CHAPTER V

INDUCED CHRONIC ADRENOCORTICAL EXCESS AND INSUFFICIENCY IN FRESHLY HATCHED WHITE LEGHORN BREED OF CHICKS: CHANGES IN THE TOTAL LIPID CONTENT AND CHOLESTEROL FRACTIONS OF SERUM, LIVER, ADRENAL AND TESTIS

To a large extent body fat acts as an energy reserve and as such is the most variable among the organic constitutents. It has been reported that the ability of birds to store lipids as an energy reserve is far greater than those of other vertebrates (Blem, 1976). The store of lipids in the avian body could serve as an important source of energy during sustained stressful conditions as well as during biological activities like migration (Griminger and Gamarsh, 1972; Bartov et al., 1980a,b),uninterrupted incubation of eggs for long periods (Oring, 1982) and molting (Williams et al., 1977). The fat content of birds varies with species, sex and age and is also strongly affected - quantitatively as well as qualitatively - by nutritional factors and neuroendocrine mechanisms. In general, the hormones inducing the fattening process in birds seem to be prolactin and corticosterone (Meier, 1977). Steroids such as corticosterone are powerful regulators of body function and they offer a mechanism by which sensory inputs are translated into biochemical processes throughout the body. Apparently, body resources can be reallocated in response to internal and external changes.

The dynamic adaptive role of endocrine system in adult vertebrates is well recognized. However, the role of the endocrine system during the ontogenetic developmental stages, more specifically the ex ovo/

neonatal period, could prove to be more dynamic and interesting. This is pertinent as the neonatal period of avian development serves to link the endocrine independent embryonic phase of development with the more hormone dependent adult phase. It is inferrable that during this period of transition, the organism might express differential sensitivity to various hormonal principles providing a developmental basis for effective adaptations at later stages of life cycle. Obviously, profound developmental consequences could be obtained by artificially manipulating the hormonal milieu during the neonatal period. Many studies have demonstrated a lipogenic influence of adrenocorticotropin and corticosterone in adult fowls and young chicks of three weeks age or more (Baum and Meyer, 1960; Nagra and Meyer, 1963; Bartov et al., 1980 a,b; Bartov, 1982; Davison et al., 1983; Buyse et al., 1987).

The present study has been undertaken to see the effects of chronic functional manipulation of the adrenal cortex (in the form of induced glucocorticoid excess by corticosterone administration and insufficiency by dexamethasone treatment) on total lipids and cholesterol contents of serum, liver, adrenal and testis in freshly hatched White Leghorn breed of chicks.

MATERIAL AND METHODS

As outlined in Chapter I.

RESULTS

Total lipid content of liver, adrenal, testis and serum

Adrenal manipulation in general did not affect the total lipid content of liver and testis. The adrenal lipid content was significantly ($P<0.001$) elevated with both doses of dexamethasone while it was depleted significantly ($P<0.05$) in corticosterone treated chicks. Total lipid content in serum was increased under both types of adrenal manipulation being more pronounced with DXM treatment. These changes are depicted in Table 1; Fig. 1.

Total, Free and Esterified Cholesterol in liver, adrenal, testis and serum

Total, free and esterified cholesterol of testis did not show any significant alterations either with DXM or corticosterone treatment. The low dose of dexamethasone increased the total and esterified fractions of cholesterol in adrenal while the high dose decreased both these fractions. Corticosterone treatment did not show any alteration except for a significant decrease in free cholesterol. The total and esterified fractions of liver cholesterol showed significant increment with both doses of DXM, with a significant reduction of free fraction in DXM(L). Corticosterone treatment increased the total cholesterol content without effecting the free and esterified fractions.

Significant increase in total, esterified and free cholesterol in the serum was indicated under dexamethasone treatment while corticosterone

Table 1 Total lipid content in liver, adrenal, testis and serum of chicks treated with dexamethasone and corticosterone for 30 days.

	mg/100 mg tissue			g/100 ml serum
	LIVER	ADRENAL	TESTIS	SERUM
CONTROL	9.529 ±0.670	16.914 ±0.740	11.265 ±0.343	1.625 ±0.037
DXM(L)	9.318 ±0.678	22.367[a] ±1.041	12.040 ±0.359	2.428[a] ±0.183
DXM(H)	10.276 ±0.516	25.018[a] ±1.169	11.257 ±0.596	2.337[a] ±0.163
CORT	10.444 ±0.382	14.674[d] ±0.602	10.925 ±0.201	1.931[b] ±0.067

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 : $P < 0.01$; d : $P < 0.05$

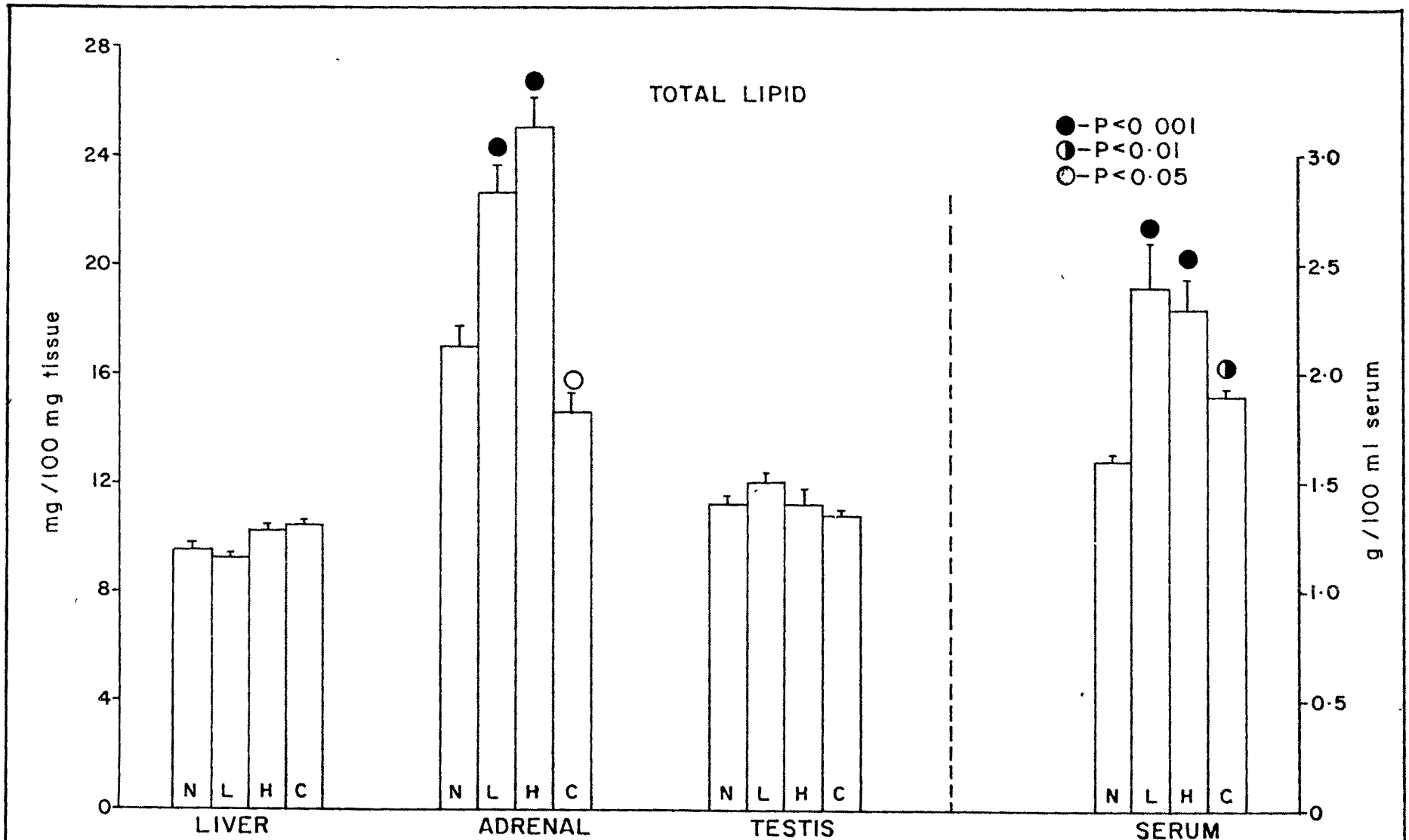

Fig. 1 Total lipid content in liver, adrenal, testis and serum of normal (N), dexamethasonised (L and H) and corticosterone (C) treated chicks.

Table 2 Total, free and esterified cholesterol contents in liver, muscle and testis of chicks treated with dexamethasone and corticosterone for 30 days.

	Total cholesterol				Free cholesterol				Esterified cholesterol			
	mg/g tissue			mg/100 ml	mg/g tissue			mg/100 ml	mg/g tissue			mg/100 ml
	LIVER	ADRENAL	TESTIS	SERUM	LIVER	ADRENAL	TESTIS	SERUM	LIVER	ADRENAL	TESTIS	SERUM
CONTROL	3.37 ±0.14	31.55 ±1.86	9.70 ±0.63	131.09 ± 5.68	1.98 ±0.11	5.26 ±0.28	2.95 ±0.14	16.48 ±0.65	1.39 ±0.11	26.29 ±1.23	6.75 ±0.52	114.61 ± 5.17
DXM(L)	4.40[a] ±0.08	41.59[b] ±2.64	9.03 ±0.60	194.78[a] ±11.62	1.65[d] ±0.10	6.21 ±0.37	3.14 ±0.29	40.34[a] ±3.81	2.75[a] ±0.16	35.38[a] ±2.34	5.89 ±0.43	154.44[a] ± 9.42
DXM(H)	4.17[c] ±0.23	24.71[b] ±1.17	10.27 ±0.68	213.92[a] ±16.20	2.03 ±0.09	5.64 ±0.18	3.02 ±0.22	42.33[a] ±2.28	2.14[a] ±0.12	19.07[b] ±0.98	7.25 ±0.58	171.50[a] ±13.30
CORT	4.03[c] ±0.12	29.66 ±2.62	8.01 ±0.52	137.13 ± 5.13	2.22 ±0.12	4.11[b] ±0.09	2.86 ±0.21	25.11[a] ±1.26	1.81 ±0.15	25.55 ±1.36	5.15 ±0.39	112.02 ± 5.82

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 : $P<0.05$

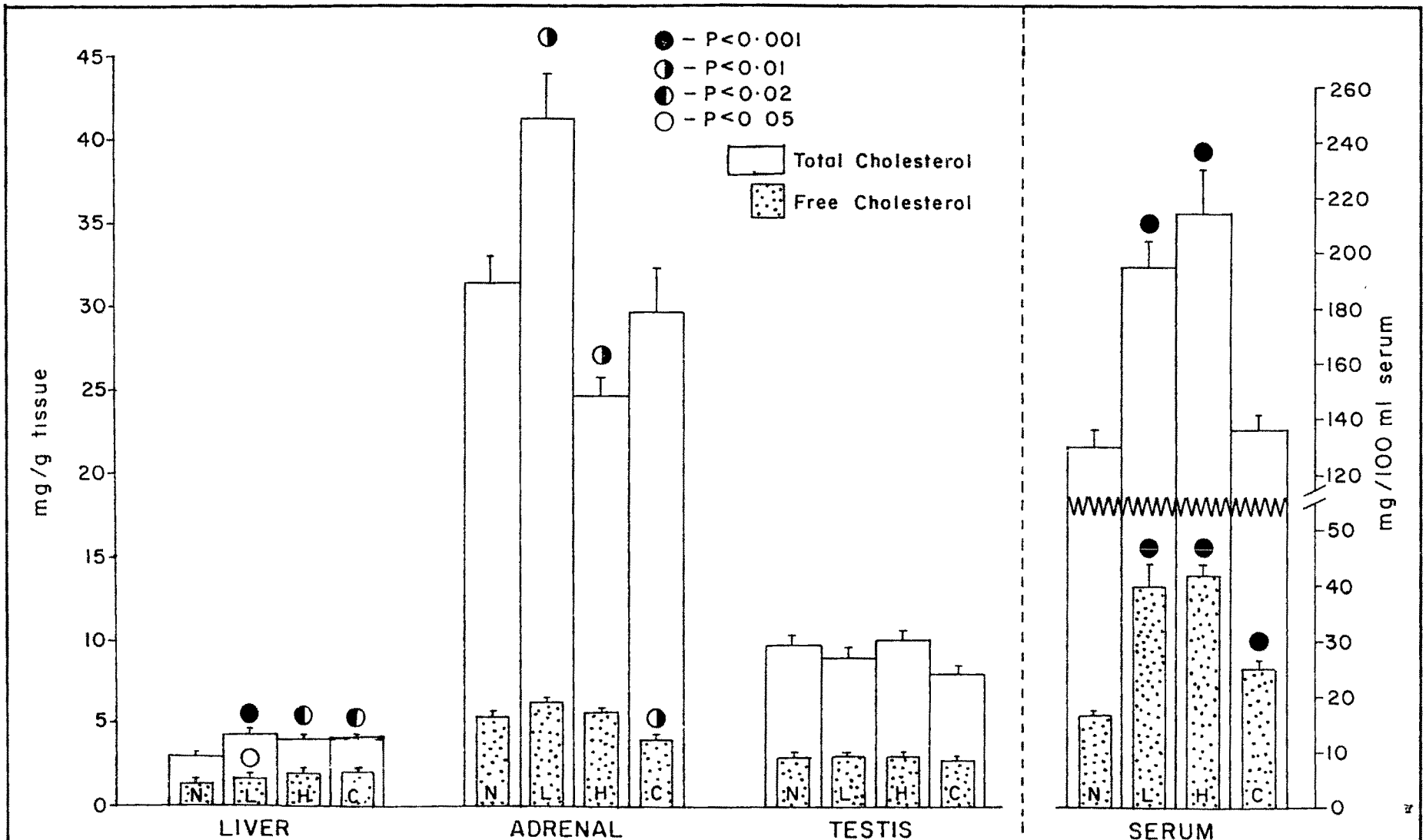

Fig. 2 Total and free cholesterol contents in liver, adrenal, testis and serum of normal (N) dexamethasonised (L and H) and corticosterone (C) treated chicks.

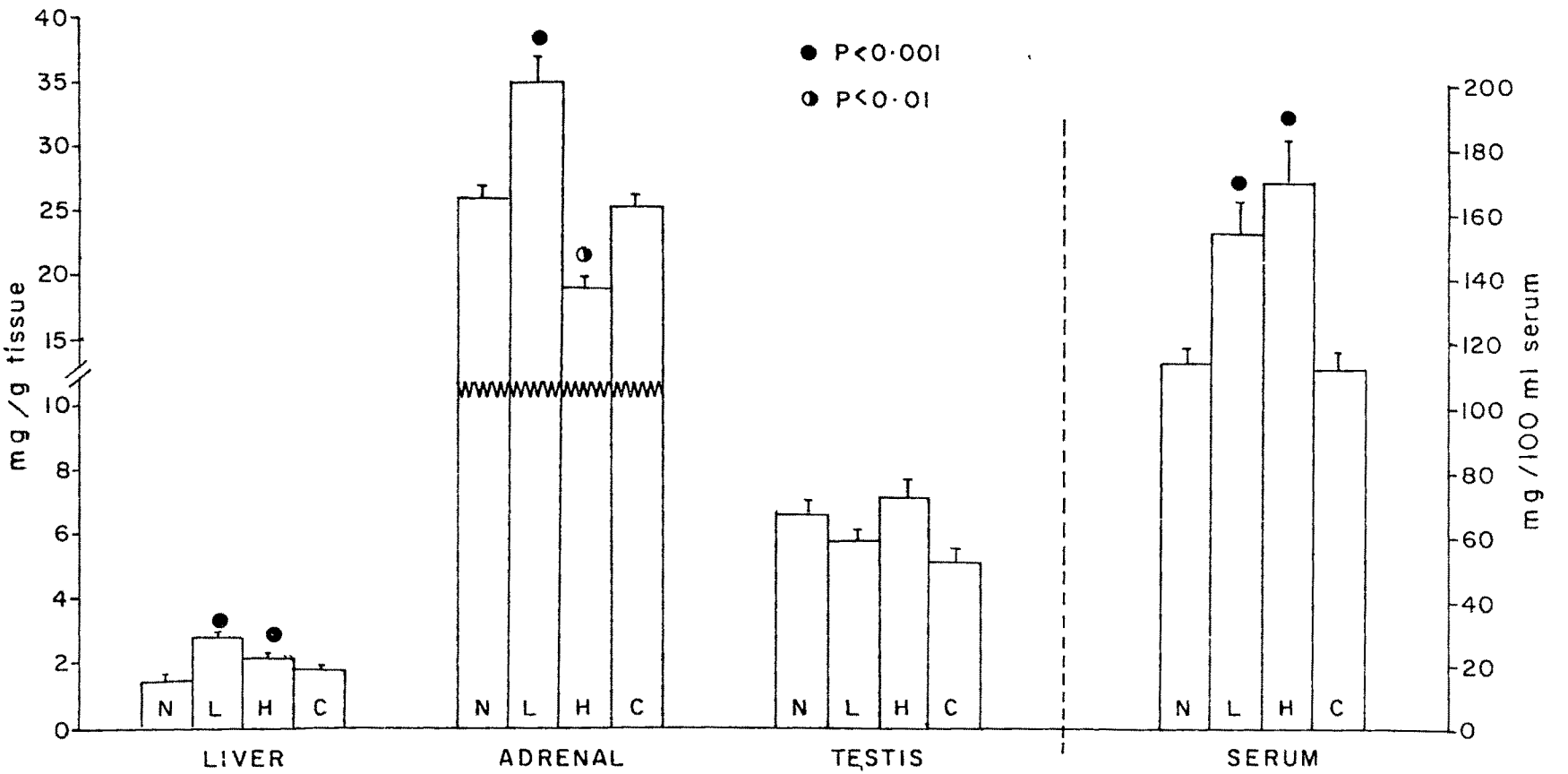

Fig. 3 Esterified cholesterol content in liver, adrenal, testis and serum of (N), dexametha-sonised (L and H) and corticosterone (C) treated chicks.

treatment induced an increase in the level of serum free cholesterol only. These changes are represented in Table 2 ; Figs. 2 & 3.

DISCUSSION

The role of glucocorticoids in inducing lipogenesis and fattening in birds in general and the domestic fowl in particular has been recognized (Baum and Meyer, 1960; Nagra and Meyer, 1963; Nagra et al., 1963; Bartov et al., 1980 a,b; Bartov, 1982; Davison et al., 1983; Buyse et al., 1987). Though a generalised increase in depot fat is demonstrated, the influence of ACTH/corticosterone on lipid and cholesterol contents of serum, adrenal and liver has been found to be variable. Number of factors such as the species, the environmental conditions, the age, the dose and frequency of treatment and even hormonal status of animals, all account for the variation. The present study has attempted to evaluate the effects of exogenous corticosterone (glucocorticoid excess) and dexamethasone (glucocorticoid insufficiency) in freshly hatched chicks chronically treated for 30 days. Though a significantly increased size of the abdominal fat pad along with increased deposition of fat in the neck region and the area surrounding gizzard and heart were observable under both treatments (relatively greater with dexamethasone), the changes in lipid and cholesterol contents of adrenal, liver, serum and testis were found to be differential.

Neither induced glucocorticoid excess, nor its insufficiency by

dexamethasone treatment, had any effect on testicular lipid and cholesterol contents. It is likely that the hypophyseal-adrenocortical axis is as yet incompetent in modulating testicular lipid and cholesterol metabolisms during the first month of neonatal chick development.

The adrenal lipid and cholesterol profiles have depicted the greatest differential response to the present experimental manipulations. Both the low and high dose of dexamethasone treatment increased the adrenal total lipid content by about 40%, while corticosterone treatment depleted the adrenal total lipid content by about 15%. In contrast, low dexamethasone dose increased the total and esterified fractions of cholesterol (30-35%) and the high dose of dexamethasone decreased both the cholesterol fractions (20-30%), while, corticosterone treatment had no effect. The two dosages of dexamethasone seem to have opposite effects on adrenal cholesterol metabolism in spite of the fact that both dosages induced cortical suppression, recognizable in terms of adrenal weight and histology (Chapter II). The increased total cholesterol and cholesterol ester contents obtained with the low dose of dexamethasone, known to suppress pituitary ACTH secretion (Kendall and Allen, 1968; Russel et al., 1969; Macharg et al., 1985; Smoak and Birrenkott, 1986; Carnes et al., 1987; De Greef and Van der Schoot, 1987; Brody and Black, 1988; Katano, 1988), are understandable in the wake of the reported depletion of adrenal stores of cholesterol in fowls treated with ACTH for a period of days (Howard and Constable, 1958; Siegel, 1962 a,b; Siegel and Siegel, 1966; Freeman and Manning, 1975). It is established that steroidogenesis in ACTH sensitive adrenal cells of mammals is modulated by the flux of cholesterol through the stored

pool of esterified cholesterol and, is essentially governed by two enzymes: Acyl Coenzyme A: Cholesterol Acyltransferase (ACAT), which esterifies free cholesterol, and Cholesterol Ester Hydrolase (CEH), which releases free cholesterol by cleaving the ester bond (Brody and Black, 1988). It is also reported that in guinea pigs, dexamethasone induced adrenal suppression leads to repression of both these enzymes (Brody and Black, 1988). In this context, a possible direct dose dependent suppressive effect of dexamethasone on ACTH release in chicks is envisaged. It is also speculated that the regulatory sensitivities of ACAT and CEH may be quantitatively related to ACTH levels, with CEH being more sensitive than ACAT with dexamethasone induced ACTH suppression. The increased cholesterol ester content obtained with the low dose of dexamethasone might indicate reduced breakdown of cholesterol ester as a result of CEH suppression brought about by the submaximal suppression of ACTH. On the contrary, the decreased ester content in chicks treated with high dose of dexamethasone suggests the suppression of both ACAT and CEH brought about by maximal suppression of ACTH. This dosage effect of dexamethasone on ACTH suppression is further indirectly corroborated by the previously reported dose-dependent effect of dexamethasone on hepatic glycogen deposition and hypoglycemia (Chapter III). The reduced total cholesterol content in chicks treated with high dexamethasone dose could also indicate a possible inhibition of cholesterol biogenesis, by way of suppression of 3-Hydroxy-3-Methyl-glutaryl Coenzyme A reductase (HMG-CoA-reductase) as well (Black et al., 1988) and/or cholesterol uptake. It is also clear from the present study that exogenous

corticosterone treatment (at least in the dosage used in the present study) is without effect on adrenal cholesterol metabolism. It is also inferable, that at the present dosage, corticosterone does not lead to any feedback inhibition of ACTH in neonatal chicks. In fact a stimulatory influence either on the hypophyseal-adrenal axis indirectly or, on the adrenal cortical cells directly, is denoted by the significantly decreased total lipid content in contrast to the significantly increased levels in dexamethasone treated chicks. The results suggest that the lipid content may serve as a better index of assessing steroidogenic activity of the adrenal glands in chicks.

Liver is known to be the principal site of cholesterol synthesis and lipogenesis in birds. The level of lipids and cholesterol in the serum is usually a reflection of their synthesis in liver and intestine and also the dietary content. In this context, a significantly increased content of both cholesterol fractions in the serum of dexamethasone treated chicks suggests increased biogenesis and spillover, alongwith probably a decreased uptake by peripheral tissues. This is reflected in the increased content of cholesterol in the liver. However, corticosterone increased the hepatic cholesterol content without affecting the serum levels, except for the free cholesterol fraction. It appears that, though, both corticosterone and dexamethasone can induce hepatic-cholesterol biogenesis, the potency of the latter seems to be of far greater magnitude. Such an increased potency of dexamethasone is also reflected in the relatively greater levels of serum total lipids in comparison with corticosterone. Though the serum total lipid was

increased in both dexamethasone and corticosterone treated chicks, the lipid content of the liver did not show any change. Obviously, the lipids being synthesized in the liver are released into the blood stream at a faster rate and are being laid as fat depots in the body. The differential potency of dexamethasone and corticosterone is further emphasized by the far greater amount of depot fat observed in chicks treated with the former. The increased fat deposition obtained with corticosterone is understandable as Nagra and Meyer (1963) have shown that glucocorticoids convert glucose carbon into more lipids and less protein and carbohydrates. This is corroborated by the recorded decreased hepatic glycogen content and hyperglycemic condition (Chapter III) and decreased tissue protein contents (Chapter IV) in corticosterone treated chicks during the course of the present study. It is also reported that corticosterone depresses T_3 levels in chicks (Decuypere et al., (1983) and the domestic fowl (Buyse et al., 1987). Propylthiouracil induced hypothyroidism in chicks has been shown to lead to a hepatic glycogen and triglyceride storage syndrome (Raheja et al., 1980). Moreover, a negative correlation between T_3 levels and carcass fat was found in chickens (Stewart and Washburn, 1983) leading to the suggestion that low levels of circulating T_3 may be associated with a higher degree of fatness and that the lipogenic effects of corticosterone may also partly be mediated by a decreased T_3 level (Ringer, 1976; Buyse et al., 1987). The presently observed fat deposition in corticosterone treated chicks and similar observations by other workers in the domestic fowl and laying hens (Nagra et al., 1965;

Bartov et al., 1980a,b; Bartov, 1982; Pilo et al., 1986) may in the above context be viewed as a mere permissive influence of lowered T_3 levels in favour of lipogenesis and fat deposition.

The far greater degree of steatogenesis and fat deposition observed in DXM treated chicks imply a similar possible lowering of T_3 levels and the prevalence of a more favourable state of fat depositon relative to the corticosterone treated groups. The previous contention that DXM treatment leads to an increased insulin : glucagon molar ratio, qunatitatively and or qualitatively (Chapter III), supports the present concept of the more favourable condition for lipogenesis and fat deposition in dexamethasone treated chicks, as insulin is known to be the potent lipogenic hormone in birds (Touchburn et al., 1981; Yanaihara et al., 1983; Griminger, 1986). The significantly higher levels of cholesterol in serum of dexamethasone treated chicks may also be relevent in this context as insulin is known to be an activator of HMG-CoA-reducatse in the liver and intestine (Mayes, 1988). It is concluded from the present observations that both dexamethasone induced adrenocortical suppression as well as corticosterone induced glucocorticoid excess result in increased lipogenesis and fat deposition, more by the former. Both dexamethasone and corticosterone seem to be capable of fat deposition not only by inducing lipogenic enzymes but also by lowering the circulating T_3 level. Dexamethasone, by reducing the endogenous corticosterone levels potentiates the action of insulin thereby leading to a greater fattening effect. Adrenal cholesterol metabolism in neonatal chicks seem to be controlled by the ACTH sensitive enzymes.