

CHAPTER VII

EFFECT OF DEXAMETHASONE AND CORTICOSTERONE ON THE
ACTIVITY LEVELS OF ATPases, PHOSPHOMONOESTERASES AND
PHOSPHODIESTERASE IN LIVER, MUSCLE AND TESTIS OF
POST-HATCHED WHITE LEGHORN BREED OF CHICKS

Previous investigations have indicated definite alterations in carbohydrate, protein and lipid metabolisms in chronic adrenocortical suppression by dexamethasone as well as by induced adrenocortical excess by corticosterone (Chapters III, IV and V). Moreover, changes associated with activity levels of glycolytic and oxidative enzymes have also been observed (Chapter VI). Hence it was thought pertinent to explore the possible alterations if any in the biochemical profile of tissues of developing post-hatched chicks in terms of activity levels of ATPase, phosphomonoesterase and cAMP phosphodiesterase under both dexamethasone induced adrenocortical suppression and corticosterone induced adrenocortical excess. Membrane bound (Na^+ - K^+ -dependent) and mitochondrial (Ca^{++} -and Mg^{++} -dependent) ATPase have been correlated with transport functions and energy transformation respectively. Though there are some reports on the effect of thyroid and adrenocortical hormones on Na^+ - K^+ -ATPase in mammals (Klein et al., 1984) and of cortisol and ACTH on Na^+ - K^+ -ATPase in fishes (Pickford et al., 1970; Langdon et al., 1984), the influence of adrenocortical hormones on ATPase activities in the avian tissues is never reported. The present study has therefore tried to evaluate the effect of chronic adrenocortical suppression or excess on ATPase activities in the liver, muscle and testis of one month old chicks.

The nonspecific phosphomonoesterases (acid and alkaline phosphatase) are reported to be involved in a number of functions such as hydrolytic activity, regulation of pyridoxal phosphate metabolism, steroid transport, vitamin B metabolism, lipid metabolism, cellular differentiation and disintegration of tissue components, metabolic activities of testis and other reproductive organs and absorption and phosphorylating mechanisms (Acid phosphatase - Burstone, 1962; Shettan, 1964; Andrews and Turner, 1966; Dipietro and Zengerle, 1967; Pearse, 1968; Klockars and Wagelines, 1969; Heinkrikson, 1969; Cohen, 1970; Blank and Synder, 1970; Serrano et al., 1976) and transmembrane transport mechanism, calcification and growth, differentiation and metabolism of DNA and carbohydrate (alkaline phosphatase - Moog, 1946; Sols, 1949; Bradfield, 1951; Rogers, 1960; Rosenthal et al., 1960; Simkiss, 1964; Rackallio, 1970).

cAMP phosphodiesterase a hydrolytic enzyme of cAMP can play a crucial role in regulating cellular cyclic AMP content, a well established second messenger for mediation of hormonal effects (Butcher and Sutherland, 1962). In general, the activity levels of these enzymes have not been evaluated in avian tissues much less under hormonal manipulations. Hence an attempt has been made presently to study the activity levels of phosphomonoesterases and cAMP phosphodiesterase in liver, muscle and testis of post-hatched White Leghorn breed of chicks under induced adrenocortical suppression (by dexamethasone) and adrenocortical excess (by corticosterone).

MATERIAL AND METHODS

As outlined in Chapter I.

RESULTS

Total, $\text{Na}^+\text{-K}^+$ - and $\text{Ca}^{++}\text{-Mg}^{++}$ - ATPases in liver, muscle and testis

In general, muscle total ATPase activity remained unchanged except for reciprocal changes of increased $\text{Na}^+\text{-K}^+$ -ATPase and decreased $\text{Ca}^{++}\text{-Mg}^{++}$ -ATPase in DXM(H) treated chicks.

Liver showed decreased total ATPase activity with DXM(L), which was essentially due to a decrease in $\text{Ca}^{++}\text{-Mg}^{++}$ -ATPase. However, both DXM(H) and corticosterone induced increase in total ATPase activity, more with the latter treatment. Both the treatments tended to increase both $\text{Na}^+\text{-K}^+$ -ATPase and $\text{Ca}^{++}\text{-Mg}^{++}$ -ATPase.

The total ATPase activity in testis was increased with corticosterone treatment which was mainly due to an increase in $\text{Ca}^{++}\text{-Mg}^{++}$ -ATPase activity. Though DXM(H) treatment did not alter the total ATPase activity in testis, there was a tendency for increase in $\text{Ca}^{++}\text{-Mg}^{++}$ -ATPase and a decrease in $\text{Na}^+\text{-K}^+$ -ATPase (see Table 1, Fig. 1).

Table 1 Activity levels of total, $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ and $\text{Ca}^{++} - \text{Mg}^{++} - \text{ATPase}$ in liver, muscle and testis of chicks treated with dexamethasone and corticosterone for 30 days.

	Total ATPase		Na ⁺ -K ⁺ -ATPase		Ca ⁺⁺ -Mg ⁺⁺ -ATPase				
	μg PO ₄ released/mg protein/10 min	μg PO ₄ released/mg protein/10 min	μg PO ₄ released/mg protein/10 min	μg PO ₄ released/mg protein/10 min	μg PO ₄ released/mg protein/10 min	μg PO ₄ released/mg protein/10 min			
	LIVER	MUSCLE	TESTIS	LIVER	MUSCLE	TESTIS			
CONTROL	33.98 ±2.73	35.19 ±2.99	49.15 ±4.86	9.58 ±0.73	10.28 ±0.86	21.00 ±2.38	24.40 ±1.89	24.91 ±2.04	28.15 ±2.31
DXM(L)	20.47 ^a ±1.38	29.52 ±2.02	47.47 ±2.76	10.86 ±1.48	9.53 ±0.35	17.93 ±1.97	9.61 ^a ±0.67	19.90 ±2.24	29.54 ±3.05
DXM(H)	47.81 ^c ±4.15	35.15 ±1.98	44.05 ±1.47	13.20 ^c ±1.07	26.40 ^a ±1.30	11.31 ^a ±1.42	34.61 ±3.17	8.75 ^a ±0.83	32.74 ±2.94
CORT	56.88 ^a ±3.49	41.51 ±6.09	74.23 ^a ±3.05	6.98 ^d ±0.64	13.44 ±1.76	16.40 ±1.85	49.90 ^a ±5.29	28.07 ±2.24	57.83 ^a ±6.46

DXM(L) : dexamethasone low dose; DXM(H) : dexamethasone high dose; CORT : corticosterone
Values are mean \pm SE of not less than 8 birds.

a : $P < 0.001$; b : $P < 0.02$; d : $P < 0.05$

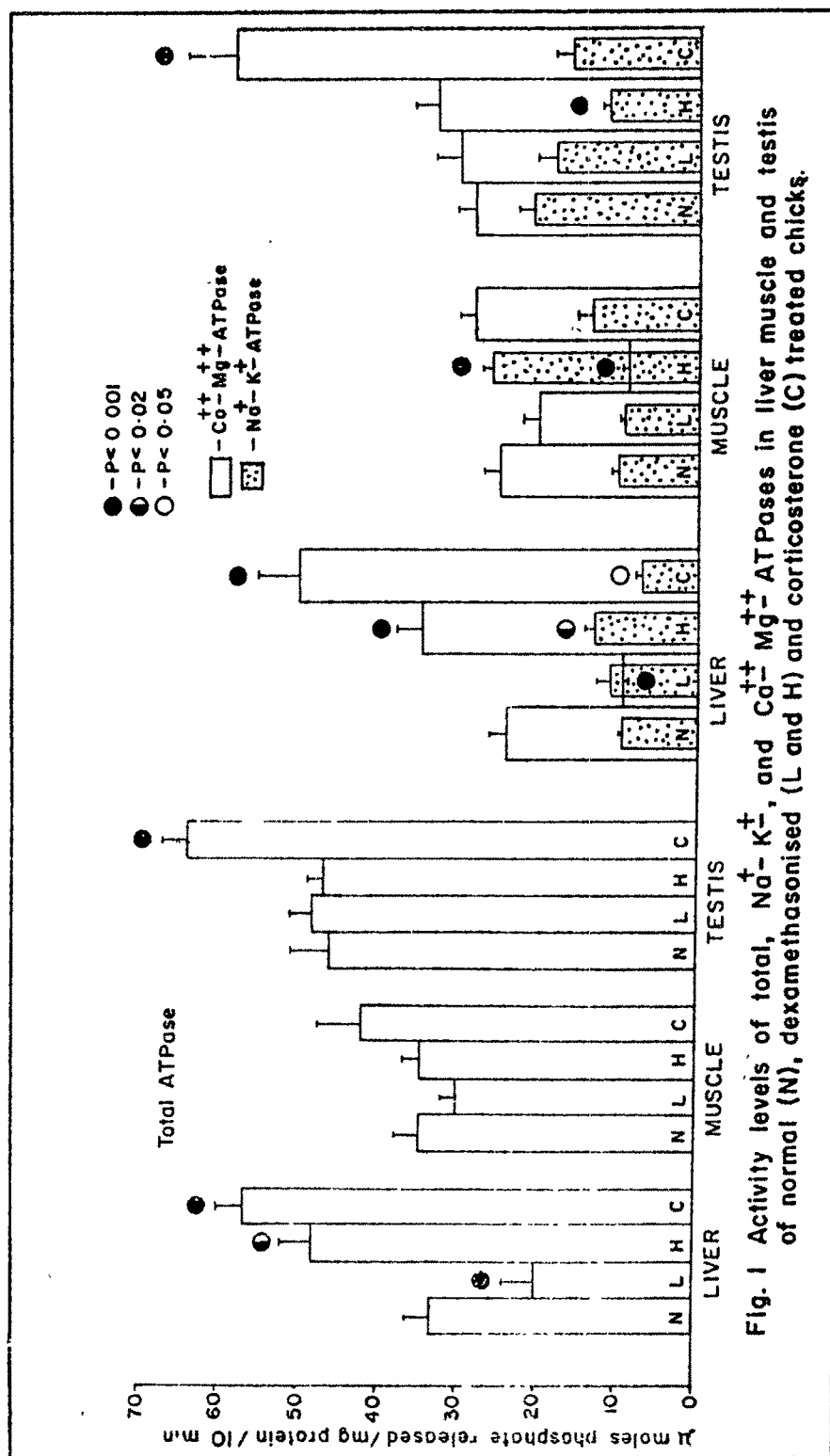


Fig. 1 Activity levels of total, Na^+-K^+ , and $Ca^{++}-Mg^{++}$ ATPases in liver muscle and testis of normal (N), dexamethasone-treated (L) and corticosterone (C) treated chicks.

Acid and Alkaline Phosphatase activity in liver, muscle and testis

The acid phosphatase activity did not register any significant change in the liver and muscle with dexamethasone treatment, however, the testis showed a differing response with an increase with DXM(H) and decrease with DXM(L). All the three tissues showed a similar significantly ($P < 0.001$) increased response with regard to acid phosphatase activity under induced adrenocortical excess. No significant change was noted in the liver alkaline phosphatase activity with all the treatment schedules, while in the muscle a significantly ($P < 0.001$) decreased enzyme activity was discernible with DXM treatments. The alkaline phosphatase in testis was found to be decreased in DXM(H) and corticosterone treatments (see Table 2, Fig. 2).

Cyclic AMP Phosphodiesterase (cAMP PDE) activity in liver, muscle and testis

The hepatic PDE activity was observed to be significantly ($P < 0.001$) decreased with DXM treatments whereas the same was found to be significantly ($P < 0.001$) increased with corticosterone treatment. Both DXM(H) and corticosterone treatments resulted in reduced cAMP PDE activity in muscle without any alteration in DXM(L) treatment. The testis did not depict any change in cAMP PDE activity under any of the treatments (see Table 3, Fig. 3).

Table 2 Activity levels of acid and alkaline phosphatase in liver, muscle and testis of chicks treated with dexamethasone and corticosterone for 30 days.

	Acid phosphatase μ moles PNP released/ 100 mg protein/30 min		Alkaline phosphatase μ moles PNP released/ 100 mg protein/30 min	
	LIVER	MUSCLE TESTIS	LIVER	MUSCLE TESTIS
CONTROL	76.97 ± 4.31	14.79 ± 1.09	43.85 ± 2.72	45.54 ± 4.05 9.47 ± 0.69 201.82 ± 20.20
DXM(L)	87.34 ± 3.96	15.64 ± 1.11	37.27 ^d ± 1.25	40.22 ± 4.63 3.10 ^a ± 0.12 192.62 ± 8.38
DXM(H)	73.11 ± 3.91	13.38 ± 0.72	55.48 ^c ± 2.74	43.23 ± 2.15 4.42 ^a ± 0.23 136.24 ^c ± 10.94
CORT	111.46 ^a ± 6.45	20.18 ^a ± 0.65	53.10 ^a ± 1.49	52.56 ± 1.41 9.65 ± 0.99 133.09 ^c ± 10.39

DXM(L) : dexamethasone low dose; DXM(H) : dexamethasone high dose

CORT : corticosterone

Values are mean \pm SE of not less than 8 birds.

a : $P < 0.001$; c : $P < 0.02$; d : $P < 0.05$

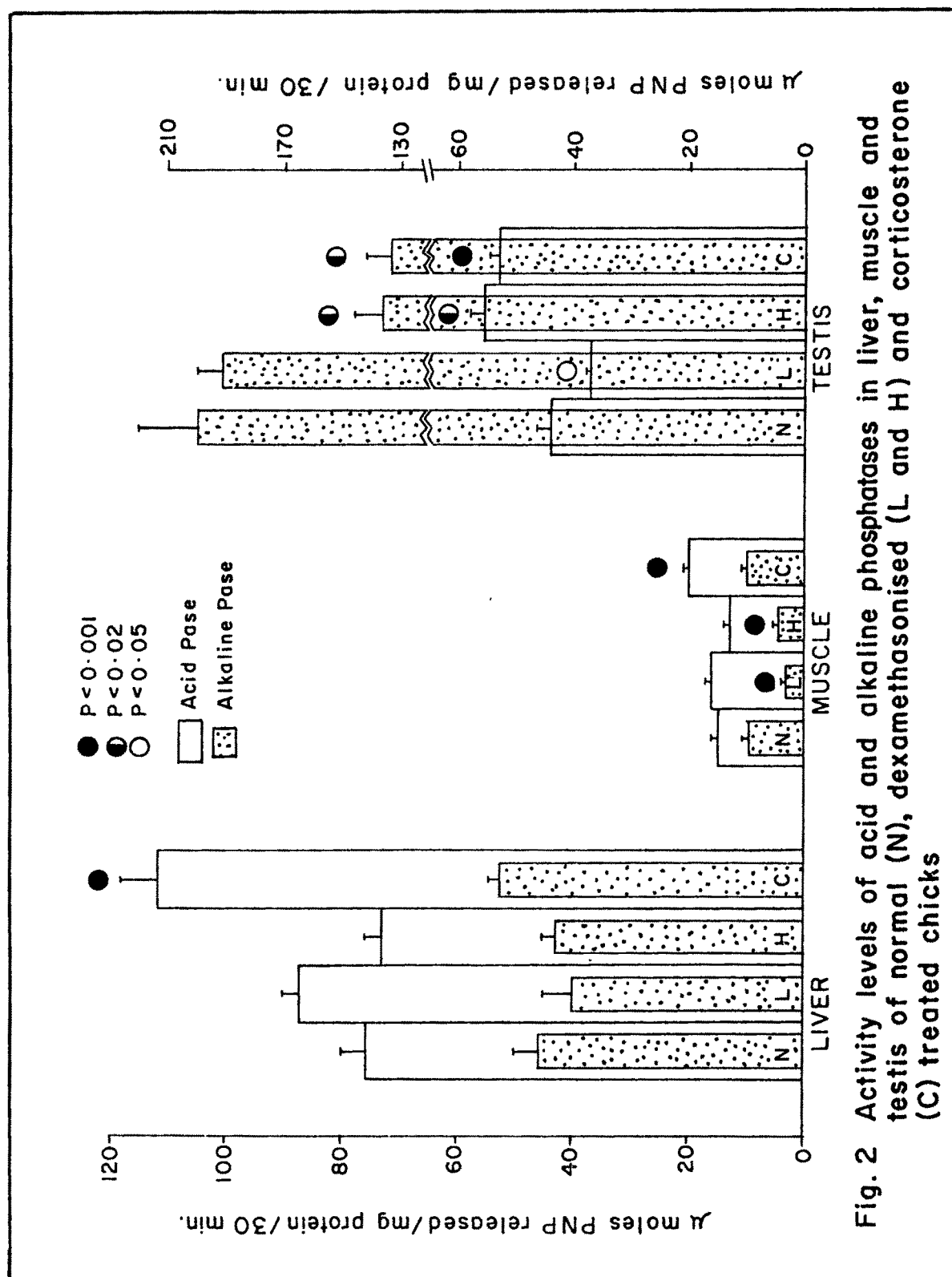


Table 3 Activity level of cAMP phosphodiesterase in liver, muscle and testis of chicks treated with dexamethasone and corticosterone for 30 days.

	cAMP phosphodiesterase µg PO ₄ released/mg protein/30 min		
	LIVER	MUSCLE	TESTIS
CONTROL	4.11 ±0.21	5.31 ±0.23	5.47 ±0.69
DXM(L)	2.79 ^a ±0.24	5.00 ±0.49	5.61 ±0.24
DXM(H)	2.61 ^a 0.20	2.21 ^a ±0.14	5.17 ±0.39
CORT	5.52 ^a ±0.34	4.61 ^d ±0.14	4.35 ±0.32

DXM(L) : dexamethasone low dose; DXM(H) : dexamethasone high dose
 CORT : corticosterone
 Values are mean ±SE of not less than 8 birds.
 a : P < 0.001; d : P < 0.05

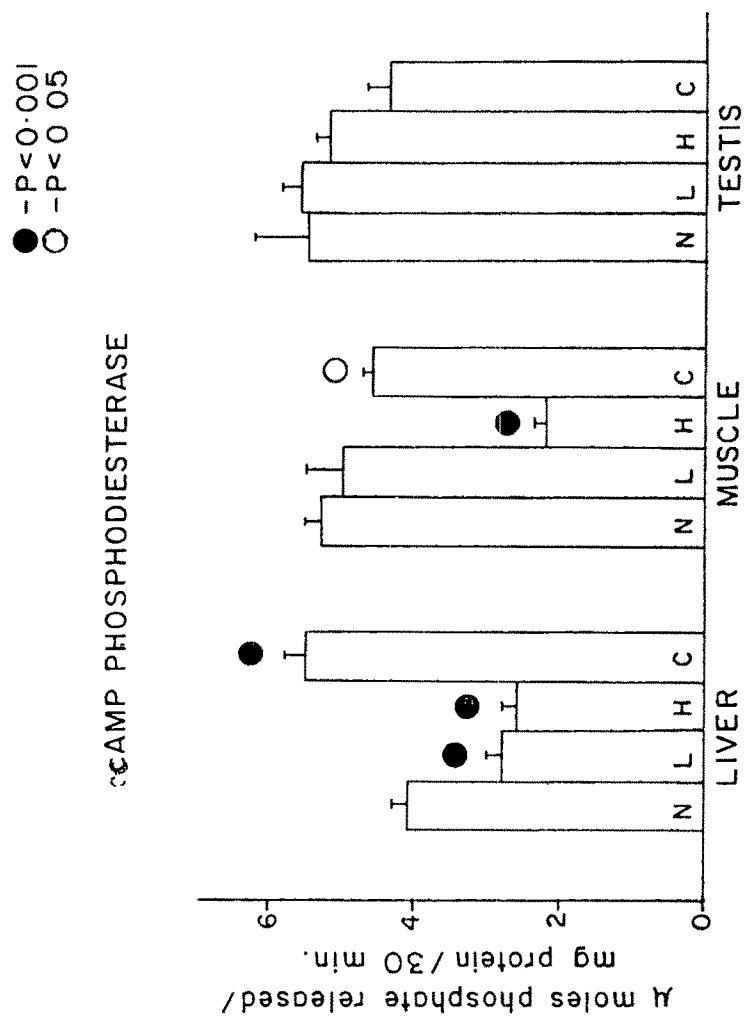


Fig. 3 Activity levels of cAMP Phosphodiesterase in liver muscle and testis of normal (N), dexamethasone-treated (L) and corticosterone (C) treated chicks.

DISCUSSION

The enzymological and biochemical profiles of avian tissues under hormonally induced dishomeostasis have received no attention in the adult conditions in general and during development in particular. The present observations deal with alterations in enzymes (not directly related to intermediary metabolism) in the liver, muscle and testis of White Leghorn breed of chicks treated chronically with dexamethasone and corticosterone for one month. The observations indicate that of the two phosphomonoesterases, acid phosphatase activity is elevated significantly in liver and muscle of corticosterone treated chicks (hypercorticalism) while in the testis it is decreased under DXM(L) (hypocorticalism) and increased under DXM(H) and corticosterone treatments. Apparently, hypocorticalism does not show any alterations in the activity of acid phosphatase in liver and muscle, while hypercorticalism has a definite stimulatory influence. In contrast, alkaline phosphatase activity was decreased in the muscle under hypocorticalism while that of testis showed significant decrease under DXM(H) and corticosterone treatments. High activity of acid phosphatase in the muscles of rat has been associated with regressive changes (see Lojda and Gutmann, 1976) and increased proteolytic activity (see Gutmann et al., 1976). In this light, the previous observation of decreased protein contents in liver and muscle in corticosterone treated chicks (Chapter IV) and the present observation of increased acid phosphatase activity in the liver and muscle of corticosterone treated chicks are correlatable. Moreover, the present observation suggests ^{that} hypocorticalism

might decrease muscle alkaline phosphatase activity in developing chicks. The changes in the testis when compared to those of liver and muscle tend to suggest a tissue specific differential effect of dexamethasone and corticosterone. The increased acid phosphatase coupled with decreased alkaline phosphatase activity under DXM(H) and corticosterone treatments might have a definite correlation with the earlier observed retardative and/or regressive alterations in the testis of DXM(H) and corticosterone treated chicks (Chapter II and VIII). More or less reverse pattern of changes in enzyme activity under DXM(L) coupled with progressive histological and histochemical changes observed earlier (Chapter II and VIII) tend to suggest a retardatory effect of adrenal corticosteroids in the progressive development and differentiation of testicular elements in the early phases of postnatal development of chicks.

Limited literature available on hormonal regulation of ATPase activity has indicated both, corticosteroids and thyroid hormones, to have a regulatory function (see Geering et al., 1982). Both these hormones have been shown to have a stimulatory influence specifically on $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ in organs involved in sodium transport. Based on such a study, Klein et al. (1984) have concluded that corticosterone and T_3 regulate rat renal cortical $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity via parallel independent pathways and that corticosterone appears to be the major regulator. However, study on hormonal regulation of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ biosynthesis in the toad bladder (Geering et al., 1982) has shown that

aldosterone has a stimulatory influence on $\text{Na}^+\text{-K}^+\text{-ATPase}$ while T_3 exerts an antagonistic effect to this response. Based on these observations Geering et al. (1982) concluded that thyroid hormone dependent induction of $\text{Na}^+\text{-K}^+\text{-ATPase}$ synthesis in various mammalian tissues may be an important factor in the transition from poikilothermy to homeothermy. Generally, stimulatory influence of cortisol on $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity et al., in the intestinal mucosa and gills of fishes has been noted (Pickford et al., 1970; Epstein et al., 1971; Forrest, et al., 1973; Saunders and Henderson, 1978). However, studies by Langdon et al. (1984) on the Atlantic Salmon suggest the pituitary-interrenal axis to be only partly responsible for increasing $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity. Convincing studies on hormonal regulation of ATPase activity on avian tissues have not been attempted. The present study on ATPase activity of liver, muscle and testis of developing chicks has revealed definite alterations in enzyme activity which are not however correlatable in a straight forward manner, and appears a bit intriguing. Based on the results, certain generalizations that can be drawn are that (1) Total ATPase activity in general increases under corticosterone treatment in all the three tissues which is essentially due to an increase in $\text{Ca}^{++}\text{-Mg}^{++}\text{-ATPase}$ with concomitant decrease in $\text{Na}^+\text{-K}^+\text{-ATPase}$ (though in the muscle, $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity was not affected). (2) Dexamethasone induced adrenocortical suppression depicts varying changes in the three tissues with

- (a) DXM(L) decreasing total ATPase activity in liver (significantly) and muscle (nonsignificantly), which is paralleled by decreased $\text{Ca}^{++}\text{-Mg}^{++}\text{-ATPase}$ activity and

- (b) DXM(H) increasing total ATPase activity in liver only with Na^+-K^+ - and $\text{Ca}^{++}-\text{Mg}^{++}$ -ATPase being affected differentially.

Though both the ATPases were increased in liver, the former was increased and the latter decreased in muscle and vice versa in the testis. These changes imply that chronic corticosterone treatment in chick increases total ATPase activity essentially by increasing the $\text{Ca}^{++}-\text{Mg}^{++}$ -ATPase, and probably also decreases $\text{Na}^{++}-\text{K}^+$ -ATPase. This is in contrast to the reported ability of corticosteroids to elevate Na^+-K^+ -ATPase activity as per the literature cited earlier. This is confirmed by the observed decrease in $\text{Ca}^{++}-\text{Mg}^{++}$ -ATPase activity under DXM(L) treatment with no change in Na^+-K^+ -ATPase activity, which is in contrast to the reports of Chignell and Titus (1966) and Jorgensen (1968) of a 47% reduction in rat renal Na^+-K^+ -ATPase activity after adrenalectomy. Obviously, the only conclusion that can be drawn is that in developing chicks, corticosterone regulation of ATPase activity is immature and differential. A direct correlation between corticosterone and $\text{Ca}^{++}-\text{Mg}^{++}$ -ATPase activity can be deduced at this stage. However, with reference to Na^+-K^+ -ATPase, it appears that while corticosterone excess suppresses its activity, corticosterone deficiency is without effect. Nevertheless the changes obtained with DXM(H) are rather enigmatic and defies any sound interpretation thus strengthening the contention that the regulation of ATPase activity by adrenal steroids is not yet fully established; or else the regulatory mechanism is different from that of the adult condition.

cAMP PDE activity can play an important role in cAMP metabolism as increased activity of this enzyme can lower cellular cAMP contents and antagonize cAMP mediated processes. Glucocorticoids have been identified as important agents modulating cAMP content by their suppressive effect on PDE activity (see Manganiello and Vaughan, 1972; Lee and Reed, 1977; Ross et al., 1977; Durand et al., 1983). This is further emphasized by the reported increase in PDE activity after adrenalectomy (see Allen and Beck, 1972). In the present study on developing chicks neither DXM induced adrenocortical suppression nor corticosterone induced excess affected the PDE activity in testis while the same was affected in liver and muscle. Both DXM(H) and corticosterone decreased PDE activity in muscle which is in keeping with the above mentioned PDE activity lowering effect of corticosteroids. However, the liver PDE activity was decreased under both DXM(L) and DXM(H) treatments and increased under corticosterone treatment. These may be construed as secondary adaptive changes characteristic of the prevailing internal milieu due to chronic adrenocortical suppression and excess. The decreased PDE activity in DXM(L) and DXM(H) treated chicks and increased activity in corticosterone treated chicks may in this context be correlated with the earlier observed increased and decreased glycogen contents respectively in DXM and corticosterone treated chicks (Chapter III). Overall, it could be concluded that DXM and corticosterone do induce tissue specific alterations in phosphomonoesterases, ATPases and PDE during early phases of postnatal development in White Leghorn chicks.