CHAPTER II

EFFECT OF DEXAMETHASONE INDUCED HYPOCORTICALISM AND CORTICOSTERONE INDUCED HYPERCORTICALISM ON BODY WEIGHT GAIN AND ORGAN GROWTH IN POST-HATCHED WHITE LEGHORN BREED OF CHICKS: A HISTOMORPHOLOGICAL STUDY.

Avian post-hatched ex-ovo phase of development is equally important if not more, as compared to the embryonic in-ovo phase. The various organs and the body as a whole differentiated during the in-ovo phase undergo both physical and physiological growth to attain the characteristic adult size, structure and functional competence. In altrisial birds this occurs to a great extent under a dependence of parental care in the initial phase while in precocial birds it occurs independently without much parental care and attention. Whether altricial or precocial, this phase of development is crucial for the establishment of adult pattern of attributes characteristic of the species. The role of endocrine secretions in regulating harmonious growth during early neonatal life cannot be overlooked.

Looked in the above perspective, the growth retardatory effects manifested by hypophysectomized young cockerels (King, 1969) and in surgically or functionally thyroidectomized ducks and fowls during post-natal development (see Assenmacher, 1973) are understandable. There are also numerous reports suggesting the influence of adrenocortical steroids on growth and development of fowls, and both lack of corticoids as well as excess have been shown to inhibit weight increase in the post-natal periods (Howard and Constable, 1958; Baum and Meyer,

1960; Greenman et al., 1961; Nagra and Meyer, 1963; Nagra et al., 1963; Bellamy and Leonard, 1965; Adams, 1968; Magdi and Hutson, 1974; Freeman and Manning, 1975; Freeman et al., 1979; Harvey and Scanes, 1979; Davison et al., 1979; Bartov, et al., 1980 a, ; Gross et al., 1980; Bartov, 1982; Davison et al., 1983; Saadoun et al., 1987; Brake et al., 1988). Most of the above studies involved the effects of acute or short term treatment with corticosteroids on growth in terms of body weight gain. However, apart from the effects of glucocorticoid lack or excess on the growth of lymphoid organs (Dougherty and White, 1945; Dougherty et al., 1964; Glick, 1957 a b; 1959, 1960a, 1967, 1972; Garren et al., 1961; Siegel, 1961; Zarrow et al., 1961; Sato and Glick, 1964, 1970; Bellamy and Leonard, 1965; Siegel and Siegel, 1966; Dieter and Breitenbach, 1970, 1971; Davison et al., 1979, 1983; Brake et al., 1988) their effects of the growth of other organs barring the isolated studies on liver and adrenal weights (Bartov, 1982; Davison et al., 1983) is totally overlooked. Similarly though both antagonistic (Greenman and Zarrow, 1961; Lorenzen and Farner, 1964; Dusseau and Meier, 1971; Martin, 1973; Wilson and Follett, 1975; Bengt, 1979) as well as parallel (Burger, 1938; Fromme-Bouman, 1962; Hohn et al., 1965; Hall, 1968; Raitt, 1968; Gorman and Milne, 1971; Moens and Coessens, 1970; Petrescu-Raianu, 1971; Jallageas et al., 1978; Ramachandran and Patel, 1986) adrenal gonad relationships have been documented to occur in adult birds, the influence of either corticosteroid insufficiency or excess on post-natal growth and development of gonads has not been studied. It is also interesting that an effective functional relationship between corticosteroids and thyroid hormones has been demonstrated in the

post-hatched periods of development (see Kühn et al., 1984). Hence in the present study an attempt has been made to study the influence of chronic functional adrenocortical insufficiency induced by dexamethasone (as numerous studies have proved the adrenocortical suppressive effects of DXM: D'Angelo, 1966; Chowers et al., 1967; Yates, et al., 1967; Dallman and Yates, 1968; Fleisher and Battenbee, 1968; Kendall and Allen, 1968; Purves and Sirett, 1968; Arimura et al., 1969; Russel et al., 1969; Sirett and Gibbs, 1969; de Kloet et al., 1974; Obara et al., 1984; Macharg et al., 1985; Radke et al., 1985; Smoak and Birrenkott, 1986; Carnes et al., 1987; De Greef and Van der Schoot, 1987; Dupouy et al., 1987; Kloeti et al., 1987; Juniewicz et al., 1987; Medleau et al., 1987; Smith and Feldman, 1987; Brody and Black, 1988; Brooks et al., 1988; Katano, 1988; Wilson et al., 1988) and excess induced by exogenous corticosterone administration on body weight gain, absolute and relative weights of organs and histo-architecture of adrenal, testis and thyroid of male White Leghorn chicks during the first month of post-natal development.

MATERIAL AND METHODS

As outlined in Chapter I.

RESULTS

Morphometric observations

All steroid treatments brought about significant reduction in body weight gain of chicks after 30 days of treatment. Whereas the absolute weight of liver and kidney was significantly reduced by DXM and

Table 1 Changes in body weight gain and absolute and relative weights of organs of chicks treated with dexamethasone and corticosterone for 30 days.

	CONTROL		DXM(L)		DXM(H)		CORTICOSTERONE	
Body weight	142.8 ± 2.3		110.0[a] ± 4.9		105.5[a] ± 3.4		125.5[a] ± 2.3	
Organ weights	ABS	REL	ABS	REL	ABS	REL	ABS	REL
Adrenal mg	24.85 ± 0.66	17.98 ± 0.67	13.20[a] ± 0.53	12.31[a] ± 0.65	13.01[a] ± 0.62	13.36[a] ± 0.61	23.73 ± 0.99	20.01[c] ± 0.49
Testes mg	36.81 ± 1.65	27.02 ± 0.80	36.55 ± 2.67	32.64[d] ± 2.12	30.05[b] ± 1.54	30.10[d] ± 1.20	23.97[a] ± 0.70	18.57[a] ± 0.45
Bursa of Fabricius mg	384.50 ±23.90	277.20 ± 9.30	116.60[a] ± 7.80	114.80[a] ± 7.50	93.60[a] ± 4.70	88.94[a] ± 4.09	198.20[a] ±17.80	148.40[a] ± 9.70
Pancreas mg	721.30 ±34.50	534.90 ±26.40	501.40[b] ±50.20	460.60 ±46.20	590.00[b] ±27.40	554.20 ±22.50	672.90 ±38.20	487.30 ±28.50
Spleen mg	178.50 ±10.42	125.15 ± 7.30	95.83[a] ± 5.63	87.12[a] ± 5.12	90.78[a] ± 6.35	86.05[a] ± 6.20	99.14[a] ±10.46	79.00[a] ± 8.34
Liver g	4.102 ± 0.243	2.650 ± 0.087	3.656 ± 0.146	3.253[b] ± 0.164	2.977[b] ± 0.180	2.689 ± 0.103	2.823[a] ± 0.123	2.317[b] ± 0.052
Kidney g	1.507 ± 0.094	1.026 ± 0.044	1.176[c] ± 0.089	1.078 ± 0.079	1.203[d] ± 0.077	1.129 ± 0.051	1.070[b] ± 0.056	0.815[b] ± 0.043

ABS : Absolute; REL : Relative
DXM(L) : dexamethasone low dose; DXM(H) : dexamethasone high dose
Values are mean ±SE of not less than 8 birds.
a : $P < 0.001$; b : $P < 0.01$; c : $P < 0.02$; d : $P < 0.05$

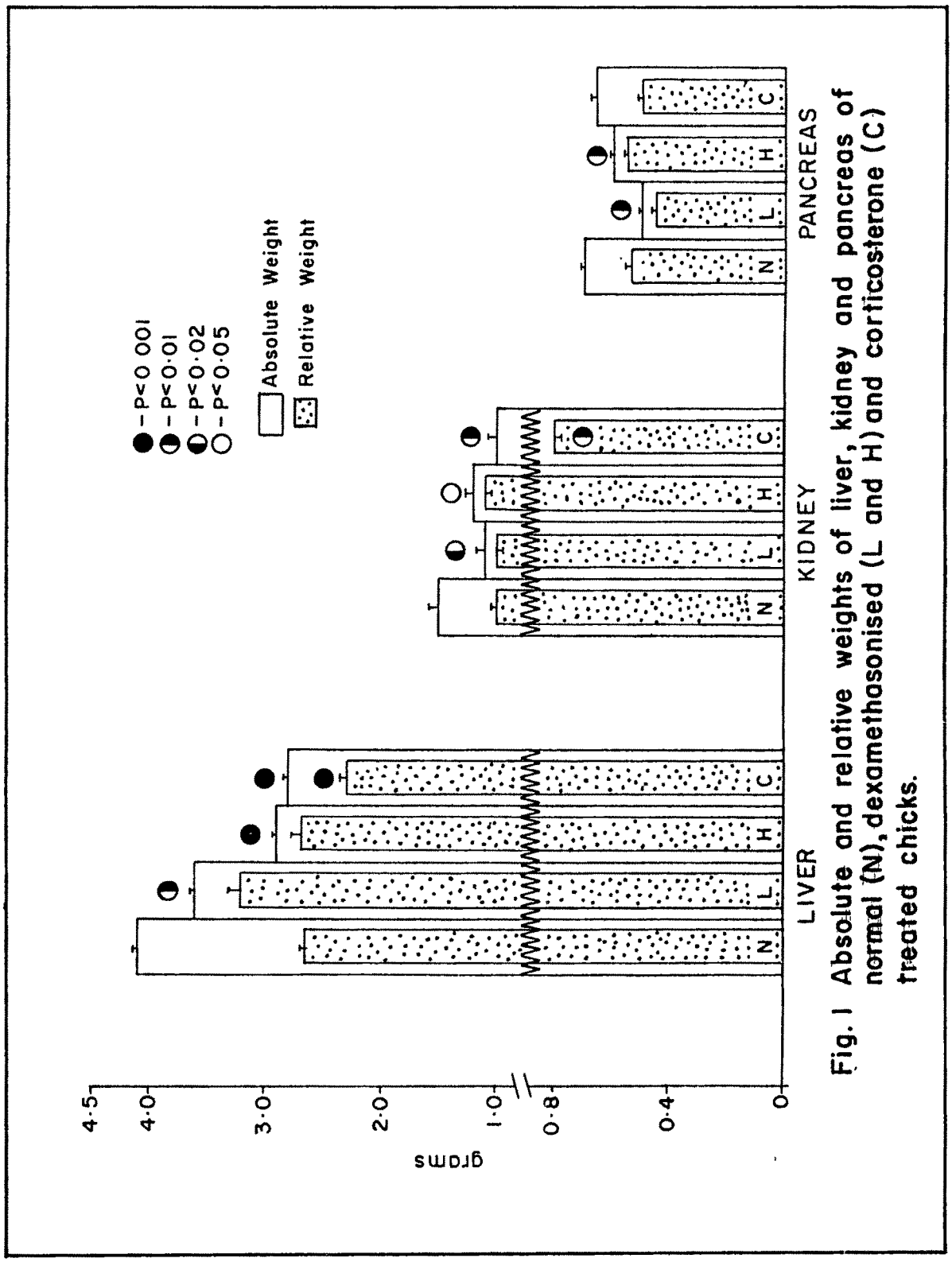


Fig. 1 Absolute and relative weights of liver, kidney and pancreas of normal (N), dexamethasonised (L and H) and corticosterone (C) treated chicks.

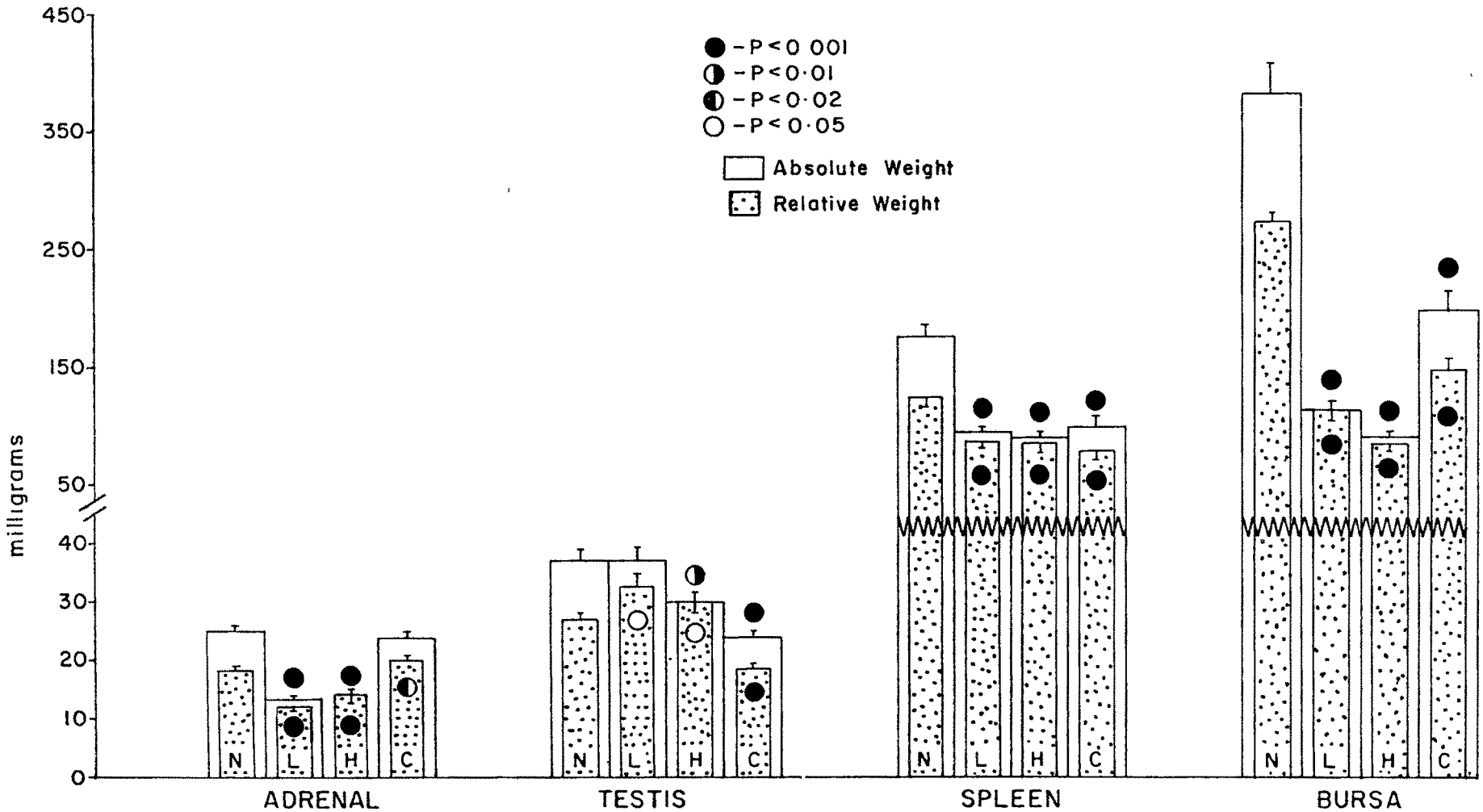


Fig. 2 Absolute and relative weights of adrenal, testes, spleen and bursa of normal (N), dexamethasonised (L and H) and corticosterone (C) treated chicks.

corticosterone, the relative weight of these organs was significantly reduced only with corticosterone treatment. The DXM treatment recorded a reduction in absolute weight of pancreas. The absoulte and relative weight of spleen and bursa of Fabricius showed similar response to dexamethasone and corticosterone in the form of significant reduction. Both DXM(L) and DXM(H) treatments brought about significant reduction in absolute as well as relative weights of adrenal, while corticosterone treatment increased the relative weight only. Both the absolute and relative weights of testes in DXM(L) treated chicks were significantly increased while only the relative weight was increased in DXM(H) treated chicks. Corticosterone treatment reduced both the absolute and relative weight of testes.(see Table 1; Figs. 1 & 2).

Histological observations

ADRENAL

Control chicks

Histological picture of the adrenal sections showed well developed cortical cells and medullary cells. The approximate ratio of the cortex to medulla was 1:1 (Fig. 1).

DXM(L) and DXM(H) treated chicks

Both the doses of DXM brought about suppression of the adrenal cortex as revealed by the histological picture. Cortex as a whole was shrunken and degenerative changes were observed. The approximate ratio of cortex to medulla was 1:2 (Figs. 2, 3).

Explanation to figures

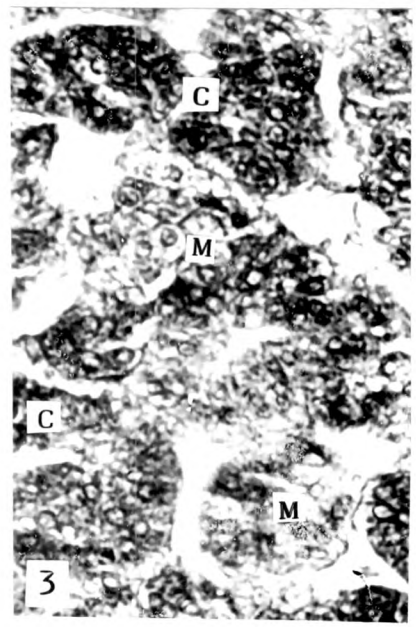
Fig.1 Histological structure of the adrenal of 30 day old chicks. Note the prominent cortex (C). M: Medulla. 400X

Fig.2 Adrenal of 30 day old chicks treated with DXM(L) showing prominent medulla (M) and regressed cortex (C). 400X

Fig.3 Adrenal of 30 day old chicks treated with DXM(H) showing prominent medulla (M) and regressed cortex (C). 400X

Fig.4 Adrenal of 30 day old chicks treated with corticosterone showing non-regressed cortex (C). M: Medulla. 400X


C
M
C
M
1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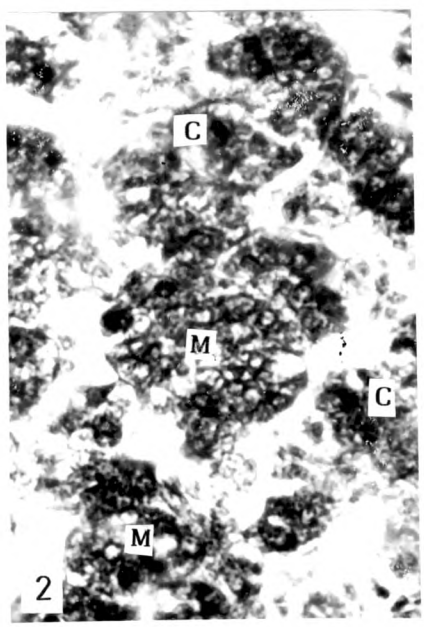
3


C
M
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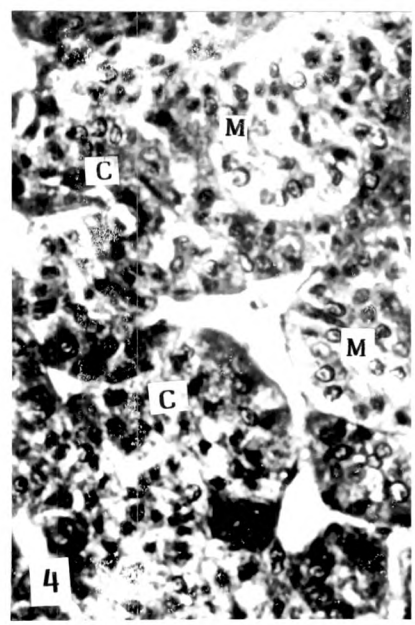
M
2


M
C
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C
4

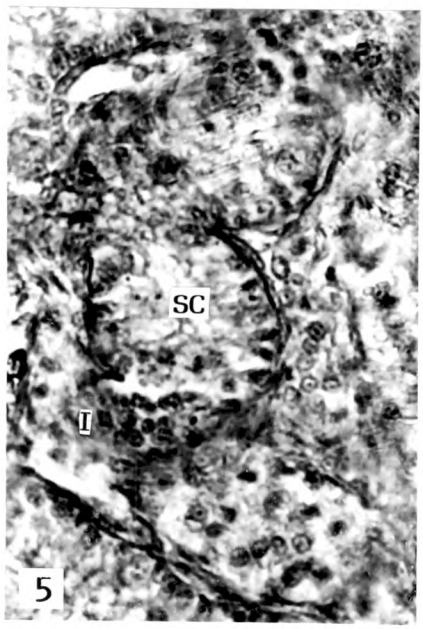
Explanation to figures

Fig.5 Histological appearance of testis of 30 day old control chicks showing poorly organized seminiferous cords (SC) and intertubular tissue (I). 400X

Fig.6 Testis of 30 day old chicks treated with DXM(L) showing well organized lumenated tubules (ST) and interstitium (I). 400X

Fig.7 Testis of 30 day old chicks treated with DXM(H) showing the presence of non-lumenated cords. SC: seminiferous cords; I: interstitium. 400X

Fig.8 Testis of 30 day old chicks treated with corticosterone. Note the poor organization of the seminiferous cords (SC) and occurrence of degenerating cords (DSC). 400X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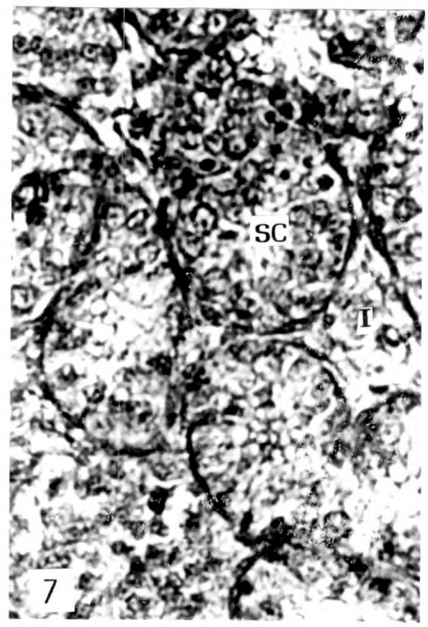
5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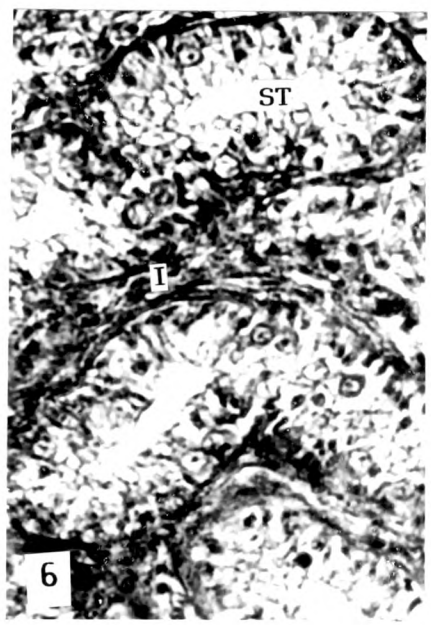
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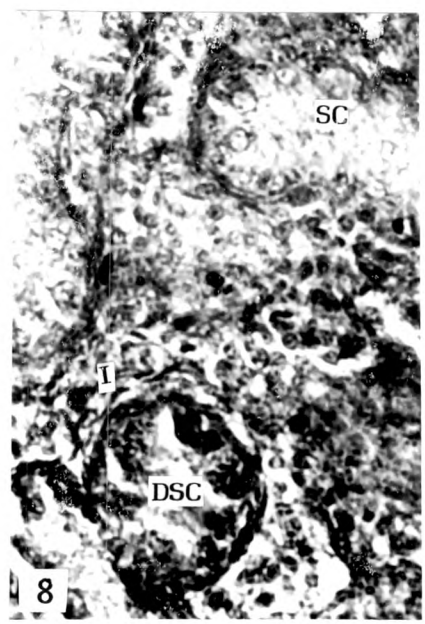

SC
I
DSC
8

Corticosterone treated chicks

The sections of adrenal of corticosterone treated chicks did not show any visible changes and were more or less similar to the control sections with an approximate cortex to medullary ratio of 1:1 (Fig. 4).

TESTIS

Control chicks

The histological profile of the control chick testis showed poorly organized tubules with more of intertubular tissue. The testis was made up of loosely arranged poorly organized seminiferous cords with no lumen. Though single layer of peripheral spermatogonial cells were observable, the interstitial cells were not clearly demarated and/or organized (Fig. 5).

DXM(L) treated chicks

The sections of the testis of low dose of dexamethasone treated chicks showed better organization of tubules and lumenation of cords. Such lumenated cords were seen to be more prominently and compactly packed. Interstitial cells were well organized and demarcated between tubules. In some tubules appearance of spermatocytes could also be noted (Fig. 6).

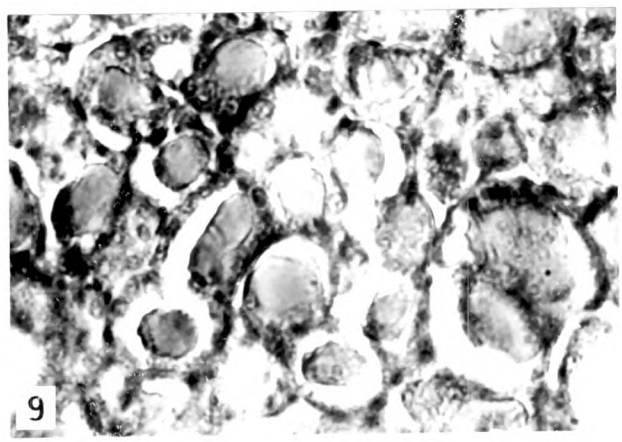
DXM(H) treated chicks

The high dose of DXM treated chick testis appeared similar to the control sections with non-lumenated cords which were loosely packed and organized. However, visible signs of spermatogonial proliferation could be discerned in many of the cords. In certain cases even cord degeneration was observable (Fig. 7).

Explanation to figures

Fig.9 Histological section of thyroid of 30 day old control chicks showing the presence of colloid filled follicles with moderate epithelial cell height. 400X

Fig.10 Photomicrograph depicting histological feature of 30 day old chicks treated with DXM(L). Note the loss of colloid from the follicles and increased epithelial cell height. 400X



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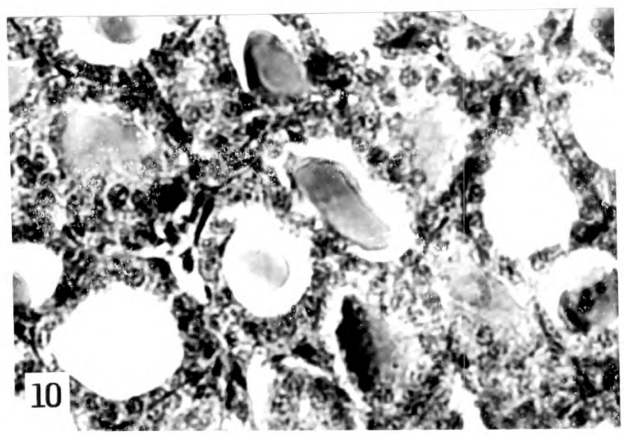

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Corticosterone treated chicks

Corticosterone treated testis showed differential changes ranging from inactive cords as seen in the control sections and sometimes better organized cords with spermatogonial proliferation. Occasionally degenerative changes in the germinal epithelium could also be discerned.(Fig. 8).

THYROID

Control chicks

The histological sections of thyroid of control chicks showed small to medium sized follicles. Most of the follicles were full of colloid material.(Fig. 9).

DXM(L) DXM(H) and corticosterone treated chicks

In all the three experimental conditions, the follicular epithelium was activated with relatively increased cell height and reduced colloidal content in the lumen (Fig. 10).

DISCUSSION

The results of the present study indicate the definite involvement of corticosteroid in the harmonious growth of organs and body as a whole of post-hatched chicks, as both corticosteroid insufficiency as well as excess have been documented to retard body weight gain, affect the weight of organs and histological organization of adrenal, thyroid and testis. The presently observed retardatory influences on weight gain have been corroborated by the reports of depressed growth in terms of weight gain by corticosterone/DXM treatment (Baum and Meyer, 1960; Nagra and Meyer, 1963; Magdi and Huston, 1974; Bartov et al.,

1980 a; Davison et al., 1983; Saadoun et al., 1987; Brake et al., 1988) in developing fowls. Of the two treatments, DXM treatment seems to have more pronounced retardatory influence [23% with DXM(L) and 26% with DXM(H)] than corticosterone treatment (12% with corticosterone) on body weight gain. Whereas the decrease in body weight gain obtained under corticosterone treatment essentially denotes the retardatory influence on body growth by hypercorticalism, that obtained under DXM treatment suggests the retardatory influence of hypocorticalism. This contention is well supported by the many reports showing the suppressive action of DXM on synthesis and release of ACTH (D'Angelo, 1966; Chowers et al., 1967; Yates, 1967; Dallman and Yates, 1968; Fleisher and Battenbee, 1968; Kendall and Allen, 1968; Purves and Sirett, 1968; Arimura et al., 1969; Russel et al., 1969; Sirett and Gibbs, 1969; de Kloet et al., 1974; Obara et al., 1984; Macharg et al., 1985; Radke et al., 1985; Smoak and Birrenkott, 1986; Carnes et al., 1987; De Greef and Van der Schoot, 1987; Dupouy et al., 1987; Kloeti et al., 1987; Juniewicz et al., 1987; Medleau et al., 1987; Smith and Feldman, 1987; Brody and Black, 1988; Brooks et al., 1988; Katano, 1988; Wilson et al., 1988). The presumed prevailing conditions of hypocorticalism (with DXM) and hypercorticalism (by corticosterone) in the two experimental groups of chicks in the present study are well corroborated by the significantly reduced relative weights of adrenals [by 31.5% in DXM(L) and 25.6% in DXM(H)] in dexamethasonised chicks and significantly increased relative weight (11.1%) in corticosterone treated chicks recorded herein. The histological observations of regressed cortical structure with a corticomedullary ratio of 1:2 in the former

and of unregressed active cortex with a corticomedullary ratio 1:1 very much comparable with the control chicks, in the latter are further evidences in this context.

In keeping with the well established antagonistic action of corticosteroids on lymphoid structures in developing and adult birds (Dougherty and White, 1945; Dougherty et al., 1964; Glick, 1957 a, b, 1959, 1960a, 1967, 1972; Garren et al., 1961; Siegel, 1961; Zarrow et al., 1961; Sato and Glick, 1964, 1970; Bellamy and Leonard, 1965; Siegel and Siegel, 1966; Dieter and Breitenbach, 1970, 1971; Davison et al., 1979, 1983; Brake et al., 1988), the presently observed significant decrement in relative weights of both bursa and spleen in both dexamethasonised and corticosterone treated chicks is self explanatory. Though functionally the two treatments are purported to induce hypocorticalism and hypercorticalism, it is presumable that DXM mimics the action of corticosterone on lymphoid structures. Obviously, the corticosterone receptors in lymphoid orgnas are nonspecific, incapable of distinguishing between native and synthetic corticoids and hence responding to both identically. Such a possible occurrence of twq types of receptors, one nonspecific, and the other specific (capable of distinguishing between native and synthetic corticoid) has been suggested by Ayyar and Ramachandran (1990) based on their observation of differential actions of DXM and corticosterone on various organs in pigeon. This aspect is further highlighted by the herein observed increased relative weight of liver (22.7%) in DXM treated chicks as contrasted with the decreased relative weight (12.5%) in corticosterone treated chicks. Apparently,

hypocorticalism promotes while hypercorticalism retards liver growth in post-natal chicks. The retardatory influences of hypercorticalism on liver growth also seems applicable to kidney, as a 20% decrement in relative weight of kidney was recorded in corticosterone treated chicks. Neither hypo- nor hypercorticalism seems to have any influence on growth of pancreas as could be noted by the similar relative weights in both DXM and corticosterone treated chicks.

Changes in testicular weight and histology studied currently reveal a stimulatory influence of hypocorticalism on testicular growth and development with a concomitant retardatory influence of hypercorticalism. This is indicated by the increased relative weight of testes in dexamethasonised chicks [by 20% in DXM(L) and 11% in DXM(H)] as opposed to decreased relative weight in corticosterone treated ones (31%). Histological observations of prominent compactly packed lumenating tubules and well organized interstitium in DXM(L) chicks and, inactive cords with signs of degenrative changes in the germinal epithelium together with poorly organized interstitium in corticosterone treated chicks, support the contention of corticosterone retarding the functional differentiation and maturation of testes in the early post-hatched phase of development. The observations of increased 3β-HSDH activity in both the tubules and interstitium with increased 17β-HSDH in the central part of the tubules and decreased lipid contents and 3α-HSDH in both the components of testis of chicks treated with low dose of DXM (hypocorticalism: Chapter VIII) provide further support to the possible favourable influence of low titres of corticosterone in early post-natal

maturation of testes in chicks. A survey of literature on relationship with reproduction and cortical activity in adult birds reveals both parallel as well as antagonistic relationships. Some sort of funtional relationship in the form of antagonistic influence of corticosterone on gonadal activity in strong migrants and favourable influence of corticosterone on gonadal activity in sedentary species or weak migrants has been suggested (see Bengt, 1979). In the case of fowl, adrenalectomy has been claimed to induce pronounced atrophy of the testes (Herrick and Finerty, 1941; Hewitt, 1947) and to inhibit the development of the right gonad in ovariectomized birds (Taber et al., 1956). On the other hand, administration of cortisone acetate has been shown to induce testicular atrophy (Selye and Freedman, 1941). Similarly corticosterone injections have been shown to depress egg laying (Greenman and Zarrow, 1961). These observations suggest that both corticosterone insufficiency as well as excess can inhibit gonadal functions in adult birds, thereby suggesting the requirement for an optimum concentration of corticosterone for maintaining normal adult gonadal functions. Based on the present observations it can be inferred that corticosterone has an antagonistic influence on male gonadal differentiation and maturation during post-hatched development of chicks. Presumably, it is a prevalance of an optimum concentration that regulates normal chronological maturation of testes. However, it is possible that decreased titres of corticosterone in post-natal phase can hasten the maturation of testes as is revealed by the lumenation of the tubules by 30 days in chicks treated with low dose of DXM as contrasted with the appearance of lumenation in normal chicks by the 6th week of development (see Johnson, 1986).

The present histological observations on thyroid also indicate a functional relationship between adrenal cortical secretion and thyroid activity in post-natal chicks. Compared to the thyroid of control chicks, those of both DXM and corticosterone treated chicks revealed increased functional activation. This represents another instance of similarity of action of both synthetic and native corticoids and could represent either a direct action at the level of thyroid or indirect one by way of increased TSH release. In mammals it has been shown that the glucocorticoid administration reduces serum TSH concentration denoting the action of corticosteroids on TSH secretion from the hypophysis (see Ingbar and Woeber, 1981). But looking at the observations made in the present study it seems that corticosteroid may exert an opposite TSH releasing influence in birds, at least in the premature post-natal phase or by a chronic administration of glucocorticoids as made in the present study. The inferred hypersecretion of thyroid hormone in both dexamethasonized and corticosterone treated chicks is however contradictory to the observations of fat depostion and speculated lower T3 titres in these chicks (Chapter V). However a valid explanation to these contradictory observations comes from the reported ability of glucocorticoid to suppress T4 to T3 conversion by way of inhibition of 5'-monodeiodinase activity in both mammals (see Ingbar and Woeber, 1981) and in post-natal chicks (see Kühn et al., 1984; Buyse et el., 1987). Overall, it can be concluded from the present observations that the responsiveness of various organs and neuroendocrine and/or physiological metabolic processes to DXM and corticosterone could be similar or differential in keeping with the

purported dual types of corticosterone receptors as inferred in the present study as well as others (McEwen et al., 1986; Ayyar and Ramachandran, 1987).