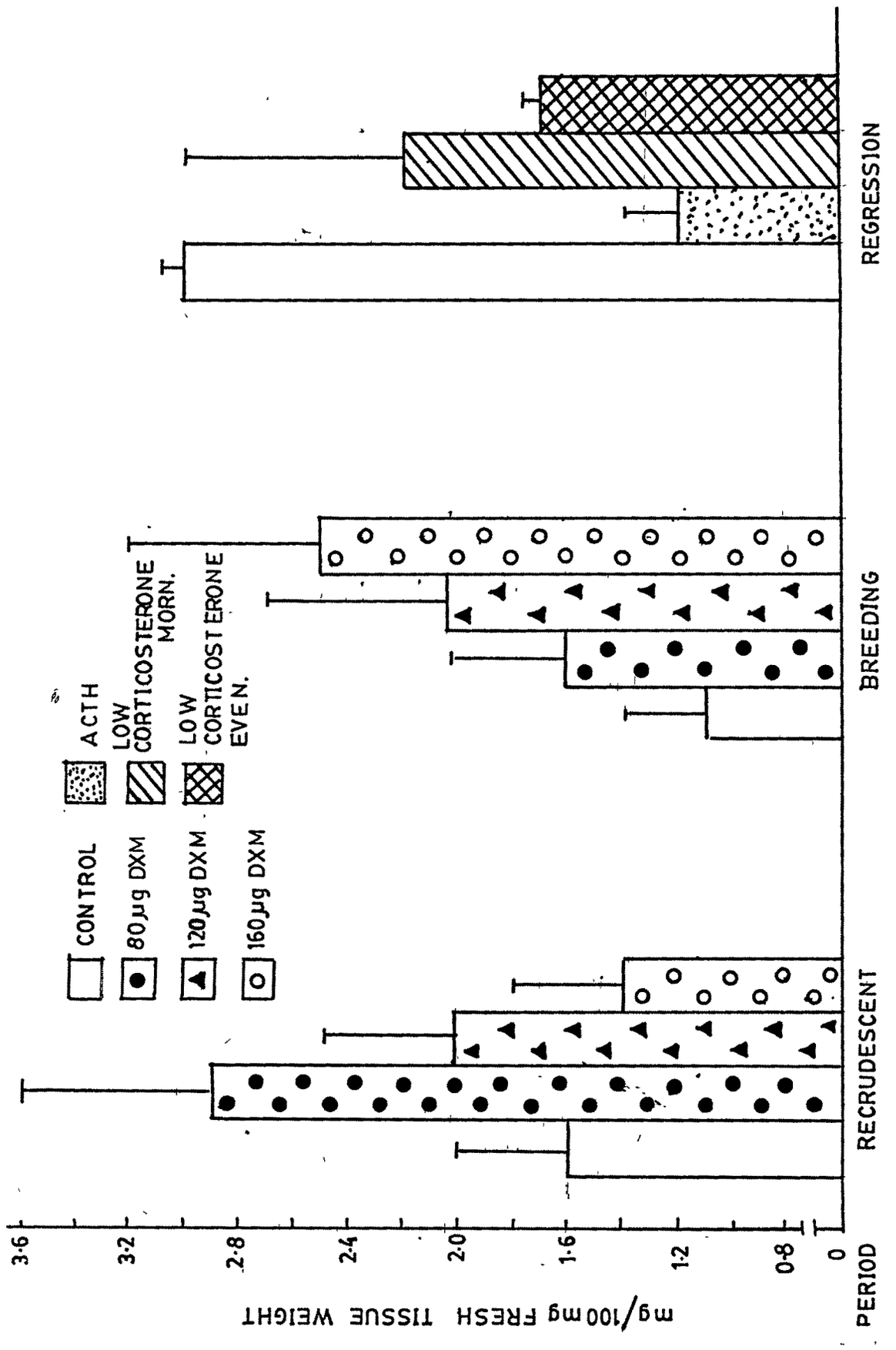

FIG. 2a. CHANGES IN HEPATIC GLYCOGEN CONTENT

TABLE 2b : SEASONAL CHANGES OF MUSCLE GLYCOGEN CONTENT (mg/100 mg FRESH TISSUE WEIGHT) IN NORMAL AND EXPERIMENTAL BIRDS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE
RECRUDESCENT	0.749 ±0.20	1.09@ ±0.27	1.02+ ±0.18	1.18+ ±0.32	-	-	-
BREEDING	1.46 ±0.29	1.35 ±0.466	1.28 ±0.35	1.08+ ±0.30	-	-	-
REGRESSION	1.68 ±0.57	-	-	-	0.54*** ±0.24	1.20* ±0.14	1.50 ±0.42

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

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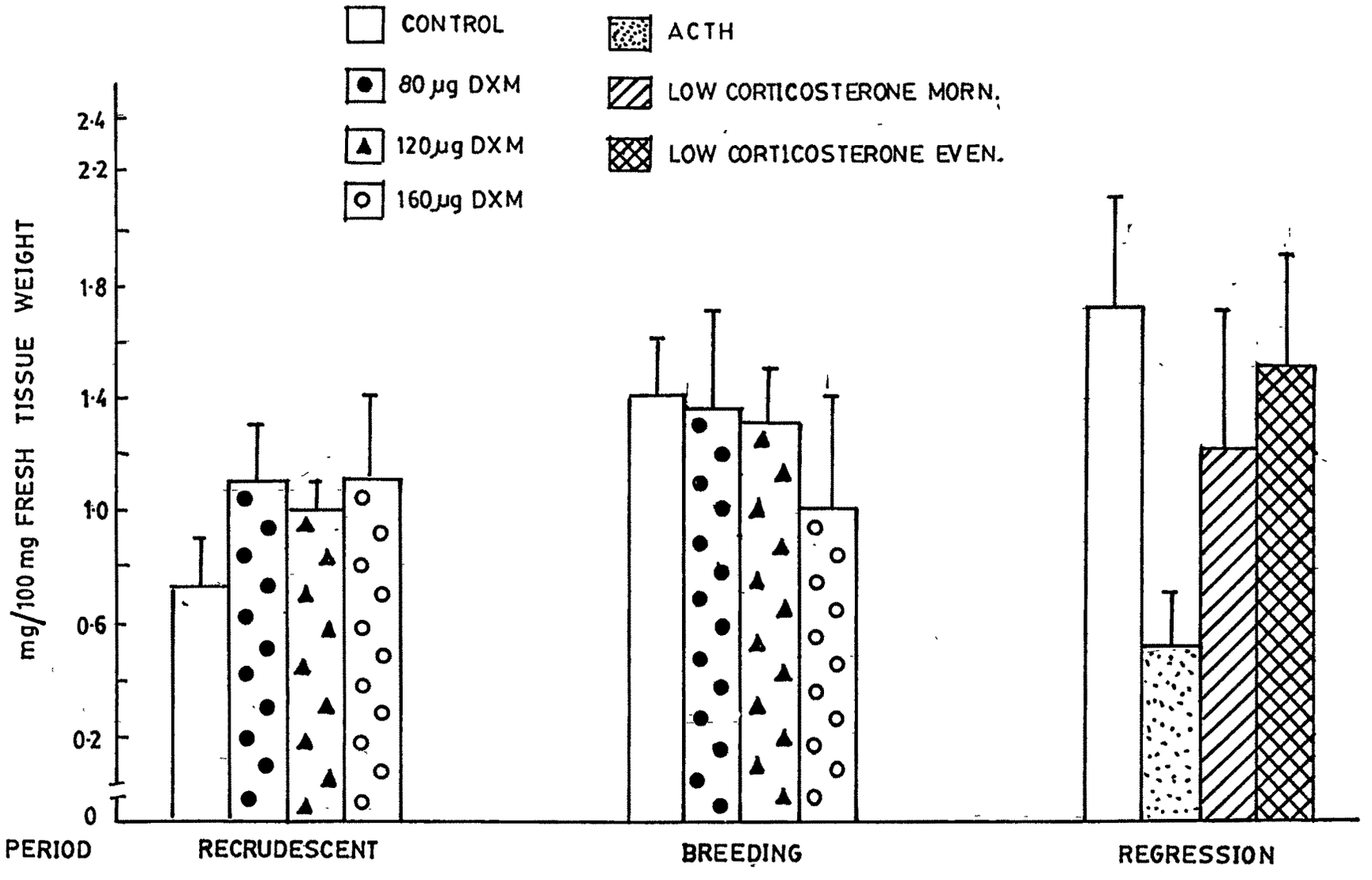

FIG. 2b. CHANGES IN MUSCLE GLYCOGEN CONTENT

TABLE-2c : SEASONAL CHANGES OF GONADAL GLYCOGEN CONTENT (mg/100 mg FRESH TISSUE WEIGHT) IN NORMAL AND EXPERIMENTAL BIRDS, C. LIVIA (± S.D.).

REPRODUCTIVE PHASES	NORMAL	DAXAMETHASONE			ACTH	CORTICOSTERONE			
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE	3µgM	3µgE
RECRUDESCENT	0.078 ±0.009	0.097 ±0.02	0.108* ±0.004	0.123** ±0.02	-	-	-	-	-
BREEDING	0.173 ±0.008	0.122* ±0.03	0.071*** ±0.02	0.054*** ±0.02	-	-	-	-	-
REGRESSION	0.065 ±0.02	-	-	-	0.241*** ±0.02	0.224*** ±0.06	0.079 ±0.01	1.99** ±0.53	2.08*** ±0.52

* P< 0.05 ** P< 0.005 *** P< 0.0005

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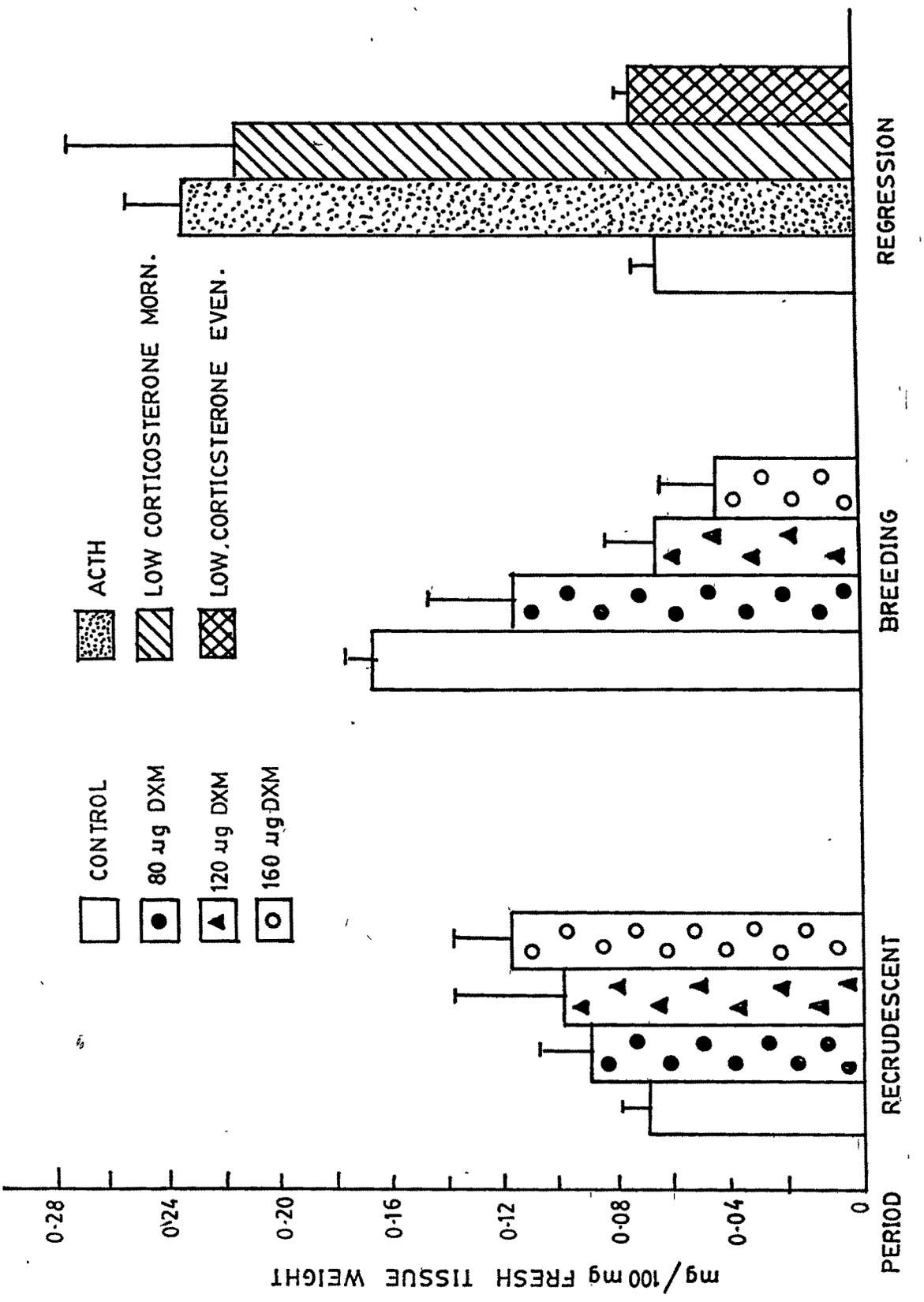

FIG. 2. CHANGES IN GONADAL GLYCOGEN CONTENT

TABLE-3 : SEASONAL CHANGES OF G-6-PASE ACTIVITY (u MOLES 'p' RELEASED/mg PROTEIN/ MINUTE) IN NORMAL AND EXPERIMENTAL PIGEONS, C. LIVIA ($\pm$ S.D.).

REPRODUCTIVE PHASES	NORMAL	DEXAMETHASONE			ACTH	CORTICOSTERONE	
		80µg	120µg	160µg	0.5 I.U.	1µgM	1µgE
RECRUDESCENT	0.183 $\pm$0.02	0.122*** $\pm$0.03	0.169 $\pm$0.03	0.106** $\pm$0.04	-	-	-
BREEDING	0.271 $\pm$0.04	0.154*** $\pm$0.03	0.181*** $\pm$0.020	0.135*** $\pm$0.02			
REGRESSION	0.091 $\pm$0.01	-	-	-	0.111* $\pm$0.01	0.106 $\pm$0.03	0.112* $\pm$0.016

* $P < 0.05$ ** $P < 0.005$ *** $P < 0.0005$

M - MORNING E - EVENING

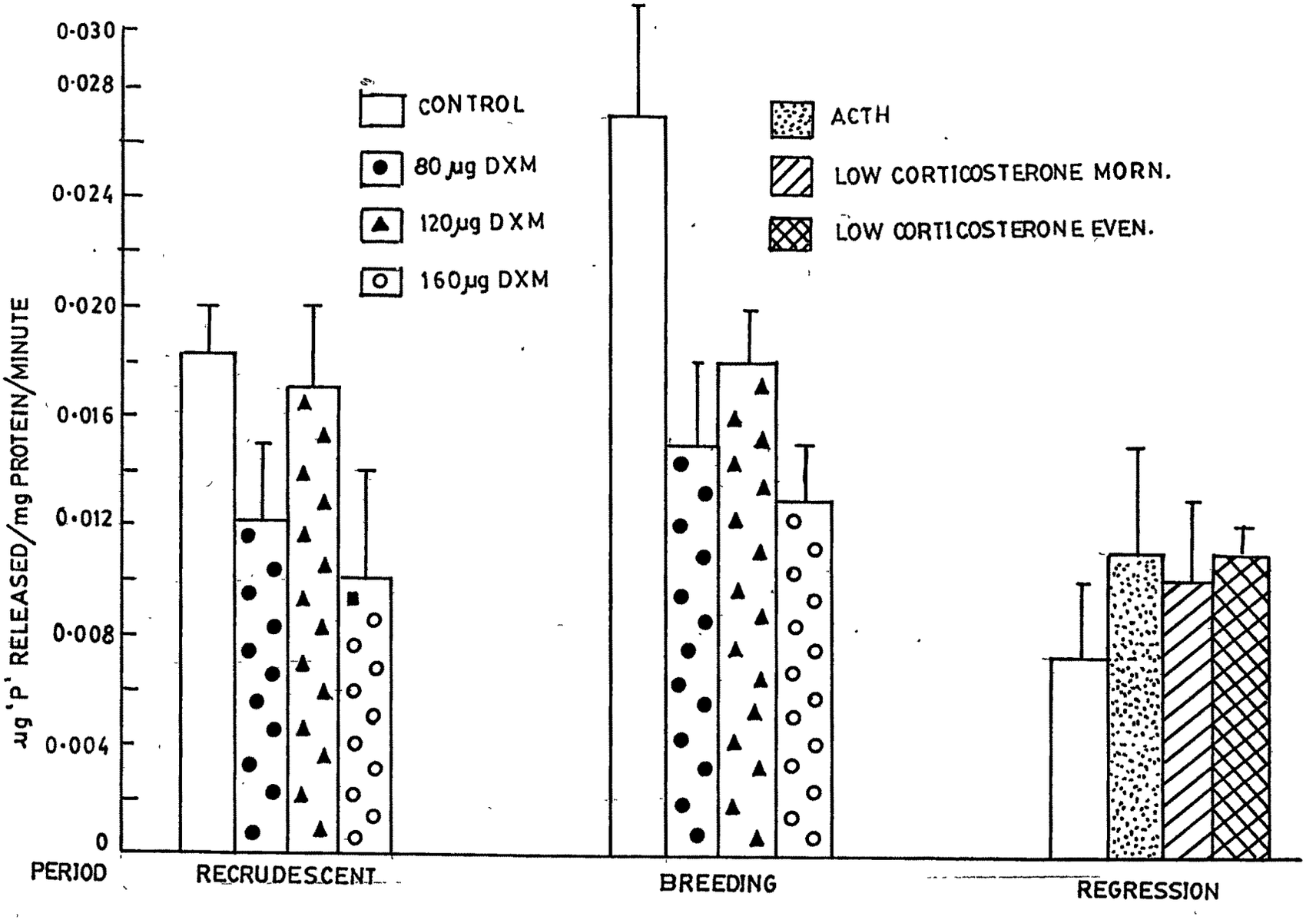

FIG.3. CHANGES IN HEPATIC GLUCOSE-6 PHOSPHATASE ACTIVITY

decrease only during the breeding period. On the contrary, ACTH and Corticosterone administration during the regression phase depicted decreased muscle glycogen content and increased gonadal glycogen content.

Blood glucose showed significant decrease in dxm treated birds during both recrudescence and breeding. A significant hyperglycaemia was the feature in ACTH and corticosterone treated birds during the regression phase. However, LCE and HCE failed to evoke any significant response. Hepatic G-6-Pase activity was decreased in all adrenal suppressed birds during both recrudescence and breeding; whereas administration of ACTH or corticosterone resulting in hyperadrenalism, increased the activity level of this enzyme in the liver during regression.

DISCUSSION

Influence of glucocorticoids on carbohydrate metabolism has been shown by several workers in various animal species (Brown et al., 1958; Greenman and Zarrow, 1961; Bellamy and Leonard, 1965; Adams, 1968; Exton, 1972). However, most of the experiments yielded diverse results essentially due to variations in season and experimental protocol which emphasize the fact that glucocorticoid actions could be modified in diverse ways. Moreover, literature dealing with the effect of glucocorticoids on

carbohydrate metabolism being scanty, the present study in this context is an attempt to gain some information about the involvement of avian adrenal glands in seasonal modulations of carbohydrate metabolism in relation to breeding. Onset of breeding activities can be expected to place a heavy demand on the energy provisions of the body leading to utilization of stored metabolites. The hyperglycaemic condition and the depleted hepatic glycogen store noted during the recrudescent and breeding months in the present study bear testimony to this thinking. Decreased energy demands with the cessation of breeding activities is denoted by the lowered blood glucose level and increased hepatic glycogen store in the regression phase. Recrudescence seems to be marked by depletion of hepatic and muscle glycogen contents. The significantly depleted content of muscle glycogen in the recrudescent phase denotes the possible participation of muscle glycogen in the energetics associated with the re-awakening of the gonads and the preparatory processes related with the onset of breeding activities. An identical observation with reference to muscle glycogen has been made by Ramachandran et al. (1987) and Ramachandran et al. (1987). Progressively depleting hepatic glycogen content throughout recrudescence and breeding indicates the importance of this organ in meeting the general energy requirements of the body during the breeding phases. Whitting and Wiggs (1978) have reported

the depletion of hepatic glycogen content during sexual maturation of brook trout. The above workers have also shown that injection of estradiol in immature female trout can induce depletion of hepatic glycogen content. In the course of the present study, glycogen content of the gonads has also been noted to undergo seasonal alterations, with the content tending to increase steadily during the recrudescent and breeding phases. Obviously, increased gonadal glycogen store along with elevated blood glucose level suggest the importance of carbohydrate metabolism in gonadal functioning during the sexually active phases. It may be relevant in this context to quote the statement of Free (1969) which says "In seasonal breeding species and those such as the avian species, exhibiting strong diurnal rhythms, we may expect the substrate requirements of the whole organ (testis) to fluctuate". Effects of seasonal variations as well as local changes associated with the spermatogenic cycle on the testicular glycogen content have been noted to occur in mammals (Wisłocki, 1949; Nicander, 1957). Further evidences in favour of carbohydrate metabolism playing a crucial role in gonadal functioning in terms of respiratory and energy producing processes, can be drawn from the reports of variations in the glycogen content of sertoli cells and spermatogonia during the spermatogenic cycle (Cavazos and Melampy, 1954; Nicander, 1957), of extensive injury to the germinal

epithelium, congestion and oedema in the interstitium and vacuolization of Sertoli cells in adult rats after insulin induced hypoglycaemia (Mahcine et al., 1960), of atropic changes in the testes in untreated cases of Diabetes Mellitus due to inefficient utilization of blood glucose (Warren and Lecompte, 1952) and of the respiratory quotient of various mammalian testis to range between 0.7 to 1.0 indicating utilization of 40-100% of the oxygen uptaken by testicular tissue for combustion of Carbohydrates (see Free, 1969). Increased hepatic glucose-6-phosphatase activity, a key gluconeogenic enzyme, noted in the recrudescent and breeding phases currently seems to correspond well with the increased adrenocortical activity (Chapter III) and decreased hepatic protein content (Chapter VIII) occurring concurrently, and the reported positive influence of Corticosteroids on gluconeogenesis (Brown et al., 1958; Greenman and Zarrow, 1961).

Seasonal experimental manipulation of adrenocortical activity in the form of suppression by dxm during the active phases and activation by ACTH/Corticosterone administration during the inactive phase have been shown to bring about gonadal involution and activation respectively (Chapters II, III). The herein observed alteration in carbohydrate metabolism under similar conditions of experimentation seems to corroborate the fact that adrenal mediated

seasonal modulation in carbohydrate metabolism does play a crucial role in controlling the seasonal cyclicity of gonads in aves. Accordingly, the data presented in the present chapter indicate alterations in hepatic glycogen content and blood glucose level in dxm suppressed birds during the active phases and ACTH/corticosterone administered pigeons during the inactive phase which tend to be diametrically opposite to those noted for the control birds during the respective phases. Hence in the breeding phases, dxm suppression led to changes which are comparable with those occurring during the non-breeding phase and ACTH/corticosterone administration during the regression phase induced changes which are comparable with those occurring during the recrudescent and breeding phases. The fall in blood glucose level in dxm treated birds and the elevation in ACTH/corticosterone injected birds are in keeping with the hyperglycaemic action of glucocorticoids (Bellamy and Leonard, 1965; Ahmed and Alim, 1977; Zbigniew et al., 1977; Freeman et al., 1980). The role of muscle glycogen in the energetics associated with recrudescence is amply indicated by the increased muscle glycogen content in dxm treated birds during the recrudescent months and by the lowered content in ACTH/corticosterone injected birds during the regression phase. The inertness of muscle glycogen during breeding is indicated by the more or less unchanged content

in dxm suppressed birds during this season. Similarly, the relationship between increased gonadal glycogen content and its activity is established by the noted decrease in gonadal glycogen content coupled with gonadal involution in dxm treated pigeons during breeding and the increased content in response to ACTH/Corticosterone administration along with gonadal enlargement during the regression phase. Obviously, corticosterone can be accredited with a role in modulating seasonal changes in gonadal glycogen content. However, the increased glycogen content in the gonads of dxm suppressed birds during the recrudescent phase may be considered essentially due to a sudden arrest in utilisation without an accompanying decrement in mobilisation. Both increased G6-Pase activity (Issebutz,1982) as well as no change (Vermier and Sire, 1978) under the influence of glucocorticoids are reported. Further, Abraham and Istran (1978) have reported that following in vivo administration of hydrocortisone hemisuccinate, intensification of gluconeogenic pathway was noted. Dunn et al. (1971) have proposed that the action of glucocorticoids which are rate limiting for gluconeogenesis takes place in extra hepatic tissues and leads to an increased flow of gluconeogenic precursors to the liver. But in the present study no glycogen deposition has been noted either during recrudescent or breeding phases denoting an increased utilization coupled with breeding activities. However

in the present, study, a definite positive influence of adrenal cortical activity on G6-Pase activity in aves is suggested by the noted reduced G6-Pase activity in dxm suppressed birds and increased activity in ACTH/Corticosterone treated birds. These changes in G6-Pase activity are in agreement with the observed hypo and hyperglycaemia respectively. Apparently, the gonadal shrinkage induced by dxm during the recrudescent and breeding months (Chapters II, III) is paralleled by hypoglycaemia and altered glycogen content of liver, muscle and gonads. Similarly, the gonadal enlargement brought about by ACTH/Corticosterone administration during regression, is paralleled by hyperglycaemia and favourable modulations in the glycogen store of the tissues. Hence in this light, a definite role of adrenal corticosteroids in modulating favourable changes in carbohydrate metabolism during the annual cyclicity of tropical wild pigeons can be inferred and may control directly or indirectly gonadal awakening, its functioning and finally its quiscence. A final conclusion that could also be drawn from the results obtained is that a low dose of corticosterone injected in the morning hours has a more favourable influence in general which tends to emphasize the inherent time specificity involved in tissue sensitivity to hormonal stimulation as well as the importance of an optimum level of the hormonal factor.

S U M M A R Y

Seasonal alterations in carbohydrate metabolism in terms of annual gonadal cyclicity and under conditions of adrenal manipulation has been evaluated in the wild pigeon, Columba livia. The recrudescent and breeding phases were marked by reduced hepatic and muscle glycogen contents, increased gonadal glycogen content, high hepatic G6Pase activity and elevated glycaemic level. Reverse set of changes were observable during the non-breeding phase which could also be brought about during the breeding phase by dxm induced adrenal suppression. Similarly, ACTH/corticosterone administration in the non-breeding phase produced alterations which were quite similar to the recrudescent and breeding phases. These changes are discussed in terms of the importance of blood glucose and carbohydrate reserves in gonadal functioning and the possibility of adrenal steroids in modulating normal seasonal changes in carbohydrate metabolism in tropical Indian pigeons.