Chapter 3

FEATHER LOSS FROM CAPITAL TRACTS RELATED TO GROWTH AND MATURITY OF THE PAINTED STORKS : DISTRIBUTION OF GLYCOSAMINOGLYCANS AND PHOSPHATASES IN THE INTEGUMENT

Progressive transformation of the capital feather tracts into an apteric zone, as a normal feature during post-hatching development and maturity of the Painted Storks (Ibis leucocephalus PENNANT); and evidences for the secretory function of the secondarily formed apterium, are reported in the previous chapter (Chapter 2). Morphogenesis of all organs and their acquisition of functions are known to be preceded by chemodifferentiation exemplified by accumulation and/or loss of chemical components, enzymes etc. (Moog, 1965). Of considerable importance, in this respect, are the glycosaminoglycane and nonspecific phosphatases, implicated in a number of biological functions including secretory activities, and development and organogenesis of the integumentary derivatives (Hamilton, 1954; Hamilton, 1965; Moog, 1965; Dapson, 1970; Shah and Menon, 1971; 1974). Whereas changes in the relative contents of various types of mucopolysaccharides noted during development and aging in vertebrates are considered as significant developmental events (Lash et al., 1974), phosphatases are known to accumulate in tissues prior to their attainment of functional maturity (Moog, 1965). Though considerable

information is available on the significance of these chemical constituents in developing feathers, practically nothing is known about their concentration and distribution pattern in avian skin accompanying transformation of previously feathered region into an apteric zone. Hence the present study was undertaken to evaluate histochemically, glycosaminoglycans and phosphatases in the progressively balding crown skin of the Painted Stork.

OBSERVATIONS

(A) Histochemistry of glycosaminoglycans :

Nestlings with natal down feathers : (upto 10th day after hatching).

All components of the skin, as named earlier (Chapter 2), especially the epidermis, stained intensely with alcian blue indicating presence of considerable amount of sulphated mucopolysaccharides; With AB-PAS staining, it appeared that most of the superficial (subepidermal) part of the dermis was non-alcianophilic but PAS positive, indicating the presence of nonsulphated mucopolysaccharides, with the deeper parts being positive for both the characteristics. Pulp cells of the developing mesoptiles were faintly stained with PAS as well as alcian blue, while feather epithelia were strongly alcianophilic.

Nestlings with mesoptile feathers (after about 10th day of post-hatching development).

Strong alcianophilia was noticed in the components of the developing contour feathers (which were to replace the mesoptiles in near future) and the interplumar epidermis. Distal parts of the developing feathers where keratinization was in advanced stage, were PAS positive; whereas, the proximal regions where keratinization was poor were alcianophilic and PAS negative. Interplumar epidermis was also faintly stained with PAS technique. Dermis exhibited only a weak alcian blue positive staining; however, increased PAS positive staining in most of the components of the dermis was noted as compared to that in the corresponding tissues during the previous stage of development.

Juvanal Storks with contour feathers (About 45 days after hatching).

The crown skin of the bird at this stage was strongly alcianophilic in most of the components including the growing feathers which were at various stages of their development. PAS positive staining was noticed only in the connective tissue components of the dermis and in the pulp cells of the feathers, though with a lower intensity in the latter.

Immature (1 year old) storks with partial feather loss from head :

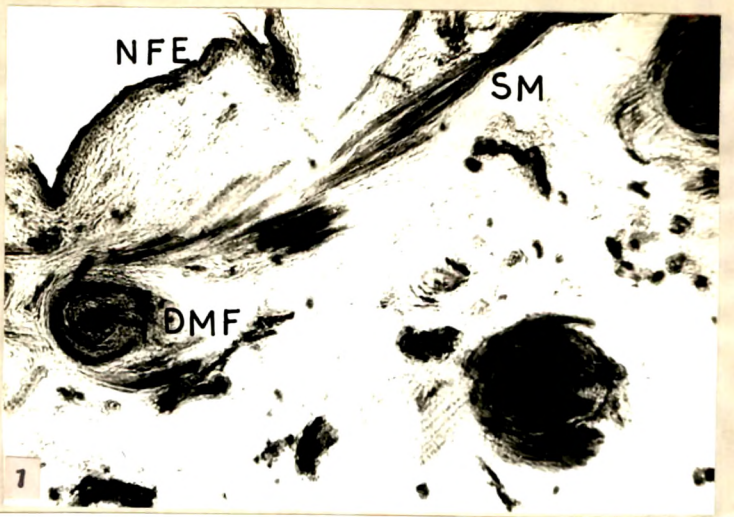
Striking morpho-histological differences observed between feathered and secondarily formed apteric zones were also reflected in the distribution of acid mucopolysaccharides.

EXPLANATION FOR FIGURES

Fig.1 Section of skin from a five day old Painted Stork showing alkaline phosphatase activity in developing mesoptiles and other components. 76X.

Fig.2 Section of capital skin from a Painted Stork chick bearing mesoptiles. Note the enzyme activity in developing juvanal feathers. 42X.

Fig.3 Section of capital skin bearing juvanal feathers. Note the enzyme reactivity in feather components. 42X.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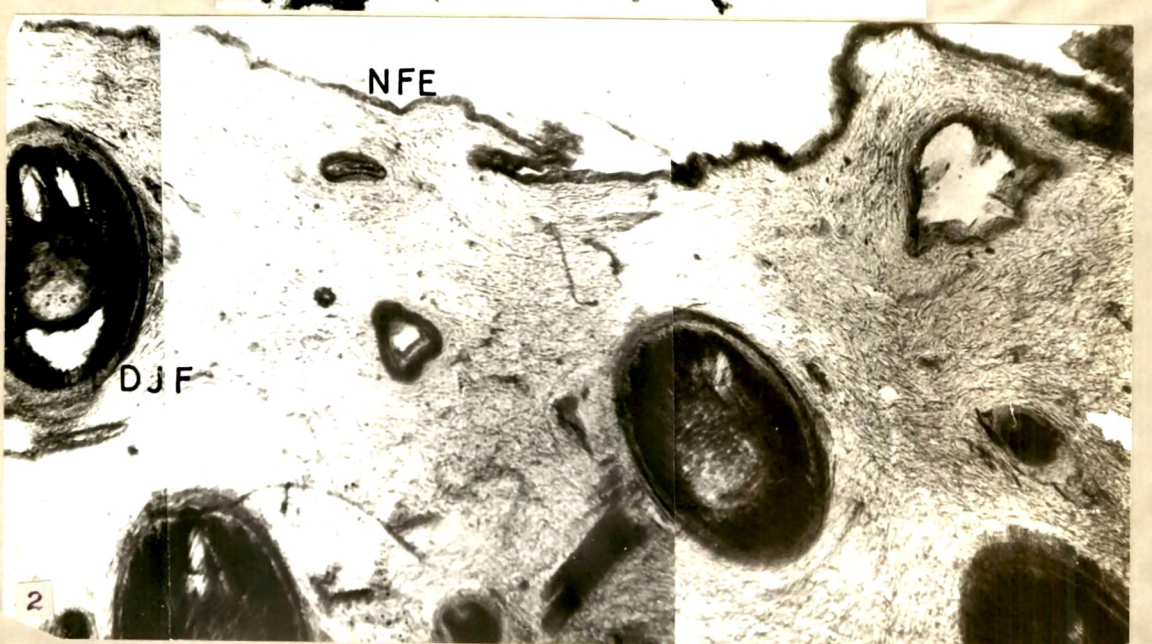
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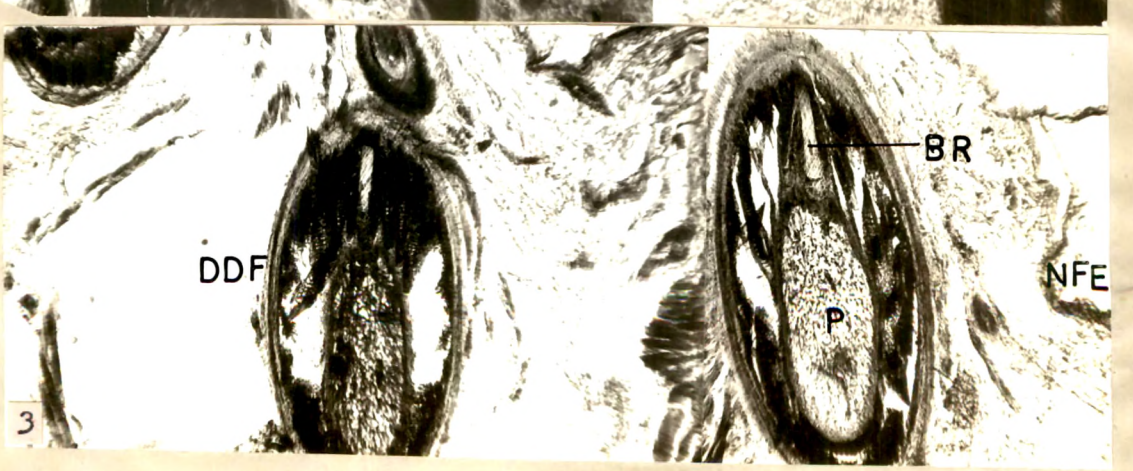
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While all the components of the skin in the feathered area, in general, were alcianophilic (with the exception of a mild PAS staining in some of the dermal components : such as follicular muscles), only the epidermis in the apteric zone was strongly alcianophilic with the dermal components being strongly PAS positive.

Adult Storks after two years with fully formed capital apterium :

In the entire crown region that had by now become apteric, only the epidermis was alcianophilic whereas dermis was intensely PAS positive. In contrast to this most of the skin components in the feathered areas of cervical region adjoining the capital apterium were predominantly alcianophilic.

(B) Histochemistry of phosphatases :

Nestlings upto 10th day after hatching with natal down feathers :

All the skin components were negative for acid phosphatase reactivity. However, strong reactivity for alkaline phosphatase was noted in the pulp and epithelial cells of the developing mesoptiles and smooth muscles of the follicles. While stratum germinativum of the inter-plumar epidermis showed low alkaline phosphatase (Alk Pase) activity, the other components remained negative to this reaction (Fig. 1).

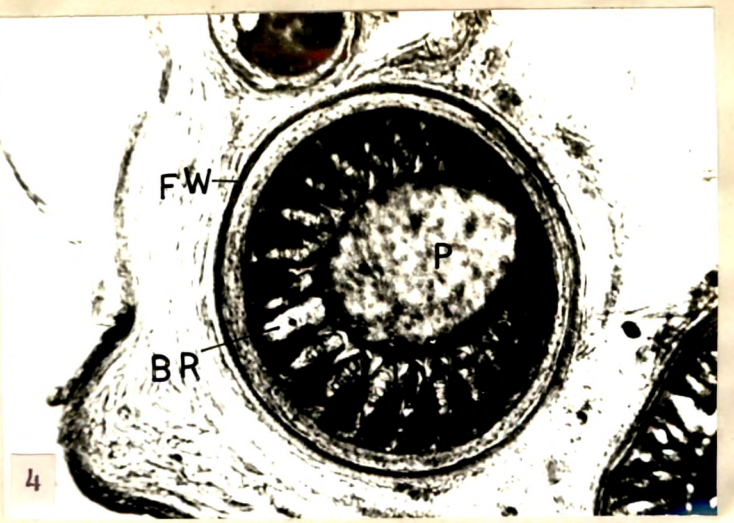
Nestling (about 10 days after hatching) with mesoptile feathers :

EXPLANATION TO FIGURES

Fig.4 Transverse section of a Juvanal feather showing alkaline phosphatase reactivity. 76×.

Fig.5 Section of skin passing through the secondary apterium as well as feather tracts of a one year old stork. Note abrupt increase in the enzyme activity in epidermis of the apterium. 13·5×.

Fig.6 Epidermis from the apterium of one year old bird, showing intense enzyme activity in the epidermis. 76×.



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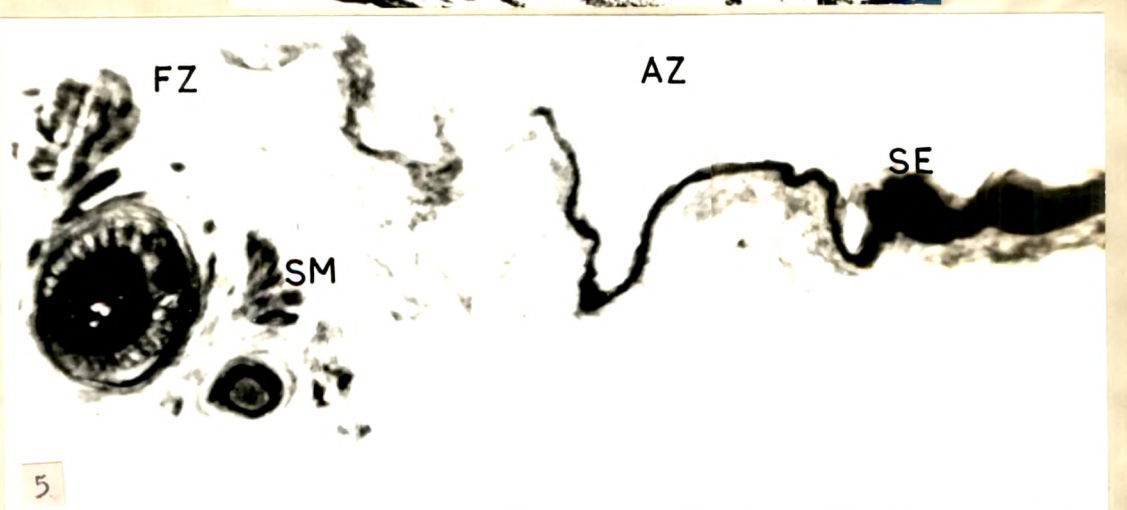

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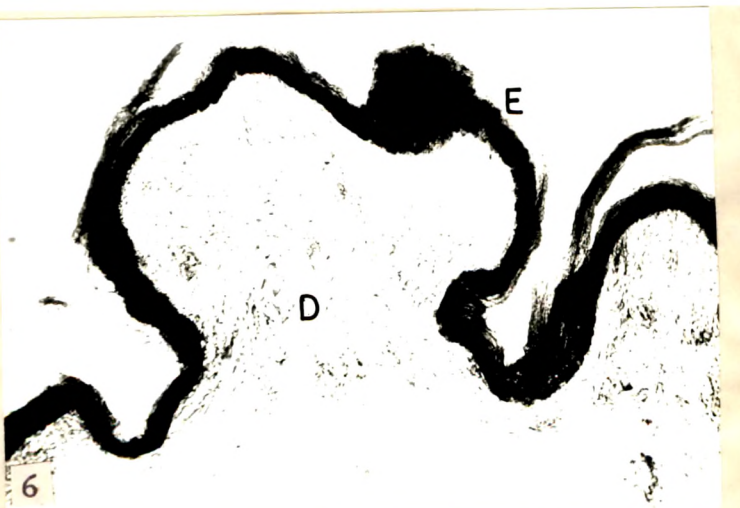

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There was no change in the distribution pattern and intensity of the reactivities of acid and alkaline phosphatases in all the components of the skin as compared to that observed in the corresponding tissues during the previous stage (Fig.2).

Juvanal Storks (About 45 days after hatching) with contour feathers :

Acid phosphatase activity was nil in the skin components, as was the case during both the previous stages - while alkaline phosphatase reactivity in the components of developing feathers, follicular muscles, interplumar epidermis and blood vessels in the dermis (Figs. 3 and 4) was quite noticeable. Nevertheless, the enzyme response was intense in the pulp cells of feathers and follicular muscles compared to that in other components of the skin.

Immature (1 year old) storks with partial loss of feathers from the capital tracts :

Acid phosphatase activity was not discernible in any of the skin components of the feathers and the apteric zones. In the feathered areas, alkaline phosphatase activity in the feather germs and smooth muscles was as noted during previous period, while it was low in the interplumar epidermis (Fig. 5). In the stratum germinativum of the epidermis of the secondarily apteric zone, the enzyme response was comparatively very high (Fig. 6). A sharp change in the enzyme distribution pattern and concentration from the feather tracts to the apterium

EXPLANATION FOR FIGURES

Fig.7 Feather follicles bordering apterium in an year old strok. Note that the muscles in anterior portion with less enzyme activity, also shows degenerative changes. 42×.

Fig.8 Photomicrograph showing transition from low to high alkaline phosphatase activity in the junction of apterium and feather tracts. 13-5×.

Fig.9 Photomicrograph showing epidermis and dermis from a portion of the secondary apterium from an adult, showing the enzyme activity. 76×.

ABBREVIATIONS

AZ - Apteric Zone; BR - Barb ridges; BVS - Blood vessels; D - Dermis; DDF- Developing definitive feathers; DJF- Developing juvanal feathers; DMF - Developing mesoptile Feathers; DMA - Dystrophic muscles; EPM- Errector ptilorum muscles; FZ - Feathered zone; FG- Feather germ; FW- Follicular wall; NFE - Nonfeather epidermis, P- Pulp; SM- Smooth muscles; SE- Secretory epidermis.

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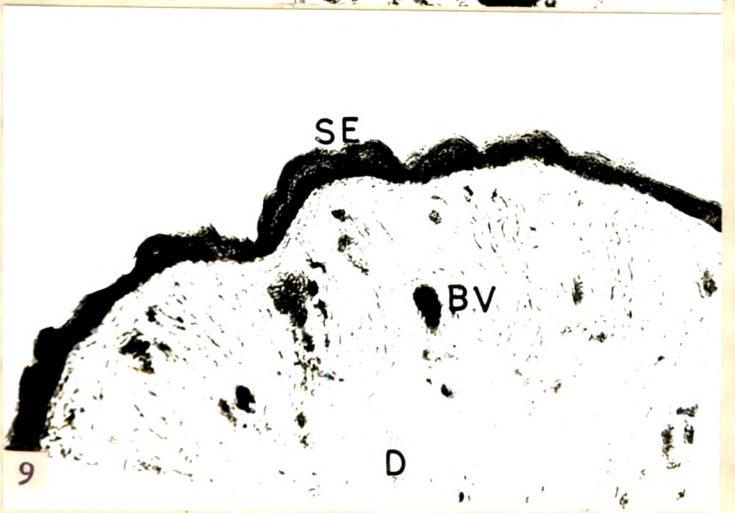

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was noticeable. Resting germs of feathers that are present in the feathered skin adjacent to the apterium (and which are destined to be lost in the subsequent moult leading to its conversion into an apterium) exhibited an intense reactivity for alkaline phosphatase. Follicular muscles in the feather tracts adjacent to the apterium which extend into the apteric zone, showed enzyme response only in parts that are very close to the follicles, while the parts which are extending deeper into the apteric zone depicted an obscure structural detail and diminished enzyme reactivity (Fig.7).

Adult storks (after the age of two years) with fully formed capital apterium :

Like in the previous stages, acid phosphatase reactivity was nil in the epidermis of capital apterium. However, a very faint, indefinite and diffused reactivity for the enzyme was noticeable in the dermis of this region. As for alkaline phosphatase, the stratum germinativum in the apteric skin showed intense histochemical response, whereas in the dermis, only the blood capillary walls were the enzyme reactive (Fig. 9). No trace of feather germs or follicular muscles could be discerned in the apteric zone even by using the histochemical localization of the enzyme as a tool. In the feather tracts of cervical region adjoining the capital apterium, the distribution pattern and intensity of alkaline phosphatase reactivity was similar to that noticed in the

feathered tracts of capital regions in the immature storks. As far as the interplumar epidermis is concerned, transition in the pattern of enzyme reactivity from feathered to apteric zone was abrupt and highly conspicuous (Fig. 8).

DISCUSSION

The present attempt to characterize glycosaminoglycans (GAG) in the integument of capital (initially feathered and subsequently becoming apteric) region of the Painted Stork during its postnatal development revealed that the relative amount of alcianophilic (sulphated) and PAS positive (non-sulphated) components of GAG undergo changes during plumage replacement and their subsequent loss related to growth and maturity of the birds. Variations in localization and intensity of alkaline phosphatase reactivity that accompany such morphological and histophysiological changes in the integument are of interest, since these two chemical components have been implicated in a variety of metabolic functions of the integument and developmental processes of cutaneous appendages in vertebrates (Koning and Hamilton, 1954; Johnson and Bevelander, 1946; Hamilton, 1965). Predominantly alcianophilic response of skin in nestling storks with natal down feathers (protoptiles) indicates that most of the GAG are sulphated mucopolysaccharides, localized chiefly in the interplumar epidermis, follicular muscles and developing mesoptile feathers which would soon replace the protoptiles. It is not surprising to note the

presence of intense alkaline phosphatase activity in these components of the skin, since GAG and the enzyme have been accredited with significant roles in the exchange and diffusion of metabolites (Laurint, 1966; Raekallio, 1970), functions that are of paramount importance to tissues undergoing development and maturation. Besides, alkaline phosphatase is known to be essential for proper feather development (Hamilton, 1965) and further, co-existence of GAG and the enzyme in developing down feathers of chick has been noticed by Koning and Hamilton (1954). It appears from the present findings that the sulphated mucopolysaccharides are more important in the epithelial cell differentiation in developing feathers. Though the PAS positive (non-sulphated) component of GAG in the skin registered an increase by the time natal downs were replaced by mesoptiles and remined so during the development of juvanal feathers (which was mainly in the dermis), the epithelial cells of the developing feathers (down and definitive) showed alcianophilia and intense alkaline phosphatase activity which are indicative of continuing morphogenitic activities in these components of the skin. Alkaline phosphatase has been implicated in synthesis of mucopolysaccharides (Kroon, 1952), while Shah and Menon (1974) have attributed a multifarious role to the enzyme during the developmental process of definitive feathers. Presently noted localization of the enzyme in the follicular muscles in the integument of the storks deserves mention, since its

activity was not demonstrable in the follicular muscles of the fowl (Scheen and Winkelman, 1960) or pigeon (Shah and Menon, 1974). The differences might probably be due to the histochemical methods used.

Shah and Menon (1971) demonstrated presence of acid phosphatase in the developing definitive feathers of pigeon. Presently noted absence of acid phosphatase in the feather follicles at all ... stages of their development is a little puzzling. It is possible that the specimens from which biopsies were obtained had feathers in the early stage of development and the enzyme probably makes its appearance in feathers at a later stage when regression of pulp occurs. Similarly, the very poor but visible response of the adult apteric skin components of dermis to acid phosphatase activity is also difficult to explain at this juncture.

The different and varied distribution pattern of the two types of glycosaminoglycans (sulphated and non-sulphated) as well as alkaline phosphatase in the feather tracts and apteric zones of the skin from capital region of immature (1 year old) and adult (2 years and above) storks in relation to feather loss is interesting. The obvious differences in morphological and histological profiles between the feathered and secondarily formed apteric zones are also reflected in the presently studied aspects of histochemistry of the skin. While the dermis in the feather tracts adjoining the apteric zone

largely showed presence of sulphated mucopolysaccharides and alkaline phosphatase in many of its components; dermis in the secondarily formed apterium showed GAG of the non-sulphated (PAS positive) type and an almost nil alkaline phosphatase activity. It is pertinent to recall here that the dermal components like smooth muscles and feather papillae which had shown intense alkaline phosphatase reactivity and presence of sulphated (alcianophilic) mucopolysaccharides lost these characteristics during the transformation of the skin into apterium.With an increase in the connective tissue mass, the dermis became more PAS positive (non-sulphated). However, when these facts are viewed in the light of other changes occuring in the dermis, namely, accumulation of lipid droplets and increased vascularity (Chapter 2), the changes in the relative contents of GAG and loss of alkaline phosphatase activity seem to be of significance concerned with the changes occuring in the functional characteristic of the apterium during its morphogenesis.

In view of the present observations on the distribution pattern of these chemical components in the apterium as well as the attributed functioning of alkaline phosphatase in exchange and diffusion of metabolites, and the implication of chondroitin sulphate and related mucopolysaccharides (AB positive - i.e. sulphated) in regulation of enzymic and other metabolic processes, it may be pressumed that the factors

(genetic or endorcrine or both) which cause the formation of the apteric zone in the capital regions of these storks, somehow alter the distribution pattern of the key tissue components as part of their chemical regulatory mechanism. Thus, it is possible that loss of feather germs and associated structures like follicular muscles, might be due to the loss of essential enzymes such as alkaline phosphatase, mediated by the quantitatively and qualitatively changing contents of the regulatory glycosaminoglycans. In the secondarily formed apteric zone, the functional state of the dermis must be undergoing changes as could be visualised from the present findings. Disappearance of AB positive (sulphated) components of GAG like chondroitin sulphate (known to show a tendency for heavy hydration) could be related to the tremendous increase in the dermal lipids accompanying transformation of capital tracts into apterium (Chapter 2). Since glycosaminoglycans in the dermal matrix determines, to a great extent, the properties of skin, it is quite likely that feather loss and subsequent exposure of the skin, necessitates changes in its physical properties too. Retention of alcianophilia and high alkaline phosphatase activity in the epidermal cells of the secondarily formed apteric zone (as opposed to negligible activity of the enzyme in the interplumar regions of the feather tracts) indicate the occurrence of active biochemical processes possibly related to lipoid secretion and carotenoid metabolism. Distribution of

both alkaline phosphatase as well as glycosaminoglycans in the skin glands of various vertebrates have been studied extensively, and their significance in secretory activities well documented (Kar, 1950; Das and Ghosh, 1959; Bhattacharya and Ghosh, 1958; Dapson, 1970; Dapson et al., 1973). Thus, the histochemical differences noted between the two regions of the skin, (viz., feather and apteric zones) offer a positive clue to their differential functional status. Besides, the changes in the type of GAG as well as the distribution and intensity of alkaline phosphatase activity accompanying the alteration of the feathered skin into an apteric one, are suggestive of a shift from one function to another.

The rich colouration of the secondarily formed apteric skin in the capital region of the stork is due to the abundant carotinoid pigments in the epidermis. Though, lipids are the solvents for these pigments, it is difficult to ascertain at present as to whether the highly enhanced secretory activity of the epidermis is solely for the purpose of colouration or for something else. In the Painted storks, the apteric crown region remains coloured all through out life becoming brighter during the breeding season. Unlike the Japanese Crested Ibis, Nipponia nippon, (Uchida, 1970), the Painted storks do not seem to transfer any colouring secretory material to other parts of the body by the brightly pigmented apteric crown which it rubs on the feathers during comfort movements or other postural movements.