POST-HATCH DEVELOPMENT OF PREEN GLAND IN THE DUCK (Anas platyrhynchos)

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I hereby declare that this thesis entitled "POST-HATCH DEVELOPMENT OF PREEN GLAND IN THE DUCK (Anas platyrhynchos)", is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Dedicated to

my mum, dad and brothers

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Introduction

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1. INTRODUCTION

Preen gland is the only true cutaneous gland possessed by birds. The gland is large and well developed in the aquatic species of birds. It is a paired gland with two ducts and a common papilla, leading the secretion of the glands to exterior (Spearman, 1973). It is also termed as oil gland, rump gland, perunctum, glandula uropygii, glandula caudae and glandula sebacae.

The preen gland is vestigeal or absent in birds like ostrich, emu, bustard, pigeon and parrot. It is prominent in the budgerigar (King and Mc Lelland, 1975).

Preen gland is a holocrine gland found in the subcutis in the rump region. Its secretion, the preen gland wax, functions as a water-repellant agent to protect the birds against wetting especially for the ducks as they spend most of the time in water. In addition, the wax makes the keratin of the feathers, beak and scales flexible. The secretion is reported to have fungicidal activity and plays a vital role in the intraspecies communication of the birds (Jacob *et al.*, 1979; Maiti and Boss, 1979). The secretion also contain traces of ergosterol in several species of birds (Farner *et al.*, 1982).

The nervous reflex initiated by preening stimulates the discharge of this fatty secretion from the gland to the papilla. The secretion is then transferred to the body and wing plumage by the beak and the head plumage. Structurally and functionally it resembles the mammalian sebaceous gland.

The secretion produced by numerous secretory tubules is collected in a common cavity from where it is transported onto the surface by two ducts opening at the tip of the papilla. The papilla is covered by a tuft of specialized feathers called uropygial circlet or wick.

The composition of secretion varies with the breeding season. The glandular activity is controlled by sex hormones, mainly the androgens (Farner *et al.*, 1982).

No precise study concerning the post-hatch development of the preen gland has been reported so far. In view of the prime importance of the gland, a detailed study on the post-hatch development of the preen gland in the duck seems to be appropriate. Moreover, the findings will add to the existing anatomical knowledge and will throw light on to the various pathological and physiological conditions affecting the gland. With this objective, a research was undertaken to study the morphology and histology of the preen gland and also to trace the morphological, histological, histochemical and micrometrical changes from the day of hatch till 150 days of age, in ducks.

Review of literature

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2. REVIEW OF LITERATURE

2.1 GROSS FEATURES

The preen glands are the well-developed cutaneous glands in ducks. These glands are reported to be better developed in aquatic species of birds (Bradley and Grahame, 1950; Rothman, 1954) including the duck (Young, 1962).

Apandi and Edwards (1964) noticed that the preen gland was embedded beneath the skin in a mass of fatty tissue, just dorsal to the levator muscle of the tail and over the dorsal surface of the free coccygeal vertebrae of chicken. They opined that the secretion of preen gland was essential for the maintenance of the water-repellant quality and structure of feathers. The large and highly developed gland of aquatic birds was to render their feathers impermeable to the watery environment.

Lucas and Stettenheim (1972) made comparative studies on the oil glands in different species of birds and found that in the turkey, the gland was smaller than that of chicken with a relatively broad isthmus. In quails, the gland was massive and extended well forward along the coccygeal vertebra. In White Pekin ducks the two lobes of the gland were widely separated.

The gland is an unpaired bilobed organ in fowl, duck and Japanese quail (Hodges, 1974; Arnall and Keymer, 1975)

According to Farner *et al.*, (1982) in the fowl, the preen gland was situated dorsal and medial to the synsacrum. The two lobes of the glandular body were recognized as eminences and the preen papilla protruded from the caudal end of the glandular body.

In fowl, the glands were located over the last coccygeal vertebra (Stevens, 1996)

Sunanda *et al.* (2001b) opined that the preen glands of ducks were paired creamy yellowish pear-shaped structures located on the dorsal surface of the coccygeal vertebrae. The glands were connected by connective tissue and a common papilla caudally. The separate ducts from each gland had independent orifices.

2.1.1 Morphometry

Thomson (1965) stated that the two preen glands in ducks were symmetrical.

According to Hodges (1974), in White Leghorn birds, the gland constituted 0.07 percent of body weight.

In most species, the weight of preen gland varied with individuals, sex, season and the living habit. Birds which swim and dive, had a large preen gland. The glands of diving ducks (*Aythya*) were relatively heavier than those of dabbling ducks (*Anas*). The land-birds like Galliformes and Passeriformes had relatively large glands, whereas the water-bird lines like the Ciconiiformes, the Columbiformes and the Strigiformes had relatively smaller ones. The preen gland weight was inversely proportional to the body weight of the bird among the Podicipedidae, Phalocrocoracidae, Anatidae and the Oscines. Smaller body had proportionately larger surface area and therefore required larger amount of secretion (Farner *et al.*, 1982).

In the Japanese quail, the preen gland was bilobed measuring 6-10 mm in diameter (Chandrasekar *et al.*, 1990).

Dhande *et al.* (1996) reported that the average combined weight of both the lobes of preen gland of quail was 396.92 ± 44.62 mg. The average length and diameter of the right lobe were 1.17 ± 0.03 cm and 0.56 ± 0.02 cm and those of the left lobe were 1.14 ± 0.02 cm and 0.55 ± 0.004 cm respectively.

Kale *et al.* (1999) observed that the preen gland was larger in broilers than layers.

Sunanda *et al.* (2001b) reported that the two glands in duck were asymmetrical. The right gland was heavier, longer, thicker and wider than the left. The two glands together constituted 0.274 percent of the total body weight.

2.1.2 Lobes

The preen glands were pear shaped in duck, oblong in Japanese quail and pea shaped in chicken (Trautmann and Fiebiger, 1957; Kato, 1968; Spearman, 1971).

In coloured Muscovy and in White Pekin ducks, the two lobes were spread apart and the papillary ends of the lobes were united by an isthmus. Each lobe was rounded at its cranial end and narrowed at the caudal end where it opened to the preen gland duct. The gland was bilobed with a strong band of connective tissue called isthmus at the caudal end. In chicken, the lobes were closely pressed together and V-shaped. The two lobes and the nipple of the gland formed a heart-shaped structure (Lucas and Stettenheim, 1972; Hodges, 1974).

In fowl, the gland was as large as a bean and in the duck and goose as large as a hazelnut (Nickel *et al.*, 1977).

Farner *et al.* (1982) described that all birds had bilobed preen gland, except in Hoopoe (*Upupa epops*) and the European nightjar (*Caprimulgus europaeus*). Hoopoe had three lobes, a small medial lobe and two lateral lobes. In the case of the ducks, the two lobes were separated from each other immediately rostral to papilla almost at right angles.

Dhande *et al.* (1996) stated that in quail, the gland consisted of two lobes separated by a median septum. Each lobe appeared tubular, rounded or oval in shape and had the size of a pea.

2.1.3 Papilla 🐳

Papilla was free of feathers except at the tip, where it possessed a tuft of down feathers called the uropygial wick in birds (Farner *et al.*, 1972).

No cross channel connected the primary cavity of one side with the cavity of the opposite lobe in ducks. Each lobe discharged all of its secretion through a single duct (Mc Lelland, 1975).

Nickel *et al.* (1977) reported that the holocrine tubular gland of fowl discharged their oily secretion into cisternae through a collecting duct system and then passed onto a single pointed papilla.

Certain species of birds such as Pelican had up to 18 orifices in the papilla (King and Mc Lelland, 1981).

Farner *et al.* (1982) explained that in birds, the papilla had at least two ducts, which opened out like a nipple. A clear division was noted between the lobes and the papilla. The external shape of the papilla was cylindrical, conical or slightly alveolar. The cylindrical shape was typical for the Anseriformes, Gruiformes, Garriformes, Piciformes and the Hoopoe and the conical for the

Podicipediformes, Charadriiformes, Strigiformes, Caprimulgiformes, Apodiformes and Cuculiformes.

Dhande *et al.* (1996) noticed that the lumen of each lobe of the gland in quails formed a duct, which passed through a nipple like structure that projected above the level of skin.

Sunanda *et al.* (2001b) noticed that the common papilla of the preen glands in duck was broad and short surrounded by skin with small feathers. It extended from posteriodorsal end of the glands and was directed dorsocaudally. The papilla had a pair of ducts, one from each lobe and they opened through small slits at its tip.

2.1.4 Uropygial Circlet

Lucas and Stettenheim (1972) noticed that an uropygial circlet arranged around the margin of the papillary tip was present in various species of birds studied. In chicken and in White Pekin and coloured Muscovy ducks, no feathers were present in the groove along the midplane of the papilla whereas, they were present in the Red-Crested Pochard breed of chicken. In the adult turkey and quail, the feathers of the circlet were broken.

Farner *et al.* (1982) described that the uropygial orifices were surrounded by a circlet of down feathers in domestic fowl, which were long and arranged like a tuft. These tufts were saturated with gland secretion. Each feather of the uropygial circlet was downy with a rachis and a shaft and had a bilateralsymmetrical formation. These feathers formed the caudal point of the dorsalcaudal tract and covered the eminentia. The number and length of the feathers varied depending on the species of birds. Some species of birds lacked circlet feathers. Chandrasekar *et al.* (1990) found that in the Japanese quail, the circlet consisted of down feathers and was arranged around the margin of the tip of the papilla.

2.1.5 Blood Supply

The arterial supply of the gland was derived from paired branches of caudal artery and the venous drainage was by branches of the caudal vein in fowl (Apandi and Edwards, 1964; Farner *et al.*, 1972).

Lucas and Stettenheim (1972) found that the caudal artery at the level of the first caudal vertebra divided into right and left branches, which supplied the preen gland in White Leghorn chicken whereas, in ducks, it was divided into three branches viz. external, internal and median branches. These branches subdivided after entering the capsule and a few of them were distributed to the papilla. Fine capillaries formed an anastomosing network around the tubules. The main vessel was the caudal vein, which emptied into the hypogastric vein.

Farner *et al.* (1982) opined that in domestic birds arterial supply to the preen gland was through a pair of branches of the caudal artery, which led away from the first tail vertebra. Before entering the gland, each artery was divided into an upper, lower and medial branch. These branches approached the capsule and finally entered the gland at various spots. Further, he noticed that the venous drainage of the gland was through a dorsal and ventral net of vessels covering the capsule. The vessels met at the neck of the gland, became two parallel veins and ran satellite to the artery. Between the second and first caudal vertebrae, they disappeared to join up with the renal portal system.

Aslan *et al.* (2000) found that the vascularization of the preen gland of both the goose and the duck was through the right, left and ventral glandular uropygial arteries, arising from the median coccygeal artery.

2.1.6 Nerve Supply

The preen gland was reported to be innervated through the fibres arising from the first and the fourth pairs of caudal spinal nerves and the sympathetic ganglion (Elder, 1954; Apandi and Edwards, 1964).

Lucas and Stettenheim (1972) and Farner *et al.* (1982) confirmed that both medullary and sympathetic fibres innervated the preen gland in chicken. They arose between the first and second caudal vertebrae and divided into three branches and distributed to skeletal muscles of the area. One branch anastomosed with the sympathetic and somatic nerves and continued as the uropygial nerve.

2.3 HISTOMORPHOLOGY

2.3.1 Capsule

Presence of smooth muscle fibres in the intertubular connective tissue was reported in chicken (Elder, 1954) and in Japanese quail (Farner *et al.*, 1972).

The lobes of the preen gland in birds were separated by an interlobular septum and were joined at their caudal ends by a band of connective tissue known as isthmus (Farner *et al.*, 1972).

The capsule of the preen gland of the adult male single comb White Leghorn chicken contained nerves and smooth muscle fibres. Elastic fibres were numerous among collagen fibres. The latter occurred as separate, uniformly spaced fibres coursing in various directions, generally toward the blind end of the tubules. The occurance of tubular structures representing the duct system was also reported in the capsule (Lucas and Stettenheim, 1972).

Mc Lelland (1975) observed that in chicken, an interlobular septum separated the two halves of the gland. Anteriorly, dorsally and ventrally, the tissue of the septum was continuous with those of the capsule. Posteriorly, they merged with the dense connective tissue of the isthmus.

Farner *et al.* (1982) found that the capsule was thicker at the rostral end of the gland and near the papilla than in the middle part of the lobes in domestic birds. The capsule was formed mainly by a dense network of collagen fibres, arranged radially toward the inner part of the lobe. However, in the European Nightjar, they recorded the presence of dense connective tissue consisting of collagen, reticular and elastic fibres in the capsule. The interlobular tissue also contained blood vessels, capillaries and lymphatic tissue.

In ducks, Sunanda *et al.* (2001c) reported that the capsule was made up of dense irregular connective tissue consisting of collagen, elastic and reticular fibres. Very thin septulae radiated from the capsule to the interior of the gland and separated closely packed secretory tubules.

2.3.2 Lobes

Kendall (1947) described the gland as compound tubular type.

In birds, preen gland was described as branched alveolar by Trautmann and Fiebiger (1957).

The stratified epithelium of secretory tubules consisted of basal, intermediate and transitional cell layers in fowl and duck (Farner *et al.*, 1972).

Lucas and Stettenheim (1972) noticed that the cells of the basal layer represented the reserve cells for the intermediate and transitional (secretory) layers. The cytoplasm of basal and intermediate layers was basophilic. Further

he observed that the preen gland in two-days-old chicks had relatively undifferentiated epithelium that lined the tubules and the cavity. Zones were not established. The basal layer was a single row of low cuboidal cells. Rarely, cells from the intermediate layer was found lying on the basal layer. Cells of both layers were basophilic. The secretory layer adjacent to the lumen had the structural features of the transitional layers.

The secretory tubules showed two zones, viz. an outer or peripheral zone, near the capsule and an inner or central zone, towards the lumen, in fowl and ducks (Ishida *et al.*, 1973).

Wagner and Boord (1975) studied the secretory differentiation in zone I and recognized a layer of basal cells and four layers of secretory cells in chicken.

The preen gland was reported to be holocrine type in fowl, duck and Japanese quail (Uva et al., 1976; Amet et al., 1982; Manna et al., 1982; Petrak 1982).

Farner *et al.* (1982) found that the multistriated glandular epithelium possessed a basement membrane in various species of domestic birds. This glandular epithelium consisted of four different layers, viz. an outer germinative layer with mitotic figures, an intermediate layer consisting of polygonal cells with spherical nuclei, a secretory layer consisting of polygonal cells and a degenerative or transitional layer characterized by pyknotic nuclei and keratohyaline granules in the cytoplasm.

The secretory tubules were arranged radially from the centre towards periphery of the gland in various species of birds (Abalain *et al.*, 1984; Abalain *et al.*, 1985; Jenik *et al.*, 1987; Carpenter and Goodridge, 1988; Fringes and Clorges, 1991).

Bacha and Wood (1990) reported the outer zone or zone I and inner zone or zone II as sebaceous and glycogen zones, in fowl.

In Japanese quail, the zone I consisted of elongated tubules lined by stratified epithelium surrounding an indistinct lumen. The zone II comprised of roughly polygonal tubules organized like typical acini with large lumen. Adjacent to the basement membrane, fusiform myoepithelial cells with centrally located, dark and ovoid nuclei were also noticed. The lumen of the secretory tubules contained cell debris and lamellar fragments in addition to the secretory products (Chandrasekar *et al.*, 1990).

Suzuki (1994) named the three layers in the zones as basal, transitional and degenerating layers in fowl.

Roeskopf and Woerpel (1996) described the gland as compound alveolar.

Kale *et al.* (1999) opined that in fowl, the lobes or adenomeres were pentagonal or hexagonal in shape. The cells of glandular epithelium contained prominent, centrally placed spherical nuclei with one or two nucleoli. The cytoplasm of secretory cells had vacuolated appearance.

Sunanda *et al.* (2001c) found that the gland was simple branched and tubular type. The basal layer of preen gland in both the zones consisted of a single layer of flattened cells in ducks, while the intermediate layer was made of a single layer of polyhedral cells in outer zones and two or three layers of cells in inner zone. Non-functional enlarged cyst-like tubules were observed in peripheral part of the gland.

2.3.3 Papilla

Bradley and Grahame (1950) reported that in fowl, the ducts were initially lined by glandular epithelium, which gradually became flattened or cuboidal type.

The keratinised stratified squamous epithelial lining of the ducts suggested that preen glands were cutaneous derivatives (Spearman, 1971).

Lucas and Stettenheim (1972) observed a system of ducts, which varied widely in diameter, in the capsular connective tissue of preen gland of chicken. They consisted of a large duct, several small ducts and small aggregates of ducttype cells without any lumen. The wall was made up of a single epithelial layer with occasional flattened replacement cells arranged on a basement membrane. The papilla of the adult chicken had no longitudinal muscles, but abundant transverse muscles were found between the ducts and the follicles.

In domestic birds, the ducts from two lobes of the preen gland were merged near the apex of the papilla, and opened into a single orifice. Lamellar corpuscles were encountered in the papilla (Hodges, 1974; King and Mc Lelland, 1975).

Farner *et al.* (1982) opined that there were three types of papillae structurally, namely the compact, delicate and the wart-like, in Passeriformes. The compact type of papilla consisted of dense connective tissue of the interlobular septum, which continued through the papilla and completely surrounded the ducts. Longitudinal and circular layers of smooth muscle were typical of this type of papilla. The arrangements of smooth muscle bundles in the papilla were of three kinds namely, the circular, longitudinal and numerous transverse bundles. Papilla of the compact type was either conical or cylindrical with a tuft. The delicate type of papilla had ducts with very wide lumen. They

were very broad at the base and became more slender toward the apex. Herbst corpuscles were present even in papillae without feather circlet. The intermediate type of papillae was very short and broad. Their ducts were broader with a distinct sphincter musculature. Wart-like papilla of the Passeriformes had a special valve-apparatus composed of dense collagenous and elastic connective tissue. The valves prevented a backflow of sebum from the papilla into the lobes. This type of papilla possessed a large number of Herbst corpuscles.

Chandrasekar *et al.* (1990) found that the subterminal portion of the papilla of the preen gland of Japanese quail revealed two ducts. It was composed of connective tissue and smooth muscle fibres arranged both in longitudinal and circular fashion.

The papilla arose from the isthmus in chicken (Banks, 1993; Dyce *et al.*, 1996).

The papilla had two ducts, each of which drained the secretion from the right and left primary cavities. The epithelium of the ducts were of columnar initially, but later became transformed into keratinized stratified squamous near the tip of the papilla. The epithelium of the initial portion of ducts was surrounded by connective tissue layer followed by longitudinally arranged smooth muscle fibres and skin (Dellmann and Carither, 1996; Sunanda *et al.*, 2001c).

2.4 HISTOCHEMISTRY

Marshall (1960) observed two distinct zones, viz. an outer sebaceous zone with high esterase activity indicating a rapid production of lipids and an inner glycogen zone characterized by the presence of glycogen, acid phosphatase and osmic acid soluble lipid, in the preen gland of domestic fowl and duck. The esterase activity was greater in the ducks than in the fowl. Apandi and Edwards (1964) reported that the secretory product of preen gland in goose and duck was characterized by the presence of calcium.

The secretion of preen gland in fowl contained both saponifiable and non-saponifiable lipids but no cholesterol. The secretory cells were rich in nonspecific esterase and acid phosphatase (Spearman, 1971).

In the glands of fowl and ducks, histochemical study showed that each gland had a peripheral sebaceous zone and an inner glycogen zone. In the sebaceous zone, the cells developed lipoid droplets as they moved towards the lumen. Then they enlarged and disintegrated, leaving corneous laminae. High esterase activity in the sebaceous zone indicated rapid production of lipids (Farner *et al.*, 1972; Hodges, 1974).

Chandrasekar *et al.* (1990) stated that in Japanese quail, the basal cells of the inner zone showed a strong positive reaction for PAS whereas, the intermediate and transitional layers showed a moderate reaction. The luminal contents in both these zones were strongly positive.

According to Kale *et al.* (1999), the PAS reaction was intense in the capsule and the septae of the gland while the reaction was weak in the preenocytes, in fowl.

Sunanda *et al.* (2001a) opined that in ducks, the Schiff's reaction was moderate in basal and intermediate cell layers and weak in transitional cell layers of both the zones. Moderate Feulgen reaction was observed in the basal and intermediate layers of both the zones.

In the preen gland of ducks, neutral mucopolysaccharides were observed in basal and intermediate cells of both the zones of secretory tubules. The

epithelial cells in both the zones were rich in lipids. The alkaline phosphatase activity was moderate in basal and intermediate cells and weak in transitional cells of outer zone. However, the activity was weak in basal and intermediate cells and intense in transitional cells of inner zone. Intense acid phosphatase activity was observed in basal and intermediate cells of both the zones, while it was weak in transitional cells (Sunanda *et al.*, 2001d).

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Materials and methods

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3. MATERIALS AND METHODS

The preen glands were collected from 44 apparently healthy ducks of various age groups and for their morphological and histological development from the day of hatch till 150 days of age were studied. Ducklings of the same hatch were procured from the University Poultry Farm, Mannuthy and reared under intensive system of management. The ducklings were not given any vaccination. Feed and water were provided ad lib.

The study was carried out in birds of different age groups, ranging from day-old to 150 days as shown in Table 1. From the day of hatch up to 30 days, the gland was collected at five days interval and thereafter at one-month interval.

2.1 Morphology

The body weight of the birds was recorded. The preen glands collected by dissection were washed in normal saline, mopped with blotting paper and examined for the gross appearance, colour and shape. The various biometric observations viz. length, width, thickness and weight of each glandand the cranial, interspace and caudal width of both the glands together were recorded. The anatomical position and relationship of the gland were studied in the culled birds from the University Poultry Farm, Mannuthy.

2. 2 Histology

Each gland and the papilla were cut across into small pieces. A few specimens were cut lengthwise to study the relation of secretory tubules with the primary cavity, ducts and the papilla. The tissues were fixed in different fixatives as follows.

- 1. 10% neutral buffered formalin
- 2. Zenker's fluid
- 3. Bouin's fluid
- 4. Chilled acetone (4° C)

Frozen sections of 20µm thickness were also taken for histochemical studies.

After fixation in the appropriate fixatives, the materials were processed for paraffin embedding. Tissues for the histological techniques were processed in high melting paraffin (MP 58-60°C) and sections of 4-5 μ m thickness were made.

The following histological staining techniques were employed on paraffin sections

- 1. Ehrlich's haematoxylin and eosin staining technique for routine histological studies (Luna, 1968).
- 2. Mallory's phosphotungstic acid haematoxylin (PTAH) method for collagen fibres (Luna, 1968).
- 3. Gomori's aldehyde fuchsin technique for elastic fibres (Carleton, 1957).
- 4. Gridley's method for reticular fibres (Gridley, 1951).
- 5. Gomori's one step trichrome method for connective tissue and muscle fibres (Luna, 1968).
- 6. Van Gieson's method for collagen (Luna, 1968)
- 7. Toluidine Blue method for metachromasia (Luna, 1968)

For the histochemical studies, the following methods were employed.

- Periodic Acid schiff's (PAS) reaction for carbohydrates (Bancroft and Stevens, 1977).
- Alcian blue method for the demonstration of acid mucopolysaccharides (Singh and Sulochana, 1996).

- 3. Best's Carmine method for glycogen (Luna, 1968).
- 4. Oil red 'O' in propylene glycol method for lipids (Luna, 1968).
- 5. Modified Gomori's method for alkaline phosphatase activity (Pearse, 1977).
- 6. Modified Gomori's method for acid phosphatase activity (Pearse, 1977).

The data on the following physical parameters were analyzed (Snedecor and Cochran, 1985) to find the significance, if any:

- 1. Age on combined weight of the preen glands.
- 2. Body weight on the combined weight of the preen glands.
- Age on the percentage contribution of combined preen gland weight to the body weight.
- 4. Age and body weight on the weight of right and left preen glands
- 5. Age and body weight on the physical parameters of right and left preen glands.

Correlation of the above parameters and the percentage contribution of right and left glands to the combined preen gland weight were also studied. The multiple regression analysis of the gland weight on the body weight and age was also done (in days).

2.3 Micrometry

Micrometry was done on the following parameters,

- 1. Thickness of the capsule in different age groups.
- 2. Width of the zone I in different age groups
- 3. Width of the zone II in different age groups.
- 4. Width of the primary cavity in different age groups.

These data were analyzed (Snedecor and Cochran, 1985) for the following to find the significance, if any:

- 1. Age on the thickness of capsule.
- 2. Age on the width of zone I and zone II.
- 3. Age on the width of primary cavity.

Correlation of the above parameters was studied.

Group number	Age (in days)	Number of ducks	Average body weight (mean ± SE)
1	Day old	4	42.795±1.657
2	5	4	55.343±1.770
3	10	4	66.050±4.417
4	15	4	83.900±3.798
5	20	4	105.865±5.456
6	25	4	148.110±3.950
7	30	4	259.822±7.022
8	60	4	1205.000±5.000
9	90	4	1510.000±5.962
10	120	4	1550.000±7.149
11	150	4	1575.000±7.078
	TOTAL	44	

Table 1. Age, number and body weight of ducks

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Results

RESULTS

4.1 GROSS OBSERVATIONS

4.1.1 Shape

The preen gland in duck was a paired organ. The two glands were separated at the anterior one third whereas, at the posterior two thirds, they were joined by an isthmus and together formed a 'V' shaped structure. The isthmus was surrounded by a skin with small feathers, which extended from the posterodorsal end of the glands and directed caudodorsally. Each gland was pear shaped and pale yellow in colour, in fresh state. Each gland had independent duct, which opened separately on to a common papilla. The papilla was short, broad and cylindrical in shape (Fig. 1). Centrally, each gland possessed a small primary cavity, which was rounded at its cranial end. The caudal end of the cavity was narrow where it continued with the duct (Fig. 2).

The uropygial circlet or uropygial wick was seen as a circlet of downy feathers at the tip of the papilla. No feathers were observed in the midplane between the two openings of the ducts. The circlet feathers made a circle around each orifice and formed a double tuft, which was shorter than the length of the gland. The right and left glands were separated from one another by an interglandular septum. Immediately rostral to the papilla both the glands were spread apart almost at right angles to each other, while the papillary ends of the glands. The tip of the papilla showed two protuberances, each with a slit like orifice at its centre.

4.1.2 Weight

The weight of the preen glands at various ages is shown in Table 2. Table 3 records the relationship between the weight of the preen gland and the body weight in different age groups.

On the day of hatch, the average combined weight of the preen glands was 0.15 ± 0.01 g of which, the right gland contributed 0.08 ± 0.01 g and left gland 0.07 ± 0.01 g. This mean weight represented 0.36 per cent of the body weight. At five days of age, there was a three-fold increase in the weight of right, left as well as the combined gland weight. The percentage contribution of preen gland to the body weight was 0.88 percent at this stage. Thereafter the gland grew less rapidly than the body. The left and right glands increased in weight progressively from day-old to 150 days of age. The preen gland weight was positively correlated with age (r = 0.954) and the body weight (r = 0.996) (Figs. 3 and 4).

A rapid growth was noticed during the first 30 days of hatch and thereafter the rate of growth was slower (Fig.5). The maximum mean weight was reached at 150 days of age $(5.31 \pm 0.23 \text{ g})$ that represented 0.34 per cent of the body weight. A slight increase in the mean weight occurred between 60 and 150 days of age. The weight of right and left glands as well as their combined weight was significantly higher after 30 days of age. However, the proportionate combined weight of the gland to body weight decreased after 30 days. A negative correlation existed between the age and the percentage contribution of the preen gland weight to the body weight (r = -0.678). The right gland was significantly heavier than the left in all the age groups studied (Fig. 6). In multiple regression analysis, when the body weight of the bird was kept constant, three percent of the total variation in the weight of preen gland was accounted for

by age and when the age of the bird was kept constant 97 percent of the variation in the weight of preen gland was accounted for by the body weight.

4.1.3 Size

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Table 4 records the length, width and thickness of the right and left glands and the cranial, interspace and caudal width of the two glands together in different age groups.

The length of the right and left glands showed an increasing trend with the advancement of age (Fig. 7). Width of both the right and left glands showed an high positive correlation with the age(r = 0.954 and r = 0.954 respectively) and body weight (r = 0.947 and r = 0.961 respectively). The right gland was significantly longer than the left.

The width of both the preen glands also increased with the advancement of age (Table 4; Fig.8). A high positive correlation existed between the width of both the right (r = 0.916) and (r = 0.912) left glands with the age and the body weight (r = 0.942 and r = 0.938 for the right and left glands respectively). The right gland was significantly wider than the left in all the age groups studied.

The thickness of the right and the left glands also showed an increasing trend with the advancement of age (Fig. 9). The thickness was positively correlated with the age (r = 0.898 and r = 0.915 for the right and left glands respectively) and the body weight (r = 0.936 and r = 0.936 for the right and left glands respectively). The right gland was significantly thicker than the left in all the age groups.

At five days of age both the right and left glands showed a two-fold increase in its length, width and thickness. Thereafter the increase was gradual up to 150 days of age. The cranial, interspace and caudal width increased gradually with the advancement of age (Table 5). These parameters also showed positive correlation with the age (r = 0.955, 0.922 and 0.876, respectively) and the body weight (r = 0.973, 0.981 and 0.918, respectively).

4.1.4. Relations

The preen glands were located on the dorsal surface of the coccygeal vertebrae one on either side extending from the caudal part of fourth coccygeal to the cranial one-fourth of seventh coccygeal vertebrae in the synsacrocaudal region (Fig. 10). The dorsal surface of the gland was convex while the ventral surface was flattened due to its relation with the tail muscles. The gland was related to the muscles levator coccygeus and a pair of coccygeus lateralis. The former ran underneath the gland while the latter flanked the gland on both sides. The gland was embedded in a cushion of fat in the subcutis.

4.1.5 Blood and Nerve Supply

The arterial supply was through a pair of branches arising from the caudal artery at the level of first caudal vertebra (Fig. 10). Then it followed along the body of the first and second caudal vertebrae and passed along the neural spines of the remaining free caudal vertebrae. Caudally, it ran alongside the furrow between the levator coccygeus and lateral coccygeus muscles. Before entering the gland, each artery divided into upper, lower and medial branches and penetrated the capsule from above, below and middle, respectively.

The venous drainage was through a pair of parallel veins, which ran satellite to the branches of the caudal artery. Between the second and the first caudal vertebrae, they disappeared to join up with the renal portal system. The preen gland was innervated by the branches of medial caudal nerve (Fig. 10). The nerve was a branch from the pudendal plexus.

4.2. MICROSCOPIC OBSERVATIONS

4.2.1. Histomorphology

The histological appearance of the preen gland did not vary much among the different age groups studied except for the day-old ducklings. However, micrometry showed significant differences.

On the day of hatch, the tubules of the inner zone and the primary cavity had an undifferentiated epithelium (Fig. 11). However, the tubules of the outer zone had all the three layers of epithelia as those of the other groups studied.

The gland was covered by a connective tissue capsule. From the capsule, numerous intertubular septae extended into the parenchyma of the gland (Fig.12). Anteriorly, dorsally and ventrally the septae were continuous with the capsule. Posteriorly, they merged with the dense connective tissue of the isthmus and the glandular wall became correspondingly thin. The capsule and the septae composed of dense irregular connective tissue composed of collagen (Fig.13) and reticular fibres (Fig. 14). Elastic and smooth muscle fibres were not detected either in the capsule or in the interglandular septum. The cellular components of the capsule were the fibroblasts and occasional multipolar neurons. The connective tissue was abundant in the zone II (Fig. 15) and also near the primary cavity. However, it was meager in zone I. The capsule and interglandular septae contained numerous blood vessels (Fig. 16). The two glands at their caudal ends were connected by an isthmus composed of collagen and reticular fibres (Fig. 17). There was no parenchymatous connection in between (Fig. 18).

From the capsule and the interglandular septa, thin strands of connective tissue fibres invaded the parenchyma and formed the supportive framework of the gland (Fig. 16). These strands surrounded each group of cells in the tubules of both the zones. The intertubular connective tissue showed blood vessels and capillaries. No basement membrane was seen between the tubular epithelium and the connective tissue.

The preen gland of the duck was of simple, branched, tubular and holocrine type. The secretory acini were made up of straight tubules with multiple branches. The tubules were arranged in a radiating manner from the centre towards the periphery of the gland. These tubules terminated near the capsule with blind ends.

The tubules showed two zones viz. an outer zone or zone I near the capsule and an inner zone or zone II towards the primary cavity (Fig. 19). The outer two thirds of the secretory tubules consisted the zone I whereas, the inner one third, the zone II. The epithelium of the secretory tubules was stratified and consisted of basal, intermediate and transitional cell layers.

The lumen of the secretory tubules contained both secretory products and cellular debris.

The zone I consisted of elongated tubules lined by stratified epithelium surrounding a small or indistinct lumen (Fig. 12). A single layer of flattened cells with darkly staining granular cytoplasm and vesicular nucleus formed the basal layer. However, in certain tubules, the basal layer consisted of more than one layer of flattened cells. In some cells clear cytoplasm was noticed around a deeply staining condensed nucleus. Next to the basal layer was a single layer of intermediate cells of polyhedral shape with irregular nucleus and slightly acidophilic cytoplasm. They were characterized by the presence of large number of small spherical lipid droplets. The irregular nucleus was either centrally placed or pushed to the periphery of the cells depending upon the size of the lipid droplets. Inner to the intermediate layer was the transitional layer. It consisted of several layers of polyhedral cells. These cells were filled with larger lipid droplets and hence appeared to be vacuolated and pale with H & E staining. The nuclei of the transitional cells were pyknotic. Towards the lumen, the cells showed disintegrative changes (Fig. 20).

In the inner zone or zone II, the tubules consisted of a single layer of basal cells followed by an intermediate layer with one or two layers of polyhedral cells. The transitional layer was made up of only a few layers of cells (Fig. 21).

Structurally, the cells in all the three layers of zone II resembled those of the zone I but the tubules in zone II possessed a wider lumen (Fig. 21).

The basophilic cytoplasm of the basal cells and the acidophilic cytoplasm of the intermediate cells gave the peripheral part of the epithelium of the secretory tubules a darker appearance as against the vacuolated and pale staining cytoplasm of the transitional cells (Fig. 21). The basal and intermediate layers of tubules in both the zones were intensely stained with Toluidine Blue (Fig. 22).

The primary cavity was lined by one or two layers of columnar epithelium with spherical nuclei (Fig. 23).

Myoepithelial cells could not be detected around secretory tubules in any of the groups studied.

A few dilated tubules were noticed randomly among the secretory tubules in ducks below 20 days of age (Fig. 24). The secretory tubules showed various stages of epithelial desquamation. Certain tubules were lined by only one or two layers of cells whereas, certain others were totally devoid of any epithelium at all.

The papilla had two ducts, each of which drained the secretion from the primary cavities of the right and the left glands separately. The primary cavity of each gland converged to form a duct near the papilla. The ducts were initially lined by one or two layers of columnar epithelium with spherical nucleus (Fig.25). Near the tip of the papilla the ducts were lined by keratinized form of stratified squamous epithelium. It consisted of a basal membrane, one or two layers of polyhedral cells with large spherical nuclei, three to five layers of large polygonal cells, four to six layers of flattened cells with pyknotic nuclei and a number of keratin layers (Fig. 26). The subterminal portion of the duct was also lined by stratified squamous epithelium but with one or two keratin layers.

At the initial portion of the duct, the epithelium was surrounded by a connective tissue layer that was continuous with the interglandular septum and followed by longitudinally arranged smooth muscle fibres and skin. The connective tissue layer was of dense irregular type and made up of collagen (Fig. 27) and reticular fibres. Elastic fibres were absent. Numerous circlet feather follicles were noticed at the tip of the papilla (Fig. 28).

The epithelium of the ducts, near the tip of the papilla, was surrounded by feathered skin. Longitudinally arranged smooth muscle fibres were noticed in the initial portion of the ducts. However, smooth muscles were absent near tip of the papilla. Smooth muscle fibres were located in the connective tissue layer without any connection to the feather follicles.

Numerous lamellar corpuscles were present near the circlet feather follicles (Fig.29).

4.2.2. Histochemistry

The capsule and the interglandular septum were strongly positive for PAS while a weak reaction was noticed in the cell layers of secretory tubules. The luminal contents in both the zones were strongly positive. The basal layer and the intermediate layer in both the zones showed moderate PAS positive reaction. The transitional layer showed a weak positive reaction (Fig. 30).

The lipid was uniformly distributed in all the three epithelial layers of both the zones. The capsule and septae were devoid of lipids (Fig. 31).

The acid phosphatase activity was moderate in the basal and intermediate layers in both the zones, whereas an intense activity was noticed in the transitional layers (Fig. 32).

The alkaline phosphatase activity was moderate in the basal and intermediate layers and weak in transitional layer of zone I. In the zone II, it was moderate in the basal and intermediate layers and intense in the transitional layer (Fig. 33). The enzyme activities were absent in the capsule and the septae.

4.2.3. Micrometry

4.2.3.1. Thickness of the Capsule

Capsular thickness gradually increased from day-old to 150 days of age (Table 6). It was positively correlated with the age of the duck (r = 0.700) (Fig. 34).

4.2.3.1. Width of Zone I, II and Primary Cavity

Width of the two zones and the primary cavity increased correspondingly with the advancement of age (Table 6). Positive correlation was noticed between the age and the width of the zone I (r = 0.845), zone II (r = 0.868) and the primary cavity (r = 0.071) (Fig. 35).

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Age in days	Weight of preen gland (g)						
	Combined	Right	Left				
Day old	0.153±0.15	0.083±0.005	0.070±0.007				
5	0.485±0.022	0.248±0.012	0.237±0.010				
10	0.538±0.034	0.275±0.018	0.262±0.016				
15	0.593±0.031	0.297±0.017	0.295±0.014				
20	0.660±0.029	0.333±0.016	0.328±0.013				
25	0.992±0.060	0.497±0.030	0.495±0.031				
30	1.350±0.032	0.677±0.016	0.672±0.017				
60	3.747±0.061	1.877±0.030	1.870±0.031				
90	5.225±0.213	2.618±0.107	2.608±0.107				
120	5.240±0.147	2.630±0.143	2.610±0.143				
150	5.310±0.231	2.660±0.105	2.650±0.104				

Table 2. Weight of preen glands at different ages (mean \pm SE)

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Age in days	Percentage weight of preen gland to body weight
Day old	0.357
5	0.876
. 10	0.814
15	0.707
20	0.623
25	0.670
30	0.519
60	0.311
90	0.346
120	0.338
150	0.337

Table 3. Perce	nt weight o	f preen glan	d (to body we	eight) at different a	ges
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Age in	Leng	th (cm)	Width	n (cm)	Thickne	ess (cm)	Cranial	Interspace	Caudal width
days	Left	Right	Left	Right	Left	Right	width (cm)	width (cm)	(cm)
Day old	0.69±0.01	0.76±0.04	0.30±0.02	0.31±0.02	0.26±0.02	0.26±0.01	1.05±0.05	0.55±0.00	0.39±0.01
5	1.16±0.04	1.17±0.03	0.54±0.04	0.54±0.02	0.45±0.03	0.47±0.01	1.65±0.06	0.92±0.02	0.37±0.01
10	1.17±0.05	1.19±0.03	0.55±0.02	0.57±0.02	, 0.46±0.01	0.46±0.01	1.60±0.02	0.76±0.06	0.40±0.03
15	1.19±0.05	1.22±0.03	0.57±0.01	0.57±0.02	0.44±0.02	0.45±0.02	1.59±0.04	0.86±0.02	0.47±0.04
20	1.42±0.08	1.39±0.07	0.61±0.04	0.65±0.02	0.54±0.03	0.54±0.01	1.72±0.05	0.85±0.03	0.59±0.02
25	1.60±0.08	1.57±0.07	0.65±0.04	0.69±0.03	0.56±0.04	0.57±0.01	1.91±0.07	0.89±0.01	0.81±0.05
30	1.76±0.05	1.75±0.05	0.77±0.02	0.77±0.02	0.64±0.01	0.63±0.01	2.21±0.16	1.19±0.11	1.19±0.06
60	2.29±0.05	.2.31±0.04	0.96±0.01	0.97±0.01	0.80±0.00	0.79±0.03	2.92±0.21	0.90±0.05	1.20±0.03
90	2.61±0.06	2.66±0.06	1.11±0.03	1.12±0.01	0.92±0.02	0.92±0.03	3.64±0.02	2.04±0.07	1.19±0.03
120	2.61±0.07	2.67±0.04	1.11±0.05	1.12±0.05	0.94±0.03	0.94±0.01	3.62±0.08	2.19±0.05	1.24±0.02
150	2.75±0.07	2.84±0.05	1.11±0.03	1.11±0.06	0.87±0.07	0.89±0.06	3.79±0.04	2.04±0.11	1.22±0.01

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Table 4. Physical parameters of preen glands at different age groups (mean \pm SE)

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Table, 5	Correlation of	nhysical	parameters of pre	en gland with ag	ge and body we	hight of bird
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Correlati on	Age	Body weight	Total gland weight	Right gland weight	Left gland weight	Right gland length	Left gland length	Right gland width	Left gland width	Right gland thickn ess	Left gland thickn ess	Cranial width	Intersp ace	Caudal width
Age		0.944	0.954	0.954	0.654	0.932	0.945	0.916	0.912	0.898	0.915	0.955	0.922	0.876
Body weight	0.94 4		0.996	0.996	0.996	0.947	0.961	0.942	0.938	0.936	0.936	0.973	0.981	0.918

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Age groups	Capsule thickness	Width (µm)						
	(µm)	Zone I	Zone II	Primary cavity				
Day old	16.00±1.11	46.29±1.35	83.96±1.31	105.62±2.12				
5	28.71±1.10	64.46±1.99	109.96±1.75	226.21±5.34				
10	39.00±1.35	93.71±1.10	135.96±1.35	303.33±5.17				
15	38.46±1.16	103.46±1.23	139.21±1.66	362.37±3.26				
20	37.92±1.08	108.87±1.31	135.96±1.35	384.58±4.35				
25	34.67±1.50	112.67±1.50	146.79±1.23	415.75±7.66				
30	39.00±0.78	119.17±2.17	153.29±2.34	482.46±6.63				
60	47.67±1.28	140.83±1.69	176.58±1.73	509.17±4.93				
90	42.79±1.23	141.37±1.80	182.00±1.75	558.46±4.46				
120	47.67±1.28	151.12±1.31	190.67±1.86	624.00±5.59				
150	46.04±1.35	156.54±1.23	191.75±2.50	634.83±5.29				

Table 6. Micrometrical parameters of preen gland at different age groups (mean \pm SE)

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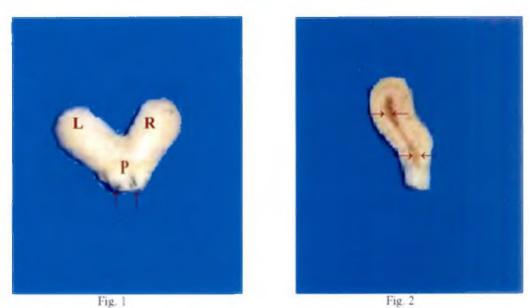


Fig. 10

Figure 1 = Photograph of the right (R) and the Left (L) preen glands from a 90 days old duck showing their shapes and the opening of the ducts (arrows) P-Papilla Figure 2 – Photograph of right preen gland cut lengthwise from a 90 days old duck showing the primary

cavity (arrows) Figure 10 – Photograph of the preen gland from a 120 days old duck showing the caudal artery (red) and

P – Papilla

U Uropygial circlet

L Left gland

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Fig. 1

medial caudal nerve (green).

R-Right gland

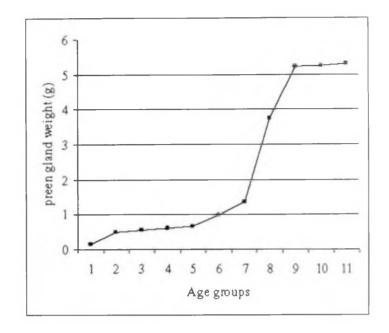


Fig. 3 Relationship between the age and combined weight of the preen gland

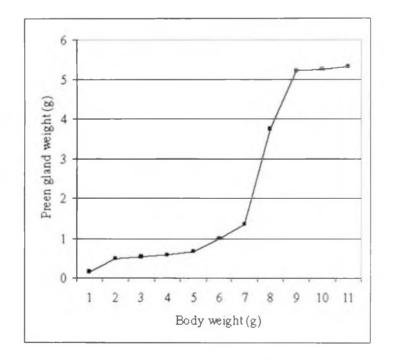


Fig. 4 Relationship between the body weight and the combined weight of the preen gland

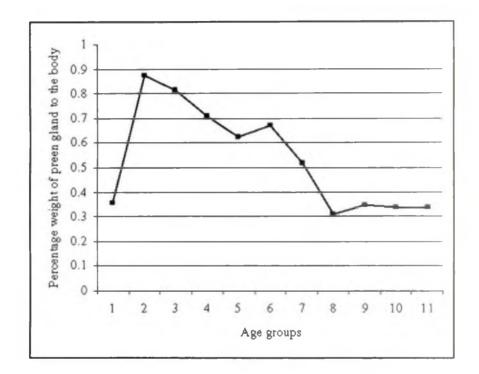


Fig. 5 Relationship between the age and percent contribution of the preen gland to the body

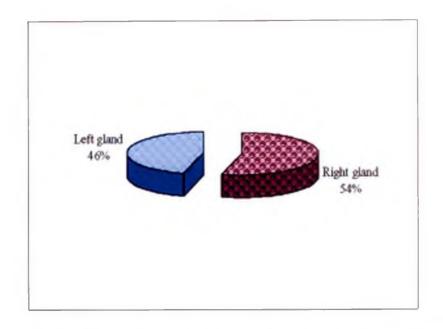


Fig. 6 Percentage contribution of the right and left preen glands to the combined gland weight

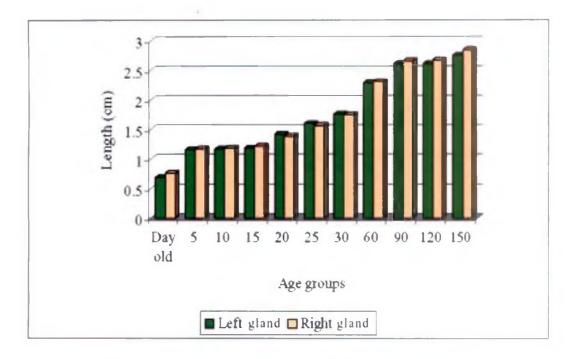


Fig. 7 Relationship between age and length of the left and right preen gland

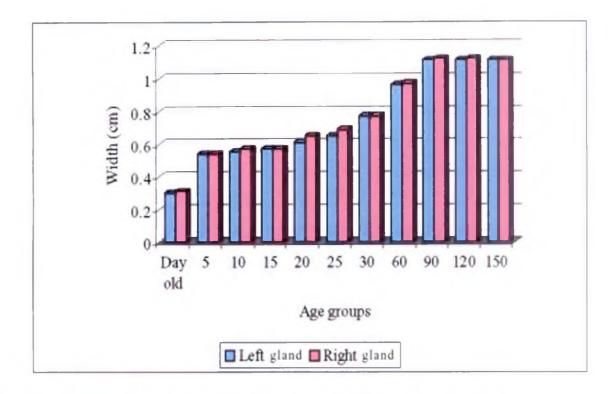


Fig. 8 Relationship between age and width of the left and right preen gland

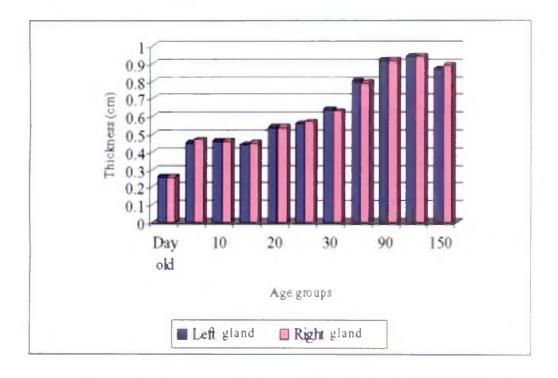


Fig. 9 Relationship between age and thickness of the left and right preen gland



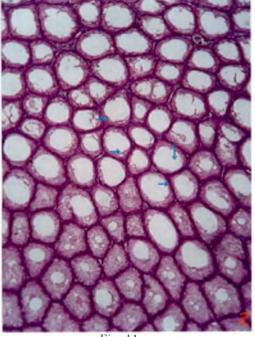


Fig. 11

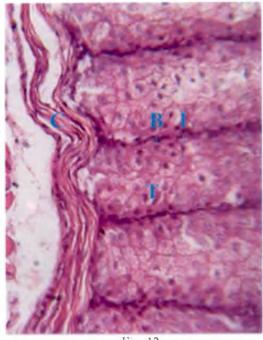
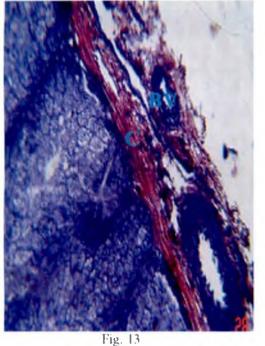


Fig. 12



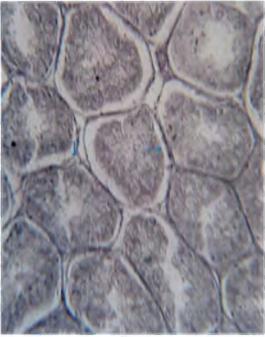
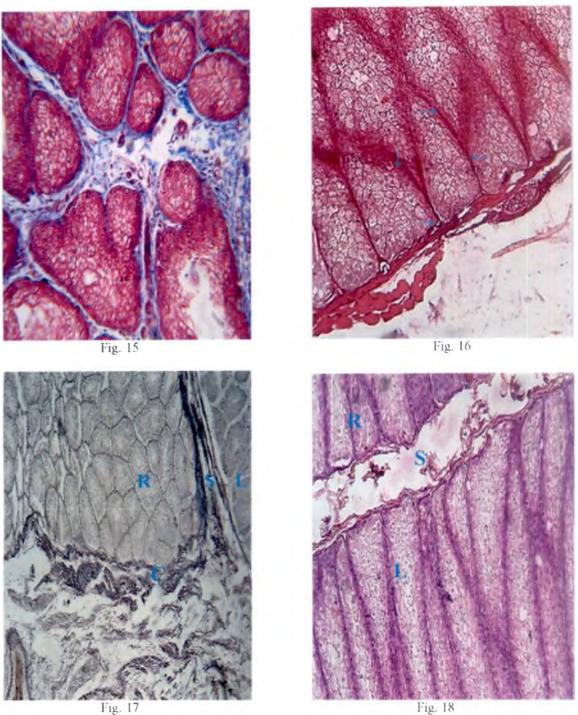


Fig. 14

- Figure 11 Section of the preen gland (day old duck) showing undifferentiated epithelium (arrows) in the inner zone H&FX100
- Figure 12 Section of the preen gland (20 days old duck) showing different layers in the tubules of outer zone

C - Capsule B - Basal layer (arrow) I - Intermediate layer (arrow) T - Transitional layer H&EX 100

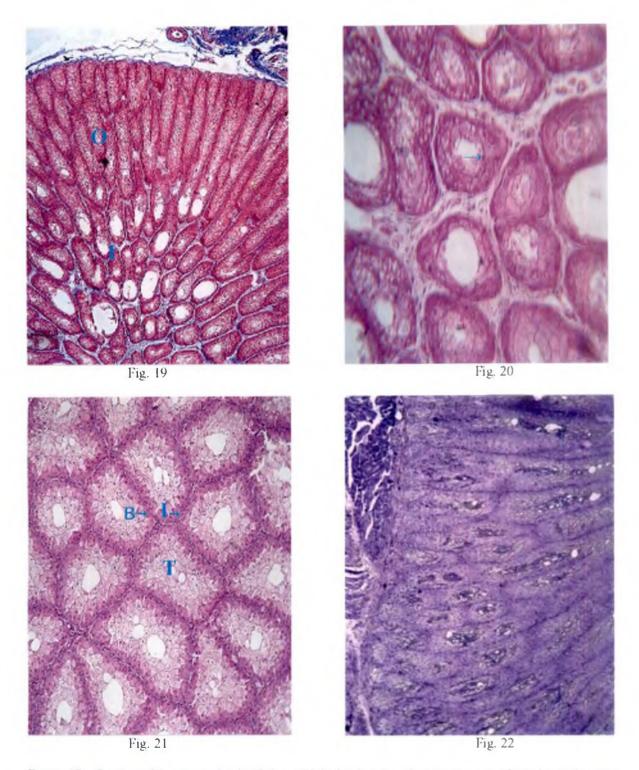
- Figure 13 Section of the preen gland (30 days old duck) showing collagen fibres and blood vessels (BV) in the capsule (C) Mallory's PTAH method X 100
- Figure 14 Section of the preen gland (60 days old duck) showing reticular fibres in the intertubular septa of outer zone (arrows) Gridley's method X 100



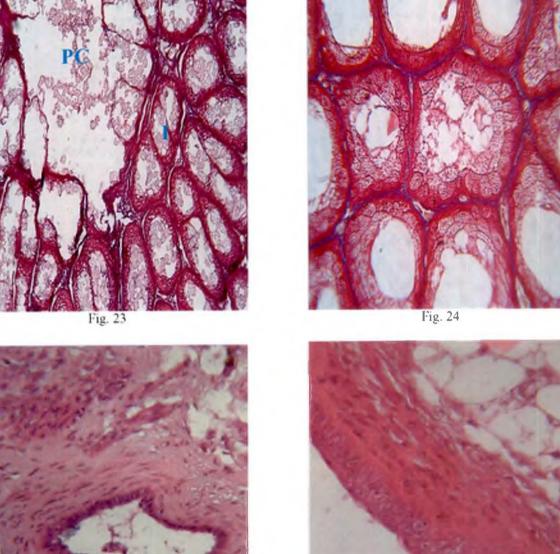
- Fig. 18
- Figure 15 Section of the preen gland (90 days old duck) showing abundance of collagen fibres in the intertubular septa of inner zone Trichrome method X 400 Figure 16 - Section of the preen gland (25 days old duck) showing the arrangement of intertubular connective tissue in the outer zone (arrows) H&EX 100

Figure 17 -Section of the preen gland (15 days old duck) showing the reticular fibres in the interglandular septum C - Capsule R - Right gland L-Left gland S = Septum Gridley's method X 100

Figure 18 - Section of the preen gland (120 days old duck) showing the interglandular septum. Note the absence of parenchymatous connection between the glands H&EX 100



- Figure 19 Section of the preen gland (10 days old duck) showing the arrangement of tubules in the outer (O) and the inner zone(I) Trichrome method X 100
- Figure 20 Section of the preen gland (5 days old duck) showing disintegration changes in the tubules of inner zone (arrows) H & E X 100
- Figure 21 Section of the preen gland (150 days old duck) showing different layers in the tubules of inner zone. Note the darker appearance of the basal and intermediate layer and lighter appearance of transitional layer B Basal layer (arrow) I Intermediate layer (arrow) T Transitional layer H & E X 400
- Figure 22 Section of the preen gland (25 days old duck) showing metachromasia of basal and intermediate layer in the tubules of outer zone Toluidine Blue method X 100



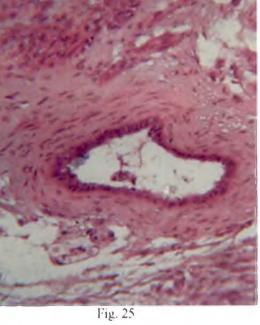


Fig. 26

- Figure 23 Section of the preen gland (20 days old duck) showing the primary cavity (PC) I-Inner zone H&EX100
- Figure 24 Section of the preen gland (5 days old duck) showing dilated tubules in the outer zone Trichrome method X 100
- Figure 25 Section of the preen gland papilla (20 days old duck) showing initial portion of a duct lined by H & E X 400 columnar epithelium
- Figure 26 Section of the preen gland papilla (30 days old duck) showing terminal portion of the duct lined by keratinized type of stratified squamous epithelium H&EX400

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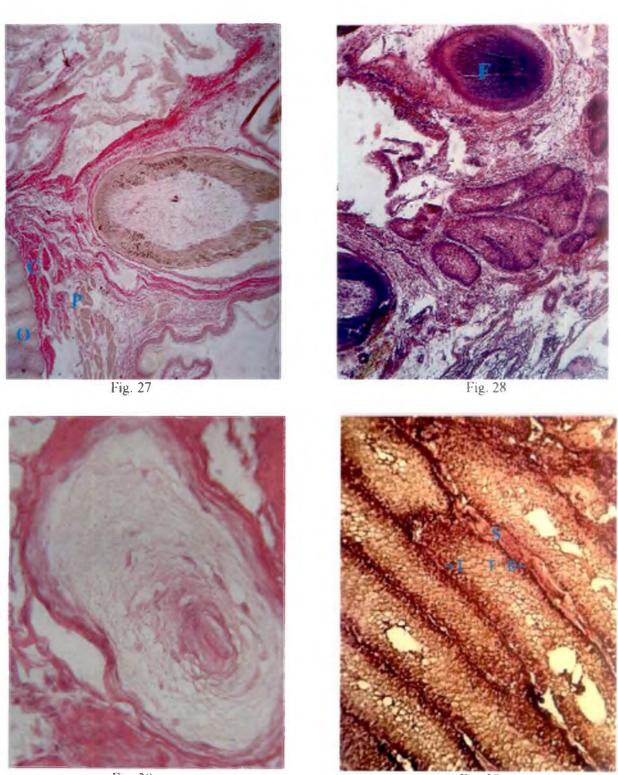


Fig. 29

Fig. 30

- Figure 27 Section of preen gland (90 days old duck) showing collagen fibres in the papilla

 C- Capsule
 O Outer zone
 P- Papilla
 Van Gieson's methodX 100

 Figure 28 Section of the preen gland papilla (60 days old duck) showing feather follicle (F)
 H & E X 100
- Figure 29 Section of the preen gland (20 days old duck) showing lamellar corpuscle in the papilla
 - H&EX 400
- Figure 30 –Section of the preen gland (90 days) showing intense PAS positive reaction in the intertubular septa (S) and basal layer (B) and moderate reaction in the intermediate (I) layer. T Transitional layer (weak reaction) PAS method X 100

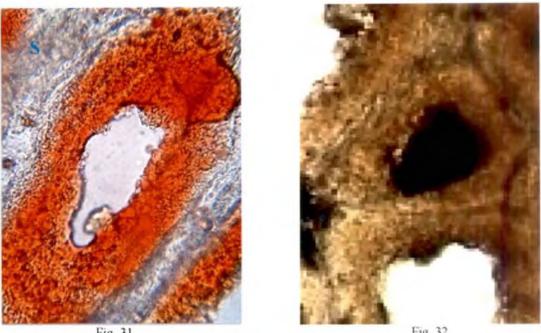


Fig. 31

Fig. 32

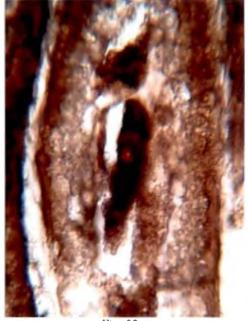


Fig. 33

Figure 31 - Section of the preen gland (90 days old duck) showing heavy accumulation of lipid in the tubules of outer zone S – Intertubular septa

Oil Red O in Propylene glycol method X 400

- Figure 32 Section of the preen gland (60 days old duck) showing intense acid phosphatase activity in the transitional layer and weak activity in the basal and intermediate layers of inner zone Modified Gomori's method X 400
- Figure 33 Section of the preen gland (150 days old duck) showing moderate alkaline phosphatase activity in the basal and intermediate layer and a strong activity in the transitional layer of inner zone. Modified Gomori's method X 400

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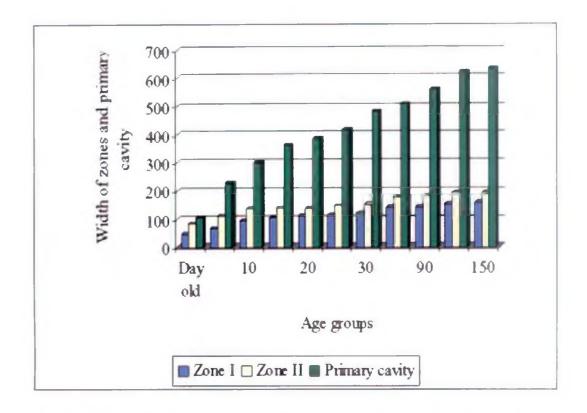


Fig. 35 Relationship between age and micrometrical parameters (µm)

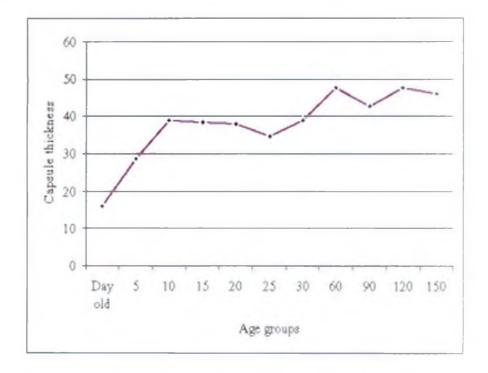


Fig. 34 Relationship between age and capsule thickness (µm)

Discussion

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5. DISCUSSION

In ducks, the preen gland was a paired organ. The glands of both sides together had a 'V' shape with the apex directed backwards. In fresh state, each gland was pale yellow in colour and pear shaped. These are in accordance with the findings of Trautmann and Fiebiger (1957) in various species of domestic birds. The glands were reported to be oblong in Japanese quail (Lucas and Stettenheim, 1972). In fowl and Japanese quail, Spearman (1971), Farner *et al.* (1982) and Dhande *et al.* (1996) reported that preen gland consisted of two lobes and a papilla. Nickel *et al.* (1977) reported that the gland was bean shaped in fowl and hazelnut shaped in duck and goose.

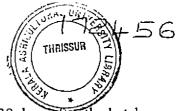
The common papilla was short, broad and cylindrical in shape as reported earlier in ducks (Lucas and Stettenheim, 1972; Farner *et al.*, 1982; Sunanda *et al.*, 2001b). Farner *et al.* (1982) reported that the conical shape of papilla was typical for the Podicipediformes, Charadriiformes, Strigiformes, Caprimulgiformes, Apodiformes and Cuculiformes.

The two glands were separated only at the anterior one-third. The posterior two-thirds were firmly joined by an isthmus. The isthmus was surrounded by a skin with small feathers, which extended from posterodorsal end of glands and directed dorsocaudally. The two glands had separate ducts, which opened independently on a common papilla. These results concur with the findings of Lucas and Stettenheim (1972) and Sunanda *et al.* (2001b) in ducks.

Each gland contained a small central primary cavity, which was rounded at its cranial end. The caudal end of the cavity narrowed where it entered the duct. Similar observations were reported by Lucas and Stettenheim (1972) and Mc Lelland (1975) in various species of domestic birds including ducks. The uropygial circlet or uropygial wick was seen as a circlet of downy feathers at the tip of the papilla. No feathers were observed in the midplane between the two openings of the ducts. These results are in agreement with the findings of Lucas and Stettenheim (1972) in chicken and White Pekin and Coloured Muscovy ducks and Chandrasekar *et al.* (1990) in Japanese quail. The feathers were present in the midplane of the papilla in Red-Crested Pochard breed of chicken while in adult turkeys the feathers were broken (Lucas and Stettenheim, 1972).

The arrangement of circlet feathers in a circle around each orifice forming a double tuft observed in the present study is as reported by Farner *et al.* (1982), in ducks. The right and left glands were separated by an interglandular septum. These two glands were spread apart immediately rostral to the papilla almost at right angles while the papillary ends of the glands were united by an isthmus. The isthmus separated the papilla from the glands. The tip of the papilla showed two protuberances, each with a slit like orifice. This agrees with the results of Farner *et al.* (1972), Lucas and Stettenheim (1972) and King (1975), in domestic fowl and Chandrasekar *et al.* (1990), in Japanese quails.

On the day of hatch, the preen gland had a mean weight of 0.15 ± 0.01 g, with the right gland weighing slightly heavier $(0.08 \pm 0.01$ g) than the left $(0.07 \pm 0.01$ g). This mean weight represented 0.36 per cent of the gross body weight. After five days of age, there was a three-fold increase in the mean combined weight and the weight of the right and left glands. The preen gland reached its maximum percentage of body weight (0.88 %) at five days of age and thereafter grew less rapidly than the body. The left and right glands increased progressively in weight from day-old to 150 days of age. The preen gland weight was positively correlated with age and the body weight. No similar results were available in the literature for comparison.



The preen gland showed a rapid growth in the first 30 days after the hatch and thereafter the growth was slower. It reached its maximum mean weight of 5.31 ± 0.23 g at 150 days of age. A slow increase in the mean weight occurred between 60 days and 150 days. The weight of the right and left glands as well as their combined weight was significantly higher after 30 days of age. The proportionate combined weight of preen gland to body weight decreased after 30 days of age. A negative correlation existed between the age and the percentage contribution of the preen gland to the body weight. This might be due to rapid structural development of preen glands during the early stages of post hatch period.

In White Leghorn birds, the percentage contribution of the preen gland weight to the body weight was found to be 0.06 percent and in mature Brown Leghorn, 0.07 percent (Hodges, 1974). The corresponding value in duck was 0.274 percent (Sunanda *et al.*, 2001b). In the present study, it was found to be 0.34 per cent of the body weight. These differences might be due to species or breed variations.

The right gland was significantly heavier than the left in all the age groups studied. This concurs with the findings of Sunanda *et al.* (2001b), in ducks.

In multiple regression analysis, when the body weight of the bird was kept constant, three percent of the total variation in the weight of preen gland was accounted for by the age and when the age of the bird was kept constant 97 percent of variation in the weight of preen gland was accounted for by the body weight. Since a greater percentage of the total variations in preen gland weight was accounted for by the body weight, it was inferred that heavier birds of a particular age have heavier glands. The physical parameters such as the length, width and thickness of the right and left glands showed an increasing trend with a high positive correlation, with the advancement of age. At five days of age, a two-fold increase in its length, width and thickness was noticed on both the right and left glands. Thereafter the increase was gradual with the advancement of age. The cranial, interspace and caudal width also increased gradually with age. All these parameters also showed a positive correlation with the age and the body weight. Similar results were not found in the available literature.

The right gland was significantly longer, wider and thicker than the left in all the age groups. Similar findings were reported in Japanese quails (Dhande *et al.*, 1996) and in ducks (Sunanda *et al.*, 2001b).

The preen glands were located on the dorsal surface of the coccygeal vertebrae one on either side extending from the caudal part of fourth coccygeal to the cranial one-fourth of seventh coccygeal vertebrae in the synsacrocaudal region. This is agreeable to the findings of Sunanda *et al.* (2001b) in ducks. However in fowl, it was located further caudally over the last coccygeal vertebra (Bradley and Grahame, 1950; Trautmann and Fiebiger, 1957; Spearman, 1973; Arnall and Keymer, 1975).

The dorsal surface of the gland was convex while the ventral surface was flattened. The gland was related to the muscles levator coccygeus, which ran beneath the gland and a pair of coccygeus lateralis muscles, which flanked the gland on both the sides. It was embedded in a cushion of fat beneath the subcutis. Similar results were reported in several species of domestic birds (Farner *et al.*, 1982) including ducks (Sunanda *et al.*, 2001b).

The arterial supply to the preen gland was through a pair of branches from the caudal artery. This agrees with the findings of Farner *et al.* (1972) and Lucas and Stettenheim (1972) in domestic birds. The course and branching pattern of the arteries into upper, lower and medial branches are in accordance with the findings of Aslan *et al.* (2000), in the preen glands of goose and duck.

The venous drainage was through a pair of satellite veins to the branches of caudal artery. Between the second and first caudal vertebrae, they disappeared to join up with the renal portal system. These results concur with the findings of Farner *et al.* (1982) in various other domestic birds.

The innervation of the glands was through the uropygial nerve. It was the direct continuation of the medial caudal nerve. The medial caudal nerve originated from the caudal plexus as reported in various other species of domestic birds (Elder, 1954; Farner *et al.*, 1982) including ducks (Sunanda *et al.*, 2001b). Lucas and Stettenheim (1972) reported that both medullary and sympathetic fibres innervated the gland, in chicken.

The histological appearance of the preen gland during the post-hatch period was almost similar except in that of the day-old ducklings. However, morphometry and micrometry showed significant differences between the age groups studied. On the day of hatch, the tubules of the inner zone and the primary cavity were lined by undifferentiated epithelium though the differentiation of the tubules into zone I and zone II was apparent. These are in partial agreement with the findings of Lucas and Stettenheim (1972) who have reported the presence of undifferentiated epithelium in both the zones of the secretory tubules on the day of hatch, in chicken. This could probably be due to the species variation.

The body of the gland was covered by a dense irregular connective tissue capsule composed of collagen and reticular fibres. The interglandular septum had a structure similar to that of the capsule. Anteriorly, dorsally and ventrally the tissue of the septum was continuous with those of the capsule. Posteriorly, they merged with the dense connective tissue of the isthmus. These are in

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accordance with the results of Farner et al. (1982) in various other domestic birds.

Absence of smooth muscle and elastic fibres in the capsule and in the interglandular septa is contrary to the findings of Lucas and Stetteneheim (1972) in fowl and Sunanda *et al.* (2001c) in ducks. They reported the presence of stray bundles of both smooth muscle and elastic fibres in the capsule and in the interglandular septa. Invasion of thin intertubular septa from the capsule into the parenchyma tally with the findings of Sunanda *et al.* (2001c), in ducks.

The capsule and interglandular septa contained numerous blood vessels. The two glands were connected only by a fibrous tissue isthmus caudally without any parenchymatous connection. The cellular components of the capsule were the fibroblasts and occasional multipolar neurons. The intertubular connective tissue was rich in reticular fibres with a few collagen fibres. The connective tissue was abundant in the zone II and near the primary cavity while it was scanty in the zone I. These results agree with the observations in various species of domestic birds (Farner *et al.*, 1982) including ducks (Sunanda *et al.*, 2001c).

From the capsule and the interglandular connective tissue, thin strands of collagen and reticular fibres invaded into the parenchyma and formed the supporting framework of the gland. These strands surrounded the group of cells in the lobules of inner and outer zones. No basement membrane was noticed between the epithelium and the connective tissue. These partially agree with the findings of Farner *et al.* (1982) in chicken, who have reported a basement membrane in between the epithelium and the connective tissue.

The preen gland of duck was of simple branched tubular and holocrine type. This concurs with the findings of several authors (Uva *et al.*, 1976; Amet *et al.*, 1982; Manna *et al.*, 1984; Abalain *et al.*, 1984; Fringes and Clorges, 1991) in various species of birds.

The gland was made up of straight branching secretory tubules. This concurs with the findings of Abalain *et al.* (1985), Jenik *et al.* (1987) and Carpenter and Goodridge (1988). These tubules were arranged in a radiating manner from the centre towards the periphery of the gland. Towards the capsule they ended blindly as reported by Apandi and Edwards (1964) and Lucas and Stetteneheim (1972) in chicken.

The epithelium of the secretory tubules was of stratified type consisting of basal, intermediate and transitional layers. These are in agreement with the reports of Farner *et al.* (1972) in Japanese quail and Mc Lelland (1975) in domestic fowl. However, Ishida *et al.* (1973) and Suzuki (1994) named these layers as basal, transitional and degenerating layers in the domestic fowl.

The division of the tubules into, an outer zone or zone I, near the capsule and an inner zone or zone II, towards the primary cavity is as reported in fowl (Ishida *et al.*, 1973), Japanese quails (Chandrasekar *et al.*, 1990) and ducks (Sunanda *et al.*, 2001c). These zones were seen along the length of the tubules. The outer two thirds of the tubules were referred to as zone I and inner one third as zone II. The lumen of the secretory tubules contained secretory products and cellular debris. Bacha and Wood (1990) designated these two zones as sebaceous and glycogen zones in the fowl.

The zone I consisted of elongated tubules lined by stratified epithelium surrounding an indistinct lumen. The basal cells comprised of a single layer of flattened cells with darkly staining granular cytoplasm and vesicular nucleus. In some tubules, the basal layer consisted of more than a one layer of flattened cells. The cytoplasm of some cells appeared chromophobic as a clear unstained area surrounding a heterochromatic condensed nucleus. Next to this was a single layer of intermediate cells, which were polyhedral shaped with irregular nucleus and lightly staining acidophilic cytoplasm. They were characterized by the presence of a large number of small spherical lipid droplets. Depending on the size of these droplets, the nucleus occupied either a central or eccentric position. Inner to this, a transitional layer with multilayered polyhedral cells was recorded. The cells were filled with larger vacuoles of lipid material and hence appeared vacuolated and pale with H & E staining. The nuclei of the transitional cells were pyknotic. The cells towards the lumen of the tubules in the transitional layer were at various stages of disintegration. These results are in total agreement with the findings of Chandrasekar *et al.* (1990) in Japanese quail and Sunanda *et al.* (2001c) in ducks. Contrary to this, Wagner and Boord (1975) reported that in chicken, the outer zone of the tubules consisted of a layer of basal cells and four secretory layers. According to Farner *et al.* (1982) described the four layers of glandular epithelium as an outer granular layer, intermediate layer, secretory layer and degenerative or transitional layer, in various species of domestic birds.

The tubules of zone II consisted of a single layer of basal cells, one or two layers of intermediate cells and a few layers of transitional cells. Structurally the cells in all the three layers of zone II resembled those of the zone I. The lumen was wider in zone II compared to that in zone I which had a little or no lumen as reported by Sunanda *et al.* (2001c), in ducks.

The basophilic cytoplasm of the basal cells and the acidophilic cytoplasm of the intermediate cells gave the peripheral part of the epithelium of the secretory tubules a darker appearance in contrast to the vacuolated and pale staining cytoplasm of the transitional layer. The basal and intermediate layers of tubules in both the zones were intensely stained with Toluidine Blue. The cells of basal layer could be the reserve cells for the intermediate and transitional cell layers as opined by Lucas and Stettenheim (1972), in fowl. The primary cavity was lined by one or two layers of columnar cells with spherical nuclei. Dhande *et al.* (1996) have also made similar findings in Japanese quail.

In the present study, myoepithelial cells were not evident around the secretory tubules. Contrary to this Chandrasekar *et al.* (1990) noticed fusiform myoepithelial cells adjacent to the basement membrane, in Japanese quails.

A few dilated tubules noticed at random in the preen gland in ducks below 20 days of age are comparable to the non functional enlarged cyst-like tubules reported by Sunanda *et al.* (2001c), in ducks. However, they recorded such tubules only in the peripheral part of the gland.

The secretory tubules showed various stages of epithelial desquamation. Some tubules were lined by one or two layers of cells whereas, certain others were totally devoid of any epithelium. These are in agreement with the results of Farner *et al.* (1982) in various species of domestic birds.

The primary cavity of each gland continued to form a duct, which opened separately on to a common papilla. These agree with the findings of Farner *et al.* (1972), in fowl and Sunanda *et al.* (2001c), in ducks.

The ducts were initially lined by one or two layers of columnar cells with spherical nuclei. The epithelium of the ducts near the tip of the papilla was lined by keratinized stratified squamous epithelium. These are in total agreement with the results of Bradley and Grahame (1950) and Dellman and Carither (1996) in fowl and Sunanda *et al.* (2001c) in ducks. The different layers of stratified squamous epithelium noticed in the present study concurs with the findings in other domestic birds (Farner *et al.*, 1982). Spearman (1971) opined that the keratinized, stratified squamous epithelium of the ducts is suggestive of a cutaneous origin of the preen glands.

In the present study, it was found that the epithelium at the initial portion of the duct was surrounded by connective tissue layer followed by longitudinally arranged smooth muscle fibres and the skin. The connective tissue layer was of dense irregular type and made up of collagen and reticular fibres. Elastic fibres were absent. The epithelium of the ducts, near the tip of the papilla, was surrounded by feathered skin. Longitudinally arranged smooth muscle fibres were observed in the initial part of the ducts. However no smooth muscle fibres were detected near the tip of the papilla. Contrary to this Farner et al. (1982) and Chandrasekar et al. (1990) reported the presence of smooth muscle fibres both in the longitudinal and circular fashion at the sub terminal portion of the papilla, in various species of domestic birds and Japanese quails. Farner et al. (1982) opined that the longitudinal muscle bundles pull the circlet feather follicles into the papilla to enable these feathers to bring into direct contact with the glandular secretion. The absence of smooth muscle fibres near the tip of the papilla as observed in the present study is in agreement with the earlier findings of Sunanda et al. (2001c), in ducks.

Numerous circlet feather follicles and lamellar corpuscles were noticed at the tip of the papilla as reported in various species of domestic birds (Farner *et al.*, 1982) including ducks (Sunanda *et al.*, 2001c).

The capsule, interglandular septum and the intertubular connective tissue were strongly positive for PAS whereas, a weak reaction was noticed in the cell layers of the secretory tubules. The luminal contents in both the zones showed a strong positive reaction. The basal layer and the intermediate layer in both the zones showed a moderate reaction for PAS. The reaction was weak in the transitional cell layer. These results are in accordance with the findings of Chandrasekar *et al.* (1990), in the Japanese quails and Kale *et al.* (1999) in fowl. However, Sunanda *et al.* (2001c) noticed only a moderate PAS reaction in the basal and intermediate cell layers, in ducks. Absence of glycogen and acid

mucopolysaccharides in the secretory tubules is agreeable to the findings of Sunanda et al. (2001c).

The lipids were distributed uniformly in all the epithelial layers of both the zones. The capsule and septae were devoid of lipids. It is in accordance with the findings of Lucas and Stettenheim (1972) in fowl and Sunanda *et al.* (2001a) in ducks. The richness of lipids in the epithelial cells is suggestive of an oily secretion from the gland. The preen gland is well developed in aquatic species of birds in which sebum together with epidermal lipids play an important role in waterproofing of the plumage. Sunanda *et al.* (2001a) opined that the sebum secreted by the gland had more water-repellant property in aquatic species of birds.

The acid and alkaline phosphatase activities were absent in the capsule and the septae of the gland as reported by Sunanda *et al.* (2001d), in ducks. The acid phosphatase activity was moderate in the basal and intermediate layers and intense in the transitional layer in both the zones. The luminal contents had a strong acid phosphatase activity. These are in partial agreement with the results of Sunanda *et al.* (2001d) in ducks who have reported a strong activity in the basal and intermediate layers and moderate activity in the transitional layer. They opined that the acid phosphatase activity is indicative of ACP dependent secretory and the lysosomal activities of the gland.

The alkaline phosphatase activity was moderate in the basal and intermediate layers and weak in the transitional layer of zone I. However, it was moderate in the basal and intermediate layers and intense in the transitional layer of zone II. Similar results were reported by Sunanda *et al.* (2001d), in ducks. They noticed that the alkaline phosphatase plays an important role in calcium metabolism and is associated with the movement of ions or organic molecules across the cell membranes.

Micrometrical studies showed that the thickness of the capsule, width of zone I, zone II and the primary cavity increased gradually with advancement of age. Positive correlation was noticed between the age and all these parameters. No similar studies are reported in literature for comparison. The increase in capsular thickness and the width of various zones could be responsible for the increase in the weight of the gland during the post-hatch period.

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Summary

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6. SUMMARY

The preen gland in ducks were paired with two glands and a common papilla. The two glands together formed a 'V' shaped structure which was pale yellow colour, in fresh state. Each gland was pear shaped with a common papilla. The papilla was broad, short and cylindrical in shape. The glands had separate ducts, which opened independently on to a common papilla.

Each gland contained a small central primary cavity. The uropygial circlet or wick was seen as a circlet of downy feathers at the tip of the papilla. The circlet feathers made a circle around each orifice and hence formed a double tuft, which was shorter than the length of the gland. The papillary ends of the glands were united by an isthmus. The tip of the papilla consisted of two protuberances, each with a slit-like orifice.

The left and the right glands increased in weight from the day of hatch to 150 days of age. After five days, there was a three-fold increase in the weight of the glands. The gland weight was positively correlated with the age and the body weight. The gland showed a rapid growth in the first 30 days after hatch and thereafter the growth was slower. A negative correlation existed between the age and the percentage of the preen gland weight to the body weight. The right gland was significantly heavier than the left in all the age groups studied. In multiple regression analysis, when the body weight of the bird was kept constant, three percent of the total variation in the weight of the gland was accounted for by the age and when the age of the bird was kept constant 97 percent of the variation in the weight of the preen gland was accounted for by the gland that the age of the preen gland was accounted for by the body weight. Therefore body weight had a greater influence on the weight of the gland than the age of the bird.

The right gland was significantly longer, wider and thicker than the left in all the age groups studied. The length, width and thickness of the right and the left glands showed an increasing trend from the day of hatch till 150 days of age. All these parameters were positively correlated with the age and the body weight. At five days of age, both the right and left glands showed a two-fold increase in their length, width and thickness. Thereafter the increase was gradual upto 150 days of age.

The preen glands were located on the dorsal surface of the coccygeal vertebrae one on either side extending from the caudal part of fourth coccygeal vertebra to the cranial one-fourth of seventh coccygeal vertebrae, in the synsacrocaudal region. The dorsal surface of the gland was convex while the ventral surface was flattened due to its relation with the tail muscles, viz. levator coccygeus and coccygeus lateralis. The arterial supply was through a pair of branches from the caudal artery and the venous drainage, through a pair of satellite veins. The gland was innervated by medial caudal nerve.

On the day of hatch, the gland had an undifferentiated epithelium lining the tubules of the inner zone and the primary cavity. Capsule was richly vascularized and composed of collagen and reticular fibres. From the capsule numerous thin intertubular connective tissue septae invaded the parenchyma and separated closely packed secretory tubules. It was abundant in the inner zone while indistinct in the outer zone. These septae contained blood vessels and capillaries. No basement membrane was noticed between the epithelium and the connective tissue. Capsule contained fibroblasts and occasional multipolar neurons. The two glands at their caudal ends were separated by an interglandular connective tissue septum. There was no parenchymatous connection between the glands.

The glands were of simple, branched, tubular and holocrine type. The secretory tubules were arranged in a radiating manner from the centre towards the periphery of the gland. Near the capsule they ended blindly. The secretory tubules were lined by stratified epithelium and that consisted of basal, intermediate and transitional layers. The tubules showed two zones, viz. an outer zone or zone I, near the capsule and an inner zone or zone II, towards the primary cavity. The zone I and the zone II were observed along the length of tubules. The former comprised the outer two-thirds and the latter, the inner one third of the gland. The lumen of the secretory tubules contained secretory products and cell debris.

The outer zone consisted of elongated tubules lined by stratified epithelium surrounding a small or indistinct lumen. Basal layer consisted of flattened cells with darkly staining granular cytoplasm and vesicular nuclei. The intermediate layer was formed of a single layer of polyhedral cells with irregular nuclei and slightly acidophilic cytoplasm. Innermost was the transitional layer comprising of multilayered polyhedral cells with vacuolated cytoplasm and pyknotic nuclei. Structurally, the cells in all the three layers of zone II resembled those in the outer zone. However, the transitional layer comprised of a few layers of cells and the lumen was wider in zone II.

The epithelium at the peripheral part of the secretory tubules was darker in appearance due to the basophilic cytoplasm of the basal cells and the eosinophilic cytoplasm of the intermediate cells as against the vacuolated and pale staining cytoplasm of the transitional layer. The cells of basal layer could be the reserve cells of the intermediate and transitional layers.

The primary cavity was lined by one or two layers of columnar epithelium with spherical nuclei. Myoepithelial cells were not evident around the secretory tubules. A few dilated tubules were noticed at random among the secretory tubules in the ducks below 20 days of age.

The papilla had two ducts, which drained the secretion from the right and left primary cavities, separately. The primary cavity of each gland formed a duct near the papilla. These ducts were initially lined by one or two layers of columnar cells with spherical nuclei. Near the tip of the papilla the lining was by keratinized stratified squamous epithelium. This epithelium consisted of a basal membrane, one or two layers of polyhedral cells with large round nuclei, three to five layers of polygonal cells, four to six layers of flattened cells with pyknotic nuclei and a number of keratin layers. At the sub-terminal portion of the duct the epithelium had only one or two keratin layers.

The epithelium at the initial portion of the duct was surrounded by a connective tissue layer followed by longitudinally arranged smooth muscle fibres and skin. Near the tip of the papilla, the epithelium was surrounded by feathered skin without any smooth muscle fibres. Numerous circlet feather follicles and lamellar corpuscles were present in the papilla.

The capsule and the interglandular septa were strongly positive for PAS whereas, a weak reaction was noticed in the cell layers of the secretory tubules. The lipids were evident uniformly in all epithelial cell layers in both the zones of the tubules. The acid phosphatase activity was moderate in the basal and intermediate layers and intense in the transitional layer in both the zones. The alkaline phosphatase activity was moderate in the basal and intermediate layers activity was moderate in the basal and intermediate layers and intense of zone I. It was moderate in the basal and intermediate layers and intense in the transitional layer of zone II.

Micrometrical findings showed that the thickness of the capsule, width of the zone I, zone II and the primary cavity increased gradually with the advancement of age. Positive correlation existed between the age and the micrometrical parameters. The increase in capsular thickness and the width of different zones could be responsible for the increase in the weight of the gland during the post-hatch period.

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POST-HATCH DEVELOPMENT OF PREEN GLAND IN THE DUCK (Anas platyrhynchos)

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ABSTRACT

Studies on the post-hatch development of the preen gland in ducks were conducted using 44 ducks from the day of hatch till 150 days of age. The project was taken up to trace the structure and development of the glands and their relationship with the age and body weight.

After recording gross relations and measurements, the material was fixed using various fixatives for studying the cellular details, arrangement of cells, connective tissue framework, micrometry and histochemistry.

The preen gland was a paired organ with a common cylindrical papilla. The two glands together formed a 'V' shaped structure. Each gland was pear shaped and pale yellow in colour, in fresh state. They were located on the dorsal surface of the pygostyle. Both the right and left glands had separate ducts. The uropygial circlet was seen at the tip of the papilla. The glands were vascularized through a pair of branches from the caudal artery and innnervated through the medial caudal nerve. The weight of the preen glands increased progressively from the day of hatch to 150 days of age. This weight was positively correlated with the age and body weight. The proportion of the gland weight to the body weight showed a decreasing trend. The right gland was slightly heavier, longer, wider and thicker than the left. The length, breadth and thickness were positively correlated with the age and body weight.

Structurally, the glands were simple, branched, tubular and holocrine type. The richly vascularised connective tissue capsule was composed of collagen and reticular fibres. Elastic and smooth muscle fibres were absent. The secretory tubules showed two zones, an outer zone or zone I, near the capsule and an inner zone or zone II, towards the primary cavity. The epithelium of the tubules consisted of basal, intermediate and transitional layers.

The papilla had two ducts, which were lined by glandular epithelium initially and keratinized stratified squamous epithelium at the tip. The glandular epithelium was surrounded by longitudinally arranged smooth muscle fibres and skin. Lamellar corpuscles and circlet feather follicles were noticed in the papilla.

Capsule, trabeculae and the parenchyma were PAS positive. Glycogen and acid mucopolysaccharides were not detected. Lipids were evident uniformly in all the cell layers. The acid phosphatase activity was moderate in the basal and intermediate layers and strong in the transitional layer. The alkaline phosphatase activity was moderate in the basal and intermediate layers and weak in transitional layer of outer zone. It was moderate in the basal and intermediate layers and intermediate layer of inner zone.

Statistical analysis showed a significant increase in the thickness of capsule, width of the two zones and the primary cavity with the advance of age.