

## CHAPTER IV

EFFECT OF INDUCED ADRENOCORTICAL DEFICIENCY AND EXCESS ON  
TISSUE PROTEIN AND ASCORBIC ACID CONTENTS IN POST-HATCHED  
WHITE LEGHORN BREED OF CHICKS

Protein constitutes one fifth to one fourth of the fat free body of mammals and birds (Mitchell et al., 1931; Griminger and Gamars h, 1972). The whole body protein is considered to be variable because of variations in body fat content which are not balanced by adjustments in water content. In birds, 20 to 30% of the body protein are localized in the feathers, while quite a bit of structural proteins are also found in bone, muscle and skin. The tissue proteins can play a functional role for provision of energy during their degradation. Protein metabolism in general, appears to be similar in amniotes. Tissue protein turnover is characterized by simultaneous synthesis and degradation of proteins and, the prevailing levels of tissue protein content are a reflection of the relative rate of synthesis and degradation. During the post-natal growth of chicks, the synthesis can be considered to proceed at a faster rate than degradation, thus leading to build up of tissue proteins and growth as a whole. Unfortunately, in birds, protein turnover studies have been limited to a few muscles in the domestic fowl (Griminger and Scanes, 1986). One of the factors that could exert a control over synthesis or breakdown of proteins is hormone(s). Again, endocrine control of protein metabolism in avian species is poorly studied (Griminger and Scanes, 1986). The major hormones considered to affect protein metabolism in vertebrates as a whole are insulin, thyroid hormones, growth hormone, androgens, glucocorticoids and estrogens.

The present study in this context is an attempt to evaluate the influence of glucocorticoids on tissue protein contents by way of induced glucocorticoid insufficiency (by dexamethasone treatment) and excess (by exogenous corticosterone administration) during the first four weeks of post-hatched development of White Leghorn breed of chicks.

Ascorbic acid (AA), the versatile vitamin has been implicated in cellular processes like electron transport, metabolism, collagen synthesis and steroidogenesis (Szent Gyorgii, 1957; Bacq and Alexander, 1961; Biswas and Deb, 1970; Chinoy, 1972 a,b). Hence, the synthesis and build up of ascorbic acid (AA) in tissues during post-hatched development, a phase of progressive attainment of functional maturity, could be of great relevance to the avian species. Unfortunately, literature on tissue AA turnover during post-hatched development of avian species is scant, bar for the restricted studies on adrenal AA contents in adult and developing chicks (Chinoy et al., 1974 a,b; Chinoy and Parmar, 1975 a,b), not to speak of its endocrine regulation. In this context, the present study attempts to estimate tissue AA contents under conditions of adrenocortical insufficiency and excess in developing chicks.

#### MATERIAL AND METHODS

As outlined in Chapter I.

Table 1 Ascorbic acid content in liver, adrenal and testis and protein content in liver muscle and testis of chicks treated with dexamethasone and corticosterone for 30 days.

	Ascorbic acid mg/100 mg tissue		Protein mg/100 mg tissue	
	LIVER	ADRENAL	TESTIS	LIVER
				MUSCLE
			TESTIS	TESTIS
CONTROL	0.0136 ±0.0009	0.1152 ±0.0028	0.0414 ±0.0038	18.64 ±0.77
				15.85 ±0.70
				12.63 ±0.86
DXM(L)	0.0146 ±0.0011	0.094 <sup>c</sup> ±0.008	0.0378 ±0.0034	21.29 <sup>b</sup> ±0.44
				19.06 <sup>d</sup> ±1.27
				14.83 ±0.79
DXM(H)	0.0141 ±0.0009	0.085 <sup>a</sup> ±0.0052	0.0340 ±0.0030	23.94 <sup>a</sup> ±1.05
				20.58 <sup>b</sup> ±1.03
				12.05 ±0.87
CORT	0.0213 <sup>a</sup> ±0.0008	0.1311 <sup>b</sup> ±0.0042	0.0557 <sup>d</sup> ±0.0047	14.89 <sup>b</sup> ±0.72
				11.76 <sup>b</sup> ±0.69
				13.97 ±0.68

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

CORT : corticosterone

Values are mean ±SE of not less than 8 birds.

a :  $P < 0.001$ ; b :  $P < 0.01$ ; c :  $P < 0.02$ ; d :  $P < 0.05$

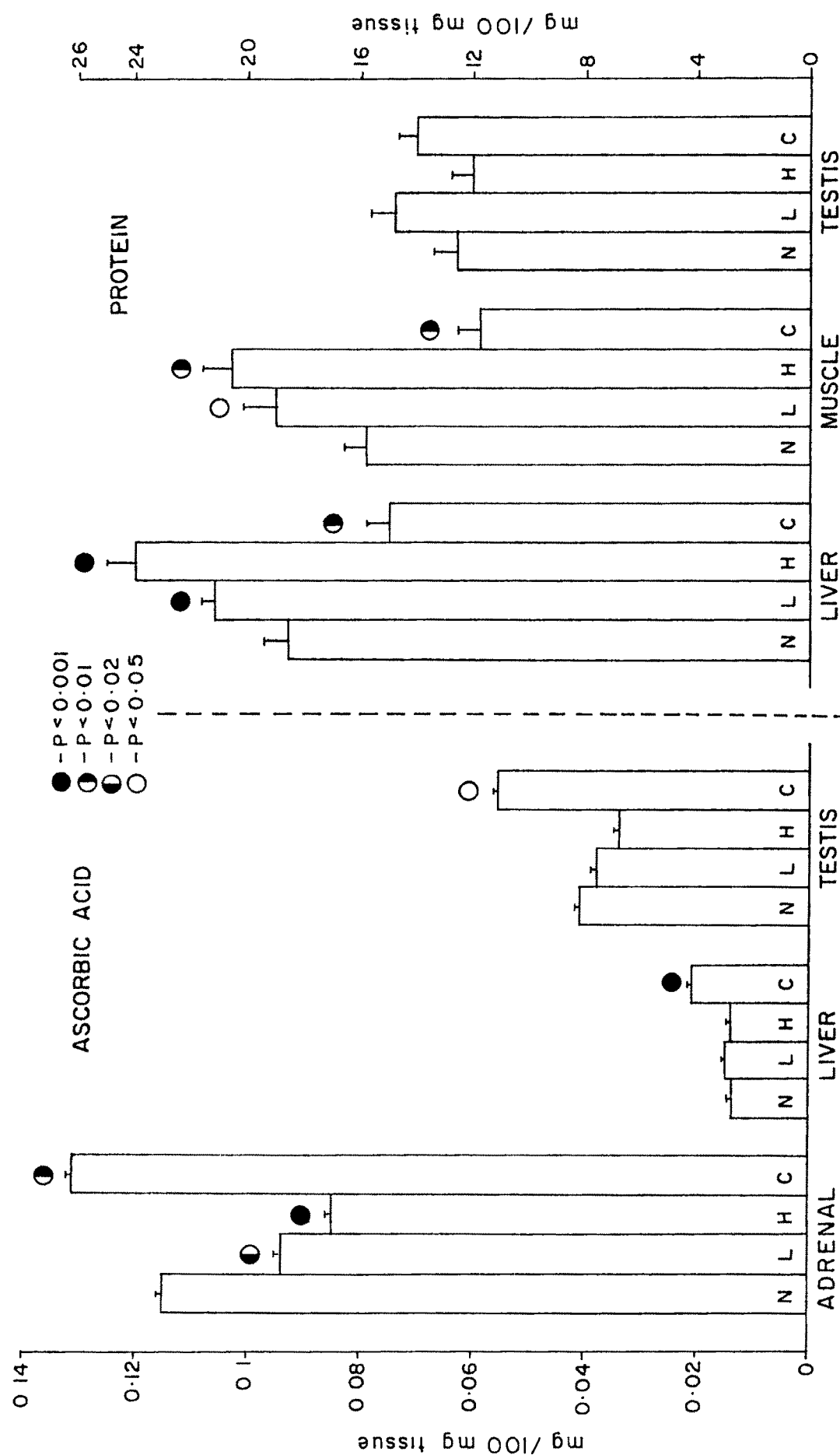


Fig. 1 Ascorbic acid content in adrenal, liver and testis and protein content in liver muscle and testis of normal (N), dexamethasonised (L and H) and corticosterone (C) treated chicks.

## RESULTS

### Protein content of liver, muscle and testis

Though the protein content of the testis was not much altered in either of the experimental groups, there was increase in the protein content of liver and muscle in adrenal suppressed chicks while the contents were decreased significantly ( $P < 0.001$ ) under induced adrenocortical excess. (Table 1, Fig. 1)

### AA content of adrenal, liver and testis

The results (Table 1, Fig. 1) showed a decrease in the AA content of adrenal and testis after dexamethasone treatment. Conversely, hepatic AA content tended to show slight increase in dexamethasonized chicks. Corticosterone treatment was found to increase the AA content in all the three tissues.

## DISCUSSION

Tissue protein turnover can serve as an index of overall body protein metabolism, a process generally related to normal growth. However, protein metabolism can be greatly influenced by many hormones, amongst which the glucocorticoid is very important. As in mammals, glucocorticoids have growth suppressant and protein catabolic roles in birds (Griminger and Scanes, 1986). Obviously, alterations in adrenocortical activity or manipulations affecting adrenocortical function should have profound influence on protein metabolism and growth.

The present study is essentially an attempt to evaluate the effect of induced glucocorticoid excess (by exogenous administration of corticosterone) and insufficiency (by dexamethasone induced ACTH suppression) on tissue protein and AA contents of chicks during the first month of development. It becomes clear from the results that chronic hypercorticalism reduced the protein content of liver and muscle while chronic hypocorticalism increased the same. This is understandable as corticosterone is reported to have profound effects on protein metabolism. Reduced biosynthesis of protein by glucocorticoids has been reported to occur in laboratory rodents (Jeanrenaud and Renold, 1960; Wool, 1960) and domestic fowl (Baum and Meyer, 1960; Nagra and Meyer, 1963).

Besides, decreased muscle protein content and depressed synthesis of DNA, RNA and proteins in tissues of chicks by cortisol have also been reported (Bellamy and Leonard, 1965). Moreover, abnormally high rate of amino acid deamination in the liver of chicks by cortisol (Goodlad and Munro, 1958; Bellamy and Leonard, 1964) and decreased nitrogen balance and increased nitrogen excretion and uric acid by glucocorticoid treatment in chickens and quail (Adams, 1968; De la Cruz et al., 1981) have also been reported. All these reports tend to suggest an overall reduction in protein content under glucocorticoid excess. A protein source for glucocorticoid induced gluconeogenesis has, in fact, been well documented for rats and mice (Rosen et al., 1959, 1963; Weber et al., 1961, 1965; Bellamy et al., 1968 a, b) and chicks (Harvey et al., 1986). Some correlation for the herein observed depletion in hepatic and muscle protein contents and gluconeogenesis

is provided by the earlier recorded hyperglycaemia in corticosterone treated chicks (Chapter III). It is realized that the rate of growth of tissue protein mass is a function of relative rates of protein synthesis and degradation and an understanding of the way in which these two processes are regulated could provide an insight into the phenomenon of development and growth. The consensus of opinion based on nutritional restriction and refeeding experiments on muscle protein turnover in growing chicks and rats, primarily favours differences in the rate of protein synthesis to be more crucial (see MacDonald and Swick, 1981). However, a perusal of the present data on hepatic and muscle protein contents shows significantly depleted levels under corticosterone treatment, though a net increase in protein content does occur (11.6 and 6.3 mg per cent respectively), from the day of hatching. Nevertheless, the increase is decidedly less than that in the control chicks. Keeping in view the catabolic role of glucocorticoids, it can be presumed that the reduced protein content observed in corticosterone treated chicks is a function of increased rate of degradation. In contrast, dexamethasone treated chicks showed increased hepatic and muscle protein contents denoting glucocorticoid insufficiency and resultant decrease in the rate of protein breakdown. It was suggested previously that dexamethasone induced adrenal suppression and resultant reduction in endogenous glucocorticoid level could increase insulin action/release by way of minimizing the glucocorticoid antagonism to insulin (Chapter III). In this light, the increased action in dexamethasone suppressed chicks could provide a more favourable influence for increased protein anabolism and decreased protein catabolism. This contention is corroborated by many



observations on protein anabolic influence of insulin, in the fowl and the duck (Langslow et al., 1970; Samsel and Ledig, 1976; Laurent and Miahle, 1978; Griminger and Scanes, 1986). With regard to testicular protein content it is clear that during the first month of chick development, testicular protein metabolism is insensitive to either induced chronic glucocorticoid excess or insufficiency.

In the present study, AA content of liver, adrenal and testis has been evaluated of which adrenal and testes are steroidogenic organs while liver is a storage organ as kidney is considered to be the organ of AA synthesis in galliform birds (Roy and Guha, 1958; Chatterjee et al., 1961). In view of the reported role of AA in cellular events such as enzyme activities, general metabolism, electron transport, collagen synthesis and steroidogenesis, to mention a few, its importance during post-natal development need not be emphasized. However, the hormonal regulation of AA metabolism has to-date remained a befuddled topic. Two of the hormones reported to exert modulations in AA turnover in tissues of rats and chicks are testosterone and corticosterone (Stubbs and Mckernan, 1967; Dieter, 1969; Dieter and Breitenbach, 1971; Majumdar and Chatterjee, 1974; Overbeek, 1985). The role of ACTH/corticosterone in overall body metabolism of AA in rats and chicks, though found significant, has not been clearly elucidated due to variable results obtained by the above investigators. In the present study, dexamethasone induced hypocorticalism decreased the adrenal AA content significantly and testicular AA content insignificantly while corticosterone induced hypercorticalism increased the AA content of all the three

organs significantly. These observations suggest that corticosterone can induce a positive AA balance in the body while lack of corticosterone can induce negative AA balance. The observation of Dieter (1969) of decrease in renal AA content (synthesizing organ) together with unchanged renal gulonate NADP oxido-reductase activity (an enzyme of AA biosynthetic pathway) and hepatic AA content in 10 days old White Leghorn cockerel treated with corticosterone for 4 days is at slight variance from the present observation. This discrepancy may have an explanation in the dose and duration of treatment schedule employed by the above investigator. It is likely that the duration of corticosterone treatment (4 days) employed in the above work may be insufficient to stimulate the AA biosynthetic enzymes. Besides, the administration of 100 µg twice a day (Dieter, 1969) as against 5-15 µg corticosterone employed once a day in the present study, could have brought about feed back suppression of ACTH and thereby nullifying the probable positive influence of corticosterone on AA synthesis and accumulation. The present findings are in agreement with the observations of increased AA content in liver and blood in response to ACTH (Stewart et al., 1953; Sinha and Lahiri, 1964) and decreased AA content of liver in adrenalectomised rats (Giovanni et al., 1957; Cuzzocrea et al., 1959). Further support to the present findings comes from the report of decreased activity of AA synthesizing enzymes, increased activity of AA catabolic enzymes and decreased AA content of liver, blood and urine in adrenalectomised rats (Nathani et al., 1971). However, Overbeek (1985), discounted the possibility of corticosterone controlling the synthesis and release of AA by liver and uptake and storage by adrenal in rats. This was based

on his observation that neither ACTH nor high dose of dexamethasone (presumed to act like corticosterone) administration in hypophysectomised rats could restore the depleted levels of AA in liver, blood and adrenal, and further that low dose of dexamethasone administered to non-hypophysectomised rats (noted to induce adrenal atrophy) did not alter the AA concentrations. Hypophysectomy induced decreased synthesis of AA in liver and decreased adrenal AA content have been reported (Salomon and Stubbs, 1961 a,b; Stähelin et al., 1965, Overbeek, 1981 a,b). According to Overbeek (1985), ACTH may be involved in maintaining adrenal size while either GH or prolactin is required for uptake and storage of AA. It was also suggested that ACTH may be involved in hepatic AA release while another pituitary factor (not studied) may be involved to stimulate AA production. Our findings of decreased AA content under both low and high dexamethasone treatment and increased AA contents under corticosterone treatment provide a clear and categorical evidence for the involvement of adrenal corticosteroids in AA biogenesis and accumulation in body tissues which is well supported by the observations of decreased activity of AA synthesizing enzymes and increased activity of AA catabolizing enzymes in adrenal-ectomised rats (Nathani et al., 1971). Further, confirmatory evidence is provided by the findings from our laboratory of decreased tissue AA contents under dexamethasone induced adrenal suppression and increased tissue AA content by corticosterone treatment in pigeons (Ayyar and Ramachandran, unpublished findings) and of decreased tissue AA contents under dexamethasone induced adrenal suppression in lizards

(Abraham and Ramachandran, unpublished findings). Moreover, considering the similarity of dexamethasone doses (low and high) employed by us and Overbeek (1985), it may be presumed that either the high dose of dexamethasone used may be insufficient to mimic the actions of corticosterone in cockerels unlike in rats or, the immature chicks are insensitive to the corticosterone like actions of dexamethasone in high dose with reference to AA metabolism. A conciliatory explanation that could be provided (taking into consideration the present results and that of Overbeek, 1985) is that corticosterone is a hormone involved in positive AA turnover while a pituitary factor like GH or prolactin may be required as a permissive factor for its expression.

Overall, based on the present findings it can be concluded that glucocorticoid excess induces protein catabolism while glucocorticoid insufficiency induced by dexamethasone favours protein anabolism facilitated greatly by the reduced antagonism to insulin. It is also concluded that, in chicks, hypercorticalism induces a net positive AA balance while hypocorticalism induces a net negative AA balance.