

## CHAPTER IV

HISTOCHEMICAL OBSERVATIONS ON 3 $\alpha$ - AND 17 $\beta$ -HYDROXYSTEROID  
DEHYDROGENASES IN EXTRA GONADAL TISSUES OF FERAL BLUE ROCK  
PIGEON COLUMBA LIVIA (G.)

Extensive involvement of liver in steroid metabolism has been ascertained through clinical as well as biochemical experiments (Baillie *et al.*, 1966). Histochemically, steroid dehydrogenases have been studied in the renal system of many chordates. As Baillie *et al.* (1966) put it, few accounts exist in the literature on histochemical localization of hydroxysteroid dehydrogenase in the alimentary tract, most of them pertaining to mammalian tissues. Notwithstanding the work done in avian gonads, literature on steroid dehydrogenases in avian tissues in general is scarce. Recently, Bhujle and Nadkarni (1974) have reported on histochemical localization of some of these enzymes in the kidney of white breasted waterhen commenting on their possible role in catabolism and excretion of sex hormones. Certain histochemical and histological characteristics of the kidney of desert sparrows have been reported (Amanova and Balbich, 1976). Though androgen synthesizing tissues show slight 3 $\alpha$ -hydroxysteroid dehydrogenase activity, (Balough, 1966), those tissues that are the target sites of androgens, such as epithelium and preputial glands, generally show

greater enzyme activity. Liver and kidneys, the organs involved in detoxification and elimination of steroids also show appreciable 3 OC-HSDH activity (Balough, 1966).

The earlier quantitative and histochemical work on gonads and other tissues of feral blue rock pigeon (Columba livia) has revealed distinct breeding and non-breeding phases (Chapters I, II and III) This chapter reports on histochemical localizations of 3 OC-hydroxysteroid dehydrogenase (3 OC-HSDH) and 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSDH) in liver, kidney and intestine of the same species during non-breeding and breeding months. The present work was undertaken to throw some light on the excretory pathway of steroid hormones and to see the possible fluctuations of enzyme activities.

#### Material and Methods

Feral pigeons were shot down in the university campus with an air rifle in the year 1976 from June to October. Pigeons of both sexes were utilized for the present study. After promptly bringing them to the laboratory a small piece of right liver lobe, first left kidney lobe and a part of ileum were taken out, blotted and fixed on cryostat chuck maintained at - 20°C. Sections (18  $\mu$ m thick) of intestine were cut <sup>where as</sup> the liver and kidney sections were cut at 12  $\mu$ m thickness on

cryostat microtome. 3 OC-HSDH was demonstrated as per the method described by Balough (1966) employing androsterone as substrate and NAD as co-factor. The pH value was adjusted at 7.7 instead of 7 as originally described since no activity was observable at pH 7. Sections were incubated for 60 min at 37°C. For the demonstration of 17 $\beta$ -HSDH, testosterone and estradiol-17 $\beta$  were used as substrates and NAD as co-factor (Kellogg and Glenner, 1960). The sections mounted on coverglass were incubated for 30 min at 37°C. Control sections for both, 3 OC-HSDH and 17 $\beta$ -HSDH were incubated without using the substrates. The results are presented in Figs 1-14.

### Results

The distribution pattern of enzyme activities in both sexes was similar. Hepatocytes exhibited a uniform cytoplasmic distribution of both the enzymes studied. Apparently no periportal, sinusoidal or peribiliary localization was discernible. In the kidney, enzyme activities were localized in the proximal and distal convoluted tubules of the cortex (Figs 3 & 9) with mild intensity in the glomeruli (Fig. 13) and absence of activity in the medulla (Figs 3 & 9). No activity was seen in serosa, subserosa and submucosa of

### EXPLANATION TO FIGURES

T.S. of pigeon (female) liver and kidney showing  
3 $\beta$  CC-hydroxysteroid dehydrogenase activity. X 75.

Fig. 1. Strong 3 $\beta$  CC-HSDH activity in hepatocytes during  
breeding season (September-October) showing  
uniform distribution.

Fig. 2. Non-breeding phase (June-July) shows feeble  
intensity of the enzyme in liver.

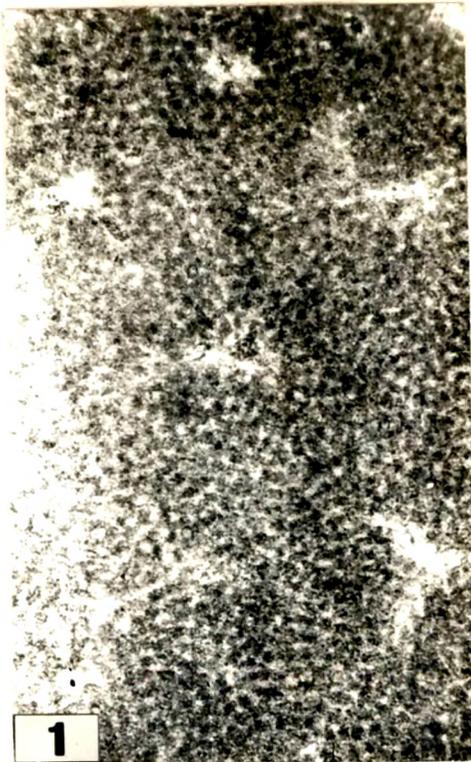
Fig. 3. Convoluted tubules of kidney showing high  
activity whereas medulla (M) reacts negatively  
(September - October).

Fig. 4. Weak intensity of the enzyme in kidney during  
non-breeding phase.

### ABBREVIATIONS

M - Medulla

G - Glomerulus



### EXPLANATION TO FIGURES

T.S. of pigeon (female) ileum showing  
3  $\alpha$ -HSDH activity. X 75.

Fig. 5. Note comparatively less activity in the intestinal  
mucosa during breeding phase.

Fig. 6. Non-breeding months (June-July ) revealing yet  
weaker activity in the intestinal villi.

T.S. of pigeon liver showing 17 $\beta$ -HSDH activity. X 75.

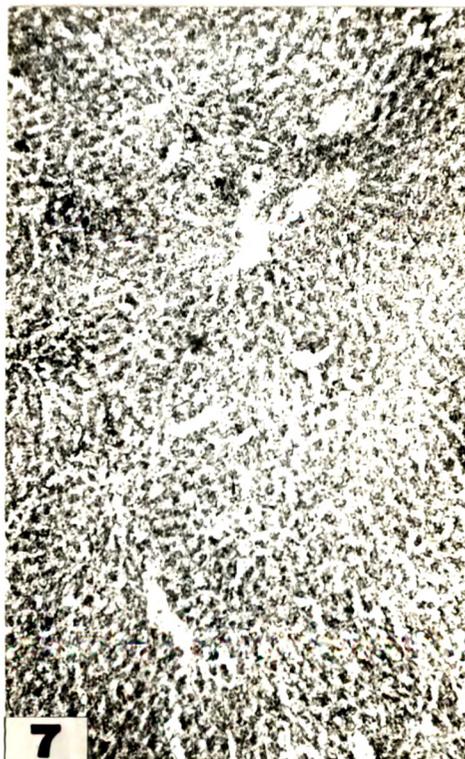
Fig. 7. Liver cells exhibit strong activity during  
breeding season.

Fig. 8. Low enzyme intensity in liver during the non-  
breeding stage.

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### EXPLANATION TO FIGURES

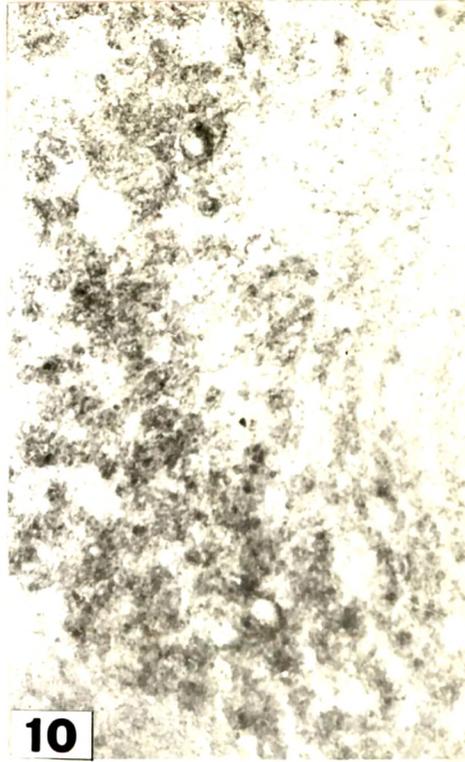
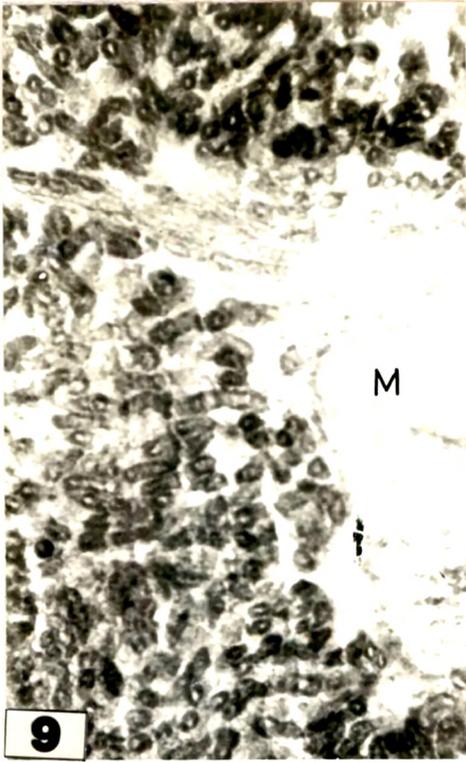
T.S. of pigeon (female) kidney and intestine showing 17 $\beta$ -hydroxysteroid ~~o~~hydrogenase activity. X 75.

- Fig. 9. Cortex of the kidney shows intense activity of the enzyme in the convoluted tubules whereas medulla (M) remains unreactive. (September-October).
- Fig.10. Feeble activity in the kidney during non-breeding months.
- Fig.11. Intestinal villi react positively during the breeding phase though the activity (as compared to that of kidney & liver) is weak.
- Fig.12. Intensity of the enzyme in ileum is very weak during June-July (non-breeding).

### ABBREVIATIONS

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### EXPLANATION TO FIGURES

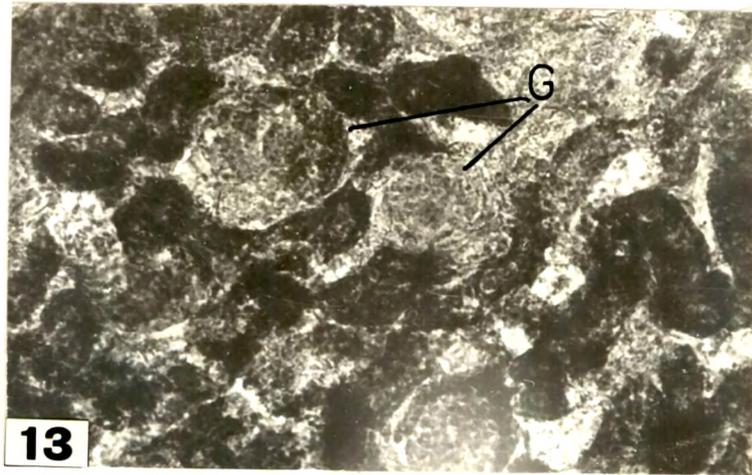
Fig. 13. Moderate intensity of  $17\beta$ -HSDH (E) in the glomeruli compared to that of the surrounding renal tubules.

Fig. 14. Diagrammatic representation of the probable pathway of androgen breakdown.

### ABBREVIATIONS

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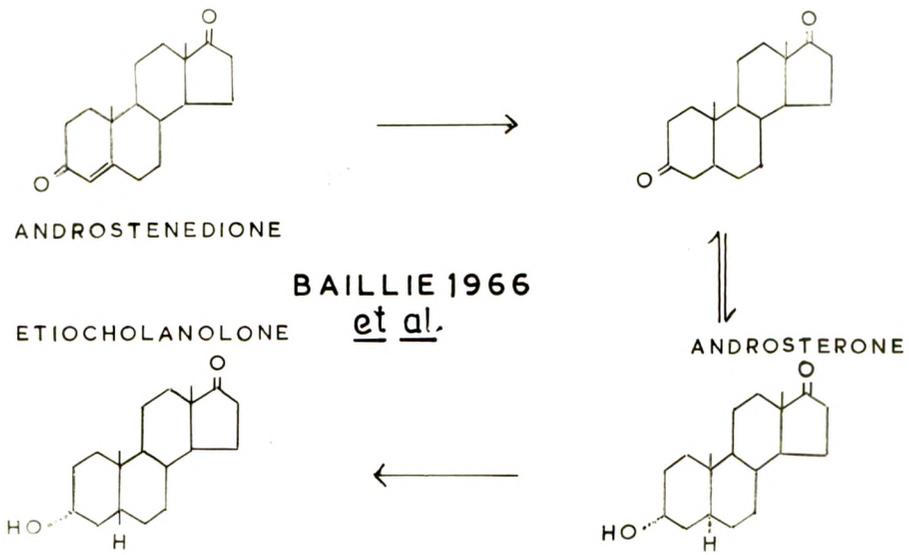


FIG.14

14

the intestine but the mucosal lining (intestinal villi) reacted positively (Figs 5 & 11). Among the 3 tissues studied, liver showed maximum activity of  $3\text{ OC-HSDH}$  followed by the kidney and ileum.  $17\beta\text{-HSDH}$  intensity on the other hand was maximum in kidney, comparatively moderate in liver and minimum in intestine. It is noteworthy that ileum showed the least intensity of both  $3\text{ OC-HSDH}$  and  $17\beta\text{-HSDH}$  in both the sexes. It was noted that tissues of female birds exhibited stronger  $17\beta\text{-HSDH}$  with estradiol- $17\beta$  as substrate than with testosterone whereas converse was true for the males.

During the months of June, July and early August (non-breeding phase), enzyme activities in all the 3 tissues were comparatively weakly localized (Figs 2,4, 6,8,10,12). From late August onwards the activity of enzymes gradually increased and became maximum during September and October (Figs 1,3,5,7,9,11) that is during the breeding phase. The distribution pattern of the enzyme did not change in non-breeding and breeding months though the intensity did vary.

#### Discussion

In 1959, Pearson and Grouse reported the first histochemical localization of  $3\text{ OC-HSDH}$  in the liver without mentioning the species. According to Vande Wiele et al. (1963),  $3\text{ OC-HSDH}$  activity present in the

liver is involved in the interconversions between  $\Delta^4$ -ketosteroids (such as androstenedione) and 3 $\alpha$ -OH-steroids (such as androsterone and aetiocholanolone) followed by their conjugation and ultimate excretion (Tomkins and Isselbacher, 1954). The possible mechanism of this biological inactivation and excretion is summarised in Fig. 14. Again, Pearson and Grouse<sup>(1959)</sup> were the first to note the presence of 17 $\beta$ -HSDH activity histochemically in rat liver. The role of this enzyme in estrogen and androgen metabolism is well known. Baillie *et al.* (1966) suggest that it is reasonable to explain the role played by 17 $\beta$ -HSDH in the interconversions between oestradiol and estrone, and, androstenedione and testosterone. In kidney, such reactions occur throughout nephron, predominantly in the convoluted tubules of vertebrates (Baillie *et al.*, 1966). and are relevant to the excretion of 17-ketosteroids and 17-hydroxysteroids. Recently, Akaishi *et al.* (1974) have observed moderate 17 $\beta$ -HSDH activity in the liver of laying white leghorn hens; commenting on its intimate interrelationship with biosynthesis of ovarian steroids.

Taking into consideration the above mentioned views on 3 $\alpha$ -HSDH and 17 $\beta$ -HSDH. Positive reactions encountered (particularly in September and October) in the convoluted tubules of the kidney, hepatocytes proper

and to a lesser extent in the intestinal mucosa, during the course of the present work indicate that these are the probable sites of sex steroid interconversions and excretion. The fact that the enzymes were not confined to any specified areas in liver (periportal, peribiliary, sinusoidal etc.) suggests that there is no regional specificity to interconversions of steroids but all the liver cells in general are involved in excretion of sex hormones. Likewise, weak intensity of both these enzymes in the glomeruli and their absence in the medullary collecting tubules revealed that these were not the active sites where reactions could be occurring. Ileum, as compared to liver and kidney, seems to be playing a rather minor role in this respect. In the Japanese quail however, Fellegiova et al. (1975) have shown by in vivo autoradiographic study that injected testosterone is metabolized mainly in the gastro-intestinal tract. The observation that among the 3 tissues studied, Kidney exhibited maximum intensity of  $17\beta$ -HSDH activity in both the sexes indicated that it is the major site of steroid metabolism prior to their excretion. In this respect liver seems to stand second. In contrast to this,  $3\text{-CC}$ -HSDH activity was found to be intensely localized in the liver followed by the kidney. This points at the principle role played by the liver in the interconversions of  $\Delta^4$ -ketosteroids and  ~~$3\text{-CC}$ -ketosteroids and~~

3  $\text{CC}$ -hydroxysteroids. Samuels et al. (1950) suggest that metabolic clearance rate of testosterone might be high in liver of fowl since conversion of testosterone to 17 ketosteroids by liver is much more rapid in the fowl than in the fish. Low levels of androsterone, dehydroepiandrosterone and aetiocholanolone have been identified in cock plasma (Furr and Thomas, 1971). These facts do lend support to the role of 3  $\text{CC}$ -functions in the processes involved in excretory pathways in avian species in general.

That the female birds showed higher 17 $\beta$ -HSDH with estradiol-17 $\beta$  as substrate than with testosterone (converse being the case in males) may be due to higher turnover rate of estrogens in females and androgens in males.

The observations reported here indicate that :

- (a) The hormone synthesis being probably at its lowest ebb during June, July and early August (Chapters I, II and III), the excretion of the same (sex steroids) was also at its minimum.
- (b) High activities of both 3  $\text{CC}$ - and 17 $\beta$ -HSDH during September and October (breeding) was due in all probability to increased circulating levels of various metabolites of sex hormones. This is substantiated by the observation that the output of steroid sex hormones during these

months was perhaps high (Chapters I, II and III).

The present findings are in conformity with the previous quantitative and histochemical data on the reproductive cycles of feral pigeons (Chapters I, II and III). It becomes evident that liver and kidney are the chief organs carrying out the interconversions of sex steroids relevant to their excretion. After the hormone inactivation has occurred in the liver and kidney, the metabolites seem preferably to be excreted via the water soluble pathway (urine). Since weak enzyme activities were observed in the ileum, it may be deduced that the fat soluble pathway for steroid hormone excretion via bile into the gastrointestinal tract may not be operative.