Localization of activities of various enzymes have been reported in the gonads from time to time, using various histochemical techniques (Gomori, 1946; Nachlas & Seligman, 1949; Niemi et al., 1962; Tice and Barrnett, 1963). Histochemical studies by Gomori (1946) and Nachlas and Seligman (1949) have shown that the Leydig cells of the testis of many species exhibit non-specific esterase activity. Huggins and Moulton (1948), using quantitative esterase estimations, demonstrated a correlation between this enzyme activity and the apparent androgenic hormone production of the testis (cited by Singh and Mathur, 1968). Niemi and Ikonen (1962) studied the cytochemistry of oxidative enzyme system in the rat testis. Ambadkar and George (1964) showed that the distribution pattern of oxidative enzyme activity appeared in four different phases in the seminiferous tubules corresponding to the gradient in the spermatogenetic wave, thereby indicating a metabolic adaptation at the subcellular level. Studies on various enzymes provide an indirect approach that could be
apparently correlated with androgenic hormone synthesis and secretion (Singh and Mathur, 1968) by the gonad.

During the recent past interest has been focused primarily on a search to find out direct criteria for assessment of the physiological state of the gonads by investigating the localization of steroid hormones in the gonads of vertebrates (Wallace, 1904; Samuel, 1943; Hisaw and Albert, 1947; Chieffi, 1961; Chieffi and Botte, 1961; Botte, 1963; Inpo disprisco et al., 1965, 1966; Lance and Callard, 1969; Te Winked, 1972). These workers have provided biochemical proof for the presence of sex steroids in gonadal extracts from a number of investigations on several species of lower vertebrates.

Although the ovarian stroma has been the subject of several investigations during recent years, very divergent opinions about the identity of its different cell-types and their development and function have been expressed by anatomists, physiologists and pathologists (cited by Guraya, 1974). As Guraya (1974) opined, the main reason for this is a general acceptance of traditionally established misconceptions and a failure to identify and differentiate the steroid gland cells properly from the relatively embryonic (or undifferentiated) and compressed stromal
elements. It is somewhat difficult to distinguish the different tissues and gland cell-types in relation to function with only routine histological techniques, therefore, application of histochemical techniques was deemed helpful in gaining better understanding of the testicular and ovarian tissue elements (viz., seminiferous epithelium, Leydig cells, granulosa and thecal cells, glandular atretic follicle, lipoglandular atretic follicle, yolk cyst atretic follicle and stromal cells) in relation to their cyclic changes.

According to present concepts, almost all the biologically active steroids are synthesized from $\Delta^5$-$3\beta$-hydroxy-steroids, pregnenolone and dehydroepiandrosterone (DHA) by means of diphosphopyridine nucleotide (NAD) dependent hydroxysteroid dehydrogenases (Samuels, 1960, Baillie et al., 1966). The enzyme systems have been demonstrated by both biochemical and histochemical means in steroid producing organs of many vertebrates; mammals (Baillie et al., 1966; Seth and Prasad, 1967; Rubin et al., 1963, 1968) birds (Chieffi and Botte, 1965; Narbaitz and Kolodny, 1964; Boucek et al., 1966; Woods and Domm, 1966; Chieffi, 1964; Botte, 1963; Gorman, 1974; Garnier et al., 1973; Bhujle and Nadkarni, 1974; Ambadkar and Kotak, 1976b, 1977;
The presence of hydroxysteroid dehydrogenases in the gonadal tissues has been shown to be associated with steroid biosynthesis in many vertebrate classes (Wattenberg, 1958; Samuel, 1960; Rubin et al., 1963; Baillie et al., 1966; Acevedo et al., 1963; Dorfman et al., 1963; Baillie and Mack, 1966). The present histochemical study of testes and ovaries regarding localization of $\Delta^5-3\beta$-hydroxysteroid dehydrogenase, a key enzyme in steroid biosynthesis, was undertaken to substantiate histological findings on testes (Chapter: 1) and ovary (Chapter: 2) of Indian house crows.

That the cytoplasm of the interstitial cells contains fatty substances, has been known since as early as 1850 when Leydig reported them in the interstitium of bat, pig, cat and mole (Leydig, 1850). Since then, Leydig cell lipids have been reported in every vertebrate group from the Agnatha to the mammalia (Marshall & Lofts, 1956). It is now known that prior to the onset of spermatogenesis in wild birds, the seminiferous tubules undergo a meta-
morphosis involving significantly quick genesis of large quantities of cholesterol positive lipids (Lofts and Morton, 1973). Histochemical studies with regard to testicular steatogenesis have been correlated with non-breeding phases or regressive testis (Marshall, 1949; Lofts and Marshall, 1959). This tubule lipid appears to contain progestagens (Lofts and Marshall, 1959) and cholesterol. Cholesterol is a precursor for steroid hormones (Bloch, 1945; Zaffaroni et al., 1951; Hechter, 1953; Hayano et al., 1956; Heard et al., 1956; Lake and Furr, 1971), like pregnenolone which could be converted into progesterone (Barllie and Furguson, 1966).

Various authors have studied testicular and ovarian lipid cycle in birds (Marshall, 1949; Marshall and Coombs, 1957; Lofts and Marshall, 1959; Hoffman, 1960; Lofts and Bern, 1972; Muninger, 1972; Lofts and Morton, 1973). It is also known that abundance of lipids in the testicular interstitium and ovarian compartments and their seasonal distribution (quantitative as well as qualitative) during different phases of gonadal development have definite bearings on the well marked seasonal reproductive periodicity in birds (Kanwar, et al., 1977).

Cyclic appearance and discharge of cholesterol and lipids both in the interstitial Leydig cells and within the
seminiferous tubules is now a known fact (Chamy, 1913; Lofts, 1960; Lofts and Boswell, 1960). The latter phenomenon has been suggested as the basis for the seasonal fluctuation in sensitivity of the germinal epithelium to gonadotropins (Lofts, 1961; Van Oordt and Lofts, 1963) and a relationship between this and the pituitary gonadotrophs (FSH producing cells) has been established (Loft and Van Oordt, 1962; Van Oordt and Lofts, 1963). A close correlation has also been shown to exist, by the same investigators, between the interstitial lipid cycle and the secretory condition of the pituitary Y-gonadotrophs which are thought to be the ICSH producing cells (Van Oordt, 1961). It was therefore thought worthwhile to study these events in Indian house crow for comparison with the already established phenomena in other avian species. Such a study would, it is hoped, also yield information about the relation of pituitary cell-types with the endocrine function of the interstitial tissue and other steroid producing endocrine gonadal elements. Recently Kanwar et al. (1977) studied the house crow for testicular lipid variation in relation to the reproductive cycle. According to these authors, Corvus splendens breeds during mid-April and mid-August. Comparison of the present study with that reported by Kanwar (1977) reveals that in Chandigar (Punjab State) an
area in northern India; crows have a longer breeding season. Due to this apparent difference in the timings of breeding period, it was therefore, thought to investigate differences in variations if any, in the lipid, cholesterol and phospholipids, in relation to reproductive cycle in both the sexes of Indian house crows.

The present investigation is also intended to establish a basis for subsequent studies of pinealectomy which may cause physiological alterations in gonadal tissues and it is hoped that study of this kind would provide, though a primary but an ideal parameter to look into the problem and to reach a probable answer.

A study of distribution patterns of succinate dehydrogenase activity has an interest in view of claims that the highest activity of this enzyme in rodent ovaries occurred at the time of the greatest luteal cell activity (Meyer et al., 1947; Padykula, 1952; Foraker et al., 1955; Pariedkans and Weber, 1968) and thecal cell activity (Zerbian et al., 1965). The present study includes findings on SHI activity (histochemically demonstrated) and its probable role in the regulation of sexual cyclicity in this bird.
MATERIAL AND METHODS

The animals of both the sexes were decapitated in the laboratory or they were shot down on the university campus and immediately brought to the laboratory. Gonads were removed and were promptly frozen. The frozen tissue was sectioned at 18 μ in cryostat maintained at -20°C; sections from the mid-region in case of testes and almost all sections of ovary were transferred onto the coverslips and fixed by momentary thawing and were semi-dried at room temperature for 4-5 minutes.

Few sections were fixed in cold calcium formal for 15-20 minutes, washed in water and treated with Pettrot 7B and Sudan Black-B, for the demonstration of neutral lipids (triglycerides) and total lipids respectively (Pearse, 1960).

Few sections were fixed in 10% neutral formalin for the demonstration of cholesterol (Schultz Reaction) and few sections were treated for histochemical demonstration of succinic dehydrogenase (SDH) activity in gonads as per method suggested by Ogata and Mov (1964).

The enzyme Δ⁵⁻Δ⁷⁻HSDH activity was demonstrated histochemically by the method of Wattenberg (1958). During the course of the
incubation, the substrate is converted to a corresponding \( \Delta^4 \)-3-Ketosteroid by the enzyme \( \Delta^5 \)-3\( \beta \)-HSDH with a subsequent reduction of NAD to NADH\(_2\). The formazan produced from the reduced factor through the action of NADH\(_2\) on nitro-blue tetrazolium (Beane and Rubin, 1965) precipitates as blue granules. The intensity of reactions, judged by the density of the formazan granulation deposited in different steroidogenic gonadal tissues, under microscopic examination was rated from (1) i.e. minimum to (5) i.e. dark dense blue granulation. Pregnenolone (3\( \beta \)-hydroxypregn-6-one-17-one) and Dehydroepiandrosterone (DHEA = 3\( \beta \)-hydroxy androst-5-ene-17-one) were added to the incubation medium as enzyme substrates. These (5.0 mg of substrate) were dissolved in 0.5 C.C. dimethyl formamide i.e. DME. In control incubations the steroids were omitted. The reaction was carried out at 37°C. Following incubation, the sections were washed in 10% formal saline to stop the reaction. Formazan, generally insoluble in water, are often soluble in lipids (Barka and Anderson, 1963) but extraction of lipids with cold acetone prior to incubation reduced the enzyme activity in the Indian house crow tissues. Such a reduction in activity after acetone treatment has also been reported for tissues from rat, rabbit, mouse, human, fish (Lery et al., 1959; Wattenberg, 1959; Wiebe, 1969), chick (Boucek et al.,
1966) and eider (Gorman, 1974). Consequently, the movement of the formazan crystals or granules was prevented by removing lipids from most of the sections with a mixture of equal parts of absolute ethanol, and diethylether for one minute, and then with 70% ethanol for 10 seconds (Boucek, et al., 1966). Finally the sections were rehydrated and mounted in glycerine jelly.

In order to follow seasonal changes in the production of steroid hormones by the testes, following histochemical criteria were used.

(1) Sudanophilia of the Leydig cells: The sudanophilia of the Leydig cell cytoplasm was graded according to the method followed by Gorman (1974), which was slightly modified from the original method as suggested by Lofts (1964) and categorized in stages as follows: Stage: 1, cells completely free of lipid droplets and cholesterol negative; and it indicates negligible (minimum possible) lipid droplets (cholesterol negative). Stage: 2, for small lipid droplets present in the cytoplasm and a slight (mild) Schultz Reaction; Stage: 3, many small lipid droplets present in the cytoplasm and slight Schultz Reaction; Stage: 4, numerous large lipid droplets present and strongly cholesterol-positive lipid droplets; Stage: 5, the cytoplasm
filled with dense large amorphous masses of strongly cholesterol positive lipid. Each testes was allocated to one of the above stages, and monthly indices obtained (as Lipid Index) by taking the average value for each month's sample.

(2) $\Delta^5-3\beta$-HSDH activity: The relative activity of $\Delta^5-3\beta$-HSDH in different testes and ovaries was measured in a similar manner to that used for lipid content. Each testis and ovary was scored on the following five point scale, monthly means again being used as indices of activity; Stage: 1, either no deposition or light blue deposition of formazan crystals in the cytoplasm; Stage: 2, a few scattered medium light blue microcrystals; Stage: 3, many medium blue microcrystals deposited; Stage: 4, large medium dark blue microcrystals present together with many smaller ones; Stage: 5, cytoplasm charged with large intense dark blue microcrystals often overlapping to form amorphous masses.

Total lipids were estimated employing the method of Folch et al., (1957), which was essentially same as that applied for mammalian tissues. Total cholesterol was extracted with 2:1 chloroform-methanol mixture (v/v), which in the presence of ferric chloride reacted with sulphuric
acid to yield a stable brown colour (Crawford, 1958). This was read at 540 nm in a colorimeter against blank and compared with a known standard. Phospholipids were measured by a method described by Dittmer and Wells (1969).

**OBSERVATION**

(A) MALE:

(a) **Seminiferous tubule lipid**: There was a seasonal accumulation and depletion of intratubular lipids. There were generally no sudanophilic droplets during June to mid-July, within the seminiferous elements, but during late July and early August small fine lipid droplets began to appear in the Sertoli cell cytoplasm. These gave a positive Schultz reaction. Throughout August there was a rapid accumulation and by the end of August the Sertoli cells were densely lipoidal and strongly Schultz positive. The cells became larger and their cytoplasm now contained numerous fine sudanophilic droplets. Intratubular lipid was maximum in mid-August, September and October. It began to disperse gradually by early November with the onset of spermatogenesis (Chapter 1). As the latter got gradually built up throughout December, the lipoidal content of the tubules decreased. Small traces of lipid
Fig. A: Seasonal variations in the index of lipid content of Leydig's cells, stromal cells, seminiferous tubule and ovarian follicular elements. For detail of score (of lipid index), see text (in section: Material and methods).
TABLE 8.1
Stowing annual variations in weight, testicular and ovarian total lipids, cholesterol and phospholipids. (Mean values are expressed in GM/lOO GM of fresh tissue).

<table>
<thead>
<tr>
<th>Month</th>
<th>Average of Total Lipids</th>
<th>Average of Cholesterol</th>
<th>Average of Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>6.982±0.009</td>
<td>0.622±0.009</td>
<td>5.278±0.009</td>
</tr>
<tr>
<td>July</td>
<td>7.285±0.010</td>
<td>0.792±0.010</td>
<td>5.203±0.010</td>
</tr>
<tr>
<td>August</td>
<td>6.921±0.012</td>
<td>0.608±0.012</td>
<td>4.601±0.012</td>
</tr>
<tr>
<td>September</td>
<td>7.164±0.016</td>
<td>0.792±0.016</td>
<td>4.550±0.016</td>
</tr>
<tr>
<td>October</td>
<td>6.805±0.017</td>
<td>0.590±0.017</td>
<td>5.117±0.017</td>
</tr>
<tr>
<td>November</td>
<td>6.982±0.018</td>
<td>0.788±0.018</td>
<td>5.186±0.018</td>
</tr>
<tr>
<td>December</td>
<td>7.261±0.020</td>
<td>0.791±0.020</td>
<td>5.443±0.020</td>
</tr>
</tbody>
</table>

Note: Mean values are expressed as %/100 of fresh tissue.

Showing annual variations in weight, testicular and ovarian total lipids, cholesterol and phospholipids.
TABLE 8.2

Showing annual variations* in histochemically demonstrable $\Delta^5\text{-}3\beta$-hydroxysteroid dehydrogenase activity in gonadal elements of Indian house crows.

<table>
<thead>
<tr>
<th>MONTHS</th>
<th>SEMINIFEROUS TUBULE ELEMENTS</th>
<th>LEYDIG CELLS</th>
<th>HEALTHY FOLLICLES</th>
<th>ATRETIC FOLLICLES</th>
<th>POST-OVULATORY FOLLICLES</th>
<th>STROMAL GLAND CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>February</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>March</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>April</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>May</td>
<td>1, 2</td>
<td>4</td>
<td>4, 5</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>June</td>
<td>1, 2</td>
<td>4, 5</td>
<td>4, 5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>July</td>
<td>1, 2</td>
<td>4, 5</td>
<td>4, 5</td>
<td>5</td>
<td>4, 5</td>
<td>4, 5</td>
</tr>
<tr>
<td>August</td>
<td>1, 2, 3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>September</td>
<td>2, 3</td>
<td>1, 2</td>
<td>1, 2</td>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>October</td>
<td>2, 3</td>
<td>2</td>
<td>1, 2</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>November</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3, 4</td>
<td>-</td>
</tr>
<tr>
<td>December</td>
<td>2</td>
<td>2, 3</td>
<td>2, 3</td>
<td>3</td>
<td>3, 4</td>
<td>-</td>
</tr>
</tbody>
</table>

*Average values for amount of formazan deposition
@Formazan depositon scored from visual microscopic examination of tissue sections as follows:
Scores: 1. negative or very light blue
2. medium light blue
3. medium blue
4. medium dark blue
5. intense dark blue
still persisted throughout the succeeding two months usually wedged between some of the developing germ cells, but by early January, only faint traces were left in some specimens whereas others possessed tubules almost completely lipid free. Thereafter there was no apparent change in the lipid content of seminiferous tubule elements till the end of February. The fine lipid droplets were very weakly cholesterol positive. Lipid distribution remained in this condition till the end of April - the so-called mid-progressive phase (Chapter: 1). During May, seminiferous tubules were more or less completely lipid free. The lipid showed no or very weak +ve Schultz reaction till mid-July, thus depicting the annual minimum lipid level in the seminiferous tubules. This was present mostly towards lumina as fine droplets, and was negative to cholesterol test. During the regressive phase i.e. August to October (Chapter: 1), when the tubules contained only resting spermatogonia, minute lipid droplets showing negative cholesterol reaction were present. It should be pointed out that in the present species intratubular lipids never accumulate to that large extent as has been reported to occur in some Amphibians and Reptiles (Lofts, 1964), but lipid concentration was slightly more than what has been reported for pigeons (Columba livia), by Ambadkar and Kotak (1986).
(b) Variations in lipids, cholesterol and phospholipids in Leydig cell: 

The interstitial tissue displayed a well-marked lipid cycle than the seminiferous lipid cycle noted herein and a seasonal variation in its response to the Schultz test. These events have been summarized along with those of the seminiferous tubules in Fig. 1, in terms of lipid index. In the specimens collected during June to mid-July, the Leydig cell cytoplasm contained none or only a few lipid droplets that failed to give a positive Schultz reaction. By the beginning of August, the whole cell cytoplasm was filled with small, discrete sudanophilic granules. After the breeding period, there was a sudden and marked increase in the lipoidal condition, and each Leydig cell was seen to contain dense amorphous masses of strongly cholesterol-positive lipids. The maximum lipid content was observable by late August and this remained fairly constant throughout September and October. Thus, during the regressive phase (Chapter: 1), the Leydig cells showed annual maximum lipid content in cytoplasm. By November, the lipid was becoming dispersed and the reaction to the Schultz test was becoming less intense. The lipid depletion continued throughout November and December, indicating a secretory activity even during these winter months. During the following four months there was a
gradual decrease reaching the annual minimum level in late May. This trend of gradual dispersion of lipids was also accompanied by a similar change in the cholesterol +ve lipid. Quantitative measurement of lipid and cholesterol content corroborated the trend in annual variations (Fig. 1) described above on the basis of histochemical findings.

Variations in the concentration of total lipid, total phospholipid and total cholesterol during different months are shown in Table: 8.1 and Fig. 1. The pattern of changes in total phospholipids was in contrast to that of total lipids. Total phospholipid content was high during the active spermatogenesis (i.e. progressive and breeding phases) when compared to the period of testicular quiescence (i.e. regressive phase which extends from August to October).

(c) Variations (histochemical) in \((\Delta^5-3B-HSDH)\); Localization of this enzyme activity was chiefly confined to the Leydig cells with a very moderate activity in the lining of the seminiferous tubules. Intensity of the enzyme activity with dehydroepiandrosterone (DHEA) as a substrate or pregnenolone as a substrate was generally similar during November to March. However, during August, September and October i.e. during regressive phase the latter substrate particularly gave comparatively intense activity. Whereas, during April to July, DHEA was
preferentially utilizable giving more intense reaction than with pregnenolone.

Table 8.2 summarizes the changes in intensity of the reaction for steroid dehydrogenases during the whole year. Enzyme activity was very weak (annual lowest) in August. No apparent increase was registered during September and October. Thereafter, a gradual rise in activity was evinced up to February, which could be considered as moderate activity. Through February to April, condition remained same. The enzyme activity was localized more in the Leydig cells and a low intensity could be found in the lining of the seminiferous tubules. The annual peak in enzyme activity was observed in the months of May, June and mid-July. The enzyme activity became relatively mild in late July, and an abrupt drop in activity was discernible from early August to October.

(d) Succinic dehydrogenase (SDH): The enzymatic activity in the testis was localized in the various cell types with the exception of the basement membrane. SDH was localized in the mitochondrial region of seminiferous epithelial elements. The granulation was comparatively heavy in spermatogonia and spermatocytes (primary and secondary) compared to other cell-types. The middle piece
of the formed spermatozoa exhibited maximum enzyme activity. The Sertoli cells too, gave strong activity. Leydig cell cytoplasm also showed moderate enzyme activity. The annual peak in activity was observed during October to February (i.e. late regressive phase and preparatory phase), thereafter gradual reduction in activity was observed reaching an annual lowest, during June to July (i.e. during breeding phase).

(B) FEMALE:

(a) Variations in lipid, cholesterol and phospholipids:

(I) Developing healthy follicles: The month-wise variations in the levels of total lipids, cholesterol and phospholipids in the ovary are presented in Table: 8.1 and Fig. A. Very small oocytes (0.25 μ) have sparse neutral lipid droplets located centrally, while in larger oocytes, the cytoplasm was with coarser droplets in peripheral regions. The follicular cells in healthy follicles were observed to have fine dispersed lipid droplets but the thecal cells were noted to possess rich deposits of fine lipid droplets and were cholesterol positive. As the follicle grew the number of lipid droplet also increased in both the layers - granulosa as well as thecal layer. In case of follicular cells coalescence of finer
lipid droplets was evident and large lipid globules could be observed. Thus, medium sized healthy follicles were having coarse lipid globules. Larger follicles (maturing ova) reaching more or less ovulable size were examined for lipid localization and content; they showed dispersion and reduction of lipid. The lipid droplets were very weakly cholesterol positive. The localization was similar to that found in medium sized follicles. Thus a gradual reduction in lipid and cholesterol content was clearly evident from late winter period, corresponding to the pursuit period and pair formation period. Schultz test for steroids gave weakly positive results. During breeding phase columnar granulosa cells were very pale red indicating negligible lipid content. The thecal cells also showed reduction in lipid droplets; both the layers were characterized by very low cholesterol positive lipids. The intensity of sudanophilic lipid and cholesterol in the follicles of the ovaries collected during late incubation period, nestling period, and post breeding period (chapter: 2) was very high. Presence of yolky follicles with yolk platelets during nest building period was common. These yolk platelets were found to possess either very weak or no lipid staining in their centres but were enclosed by strongly sudanophilic envelopes.
(II) Atretic follicles: Dispersed atretic follicles of variable size were in all cases lipid (sudanophilic and cholesterol positive) laden. The follicles, when of smaller sizes, were seen as small yellow follicles with naked eye and the frequency of occurrence was very high during incubation and nestling periods. Lipid was plentiful in the granulosa as well as theca interna. The droplets of lipid were very closely packed filling the complete follicle. Large atretic follicle revealed a broad band of lipid in the peripheral regions with few smaller and scattered lipid droplets in the centre. Yolky atretic follicles were loaded with lipids. In later stages of follicular atresia the lipid droplets were coarser and more distinct and considerably more numerous in thecal cells while in granulosa, lipid was present in varied amounts and vacuoles were often frequent and large. In the terminal stages of atresia the positive reaction to lipid appeared to be exclusively thecal in localization. The cholesterol positive lipid was very less in initial stages but gradually increased towards terminal stages of atresia.

(III) Post-ovulatory follicles (P-OF): Staining of P-OF demonstrated the presence of lipids in small droplets or in larger masses. In initial stages of resorption of
The granulosa lipid was sparsely scattered. In somewhat more advanced stages, greater concentrations were present, particularly near the central parts. Thecal cells too contained lipid. The thecal gland cells, which were transformed into prominent islands of cells, usually were even richer in lipid (sudanophilic as well as cholesterol positive) than the granulosa. Scattered cells in theca externa also contained lipid. Gradually these cells showed diminution of lipid.

(IV) Stromal cells: Dispersed stromal cells heavily laden with lipids (sudanophilic as well as cholesterol +ve) were observable all throughout the year cycle. The intensity of lipid staining was generally similar to that found in thecal gland cells. Stromal gland cell islands were very heavily charged with lipid toward nest building period, but on the contrary cholesterol positive lipid showed much reduction in the amount. Cholesterol +ve lipids were showing intense reaction during nestling period i.e. post-breeding phase. It was also been noticed that cholesterol +ve lipids were present from this phase to the phase of early pursuit period of the next breeding season. Thereafter a decreasing tendency was evident up to nest-building phase (chapter: 2). During mid-nest building period cholesterol +ve lipid showed abrupt reduction and it was
maintained at the same condition till early incubation period. It has also been noticed that smaller islands were observable during nestling to winter period, and then the number and size of the islands increased toward pursuit period reaching a maximum in mid-nest building period. Thereafter, once again, a very slight reduction in the number and size was noticed, and it was reduced too much by late incubation or early nestling period. This variation was clearly discernible when one looks to the localization of lipids in stromal tissue of the ovary.

Quantitative estimation of lipids and cholesterol paralleled the trend in annual variations (Fig. A Table: 8.1) described earlier on the basis of histochemical findings.

Concentration of phospholipids was high during mid-nestbuilding to laying period and then the level gradually fell down in August. It remained at that annual low level during incubation, nestling, and post-breeding periods. Thereafter, a gradual rise was discernible which went abruptly high in early nest-building period. The overall concentration was maximum during laying period.
(b) $\Delta^5$-3\(\beta\)-HSDH:

Of the two substrates used, DHEA was seen to be the preferred one, resulting in heavy formazan deposition during April to July. Incubation with pregnenolone as substrate resulted in a weak reaction during the same period. In a few cases, chiefly during August, September and October, pregnenolone could be utilized by ovarian tissue to a considerable degree. Atretic follicles as well as developing healthy follicles of various sizes depicted $\Delta^5$-3\(\beta\)-HSDH activity throughout the yearly cycle. Although there were positively reacting thecal as well as granulosa cells in almost all of the follicles at any stage, the heavy formazan deposition was more apparent in thecal cells.

(I) Healthy Growing (Developing) Follicles: The smaller healthy developing follicles did show $\Delta^5$-3\(\beta\)-HSDH activity in thecal as well as granulosa cells but the formazan deposits were diffused and the intensity of the $\Delta^5$-3\(\beta\)-HSDH activity was very low. As such minute follicles were numerous during August, September and October, overall enzyme activity was at an annual lowest mean score of 1 (August) or 2 (September and October). During winter phase/period (chapter 2) the mean score of $\Delta^5$-3\(\beta\)-HSDH activity increased to 3 (medium blue). Moderate activity was thus evident from
November to February. Even during this period (i.e. Winter) the light formazan deposits (Score: 2; medium light blue) occurred consistently in the granulosa whereas moderate granulation (Score: 3) appeared in thecal cells. The ovarian follicles having been developed to a higher degree during March and April—where the thecal as well as granulosa layers were well formed (i.e. increased in thickness)—showed the mean score 4. Intense enzyme activity was discernible in the ovarian follicles of nest-building and laying periods. These follicles were yolk-laden and either of ovulable size or slightly smaller. In such follicles the thecal cells showed relatively more intense activity than the granulosa cells.

(II) Post Ovulatory Follicles (P-OFs.): Granulation was dense in the granulosa cells of P-OFs (intensity score 5), rather than the thecal cells. It was observed that $\Delta^5-3\beta$-HSD activity was much stronger with pregnenolone as compared to that with DHEA as the substrate. It is noteworthy to mention that the $\Delta^5-3\beta$-HSD activity was comparatively stronger in atretic follicles when the same was compared with that found in P-OFs.

(III) Atretic Follicles: Atretic follicles showed relatively stronger activity (intensity score 4) of $\Delta^5-3\beta$-HSD and the intensity was much stronger than that noted in
growing healthy follicular elements. Atretic follicles thus exhibited relatively high $\Delta^5$-3\beta-HSDH activity and this was apparent, especially during incubation and nestling periods. The same was also true for follicles in all stages of degeneration. However, the intensity was similar to that observable in stromal gland cells of the same section of ovary. A clear cut annual cycle of $\Delta^5$-3\beta-HSDH activity was not observable, as these follicles were relatively short lived. It should be pointed out that the enzyme activity was comparatively strong in the initial stages of degeneration; reaching low levles towards terminal stages of atresia. Thus it could be said that the increase in $\Delta^5$-3\beta-HSDH activity (in a given or observed sample of ovary) registered by the atretic follicles, depend largely on the stages of degeneration and the population density of such follicles in the stroma of the ovary. Considering the above fact, a generalization can be made that the $\Delta^5$-3\beta-HSDH activity was intense (intensity score: 5) during May to July, and thereafter though the number of atretic follicles was increased, the activity of $\Delta^5$-3\beta-HSDH decreased. This was probably due to their terminal stages in atresia when they became densely loaded with lipids and cholesterol positive lipid. The activity in atretic follicles during November to March, was more or less of
moderate nature, registering a gradual increase in April and reaching an annual maximum during May onwards. Fall in activity was noted from early August and remained more or less at the same level till October.

(IV) Stromal Gland Cells: The activity index showed a trend during an annual cycle, similar to that described for atretic follicles, registering an intense activity during May to July and the lowest during August to October.

(c) Succinic Dehydrogenase (SDH):

The SDH activity was localized in various cell types with the exception of the basement membrane. Formazan granulation was intense in granulosa as well as thecal cells.

DISCUSSION

The involvement of the testes and their hormone in sexual and aggressive behaviour is well known (Watson, 1970). Since the Leydig cells of the testis are known to be the major site of androgen synthesis within the testes (Woods and Domm, 1966), an index of their relative abundance provides a measure of the potential production of androgens at specific times of the year (Threadgold, 1956; Selander and Hauser, 1965), but provides no information on the
relative activity of the tissue at different times of the year. Tam et al. (1969) have shown that, in the Cobra, *Naja naja*, changes in the lipid histochemistry of the Leydig cells closely paralleled changes in the capacity of the testis to convert Progesterone - 4-CH$_2$ to androgens.

In view of the conformity of the patterns of Leydig cell lipid cycle in seasonal vertebrates, it seems justifiable to believe that accumulation of lipids by the cells indicates low levels of androgen synthesis, and dispersion of lipids points to increase in the rate of androgen production.

The cycle of Leydig cell abundance in house crow, was distinctly unimodal, with a peak in June and July (Chapter:1). Evidently there was only one potential annual peak in the synthesis and release of androgenic hormones. That the single peak of Leydig cell abundance did correspond to the period of increased androgen synthesis is supported by parallel cyclic changes in Leydig cell lipid content and $\Delta^5-3\beta$-HSDH activity.

Such a single peak of cell abundance was associated with a decrease in cholesterol positive lipid content and with an increase in $\Delta^5-3\beta$-HSDH enzyme activity. Whereas, decrease in the number of Leydig cells was accompanied by an
accumulation of cholesterol positive and total lipid and by a fall in $\Delta^5$-3$\beta$-HSDH enzyme activity. The seasonal changes in the abundance of Leydig cells were paralleled by seasonal changes in the activity (steroidogenic) of the cells; the two cycles running concurrently.

The seasonal pattern of display of pair-bond formation (Chapter 2) essentially occurred in phase with the period of active androgen production, as inferred from the abundance of Leydig cells and their relative activity. One may conclude that seasonal changes in the rate of display were due, at least in part, to seasonal changes in the production of androgens by the testis.

The interpretation of the results on the basis of pituitary gonadotropic activity, carried out with histological techniques, is difficult, since they give an estimate of hormone content at one instant of time without providing information upon rates of synthesis and/or release. However, a consideration of the cycle of pituitary gonadotropin in relation to gonadal development provides some insight into the phases of synthesis, storage and release of the pituitary hormones (Chapter 6). There is evidence that the avian testis, as in other vertebrates (Burgos and Ladman, 1957; Lofts, 1961), is regulated by
two gonadotropic hormones (Lofts and Murton, 1973); an FSH-like factor stimulating the interstitial and inducing spermiation. The cyclical variation in the interstitial cells of the species under investigation probably reflected a seasonal variation in the secretion of the latter's hormone by the anterior pituitary.

The decline in gonadotropin content following breeding was associated with the regression of the gonads, and thus one can conclude that synthesis was at a low rate and that the hormones were being released, if at all in small amounts. The fall in gonadotropin content during August, September and October was also responsible for the pre-winter decline in Leydig cell abundance and secretary activity and the arrest of spermatogenesis beyond the early stages. However, by the end of October the new generation of Leydig cells was being established, and was becoming lipoidal. It may be suggested, on this basis, that LH (Luteinizing Hormone) secretion was occurring at this time. Increased development and activity of the Leydig cells during the following months (November to May), together with concurrent renewal of spermatogenesis was associated with an increase in pituitary gonadotropin content and its release. During June and early July increased gonadotropic potency lead to maximum development of the endocrine and
gametogenic functions of the testis. At this period (June and early July), ICSH (interstitial cell stimulating hormone) output is presumably cut-off or depressed, and the intratubular lipid is dispersed by FSH secretion. It is possible that high summer temperatures might exert their influence via the pituitary. They probably stimulate maximum FSH and LH secretions with subsequent high spermatogenic activity. Similar opinion has been also given by Lofts (1961) and Lofts and Van Oort (1962).

Variations in the histochemical activity of $\Delta^5-3\beta$-HSDH occurred in the ovarian tissues during different stages of the reproductive cycle presumably as a result of differing stages of sexual maturity induced by long (stimulatory?) and short (non-stimulatory?) day-length photoperiods.

Positive reaction for $\Delta^5-3\beta$-HSDH activity has been reported in the granulosa and thecal cells of the pre-ovulatory, post-ovulatory and atretic follicles and in clusters of interstitial cells of the chicken, Japanese Quail and Pigeon (Chieffi and Botte, 1965; Woods and Domm, 1966; Novbaitz and de Robertis, 1968; Sayler et al., 1970; Ambadkar and Kotak, 1976). The pattern of histochemical distribution of $\Delta^5-3\beta$-HSDH in Indian house crow was similar to that
described in chickens and pigeons by the workers cited above. Although few assays have been made on individual ovarian tissue components, to determine which steroids are produced, there is ample evidence that the avian ovary secretes estrogen, androgens, and progestins (van Tienhoven, 1961). The consensus of biochemical and histochemical findings indicated that androgens are produced in the interstitial (stromal gland) cells that arise from the ovarian stroma (Marshall and Coombs, 1957; Woods and Domm, 1966), and oestrogens are produced from interstitial cells derived from the theca interna of atretic follicles (Chieffi, 1965; Chieffi and Botte, 1965). Estrogens are also produced in the granulosa cells of the pre-ovulatory and atretic follicles (Chieffi and Botte, 1965; Botte et al., 1966) and in theca interna of the pre-ovulatory follicles (Botte et al., 1966). Developing follicles (Fraps, 1955) and atretic follicles (Marshall and Coombs, 1957) have been suggested as sources of progestins.

In Indian house crow, the rate of gonadal growth is a good indication of the gonadotropic content in the anterior pituitary (Chapter: 6). A rapid increase of gonadotropic potency occurs, corresponding in time with the rapid increase in ovarian weight, under stimulatory photoperiods (i.e. long day-length period). Under non-stimulatory day-
length conditions, both ovarian weight and gonadotropic potency remain low. The present findings show that the activity of the $\Delta^5-3\beta$-HSDH in ovarian and testicular tissues reflects increases in ovarian/testicular weight and gonadotropic potency when the birds were under long days. Under the stimulatory light regimen, a definite increase in steroidogenic potential occurred in testicular/ovarian tissues during June and July. This period coincides with the phase of rapid yolk deposition which immediately precedes ovulation (Chapter 2). Since estrogen immediately mobilizes yolk precursors and facilitates yolk deposition in the presence of gonadotropins (Clavert, 1958), ovarian and oviducal development with subsequent oviposition. Under these conditions, gonadotropic potency, ovarian and testicular growth, and steroidogenic potential of ovarian hormone-producing tissues are synchronized so that steroid hormone production reaches a peak immediately before oviposition. The increase in enzymatic activity at that time may reflect a change in the function of hypothalamo-hypophyseal system.

Under non-stimulatory (short-day length regimen) conditions, a strong dehydrogenase reaction was seen in interstitial cells and in the cells of theca interna. These observations indicate that the potential for steroid
synthesis is present even under short day length periods. It is apparent from the data and work done on chick embryos (Chieffi, 1965; Boncek et al., 1966; Woods and Weeks, 1969) and Japanese Quail, Coturnix coturnix japonica (Sayler et al., 1970) that $\Delta^5-3\beta$-HSDH activity can be demonstrated in embryonic, prepubertal and light inhibited avian ovaries.

The results presented demonstrate that photoperiod is an important factor in the cellular distribution and onset of the steroid-producing enzyme $\Delta^5-3\beta$-HSDH. Although histochemical methods do not permit exact quantification (Lobel et al., 1962; Christensen and Mason, 1965), yet there is an evident correlation between histochemical and biochemical results for hydroxysteroid dehydrogenase activity (Dean and Rubin, 1965; Davies et al., 1966). Histochemical procedure reveals changes in enzyme patterns that could be easily related to the stages of follicular growth and maturation.
EXPLANATION TO FIGURES

Figs. 1 to 8 Photomicrographs of T.S. of testes of Indian house crow.

PLATE : I

Fig. 1. T.S. of testes (from non-breading phase: September) showing seminiferous and Leydig's cells heavily charged with cholesterol. X 30.

Fig. 2. T.S. of testes (from breeding phase: June) showing reduction in the distribution of cholesterol in both the elements: seminiferous/elements and Leydig's cells. X 200.

Fig. 3. T.S. of testes from non-breading phase showing lipid distribution (stained with Sudan Black). Note the Leydig's cells heavily loaded with lipids. Few very fine scattered lipid with droplets are also present in the seminiferous tubule elements. X 50

Fig. 4. T.S. of testes from breeding phase. Note the reduction in lipid content in both the seminiferous and Leydig Leydig's cells. X 200.

Fig. 5. Note feeble $\Delta^5 - \Delta^3$ - hydroxysteroid dehydrogenase ( $\Delta^5 - \Delta^3$ - HSDH) activity in Leydig's cells and complete absence of the activity pattern in Seminiferous tubule elements. X 50
Fig. 6. Note the stronger activity (intensity) of $\Delta^5 - \Delta^2$ - HSDH in T.S. of testes from breeding phase. X 250

Fig. 7. Succinic dehydrogenase (SDH) activity (mitochondrial) in testes from non-breeding phase. X 50

Fig. 8. SDH activity in testes (from breeding phase) showing the presence of **mitochondria** and Enzyme in both the Leydig's cells and seminiferous tubules. X 250.

**PLATE : II**

Fig. 9 to 16 Photomicrographs of ovary of Indian house crow.

Fig. 9 Lipids stained with sudan black B. Thecal, granulosa and stromal glands cells to depict heavy content of lipid. X 150.

Fig. 10. Neutral lipids stained with Fettrot - SBB. Note reduction in the lipids in all ovarian compartments during breeding phase. X 250. X 50.

Fig. 11. Heavily laden with Cholesterol, the ovarian compartments ( thecal, granulosa and stromal gland cells ). Note atretic follicle very heavily charged with cholesterol. X 50.
Fig. 12. Showing reduction in the cholesterol content in ovarian compartments except for stromal gland cells, X 50.

Fig. 13. Weak intensity of the $\Delta^5 - 3\beta$ - HSDH during non-breeding period in the ovarian compartments. X 50.

Fig. 14. Showing high activity of $\Delta^5 - 3\beta$ - HSDH in thecal, granulosa cells. X 50.

Fig. 15. Various atretic follicles showing $\Delta^5 - 3\beta$ - HSDH activity. X 50.

Fig. 16. A single atretic follicle heavily laden with cholesterol. X 50.