CHAPTER VIII

SEASONAL ALTERATIONS IN cAMP CONTENT AS EXEMPLIFIED BY PHOSPHODIESTERASE ACTIVITY IN LIVER AND GONADS OF NORMAL AND ADRENAL MANIPULATED PIGEONS, COLUMBA LIVIA

Since its discovery, cAMP has gained wide recognition as an essential link in the specific cellular response to the various stimuli that impinge upon the cells in vivo. According to the 'second messenger' hypothesis, cAMP acts as the second messenger and is derived from ATP intracellularly by a membrane bound enzyme adenylate cyclase in response to the first messenger (hormone and/or any other agent), combining with the appropriate receptors. cAMP thus produced in turn mediates various cellular activities such as division, metabolic alterations, synthesis of macromolecules, differentiation etc. Importance of cyclic nucleotides in various cellular mechanisms is being increasingly realised and has been reported by several workers involving cell division (Thomas et al., 1973; Berridge, 1975; Carlone and Foret, 1979), cell differentiation (Friedman, 1976; Miller, 1977; Taban and Cathieni, 1978), macromolecule synthesis (Sharma and Talwar, 1970; MacManus et al., 1972, 1973; Dokas, 1973; Short et al., 1975), metabolic alterations (Rall et al., 1964; Drummond et al., 1969; Beriz et al., 1977; Ishibashi and Catton, 1978) and reproductive processes (Menon and Gunaga, 1974).

It is now a well established fact that the approach of breeding season in a seasonal breeder is marked by changes in many organs as well as general body metabolism which are triggered by subtle alterations in the circulating levels of various hormones. Gametogenesis represents a conglomerate of various tissue functions such as cell division, differentiation, synthesis of macromolecules and metabolic alterations all of which require the participation of cAMP as the second messenger and hence the gonads are ideal for studying functionally correlatable changes in cAMP content. Although the cAMP level is known to undergo alterations in response to hormonal and/or other factors, there are fewer reports regarding the actual involvement of cAMP in reproductive processes, Menon and Gunaga (1974) have reported the involvement of cAMP in reproductive processes right from hypothalamic control to embryogenesis and differentiation. However the seasonal variations of cAMP with respect to annual gonadal cyclicity or in relation to many hormones which influence gonadal functions have not received much attention especially in birds. Needless to say that the activity levels of phosphodiesterase can give an indirect estimate of the prevailing concentration of cAMP in any tissue (Butcher and Sutherland, 1962). Due to this fact and owing to the practical limitations in directly assaying cAMP, it was thought pretinent to estimate the levels of phosphodiesterase activity in the

gonads of tropical wild pigeons, Columba livia on a seasonal basis. Liver being the centre of metabolic homeostasis, adaptive functional changes in relation to annual gonadal cyclicity can be expected and hence the levels of the enzyme in liver also was thought pertinent to be assayed. As the present study had indicated a parallel adrenal-gonad axis, (Chapters II and III), the enzyme activity was also assessed in terms of adrenal suppression and activation respectively.

MATERIALS AND METHODS

As Outlined in Chapter I

RESULTS

The levels of phosphodiesterase activity in the gonads and liver of normal and experimental birds are given in figs. 1 & 2 and tables 1 & 2.

Changes in Normal Birds

In general, the phosphodiesterase activity was found to be relatively higher in gonads than in liver. Seasonal changes in enzyme activity in normal birds appeared to be similar in both liver and gonads with maximum activity being during the breeding months and minimum during the

TABLE-1 : SEASONAL CHANGES OF HEPATIC HOSPHODIESTERASE ACTIVITY ('µ' MOLES 'p' RELEASED/mg PROTEIN/15 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	0.618 ±0.22	0.725 ±0.06	0.960* ±0.19	0.844* ±0.10	-	-	-
BREEDING	0.662 ±0.081	0.751* ±0.10	0.765* ±0.10	0.827+ ±0.14	-	-	-
REGRESSION	0.513 ±0.104	-	-	-	0.602* ±0.106	0.611* ±0.094	0.675* ±0.07

+ $P < 0.01$ * $P < 0.05$

M - MORNING E - EVENING

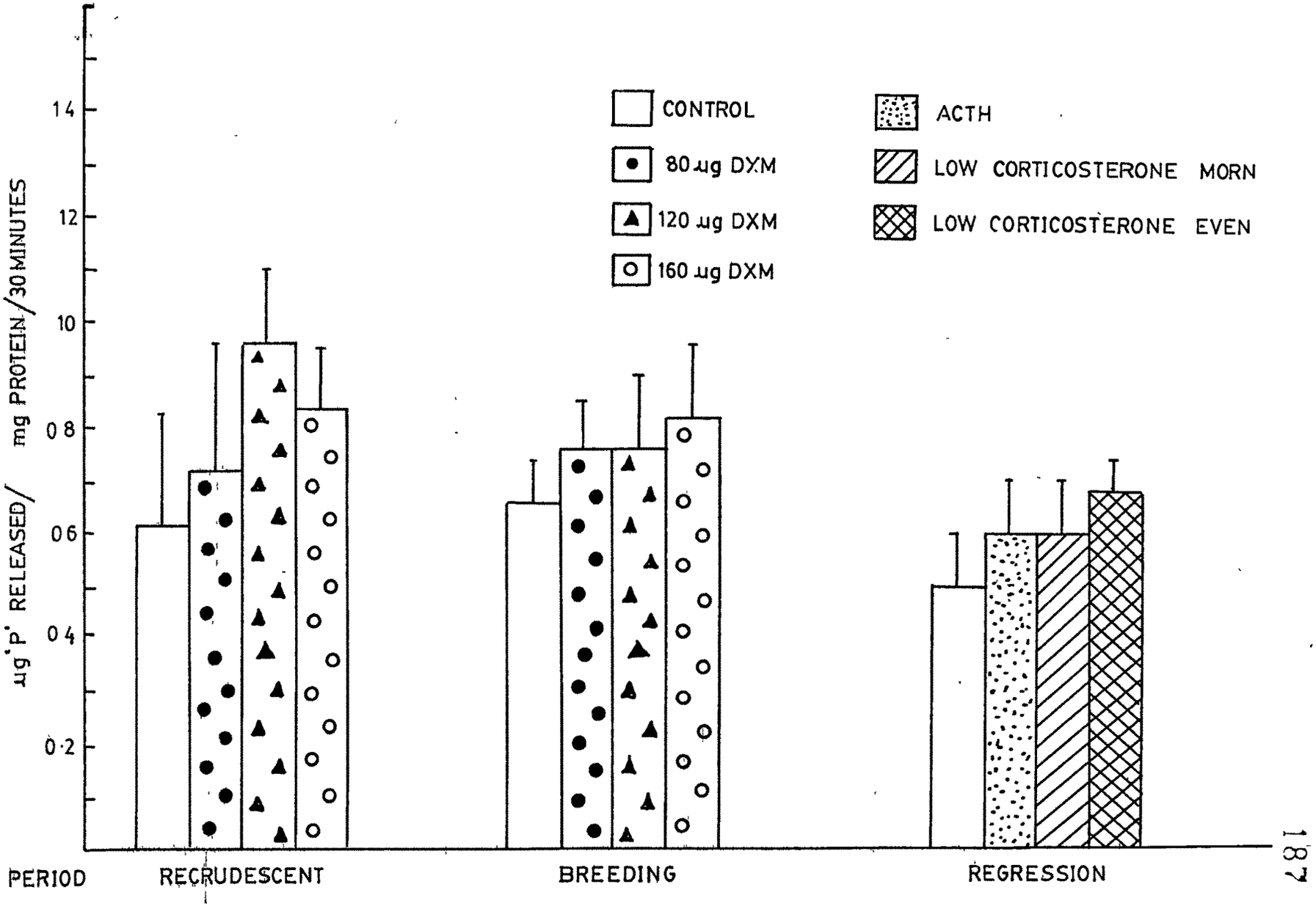

FIG. 1. CHANGES IN HEPATIC PHOSPHODIESTERASE ACTIVITY

TABLE-2 : SEASONAL CHANGES OF GONADAL PHOSPHODIESTERASE ACTIVITY ('µ' MOLES 'p' RELEASED/mg PROTEIN/15 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	1.216 ±0.24	0.613*** ±0.235	0.859+ ±0.12	0.404*** ±0.087	-	-	-
BREEDING	1.078 ±0.29	0.87+ ±0.19	0.577** ±0.102	0.691* ±0.172	-	-	-
REGRESSION	0.832 ±0.211	-	-	-	0.825 ±0.17	1.30+ ±0.44	1.011 ±0.12

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

M - MORNING E - EVENING

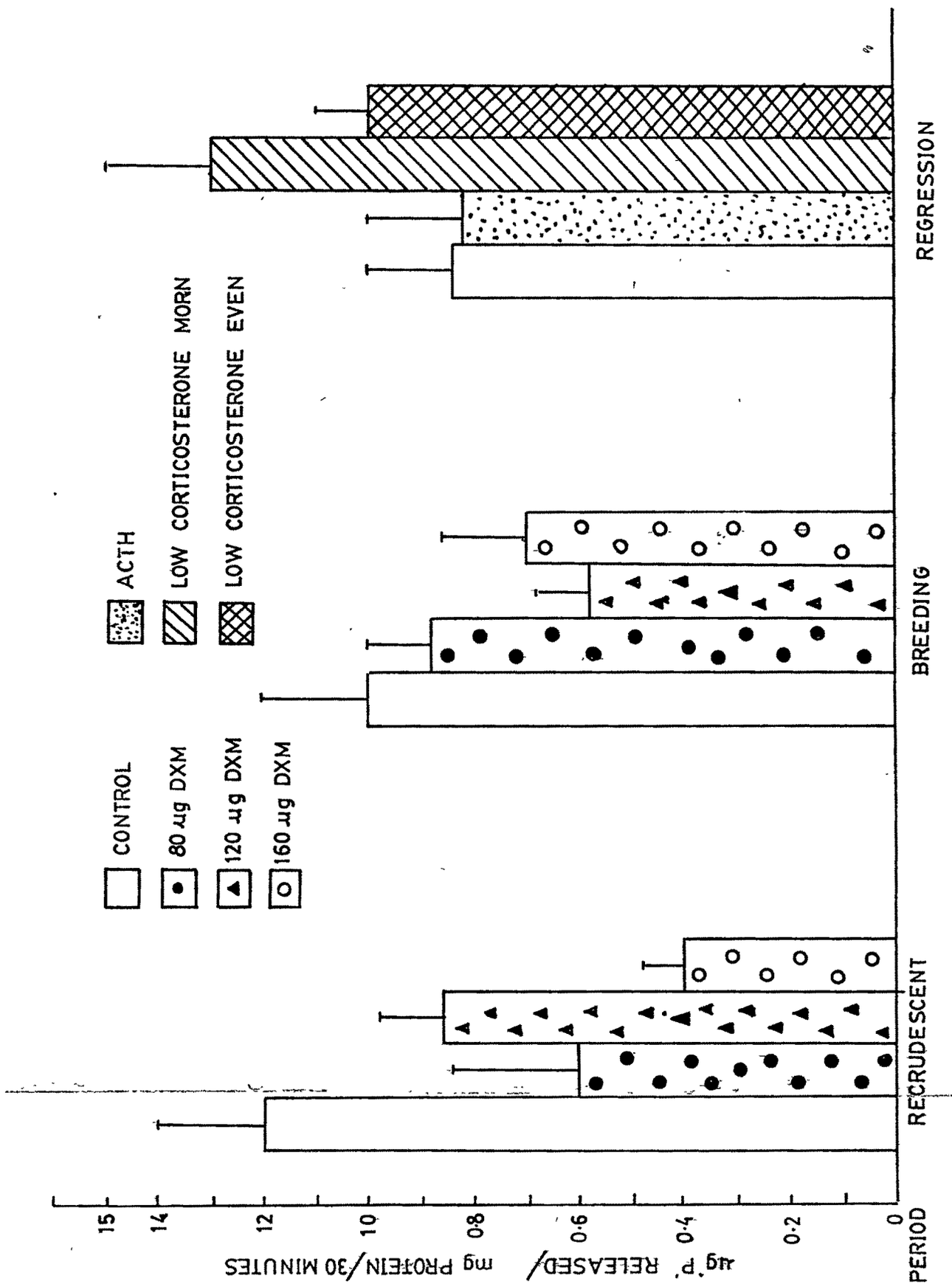

FIG. 2. CHANGES IN GONADAL PHOSPHODIESTERASE ACTIVITY

non-breeding months. The increase in hepatic phosphodiesterase activity on a percentage basis was about 20% from regression to recrudescence and a further increase of about 7% from recrudescence to breeding. Gonads too depicted an increase in phosphodiesterase activity from regression to recrudescence to the tune of about 46% which remained more or less steady during the breeding phase.

Changes Under Experimental Conditions.

Experimental manipulation of adrenocortical activity was noted to bring about differential changes in phosphodiesterase activity in the liver and gonads. Whereas chemical adrenalectomy led to increased phosphodiesterase activity in liver, gonads depicted an opposite change, during both recrudescent and breeding phases. Adrenal activation by ACTH failed to bring about any significant change. However, administration of corticosterone did result in significant changes in both the tissues. Both hepatic and gonadal phosphodiesterase activity was found to increase under the influence of exogenously administered corticosterone(both LCM and LCE) towards levels characteristic of the recrudescent phase in normal birds.

DISCUSSION

The intracellular levels of cAMP, the second messenger for many hormonal agents, are regulated by both adenylate cyclase, the synthetic enzyme, as well as phosphodiesterase the degradatory enzyme. The relative importance of the two is difficult to judge as the mode of regulation might depend on many aspects of tissue specific functions. Though the role of phosphodiesterase is essentially to lower cAMP level by bringing about its breakdown, an increase in phosphodiesterase activity should not be viewed with the singular idea as a mechanism for lowering cAMP content, as an increase in phosphodiesterase activity can also occur immediately subsequent to an increased cAMP content. In this context, whereas a decrease in phosphodiesterase activity below the basal level can be looked upon as a regulatory mechanism, the increase in phosphodiesterase activity above the basal level could in all probability be due to raised cAMP levels by adeylate cyclase action.

Since gonadal functions are mainly controlled by gonadotrophic hormones and other hormones involving cAMP mediated actions, variations in the levels of cAMP on a

seasonal
basis can be expected in the gonads of seasonal breeders. Obviously, increased cAMP content during the breeding season can be presumed, with a loweved level during the reproductively inactive non-breeding phase. So in a seasonally breeding animal, the prevailing level of cAMP in the non-breeding season can be considered as a basal level from which it increases to a higher level by hormonal stimulation during the breeding season. The present study on phosphodiesterase activity in the gonads and liver of tropical wild pigeons has revealed lower levels of enzyme activity during the non-breeding season and increased levels during the recrudescent and breeding phases. Based on the presumed seasonal changes in the cAMP content and the relationship between cAMP and phosphodiesterase outlined as above, the increased level of phosphodiesterase activity in the recrudescent and breeding phases recorded herein would indicate a change essentially associated with increased cAMP content rather than a specific regulatory mechanism to decrease the cAMP content. In this light the lowered levels of phosphodiesterase activity during the non-breeding season would represent the basal level of enzyme activity in keeping with the decreased cAMP mediated activities. Whereas the increased gonadal phosphodiesterase activity during the breeding phase would suggest occurrence of many cAMP

mediated activities such as cell proliferation, differentiation, macromolecule synthesis and metabolic alterations (Berridge, 1975; Short et al., 1975; Miller, 1977; Beriz et al., 1977; Ishibashi and Catton, 1978), all events of crucial significance related to gamete formation and differentiation, the observed parallel increase in hepatic phosphodiesterase activity could denote increased cAMP dependant biochemical and metabolic alterations associated with the establishment of a propitious physiological state conducive for gonadal functioning. The reported stimulatory role of calcium on phosphodiesterase activity (Berridge and Rapp, 1977) and the increased hepatic calcium content observed during recrudescence and breeding in the pigeon (Patel, 1982), are in agreement with the presently recorded increased hepatic phosphodiesterase activity during the breeding months.

The decreased phosphodiesterase activity in the gonads of birds rendered adrenal insufficient by dxm during the breeding months, and the increased phosphodiesterase activity after corticosterone treatment during the regression phase are confirmative of the seasonal alterations in the cAMP content presumed to occur in the control birds. It is very clear now that both FSH action on sertoli cells as well as LH action on leyding cells are mediated by the generation of cAMP and the subsequent activation of protein kinase activity (Means, 1975;

Means et al., 1976). Though the role of cAMP in mediating the actions of increasing titers of gonadotropic hormones during the recrudescent and breeding phases of a seasonal breeder can be expected, the inhibitory effects of increased cAMP content on a prolonged basis on gonadal functions, particularly on gametogenesis, cannot be overlooked as Braun and Shizowa (1973) have reviewed the inhibitory influence of increased cAMP content on cell function and Aoyogi et al. (1981) have demonstrated the inhibitory effects of increased cAMP content on epidermal growth and mitotic index. Viewed in this context, an increased phosphodiesterase activity in conjunction with elevated cAMP content as has been noted in the present study would be of functional significance in either overcoming the prolonged action of high cAMP content or in balancing the cAMP content at a higher optimal basal level. Such a regulatory role of phosphodiesterase, as is being inferred from the present study can be presumed from the reported presence of phosphodiesterase in testicular cytosol of rats (Purvis et al., 1981) as well as its increase under the influence of both FSH and dibutyryl cAMP, essentially a consequence of raised intracellur cAMP content in the sertoli cells (Conti et al., 1981). In the present study, dxm induced adrenocortical suppression during the recrudescent and breeding months has been noted to lower the phosphodiesterase activity in the gonads to levels characteristic of inactive gonads during the regression phase.

Moreover, corticosterone administration during the non-breeding phase was marked by increased phosphodiesterase activity to levels characteristic of the active gonads. These observations together with the noted gonadal involution under dxm treatment and gonadal activation under ACTH/corticosterone administration (Chapters II and III) suggest the positive influence of corticosteroids on seasonal gonadal cyclicity in pigeons. A direct interrelationship between adrenal and gonad in this respect in pigeons can be considered feasible and is supported by the observations of favourable biochemical alterations in conjunctions with histomorphological changes of the gonads under altered adrenocortical activity (Chapters III, IV, VII and X).

A discordant observation is the increased hepatic activity in dxm treated birds unlike the decrement in gonadal phosphodiesterase activity. It is also evident that dxm induced increase in hepatic phosphodiesterase activity is more significant than the corticosterone induced increase. This indicates the possibility of dxm mimicking the actions of corticosterone in hepatic tissue, and strengthens the previous concept of presence of specific and non specific type of corticosterone receptors (Chapter II, Ballard et al., 1974). The report of Rousseau et al. (1980) of the three fold increase in plasma membrane alkaline phosphodiesterase activity induced by dexamethasone is revelant in this context. It is quite likely

that dexamethasone is more potent in inducing phosphodiesterase activity in the hepatic tissue and in the present case must be occurring as an independant effect unrelated to changes in cAMP content. The consequent decrease in cAMP content could influence hepatic functions as has been noted with reference to metabolic alterations in the current study (Chapter VII, VIII).

S U M M A R Y

A quantitative evaluation of cAMP phosphodiesterase activity in liver and gonads of normal and adrenal manipulated birds has been carried out on a seasonal basis. In general, phosphodiesterase activity was higher during the recrudescent and breeding phases and low during the regression phase. Adrenal suppression during the active phases reduced gonadal phosphodiesterase activity to the regression level. Similarly, administration of ACTH or corticosterone during the quiescent phase of gonads increased phosphodiesterase activity in both the organs along with gonadal enlargement. High levels of phosphodiesterase activity in the active gonads are correlated with the increased cAMP mediated mechanisms in operation which would require increased phosphodiesterase activity. This is confirmed by the observation of reduced phosphodiesterase activity in the regression phase as well as under adrenal suppression induced involution of gonads.