Chapter 11

SECRETORY FUNCTION OF AVIAN EPIDERMIS

Though most of the standard books on vertebrate anatomy including some recent editions maintain, that with rare exceptioms glandular derivatives of avian integument are restricted to the uropygial gland, there are ample evidences to the contrary. The fact that avian epidermis actually produces lipoidal substances functioning similar to the sebum in mammals has been noticed independently by different workers (Matoltsy, 1969; Lucas, 1969; 1970; Freinkel, 1972; Jacob, 1978). Historically, though, it was Vericak (1939) who found that such lipid bodies are secreted by the web and soft skin parts of the fowl, his findings remained unnoticed till about 1973. Lucas and Stettenheim (1973) unequivocally established that the whole of the avian epidermis is involved in secretory activity and they proposed that whole epidermis should be considered as a gland. Shah and Menon (1973) and Jacob (1978) found such secretory activity in the interplumar epidermis of the pigeon intergument. Freinkel (1972) established that lipogenesis is an active feature of the process of maturation of avian epidermal cells, and termed them as "Keratino-sebocytes". Thus, lipoidal secretion, as an important function of avian skin, has become quite evident in the recent years.

On the basis of the present investigations on suppression of feather development, it has been realized that there exists an inverse relationship between two major functions of the avian integument - viz., production of feather, and lipoidal secretion (Chapters 2,9). With these facts in view it was thought worthwhile to study more instances where loss or modification of cutaneous appendages (in birds) occur as a normal feature of development or while attaining maturity, where alterations in the epidermal secretory function of the specific body region may take place. The Sarus Crane (*Grus antigone antigone*) and the Indian White Ibis (*Threskiornis melanocephala*) were chosen for the present study. In case of the Sarus Crane, the head and neck, originally covered by buff coloured contour feathers in the young stage, become apparently bare and coloured grey in the capital, and reddish in the facial and anterior neck regions in the adults. The skin in the facial and upper neck regions bears papilliform outgrowths along with bristles. A close look at the skin on the neck reveals three distinct 'zones', (1) an anterior zone where well developed bristles and papilliform skin outgrowths are interspersed, (2) the middle zone having well developed papiliform outgrowths, but has poorly formed small bristles, and (3) the posterior zone where the papiliform outgrowth gradually gets reduced in size and number, and feathers replace bristles to merge with skin bearing white

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EXPLANATION FOR FIGURES

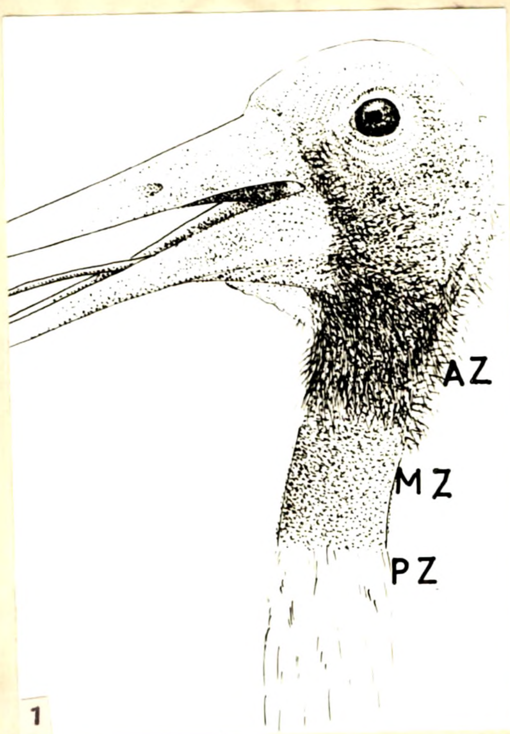
Fig.1 Drawing of the head and neck of Sarus Crane depicting the morphologically distinct 'zones'.

Fig.2 (a) Bristle with aftershaft from anterior cervical region

(b) Smaller bristle from the same region. Note short Shaft and after feather.

(c&d) Bristle and bristly feather from the middle zone.

(e) Contour feather from the posterior zone.



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contour feathers in the posterior half of the neck. Since the three 'zones' are, more or less distinct from each other, and are formed as a result of qualitative changes in ptylosis during the attainment of maturity, it would be of interest to note the differences, if any, in the secretory activity of the epidermis from the three zones. The Indian White Ibis (Threskiornis melanocephala) is a species with young ones having fully feathered head and neck which on attaining adulthood have a secondarily formed apterium spanning the entire capital and cervical regions. Here the feather loss is on a much larger scale as compared to that seen in the Painted Stork described in Chapter 2.

OBSERVATIONS AND DISCUSSION

Morphology : (Figs. 1, 2, and 3)

In an adult Sarus Crane (Grus antigone antigone), the anteriormost zone of the neck bearing red papilliform outgrowths extend from the occipital region to about 6.5 cms down the upper neck.

The middle zone where only red papilliform outgrowths are evident and small bristles, though present, but apparently scanty, extends about 3.5 cms after the first zone, and later it merges into the third or the posterior zone, where skin bears contour feathers and fewer papilliform integumentary outgrowths (Because of its gradual merging

EXPLANATION FOR FIGURES

Fig.3 A close-up surface view of the skin from the neck region. Note the anteriou bristly zone (AZ), papilliform middle zone (MZ) and posterior Zone (PZ) with contour feathers.

Fig.4 Histological features of a bristle follicle. Haematoxylin eosin. 76 X.

Fig.5 Histological features of papilliform outgrowth in the middle zone. Note numerous blood vessels in the dermis. Mallory's triple stain. 76 X.

Fig.6 Histological features of a contour feather folicle from posterior zone. Mallory's triple stain. 76 x.


MZ
PO
PZ
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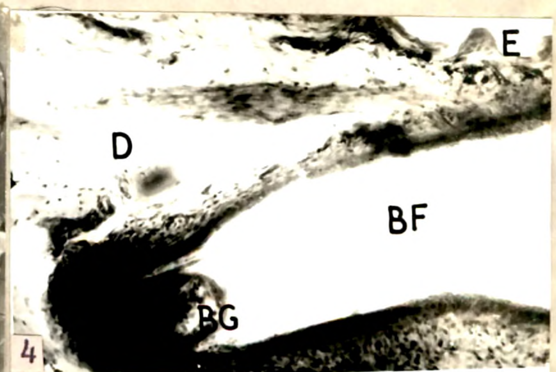
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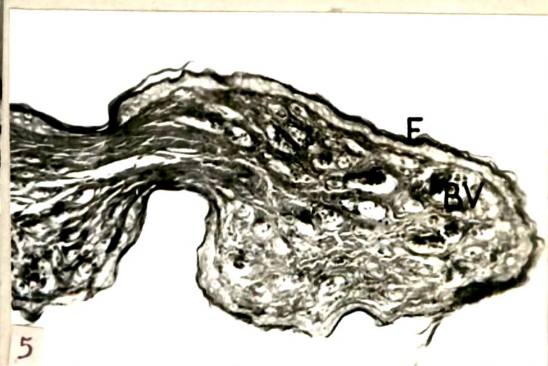
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with the feathered neck region it is not possible to give measurements of the last zone) (Fig. 1 and 3). In the anterior facial region, very fine bristles are present and they grade into coarse 'hair' like bristles directed posteriorly.The genal tracts bear many intermediary forms of bristles between the typical type and semibristles or feathers. Such types are noted even within a single row. The arrangement of bristle bears similarity to the pattern of arrangement of contour feathers. Many of the bristles possess after shafts with varying degrees of development. Small bristle feathers with bifid or forked tips bearing barbs are interspersed amongst the typical bristles. The grey auricular patch bears feathers with well developed barbs, but not interlocked by means of barbules, and hence the vane is not pennaceous. The rachis is also stiffer than that of the ordinary contour feathers and lightly pigmented. Their structure conform to the typical 'auricular feather' type of bristles in birds, described by Lucas and Stettenheim (1972). Varying degrees of structural modifications amongst these feathers and bristles are seen in front of the auricular tracts. Most of these possess a well developed aftershaft. In some cases, denser clumps of barbs are present on the aftershaft as compared to the main feather.

The papilliform outgrowths of the interplumar skin in general are smaller in the facial region than those present in the cervical region. In the anterior zone of the cervical region, most of the bristles do not possess barbs, and even the basal barbs or an aftershaft are absent. Thus these bristles appear to have a simple hair like structure. Longest bristles are about 1.6 cms in length. Interspersed with these are various small and fine briestle feathers which on a cursory examination appear as filoplumes. However, these are definitely bristles and some of them have a bifid or multi-branched tip bearing numerous small barbs (Fig. 2a and b).

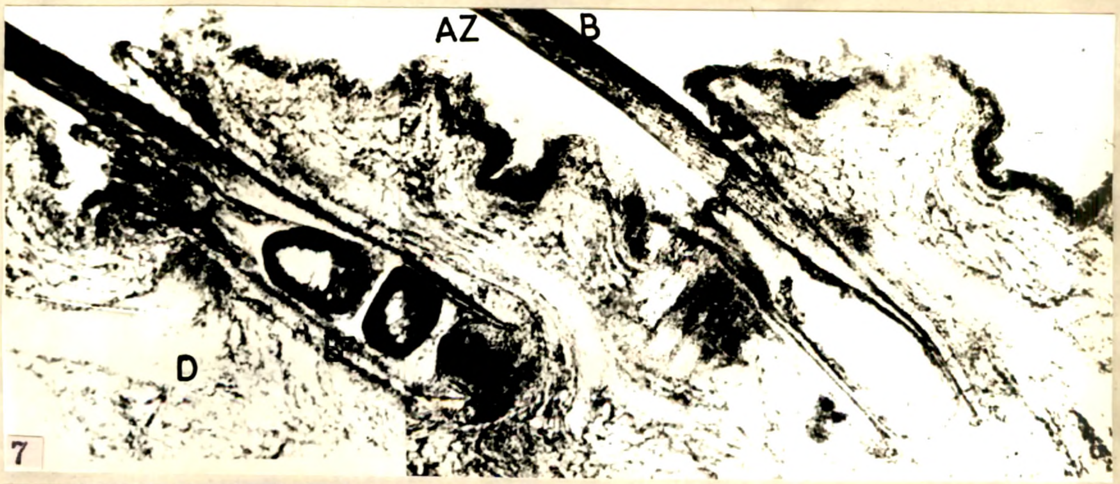
In the middle zone, the papilliform outgrowths are large and numerous, and overlap each other apparently without a precise pattern of arrangement. Papilliform outgrowths are generally not uniform in size and shape. They may be spatulate, pointed or bifid. Interspersed among these are short thin bristle feathers, noticeable only on close examination. Most of these appear to be semibristles with darkly pigmented rachis and well developed barbs bearing barbules (Fig. 2c & d). The semibristles grade gradually into contour feathers (Fig. 2e) present in the next zone. So graded contour feathers do not have pennaceous vanes and in this respect they resemble the auricular feathers. However, their barbs are much closer to each other than those seen in the auricular feathers. The average length of the contour feathers here is about 1.5 cms.

The present observations reveal that the cutaneous

EXPLANATION FOR FIGURES

Figs. 7,8, 10: Sections of skin from the anterior, middle and posterior zones respectively, showing neutral lipids in the epidermis. Fat Red 7 B staining. 76 x.

Fig. 9 Epidermis from the middle zone showing secretory lipoid globules stained with Fat Red 7B. 500 x.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MZ
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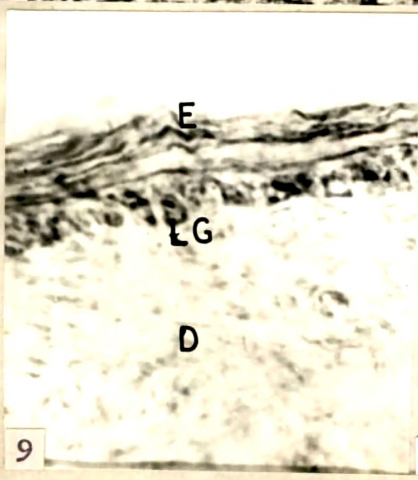
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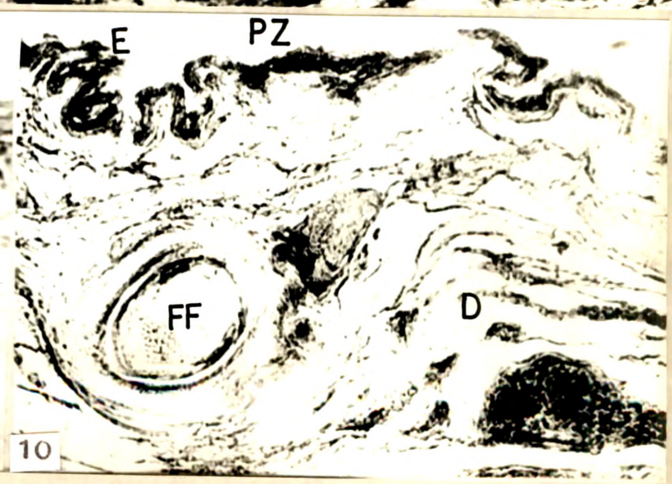
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appendages that are seen in the three distinct 'zones' of the neck in the Sarus Crane, show morphological gradation between contour feathers and typical bristles. Some of these bristles are bifid or have multibranched tips bearing barbs. It could also be seen that feathers collected from the head of an immature Sarus Crane (bristles would be produced from the same follicles when the bird attains adulthood) were essentially similar in structure to the contour feathers noticed in the posterior zone of the neck of adult birds. On the basis of these observations, it can well be surmised that bristles are only modified contour feathers. This contention finds support in the work of Stettenheim (1972). An interesting question in this regard could be the mechanism that underlies the change in form of the product of follicular activity - from a well designed feather to a relatively simpler structure such as a bristle. Presently, author is not in a position to offer any explanation for the problem but can suggest that the answer may lie in the genetical programming involving metabolical-morphological modifications.

Histology and Histochemistry (Figs. 4 to 10)

Histologically, the follicles of bristles and contour (Figs. 4 and 6) feathers show a good deal of similarity in their general features except for the concentration of melanocytes and poor development of follicular muscles in the former. The number of feathers or bristles per unit area of the neck skin was not found to vary and hence it could be assumed that quantitative changes in the number of follicles do not accompany changes in the type of feathering of the head and neck of Sarus Crane during post-hatching development and maturity. Papilliform outgrowths (Fig. 5) on the neck skin has a core of dermal tissue covered by epithelial cells. The basal epithelial layers are 2-3 cells thick, while the stratum corneum though relatively thin has several layers of cornified cells. The dermal core is richly vascularised, especially at its peripheral regions subjacent to the epithelial cells. Such disposition of the blood capillaries can be considered important from the point of view of thermoregulation. Development of these papilliform outgrowths in the interplumar region brings in a significant increase in the surface area of the bare skin, which due to its high vascularity provides an effective mechanism for dissipation of body heat. Besides, the pigmentation of the papilliform skin would also have a certain bearing on the social life of these cranes, being important in visual signalling. Histological observations reveal that differences do exist in the nature of connective tissue in the dermis of the skin of the three distinct zones. With Mallory's triple stain, much of the dermis stained red in the anterior zone bearing bristles suggesting presence of high concentration of amyboids but such property of the dermis gradually decreases from the anterior to the posterior zone.

As the distribution pattern of lipids is concerned, much of the neutral lipids were noted to be localised in the epidermis. Within the dermis, except the adipose tissue, only a moderate amount of neutral lipids were discernible in the connective tissue, smooth muscles and feather germs. Staining with Sudan black showed that the dermis was poor in its general lipid content as opposed to the epidermis. An uniform staining of all components of skin with Nile Blue Sulphate indicated that the acidic lipids are present mostly as structural components of the integument. It is noteworthy that within the three different 'zones', the neutral lipids were relatively more in the epidermis of the anterior zone and least in the posterior one. In the middle and anterior zones, especially in the former, lipoid globules were seen in the cells of basal epidermal layers (Figs. 7,8,9, and 10) denoting active secretion of lipids by the epidermal cells. Secretion of lipoid substances by avian epidermis is by now well established (Lucas, 1968; Lucas and Stettenheim, 1972; Shah and Menon, 1972; Freinkel, 1972). On the basis of the studies on secondarily formed apterium in the capital region of the Painted Stork (Chapter 2), it has been suggested that loss of feather producing function enhances secretory (holocrine) activity of the integument. In light of these observations and the present study on Sarus Crane, it is tempting to generalise that secretory activities of epidermis

EXPLANATION FOR FIGURES

Fig.11 Section of Skin from the Capital region of Indian White Ibis stained to show presence of Nuteral lipids. Note the liquid droplets oozing out of epidermal cells after application of slight pressure on the slide (Arrow) 76x.

ABBREVIATIONS

AZ- Anterior zone; B- Bristle; BF- Bristle follicle; BV- Blood vessels; BG- Bristle germ; D- Dermis; E- Epidermis; FF- Feather follicle; LG- Lipoid globules; MZ- Middle zone; PO- Papilliform outgrowth; PZ- Posterior zone; SM- Smooth muscles.


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D
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in avian integument is inversely related to the degree of its feathering. Thus the epidermis from glabrous regions is most secretory, while that in the feathered region is least, and of intermediate state where modified feathers having lesser degree of structural complexities (bristles) are present.

Indian White Ibis, (*Threskiornis melanocephala*)

Histology and Histochemistry :

The stratified epidermis of the secondarily bare skin of head and neck of the Indian white Ibis (*Threskiornis melanocephala*) has a fairly heavy concentration of melanin pigment. Melanocytes are discernible in the dermis subjacent to the epidermis with ramifying processes in the intercellular spaces of the epidermis. No trace of feather follicles are seen in these areas of the skin. Like in Sarus Crane, the dermis is poorer than the epidermis with regard to neutral lipid content. On applying slight pressure on the mounted sections, oil droplets are noticed to be squeezed out of the epidermal layers (Fig. 11). These are neither artifacts nor derived from the dermal fat deposits, as they are always stained red/pink with fetrot 7 B and aqueous Nile Blue Sulphate (specific staining properties of triglycerides), and as no adipose tissue is seen immediately subjacent to the epidermis. Present observation indicates a high degree of lipoid synthesis and its accumulation in the epidermal cells in the secondarily bare skin of the capital and cervical regions of the Indian White Ibis. By desquamation of the outermost lipid loaded epidermal cells, the lipoid substance(s) gets spread over the skin. Thus the functioning of the whole of the bare skin is much like an open holocrine gland. The present observation also confirms the earlier contention that apteric regions that are secondarily formed as a result of permanent loss of feathers, tend to show an enhanced secretory activity (Chapter 2). This contention is further borne out by the observations of Lucas (Personal communication) that in pheasants too, the non-feather epidermis has a much higher lipid content than in the feather tracts.

Formation of an apterium from the previously feathered regions of the head and neck in case of the White Ibis seems to be functionally correlated with the habitats and feeding habits of the birds. Ali (1954) reports that while probing into the bottom mud in shallow waters, the head and neck of these birds is sometimes completely immersed, though momentarily. The oil bearing epidermis would then be of obvious adaptive value, preventing the skin from remaining water soaked apart from maintaining flexibility and integrity in spite of being exposed or uninsulated. Besides these points the bare skin would help in thermolegulation. Present observations also underscore the fact that such functional modifications of the avian integumentary system are of more common occurrence than was hitherto suspected. The role of pigmentation of the bare skin in social communication and its significance in social life of these birds also cannot be overlooked, considering that the same areas of skin are not coloured black when they were feathered in the subadults.

SUMMARY

Chapter 2 : Histological and histochemical observations with reference to distribution of both acidic and neutral lipids have been made in the secondarily formed capital apterium of the crown region of the Painted stork- Ibis leucocephalus. The storks to begin with after hatching show the presence of feathers as well as the associated epidermal and dermal components. However, in the one year old immature birds, partial defeatheration of the head sets in. The defeathered areas are marked by absence of feather follicles along with a loss of their follicular muscles. An increased vascularity and reduction in the number of adipocyte are also observable. Hyperplasia in the apteric epithelium leads to the development of a thick stratified epidermis. The skin of the bare region assumes a yellowish tinge. By about two years of age, the capital apterium gets well established with a highly stratified and wrinkled epithelium. An interesting aspect of the development of capital apterium is the increased content of lipids (both neutral and acidic) in both the epidermal and dermal components. The histological and histochemical observations recorded herein tend to indicate the possible involvement of a process of programmed cell death leading to loss of feather follicles and feathers in association with the acquirement of an increased functional competence of the

apteric zone in secreting large quantities of lipids. Such a lipid rich capital apteric zone in storks might help them in diverse and ritualised social displays used for communication as the flexibility of the secondarily formed apteric zone is very high. It may be presumed that the transformation of a fully feathered area into a permanent apteric zone is coupled to a loss of one function i.e., feather formation and the acquirement of another functional activity i.e., the holocrine secretion.

Chapter 3 : Possible involvement of chemodifferentiation in the morphogenesis of the crown apterium was investigated by studying the distribution pattern of glycosaminoglycans and phosphatases during the transformation of the feathered crown part of *Ibis leucocephalus* into a nonfeathered balded region. The investigations have yielded certain changes in the distribution pattern of these chemoregulatory substances. The most significant change was the total disappearance of alcianophilia i.e., sulfated mucopolysaccharides and alkaline phosphatase activity in the various dermal components of the apteric zone which could thus be correlated with the process of programmed cell death leading to the loss of feather papillae, follicles and muscles. The loss of dermal components and the accumulation of collagenous connective tissue material are paralelled by an increase in nonsulfated mucopolysaccharides as marked by the increased PAS positive reaction of the dermis.

Unlike the dermis, the epidermis of the region did not depict a changing pattern and was found to retain both its alcianophilia as well as high alkaline phosphatase activity and is being correlated with its functional involvement in lipid elaboration and carotenoid metabolism which lend the skin of the apteric zone its rich coloration and flexibility both of utility value in social displays during breeding season.

Chapter 4 : In order to understand the functional involvement of thyroxine in feather development, the distribution pattern and intensity of certain enzymes such as phosphatases, LDH, G-6-PDH, SDH and MDH were studied histochemically in the feather forming tissues of the pigeon, Columba livia, under experimentally induced athyreosis. Experimental athyreosis as induced by thiouracil treatment led to a decreased activity of alkaline phosphatase,G-6-PDH, SDH and MDH. The fall in the activities of these enzymes is marked by a delayed growth and maturation of feathers. The fall in the activity of these enzymes during the athyroidic condition as well as the observation of a return to normalcy of the activity of all these enzymes under replacement therapy, indicate that thyroxine may have a controlling influence over the processes of cell proliferation and differentiation (associated with feather growth and maturation) by its regulatory effect on the activity of enzymes. LDH and acid phosphatase, however, did not show much change in the absence of thyroxine and hence could be considered to be independent of the regulatory influence of the thyroid principle.

Chapter 5 : A histomorphological analysis of the incubation patch skin of the house sparrow, Passer domesticus has been carried out so as to not only understand the sequential series of events involved in the formation of the patch but also to assess the fate of the feather forming tissues such as papillae, follicles and their associated muscles. The essential events involved in the metamorphosis of incubation patch during the breeding period are, defeathering, increase in thickness of both the epidermis and dermis (due to hyperplasia), development of an edematous condition and, attainment of hypervasuclarity. With regard to the fate of feather papillae, contrary to the existing view, the present observations have revealed the persistance of feather papillae even in a fully formed patch skin though in a regressed and reduced shape and form. Some sort of differential regulatory influences can be inferred looking into the differential behaviour exhibited by the patch skin epidermis from the underlying feather germ epithelium.

Chapter 6 : Since the proliferation and differentiation of integumentary components, like other organs are known to involve morphogenetic interaction between the epidermis and dermis controlled by the extracellular dermal substances like glycosaminoglycans and collagen, possible changes in the distribution pattern of these two substances were investigated with histochemical techniques during the morphogenesis and regression of the incubation patch in the ventral skin of Passer domesticus. The development of the incubation patch skin was marked by an increased alcianophilia of the epidermis and the feather papillae with a concomitant increased PAS reactivity in the immediately adjacent subepidermal part of the dermis. Neither did the epitheliem of the feather papillae exhibit alcianophilia nor did the feather germs exhibit PAS reactivity. The presence of PAS positive collagen material in the dermis and alcianophilic GAG in the patch skin epidermis may be thought to involve in some sort of interaction leading to hyperplastic and stratified nature of the epidermis noticed. Based on this rationale, the dormancy of the feather follicle noted in the patch skin may be explained by the absence of both the inducible collagen material in the feather papillae as well as the target cell specific GAG substance in the feather epithelium.

Chapter 7 : Distribution pattern of acid and alkaline phosphatases in the ventral skin of the house sparrow, Passer domesticus has revealed the presence of acid phosphatase in all the integumentary structures including the epidermis, dermis, feather papillae and follicles. On the other hand alkaline phosphatase activity was restricted only to the feather germs. During the defeathered incubation patch stage (during the breeding period) both the enzymes were noted to be present in high concentrations in all parts of the skin excepting for the feather germs which depicted a negative response towards both the enzymes. With the regression of the patch and refeathering of the ventrum, both the enzymes settled down to their respective original nonbreeding pattern of distribution in all components of the skin. Along with this, the activity of both the enzymes reappeared in the feather germs. The disappearance of both the phosphatases from the feather germs during the patch formation can easily be correlated with the regressed, dormant state of the feather germs and the follicles. Increased enzyme complement in all other parts at the same time could be correlated with increased metabolic activity, metabolite transfer, lipid synthesis, development of edematous condition, attainment of hypervascularity etc., accompanying the formation of the incubation patch.

Chapter 8 : In order to correlate the structural metamorphosis of the incubation patch in the house sparrow with its functional status, possible changes in the metabolic profile involved is investigated by studying the distribution of enzymes like LDH, MDH and SDH. Of these enzymes, LDH was found to be most active in the various skin components during the breeding period, thus indicating active anaerobiosis to be the dominant feature of the sparrow skin, excepting for the feather germs where SDH and MDH - the TCA cycle enzymes were also active. The formation of the incubation patch during the breeding period was marked on the one hand by increasing activity of all the three enzymes in the patch skin components and on the other by reduced activity of the enzymes in the feather germs. This pattern ultimately reverted back to the original condition with the regression and reversion of the patch skin back to the original feathered (normal) condition. Increase in activity of all the three enzymes in the fully formed patch is indicative of the high state of metabolic activity generating considerable amount of heat (probably required during the incubation of eggs) as well as energy required for the active cellular proliferation associated with the stratification of epidermis. Fall in the activity of all the three enzymes in the feather germs is indicative of a low gear metabolic activity correlatable with the functional inactivation of the germs characteristic of patch skin. Activation of the germs and refeathering of the patch skin seem well paralleled by the reappearance of all the three enzymes in increasing concentration. Similarly with the fall in energy requirement during regression of the incubation patch, the activity of LDH, MDH and SDH also settle down to the preincubation status.

Chapter 9 : Lipid metabolism in relation to the incubation patch of the house sparrow, *Passer domesticus* was evaluated by studying the histochemical localization of enzymes such as G-6-PDH, α-GPDH and BDH, and also the distribution of neutral and acidic lipids. Operation of HMP pathway at a moderate level in the normal skin could be discerned by the moderate activity of this enzyme in all parts of the skin. However, low levels of α-GPDH and BDH at the same time are indicative of the nonparticipation of lipid catabolic process in the integument. Presence of G-6-PDH in the normal skin can also be correlated with the observed presence of lipids. With the formation of incubation patch, this region of the ventral skin depicted an increased localization of G-6-PDH and high lipid content, which are by themselves self explanatory. Increased presence of G-6-PDH and α-GPDH could be evidenced in the feather germs only during the regressive phase of the incubation patch, whence the germs are activated and feather development is induced. This observation coupled with the appearance of lipids in the feather germs are taken to indicate the functional involvement of lipids in forming the structural framework of the developing feathers. Continued low level of BDH activity once again emphasizes the adaptive value of the avoidance of lipid catabolism in the skin. Presence of high content of lipids in the patch skin is also suggestive of the protective value that it affords to the exposed patch skin in terms of desiccation and cracking.

Chapter 10 : It is well established that thyroxine can induce moulting and control feather development in birds. Further, the role of sex steroids and prolactin in rendering the feather germs refractory and unresponsive to thyroxine is also known. In this light, the possibility of overcoming the refractoriness of the feather germs of the brood patch skin, and induction of refeathering were examined by thyroxine injection in the house sparrow, Passer domesticus. The experiment has proved the possibility of overcoming the refractoriness of the feather germs by high dose thyroxine treatment. Under the high dose of thyroxine injection the germs not only overcome the suppressive influence but an actual refeathering along with moult of other body feathers also occured. Concurrent studies on the histoenzymological profile of the patch skin also showed an increaased activity of almost all the enzymes in the feather germs. This has led to the surmise that the mode of action of thyroxine essentially involves a stimulatory influence on metabolic events in the feather forming tissues, thus leading to the induced refeathering of the patch skin. A detailed review and discussion of the aspect is undertaken in the text of the chapter.

Chapter 11 : In a number of birds loss of feathers from specific regions of the body is followed by high lipid content leading to the contention that loss of one function (i.e. feather formation) is compensated by acquirement of another function (*i.e.*, lipoidal secretion).This problem was examined by histomorphological and histochemical techniques in two birds - the Sarus Crane, *Grus antigone antigone* and the Indian White Ibis, *Threskiornis melanocephala*. In the former, the head and cervical regions of the adult birds are known to undergo defeathering and can be divided into three zones; an anterior zone with red papilliform outgrowths and bristles, a middle zone with only papilliform outgrowths and a posterior zone bearing countor feathers and fewer papilliform outgrowths. The transformation of normal countour feathers into bristles does not seem to involve any quantitative change in the number of follicles but appears to involve qualitative changes marked by an increased concentration of melanocytes and poor development of follicular muscles. On the other hand in Ibis, its head and neck become secondarily apteric and bare. Histological and histochemical observations made in these regions of the two birds indicate the presence of lipoidal substances in substantial quantities especially in the epidermis. These observations tend to strengthen the contention that the secretory activities of avian integument are inversely related to the degree of its feathering. Elaboration and accumulation of lipoidal material appears to be the characteristic functional feature of all secondarily formed apteric regions of birds. A number of functions could be ascribed to these secretory activities, like adaptive value in feeding habits, flexibility, thermoregulation, as well as in social communication as these areas are usually coloured. A detailed discussion is given in the text.