OBSERVATIONS ON 17β-, 3β- AND 3α-HYDROXYSTEROID DEHYDROGENASES IN THE UROPYGIAL GLAND OF HOUSE SPARROW (*PASSER DOMESTICUS* L.): A HISTOCHEMICAL PROFILE

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ABSTRACT

Activities of 17β-, 3β- and 3α-hydroxysteroid dehydrogenases were studied histochemically in the preen gland of *Passer domesticus* L. 17β-hydroxysteroid dehydrogenase showed uniformly distributed high activity, particularly with testosterone as substrate, whereas, 3β-hydroxysteroid dehydrogenase failed to show any activity. Maximum intensity was seen with respect to 3α-hydroxysteroid dehydrogenase in the bordering cells of the lumen (*i.e.*, fully differentiated cells); the localization in the rest of the gland was minimum.

The relative importance of these enzymes in steroid hormone metabolism in the gland is discussed.

INTRODUCTION

THE avian uropygial gland, also otherwise termed the oil gland or preen gland, is a bilobed sebaceous gland lying between the dorsal skin and muscles at the base of the tail. Fundamental literature on this organ is the monograph by Paris[1] and the reviews by Elder[2] and Ghosh and Bhattacharyya[3]. The gland is innervated by the first pair of caudo-spinal nerves and sympathetic fibres[3,4]. Structurally the secretory units are comprised of a large number of alveoli, the cells of which exhibit varying states of division and differentiation[3]. Interestingly, the gland is absent in non-flying birds and well developed in aquatic birds[3]. The chemistry of the secretion has been elucidated and it has been found to be rich in lipids, particularly wax esters, with varying degree of other lipid classes[3–8].

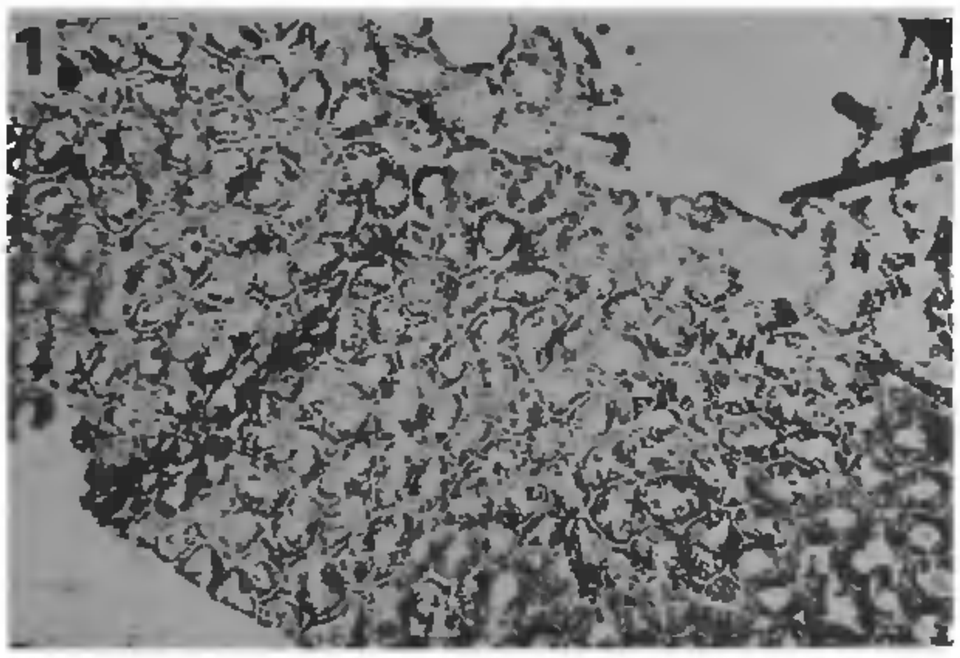
Some recent findings indicate that this gland possesses the capacity to convert labelled progesterone into 17-hydroxy progesterone, testosterone and androstenedione[9]. Besides this, the presence of androgen and estrogen receptors have also been reported in the preen gland of male ducks[10]. In case of the male house sparrow, the gland becomes hyperactive during the breeding phase[11]. Keeping these views in mind, an attempt has been made to study the involvement of steroid sex hormones in the preen gland of house sparrow. The study was undertaken by localizing some important enzymes concerned with sex hormone synthesis and breakdown, employing histochemical means. This could indicate whether the gland is a steroidogenic site or a target site of steroid hormones.

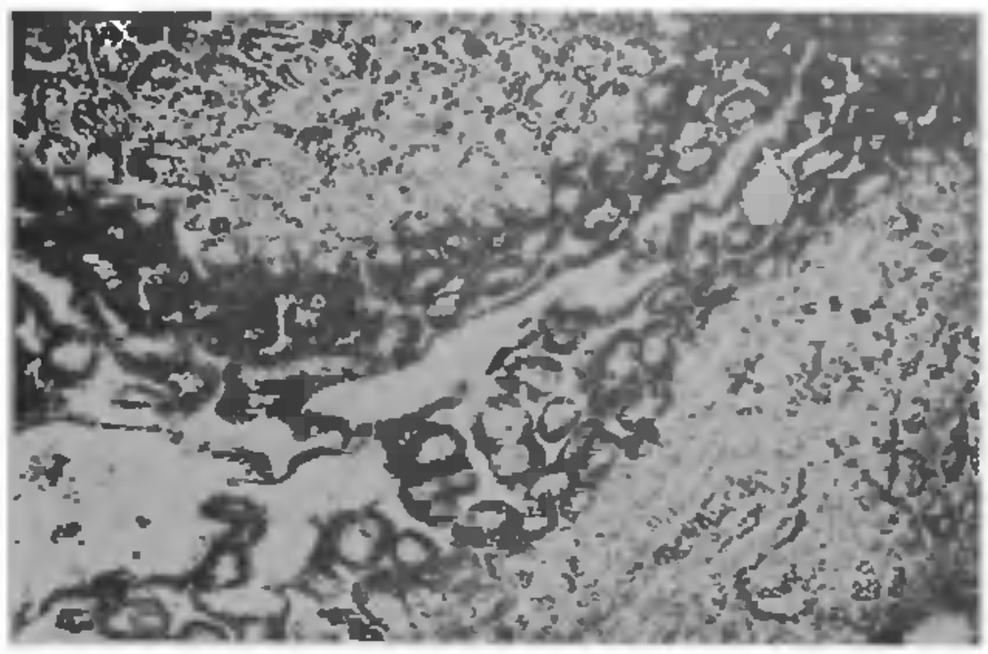
MATERIAL AND METHODS

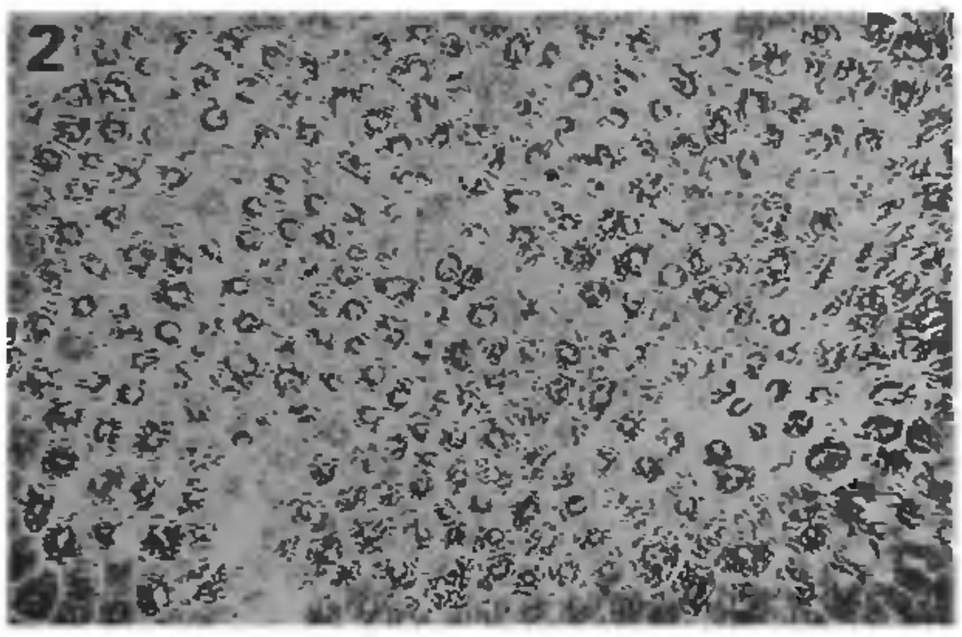
Investigations were made on the house sparrow *Passer domesticus* L.; both the sexes were studied. The birds were shot down during February and March 1979 when they were nesting and the gonads were fairly well developed. The preen glands were taken out and fixed on cryostat chucks maintained at − 28° C. Sections (12 μm) were cut on the "American Optical" Cryo-Cut Microtome. The high lipid content necessitated the employment of low temperatures to freeze the tissue. Sections, taken up on cover glasses, were processed for the demonstration of the enzymes. These were incubated at 37° C for 30 min. for demonstrating 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity, utilizing both testosterone (T) and estradiol-17β (E) as substrates and NAD as cofactor[12]. The same incubation temperature and time were employed to demonstrate 3β-hydroxysteroid dehydrogenase (3β-HSDH) activity using pregnenolone and dehyroepiandrosterone as substrates (P and DHEA respectively) and NAD as cofactor[13]. In the case of 3α-hydroxysteroid dehydrogenase (3α-HSDH), the sections were incubated at 37° C for 60 min using androsterone (A) and NAD as substrate and cofactor respectively[14]. The pH was adjusted to 7·7 and not 7·00 as originally mentioned[14] since practically no activity (3α-HSDH) was seen at pH 7·00. (Original method[14] has been reported in case of mammalian tissues and it is likely that avian tissues have higher pH optimum for the same enzyme[15].) Sections were then thoroughly washed, post-fixed in 10% formalin, rewashed and mounted in glycerine jelly. Control sections in all the cases were incubated in the media devoid of steroid hormones.

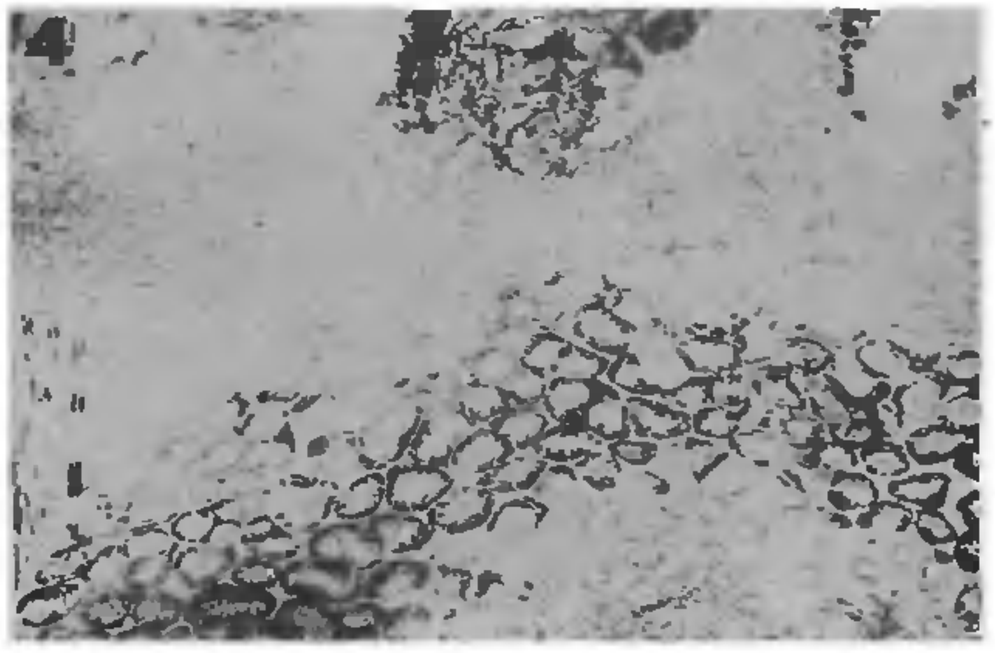
RESULTS AND DISCUSSION

Histologically, the gland has been demarcated in various cell types[3]. These include small, flat, dividing cells occupying the basal region of the alveoli or tubules, inner to which are some rows of large and polyhedral differentiating cells. In the centre are the fully differentiated cells followed by the innermost disintegrating









FIGS. 1–4. Photomicrographs of the preen gland of male house sparrow showing hydroxysteroid dehydrogenase activity × 60. Fig. 1. Uniformly high 17β-HSDH (Testosterone) activity. Fig. 2. Feeble activity of 3β-HSDH (Pregnenolone). Fig. 3. Note intense 3α–HSDH (Androsterone) activity in the central cells (lying in the immediate vicinity of lumen); the surrounding area exhibits weak reaction. Fig. 4. 3α–HSDH (control).

cells bordering the acinar lumen. The gland is chiefly involved in plumage maintenance.

Positive reactions of 17β-hydroxysteroid dehydrogenase in the preen gland has been reported in case of chicken[3] but there is paucity of information in other avian species. In the case of mammals, the interconversions between androstenediol and DHEA, testosterone and androstenedione, and, estradiol and estrone are known to be catalyzed by 17β-HSDH[16]. This enzyme has also been localized in the liver of hens[17] and liver and kideny of both sexes of feral pigeons[15]. During the present investigation, 17β-HSDH (T) activity was high in the case of male birds (Fig. 1) and only slightly less in the females (+++ and ++, respectively). However, with estradiol-17β as substrate, the enzyme activity on the average remained more or less same in both sexes, varying between moderate to high (++, +++). This indicated that in both the sexes, all the cell types of the gland have a uniform capacity to interconvert 17-hydroxysteroids to 17-ketosteroids.

Synthesis of sex steroids usually involves cholesterol to pregnenolone conversion. From the latter compound, a bifurcation begins. In this biosynthesis, Δ^5-3β-HSDH is one of the key enzymes involved in catalysis[15]. Its presence in a tissue would indicate a strong evidence for sex hormone biosynthesis. For example, it catalyses the conversion of pregnenolone to progesterone and dehydroepiandrosterone to androstenedione; both of these reactions are characteristic of proandrogen biosynthesis leading ultimately to the formation of testosterone and estrogens. During the course of the present investigation, no 3β-HSDH activity was detected with either pregnenolone or DHEA in either sex (−, Fig. 2). This suggested that the uropygial gland is not the site of steroidogenesis. The finding implied that in all probability, there is an absence of *de novo* synthesis of androgens and estrogens in the preen glands of both sexes of the sparrow.

Though androgen synthesizing sites show feeble 3α-HSDH activity, the target sites of androgens (*e.g.*, the preputial glands of rats) show greater activity[11,18]. This enzyme involves interconversion between Δ^4-ketosteroids (*e.g.*, androstenedione) and 3α-hydroxysteroids (*e.g.*, androsterone)[18]. In the present study, the cells surrounding the lumen, *i.e.*, fully differentiated cells, revealed maximum 3α-HSDH (A) activity which appeared intensely blue (++++) whereas the rest of the gland showed feeble (+) reaction (Fig. 3). It pointed that interconversions of androgens at 3α-

position take place in the immediate vicinity of lumen. The rest of the cell types of the preen gland does not seem to partake actively in androgen breakdown. Higher 3α-HSDH activity in the central cells of chicken uropygial gland has also been reported[3]. *It is possible that such androgen catabolism occurs* just prior to the lipid secretion in the lumen. Thattacharyya *et al.*[11] have commented that the structural and functional maturation of this gland may be dependent upon androgens, estrogens and corticosteroids. Various androgens (testosterone, androsterone, aetiocholanolone) and estrogens have been *quantified in avian species (e.g., in fowl and pigeon)*[20,21].

Taking into consideration the above discussion, it seems likely that :

(i) interconversions of sex steroids at C-17 position occur uniformly throughout the gland, (ii) the gland is not involved in steroidogenesis; (iii) androgen metabolism involving 3α position occurs predominantly in the cells bordering the lumen.

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