CHAPTER VIII

HISTOCHEMICAL OBSERVATIONS ON STEROID DEHYDROGENASES AND LIPIDS IN THE TESTIS OF DEXAMETHASONE AND CORTICOSTERONE TREATED WHITE LEGHORN BREED OF CHICKS

Production and secretion of steroid hormones by embryonic gonads of vertebrates have been documented (see Bhujle et al., 1979). Evidences for steroidogenic ability by avian embryonic gonads come from the reported detection of steroids in amniotic and allantoic fluids and blood of chick and quail (Stoll and Maraud, 1956; Ozon , 1965 , 1969) and, the reports of conversion of radioactive precursors into steroids by cultured embryonic gonads (Weniger et al., 1967; Akram and Weniger, 1969; Weniger, 1970; Galli and Wassermann, 1972 , 1973; Guichard et al., 1973 a, b , 1977). Moreover, identification of steroidogenic cells in the embryonic gonads of birds has also been made (Narbaitz and Kolodni, 1964; Chieffi et al., 1964; Sheib and Haffen, 1968 , 1969; Sheib, 1973; Bhujle and Nadkarni, 1977). A number of other investigations have provided evidence for the establishment and functioning of hypothalamo-hypophysio-gonadal axis during the last third of embryonic development in chicks (see Woods and Brazzil, 1981). Apparently, enough attention has been focussed on steroidogenic capacity of avian embryonic gonads. However, literature on biogenesis and metabolism of steroids during avian post-natal development is sparse. There are reports indicating influence of dexamethasone and adrenal corticosteroids on gonadal functioning and LH release in mammals (Baldwin and Sawyer, 1974; Saez, et al., 1977; De Greef and Van der Schoot, 1987) and

influence of corticosterone on *in vitro* metabolism of testosterone in the comb and brain of young male chickens (Deviche *et al.*, 1982).

In this context, the present study is an attempt to evaluate the effect of induced chronic hypercorticalism and hypocorticalism on the steroidogenic abilities of testes of chicks at the end of thirty days of development/treatment.

MATERIAL AND METHODS

As outlined in Chapter I.

OBSERVATIONS

Control chicks

Both neutral lipids and total lipids were intensely localized in the interstitial cells of the control chick testes. In the tubules, total lipids were preponderant with very little neutral lipids (Figs. 1, 1a). The control testes sections showed both 3β-hydroxy steroid dehydrogenase (3β-HSDH) and 17β-hydroxy steroid dehydrogenase (17β-HSDH) activities in the seminiferous cords. The poorly organized interstitial tissue showed intense 3β-HSDH activity and poor 17β-HSDH activity (Figs. 5, 5a, 9). A much greater response of 3β-HSDH activity with dehydroepiandrosterone (DHEA) as substrate in comparison to

pregnenolone was discernible. The 17β-HSDH response was similar with both estradiol and testosterone as substrates. Though a moderate 3∝-HSDH activity was observable in the interstitium, the tubules showed poor activity (Fig. 13).

DXM(L) treated chicks

The sections of testis of chicks treated with low dose of DXM depicted reduced intensity of neutral and total lipids in the interstitial cells with almost negligible localization in the tubules (Figs. 2, 2a). The 3β-HSDH activity with pregnenolone as substrate was also intensified and equalled that of DHEA. The activity of 3β-HSDH was increased in both the tubules and interstitium while that of 17β-HSDH activity was increased (with both the substrates) only in the central part of the tubules in chicks treated with low dose of DXM (Figs. 6, 6a, 10, 10a, 10b). The localization of 3∝-HSDH decreased in both the components of the testis when compared to that of controls (Fig. 14).

DXM(H) treated chicks

The interstitial cells showed increased lipid content (both neutral and total lipid) even more than in the control. Localization of both types of lipids was evident in the tubules (Figs. 3, 3a). The 3β-HSDH activity was overall less as compared to DXM(L) treated chicks with less well organized tubules. Though the interstitium was prominent, the enzyme activity in the interstitium as well as in the tubules was

moderate (Fig. 7). The enzyme activity with pregnenolone as substrate was less than that with DHEA. The 17β-HSDH activity was less than that in the controls with moderate activity in the interstitium with testosterone as the substrate (Fig. 12). The 17β-HSDH activity with estradiol as substrate showed low activity in the tubules with visible activity in interstitium. The 3∝-HSDH activity depicted intense localization in the interstitium and low activity in the tubules, but the activity in both the components was more as compared to that of controls (Fig. 15).

Corticosterone treated chicks

Both total lipids and neutral lipids were increased in the interstitial cells. The localization of both types of lipids was evident in the tubules (Figs. 4, 4a). The pattern and intensity of localization was similar to that of DXM(H) except for the slightly less intensity of localization. The testis of corticosterone treated chicks depicted moderate 3β-HSDH activity in the tubules with poor activity in the interstitial cells and was very much comparable to DXM(H) and control sections (Figs. 7, 8). The activity with pregnenolone as the substrate was very low when compared that with DHEA. The 17β-HSDH activity with both the substrates (testosterone and estradiol) showed weak activity in the tubules and slight activity in the interstitium which was evidently lower than in the controls (Fig. 11). With regard to 3∝-HSDH activity, the tubules showed weak activity with moderately high activity in interstitium (Fig. 16). Overall, the appearance of enzyme activity was slightly more than in the controls but less than in DXM(H).

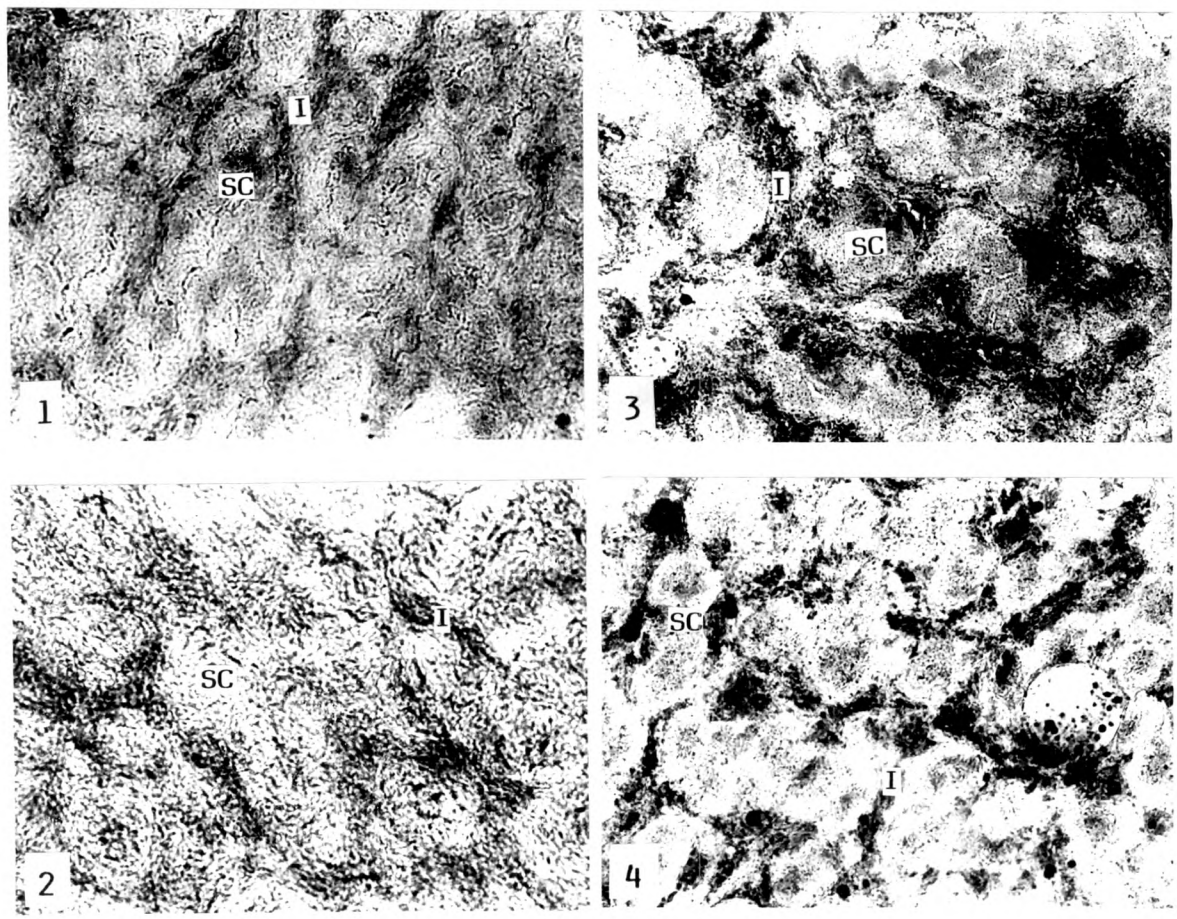
Explanation to figures

Fig.1 Control testis section of 30 day old chicks depicting intense localization of Sudanophilic lipids in the interstitium. Note the presence of lipids in the seminiferous cords (SC) also. 200X

Fig.2 Testis section of 30 day old chicks treated with DXM(L) showing reduced localization of Sudanophilic lipids. Note moderate localization in the interstitium (I) and absence of lipids in the cords (SC). 200X

Fig.3 Testis section of 30 day old chicks treated with DXM(H). Note the increased lipid content in both the interstitium (I) and cords (SC). 200X

Fig.4 Testis section of 30 day old chicks treated with corticosterone showing very much increased lipid content in both the interstitium (I) and cords (SC). 200X


I
SC
1
I
SC
3
SC
I
2
SC
I
4

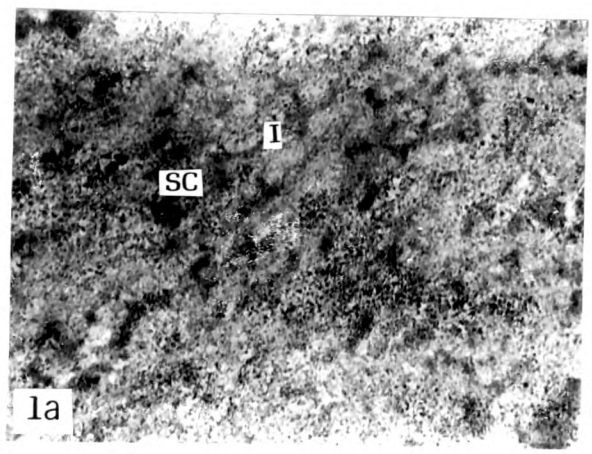
Explanation to figures

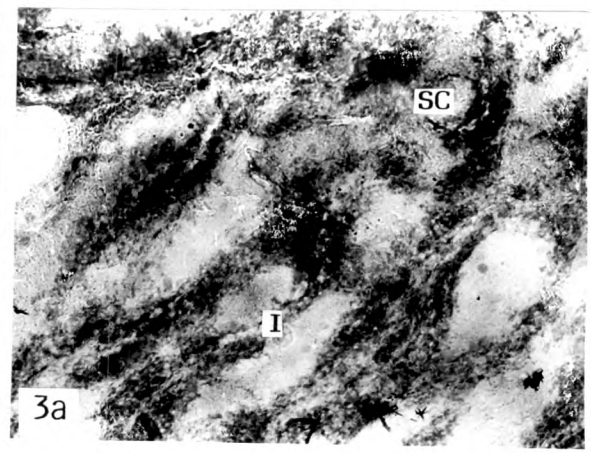
Fig.1a Control testis of 30 day old chicks depicting stronger localization of neutral lipids in the interstitium (⟼) and milder presence in the cords (SC). 200X

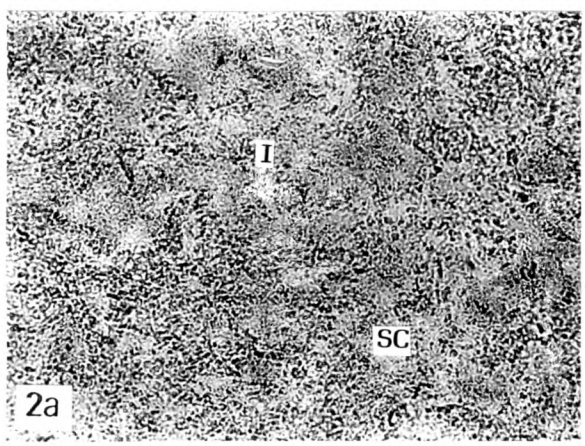
Fig.2a Testis section of 30 day old chicks treated with DXM(L) showing reduced localization of neutral lipids. Note moderate localization in the interstitium (I) and absence of lipids in the cords (SC). 200X

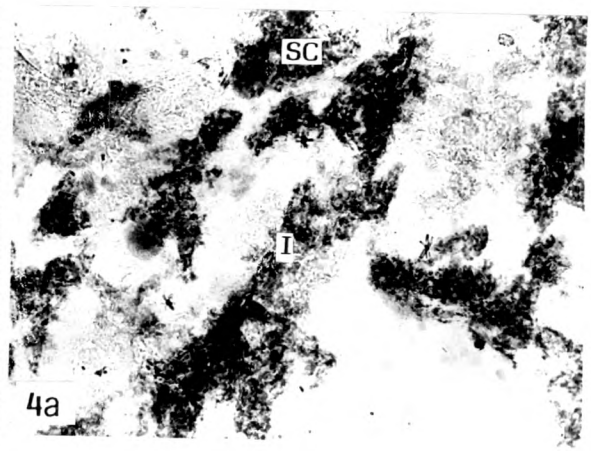
Fig.3a Testis section of 30 day old chicks treated with DXM(H). Note the increased neutral lipid content in both the interstitium (I) and cords (SC). 200X

Fig.4a Testis section of 30 day old chicks treated with corticosterone showing very much increased neutral lipid content in both the interstitium (I) and cords (SC). 200X


I
SC
1a


SC
I
3a


I
SC
2a

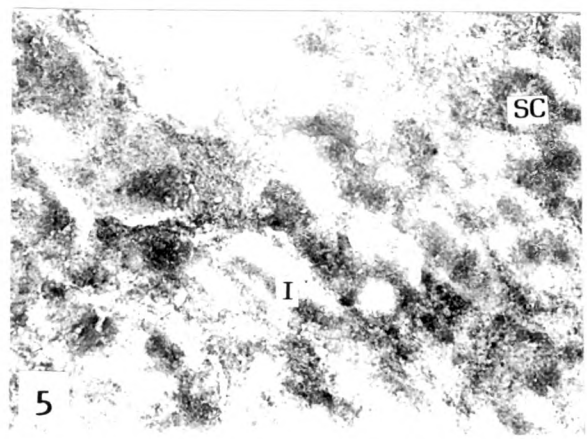

SC
I
4a

Explanation to figures

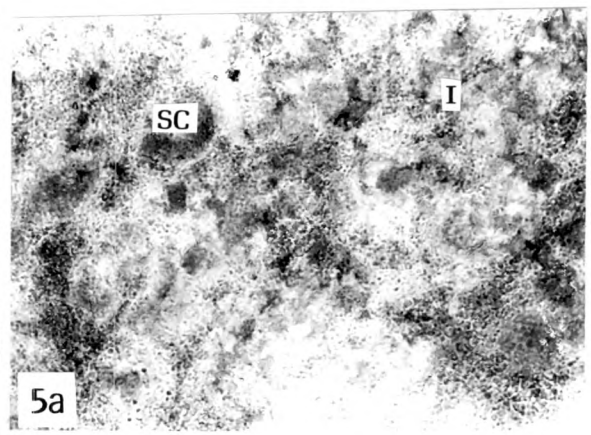
Figs.5 & 5a — Sections of testis of 30 day old control chicks showing strong 3β-HSDH activity with dehydroepiandrosterone and pregnenolone as substrates respectively. Note the enzyme activity in both the cords (SC) as well as interstitium (I). 200X

Figs.6 & 6a — Testis of 30 day old chicks treated with DXM(L) demonstrating increased 3β-HSDH activity in both seminiferous cords (SC) and interstitium (I) with dehydroepiandrosterone and pregnenolone as substrates respectively. 200X

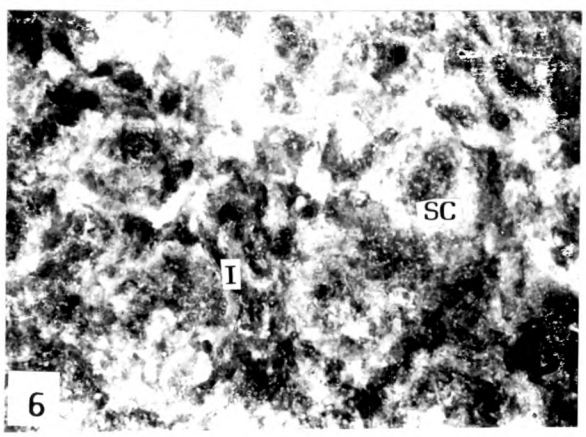
Figs.7 & 8 — 3β-HSDH activity using DHEA as substrate in the testis of 30 day old chicks treated with DXM(H) and corticosterone respectively. Note the mild localization of the enzyme both in cords (SC) and interstitium (I), very much comparable to the control testis. 200X



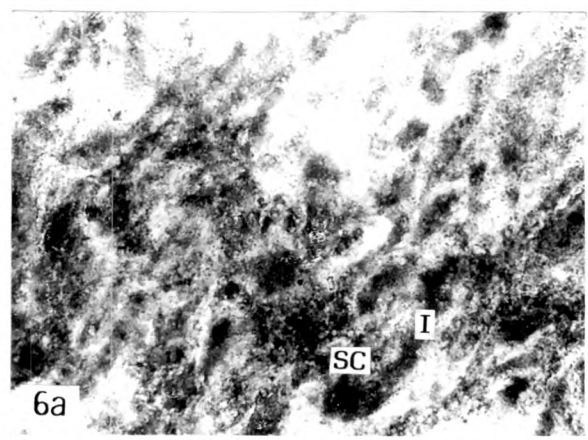
5



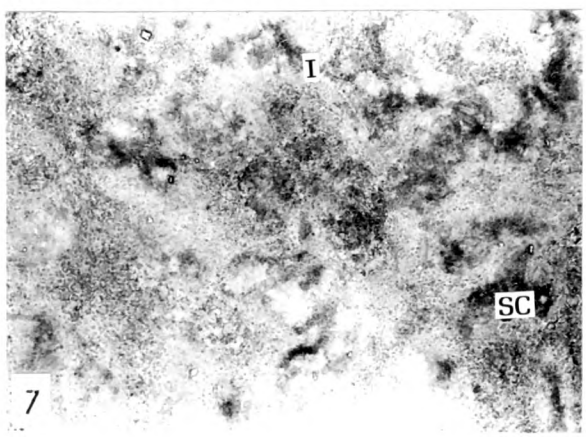
5a



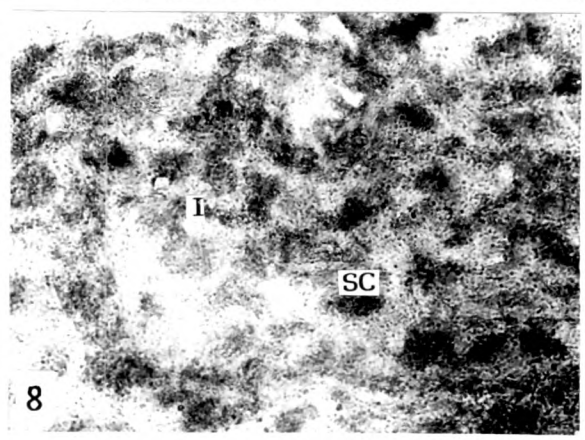
6



6a



7



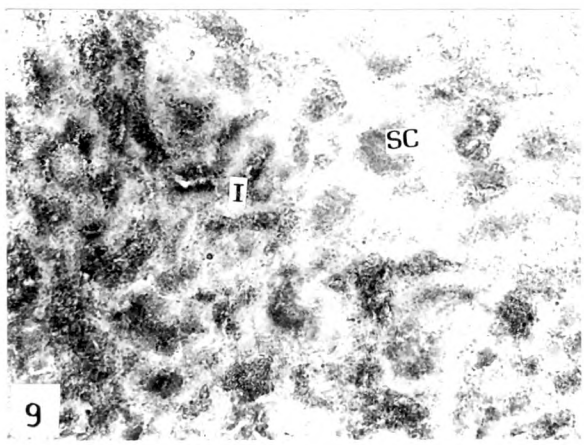
8

Explanation to figures

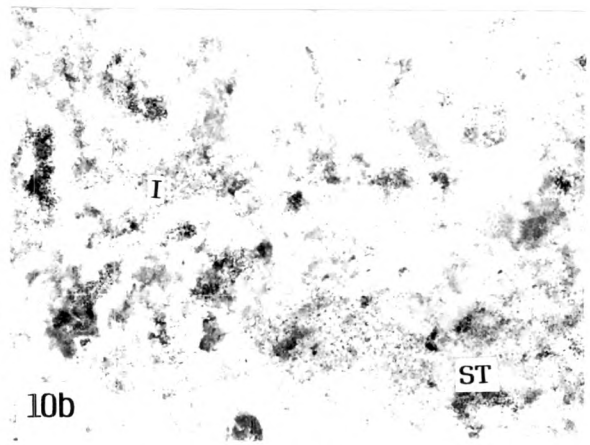
Fig.9 — 17β-HSDH activity in the control testis of 30 day old chicks showing presence of the enzyme in the cords (SC) and weaker localization in the interstitium (I). 200X

Figs.10,10a & 10b — Photomicrographs of sections of testis of 30 day old chicks treated with DXM(L) showing intense 17β-HSDH activity in the cords/tubules (SC/ST) and poor activity in the interstitium (I). Note the chronological decrement in enzyme activity in the seminiferous components during transformation from solid cords (10, 10a) to lumenated tubules (Fig.10b). 200X

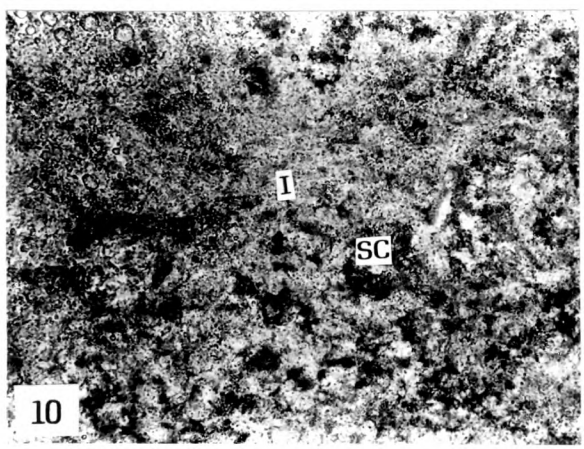
Figs.11 & 12 — 17β-HSDH localization in the testis of 30 day old chicks treated with DXM(H) and corticosterone. Note the weak enzyme localization very much comparable to the control testis. 200X
SC: seminiferous cords; I; interstitium.



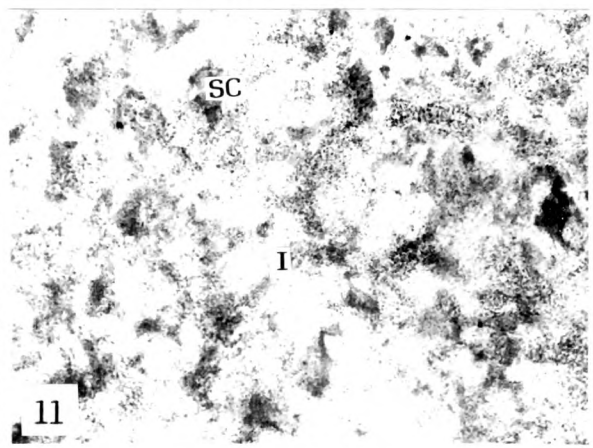
9



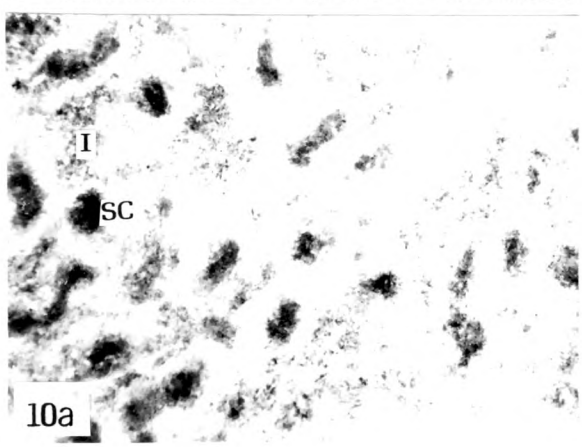
10b



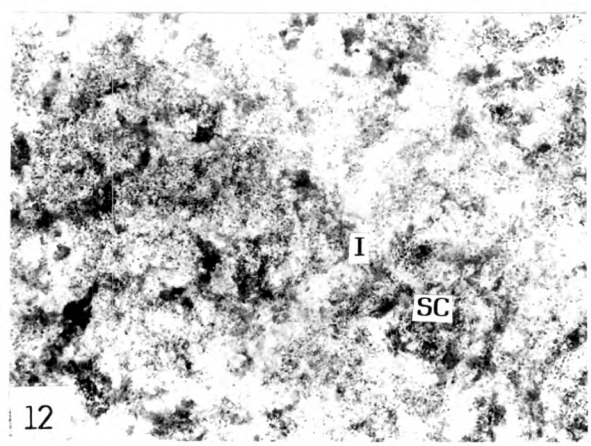
10



11



10a



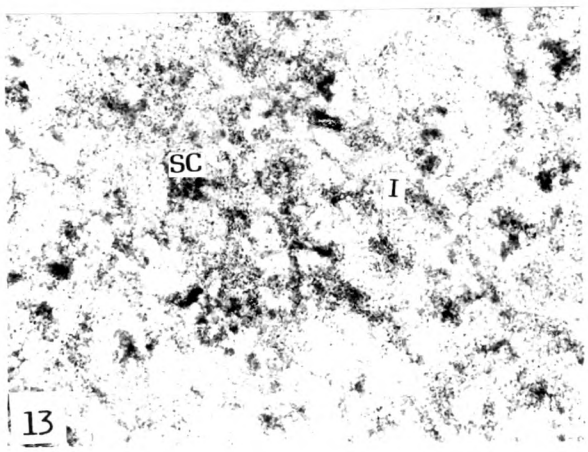
12

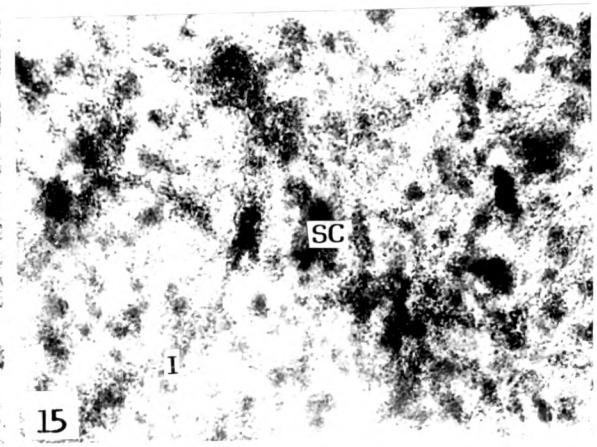
Explanation to figures

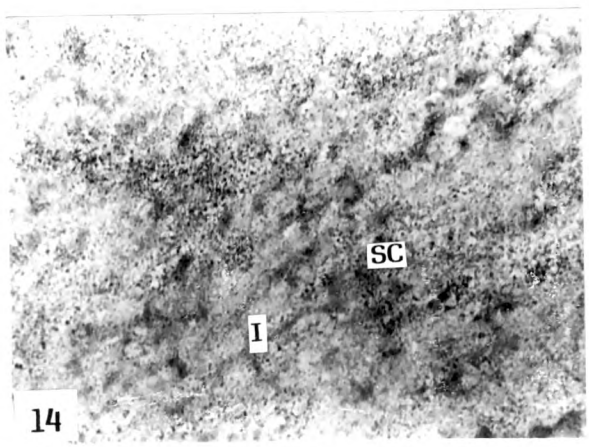
Fig.13 Testis of 30 day old control chicks showing 3∝-HSDH activity. 200X
SC: seminiferous cords; I: interstitium.

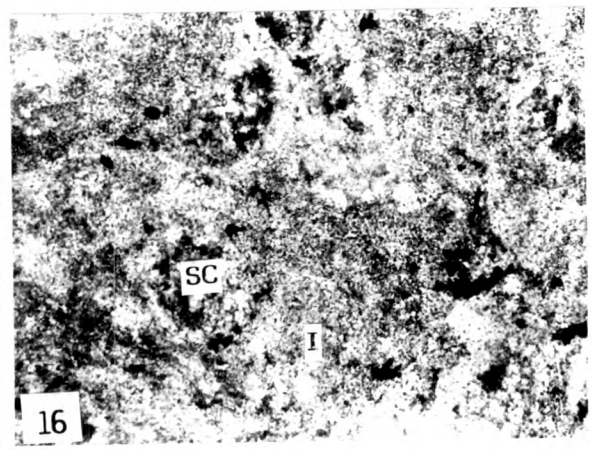
Fig.14 Photomicrograph showing decreased 3∝-HSDH activity in the testis of 30 day old chicks treated with DXM(L). 200X
SC: seminiferous cords; I; interstitium.

Figs.15 & 16 Photomicrograph showing increased 3∝-HSDH activity in the testis of 30 day old chicks treated with DXM(H) and corticosterone respectively. 200X
SC: seminiferous cords; I: interstitium.


SC
I
13


SC
I
15


SC
I
14


SC
I
16

DISCUSSION

The results of the present study indicate presence of both 3β-HSDH and 17β-HSDH in the seminiferous cords of one month old chicks. However, the poorly organized interstitial tissue shows intense 3β-HSDH activity with poor 17β-HSDH activity. The greater response of 3β-HSDH with DHEA as substrate in comparison to pregnenolone together with the above observations suggest production of the weak androgen, androstenedione essentially through the Δ^5 pathway. This is in contrast to the reported domination of Δ^4 pathway for steroid hormone biogenesis by the embryonic gonads of the pigeon (Bhujle et al., 1979). The present inference of production of androstenedione principally by the one month old post-hatched testis of the chick is supported by the 7 times more plasma androstenedione level than testosterone in the immature cockerel (see Johnson, 1986). Increased testicular androstenedione content has also been reported for 15 day old chick embryos (Galli and Wassermann, 1972) and incidentally this characteristic has been related to sexual immaturity in mammals (Karg and Strück, 1966). Moreover, the reported increase in the testicular progesterone content during the first 28 days after hatch in the chick (Tanabe et al., 1979) suggests production of progesterone, by the weak Δ^5 pathway as noted in the present study. Chronic treatment with DXM(L) resulted in better organization and compact packing of the seminiferous cords, accompanied by lumenation and transformation of cords into tubules and greater differentiation of interstitial cells (Chapter II). These histological alterations are paralleled by increased 3β-HSDH activity in both the

tubules and interstitium, increased 17β-HSDH activity in the central part of the tubules and, decreased lipid contents and 3∝-HSDH activity in both the components of the testis. It is inferable that lumination of the seminiferous cords and the resultant transformation into seminiferous tubules is associated with increased intratubular 3β- and 17β-HSDH activities. A possible role for testosterone synthesized locally within the central part of seminiferous cords (albeit in small quantities) prior to and concomitant to degeneration of the central germ cells and resultant formation of seminiferous tubules in avian testis is assumable in the context of the present observations; a process which is greatly facilitated or enhanced under DXM treatment. The present observations also indicate that DXM(L) induces increased 3β-HSDH activity in the interstitium as well, with both the Δ^4 and Δ^5 pathways being more or less equally active. The increased production of progesterone and androstenedione by the interstitial cells coupled with reduced breakdown of androstenedione (due to reduced 3∝-HSDH) could contribute to a local build up of these two steroids leading to organization and differentiation of the testicular components during post-natal development. It is presumable from these that androstenedione and probably also progesterone could have a tubular differentiation and organization capacity during the post-hatched avian development.

It is debatable as to how DXM can accelerate or hasten these processes in the post-hatched avian testicular development. Interestingly, neither DXM(H) nor corticosterone could induce such changes and the activities of 3β- and 17β-HSDH remained similar to that of controls with concomitant increase in 3∝-HSDH activity. These observations suggest

no enhancement in either androstenedione or progesterone production but increased degradation of androstenedione under DXM(H) and corticosterone treatments. Increased lipid content together with unchanged 3β- and 17β-HSDH activities and increased 3∝-HSDH activity, bear testimony to the fact that normal testicular differentiation and development are retarded under these treatments. It is presumable from these observations that DXM(L)-induced hypocorticalism is somehow linked with early maturational changes affecting the post-hatched chick testis development; while hypercorticalism or DXM(H) might retard the same. In this context, it is assumable that hypocorticalism might provide favourable milieu for early development in the immature male chicks. The possibility of DXM increasing LH secretion from the pituitary at this stage of development is overruled by the fact that 17β-HSDH activity was not increased under this treatment and thus did not lead to significant testosterone production. A possible direct local action of DXM on the testis can be overruled as the higher dose of DXM failed to induce the changes associated with the low dose. Obviously, the DXM(L) induced changes are ascribable solely to the prevailing hypocorticalism. It is to be further presumed that DXM(H) also though could bring about adrenocortical suppression (Chapters II-IV) was however exerting a corticosterone like action in the testis at the concentration employed. The differential effects of low and high doses of DXM on ovarian function in terms of ovulation in the domestic hen (inducing action at low doses - Rzasa et al., 1983) and oocyte growth, recruitment and vitellogenesis in the frog (no effect at lower doses and impairment effect at high doses - Kupwade and Saidapur, 1987)

are relevant in this connection. An earlier study with testicular subcellular fractions of the chicken had indicated the enzymes concerned with steroidogenesis to be located in the microsomal fraction (Nakamura and Tanabe, 1972). It is logical to presume that hypercorticalism or DXM(H) may be exerting an antagonistic physiological and/or genetic regulatory effect on microsomal enzymes involved in steroid metabolism in the early phases of the post-hatched developing chicks. In this context it is also speculatable that DXM(L) induced hypocorticalism may be providing a permissive influence for the low titers of circulating gonadotropins in the immature cockerels (Sterling et al., 1984) to act on the seminiferous cords and hasten their transformation into tubules. Interestingly, such transformation of seminiferous cords into tubules has been reported to occur 30-40 days after hatching in the pigeon (Bhujle et al., 1979).

In the wake of the reported inhibitory action of corticosterone on conversion of testosterone into active 5α- reduced metabolites and concomitant enhancement of conversion into inactive 5β- metabolites in the peripheral tissues of young male chicken (Deviche et al., 1982), it is debatable as to whether similar mechanisms may also be operative in the testis of post-hatched immature chicks. The present observations also suggest that the seminiferous compartment of immature chicks during the first month of post-hatched development has the potency to synthesize steroids (albeit in small quantities - the functional significance of which is already discussed) when the interstitium has not yet achieved the full potency. This is in contrast to the observations of Bhujle et al. (1979) in the pigeon but is in conformity with the findings

of Botte and Rosati (1964), Chieffi et al. (1964) and Woods and Domm (1966) of both the tubules and interstitium being sites of androgen synthesis in the male domestic fowl.