Chapter 6

INCUBATION PATCH FORMATION IN HOUSE SPARROW, PASSER DOMESTICUS : POSSIBLE ROLE OF GLYCOSAMINOGLYCANS (GAG)

Formation of the incubation patch, viewed from the point of view of developmental biology, involves two distinctly diverse events. While, there is active proliferation and stratification of epidermis in the interplumar region of the ventral skin, the epithelial cells of the feather germs possibly under some inhibitory influence fail to multiply, resulting in a prolonged dormancy of these cutaneous structures. Such a differential activation or suppression of the developmental events in the epithelial cells from the two loci could well be due to metabolic differences reflected in the enzymological profile of the tissue. Moreover, it is logical to assume that inherent differences also exist between that of interplumar epidermis and the feather epithelium; hence, histochemical localization of certain important chemical components and evaluation of possible qualitative differences present therein would be of value in analysing metabolic differences in these two regions. Functional differentiation of epithelial cells in a number of organs and organ systems depends on an essential event of epithelio-mesenchymal tissue interaction. Examples of organs

where such a phenomenon occurs, are salivary glands, kidney, pancreas, lung, lens, liver, mammary glands (Kratochwil, 1963), limbs, thymus, thyroid (Hilfer, 1967) and skin (Sengel, 1976). Though such epithelio-mesenchymal interactions are required for epithelial morphogenesis, it is clear from transfilter-organ-culture experiments that direct tissue contact is not required (Grobstein, 1967). This finding led to study of the role of extracellular materials at the interface between the two interacting tissues, in moderating morphogenetic interactions. The major molecular species of these substances have been identified as mucopolysaccharides (Glycosaminoglycans - GAG) and collagen (Bernfield and Wessels, 1970). The surface associated mucopolysaccharides (MPS) are involved in the branching morphogenesis of the salivary glands. The possibility of a specific combination of macromolecules including MPS collagen and other extracellular glycoproteins influencing the pattern of morphogenesis has also been suggested (Spooner, 1974). Acid MPS (GAG) could bind calcium at the cell surface, and a unique array and distribution of these interface materials might then provide differential calcium availability to the epithelium and bring in a differential developmental response in this tissue (Spooner, 1974). With these facts in view it was thought desirable to study the possible alterations in distribution pattern of GAG in the ventral skin of female house sparrows which might underlie the phenomenon of incubation patch formation, its maintenance, and regression to return to

the non-functional (as far as heat transfer is concerned) feathered condition.

OBSERVATIONS

Appreciable changes were noticed in the intensity of staining as well as relative distribution of the two types of glycosaminoglycans (alcianophilic and PAS positive) during the various phases of incubation patch formation in House Sparrow. In general, glycosaminologycans of both alcianophilic as well as PAS positive types were noticeable in the ventral skin of female birds during the non-breeding season (Fig.1). Here the epidermis, deeper parts of dermis and feather germs were predominantly alcianophilic, whereas the more superficial layers of the dermis (subepidermal parts of dermis) were PAS positive. Alcianophilia was more pronounced in the prospective patch areas of the skin than in the adjoining skin bearing feather tracts, while PAS positive staining was comparatively lesser in the former than in the latter.

During the initial stages of patch formation (i.e. while defeathering of the ventrum was in progress) an almost two-fold increase in the intensity of staining of PAS positive component of the glycosaminoglycan was noticed in the patch skin. However, the concurrent increase in alcianophilia was not of an appreciable extent. Thus most of the dermis in the patch region appeared to be PAS positive. With the completion of defeathering, (Fig. 2) increased vascularization of the dermis

EXPLANATION FOR FIGURES

Fig.1 Photomictograph of V.S. of ventral skin in non-breeding season. Note feather follicles (FF) and adipose tissue (AT). Stained with Alcian Blue-PAS technique. 160X.

Fig.2 Photomicrograph of section of skin from ventrum after defeathering. Note feather germ (FG) and adipose tissue. Alcian Blue-PAS staining. 60X.

Fig.3 Photomicrograph of section of fully formed incubation patch. Note wrinkles on the skin, epidermal thickness, loss of dermal compactness and increase in blood vessels (BV). Feather follicle and adipose tissue are evidently seen. Alcian Blue staining. 60X.

Fig.4 Photomicrograph showing presence of feather germ in the fully formed patch. Alcian Blue - PAS staining. 60X.

Fig.5 Photomicrograph depicting the PAS positive zone (PPZ) subjacent to the hyperplastic epidermis. Alcian Blue - PAS staining. 160X.

OBSERVATIONS

ALZ- Alcianophilic zone; AT- Adipose tissue, BV- Blood vessel, D- Dermis, E- Epidermis, FF- Feather follicle, FG- Feather germ, FW- Follicular wall, PPZ- PAS positive zone, SC- stratum cormeum, SG- Stratum germinatum, SM- smooth muscles.

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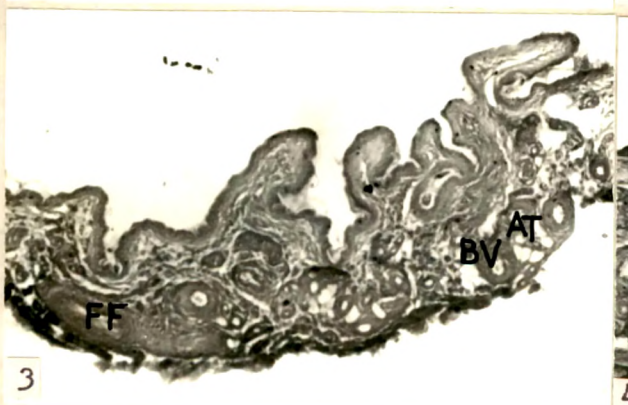

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and additional stratification of the epidermis were both evident; however, the intensity of staining and the distribution pattern of GAG remained almost the same in all the components of the skin as was noticed during the earlier phase. Nevertheless, the band of dermis subjacent to the epidermis became less alcianophilic and thus appeared to be distinctly PAS positive.

In the fully formed patch, the hyperplastic epidermis became strongly alcianophilic while the subepidermal region of the dermis showed a further increased PAS positive response (Figs. 3, 4 and 5). However, the feather germs that were predominantly alcianophilic, achieved an enhanced state of alcianophilia, representing the highest peak level in the fully formed incubation patch. The walls of blood vessels in the dermis were both alcianophilic as well as PAS positive. The epithelial cells of the follicular wall and those of the interplumar epidermis were highly alcianophilic in contrast to those covering the quiescent feather germs in the patch skin. The dermal papillae presented only a weak PAS positive response.

The concentration and distribution of GAG in all parts of the ventral skin (patch as well as the adjoining feathered skin) remained so till the patch began to regress. During the regression phase, a notable decrease in the intensity of staining of alcianophilic as well as PAS positive components of the GAG was noted, though the distribution pattern of these

did not show any change. During refeathering of the patch, the GAG concentration and distribution did not differ much from what was seen during regression phase.The epidermis as well as deeper layers of dermis returned to their normal alcianophilic state which was more or less like that seen in the normal feathered ventral skin during the non-breeding season.

DISCUSSION

A general increase in the concentration of GAG in the ventral skin, concomitant with the formation of the incubation patch highlights the functional involvement of GAG in the process. Such alterations in GAG concentration under hormonal influence is known to occur in target areas of avian integument like combs and wattle (Szirmai, 1956a and b) and in many mammals, especially in the regions of skin (like sex skin) in monkeys, that are the target organs for steroid hormones (Bentley, 1970). Besides, a number of other hormones are also capable of modifying the integumentary GAG content. This contention finds support in the report of reduced staining response of the skin to PAS technique in diabetic human subjects as opposed to healthy individuals (Rosenquist, 1977). Since much of the physical and physiological properties of the skin are determined by the dermal matrix (integral components of which are the GAG), it seems fruitful to think in terms of the possible modifications in the integumentary functions

with alterations in the GAG composition affected through hormonal status.

The increase in alcianophilia of the patch skin, especially that of the deeper layers of the dermis, reaching the maximum intensity in a fully developed edematous patch could mean increase in the sulphated mucopolysaccharides like chondroitin sulphates which are significant in collagen production. The lack of PAS staining and presence of high alcianophilia in this region suggests that possibly hyaluronic acid also might be increasing, which would be significant in augmenting retention of water in the tissues and inducing an edematous condition. Possibly, the edema of the denuded patch skin provides a 'cushioning effect' during incubation.

Since the non-sulphated mucopolysaccharides (PAS positive components of GAG) are regarded to have a stimulatory effect on proliferative activities of cells, and since the superficial layers of the dermis are strongly PAS positive in the incubation patch, it is tempting to believe that this component of the skin stimulates epidermal hyperplasia which is evident at this stage. Simultaneously observed low PAS positive reactivities in the feather papillae (which are in a quiescent state) present in the patch skin could be considered as one of the factors that is involved in the suppression of proliferation of feather epithelial cells; which ultimately results in a temporary dormancy of the feather follicles.

Sengel and his co-workers (see Sengel, 1976) through their elaborate experimentation on avian integument, have established that dermis influences the pattern of epidermal growth and differentiation. Importance of GAG in induction processes during morphogenesis of avian integument has been highlighted by Carinei et al., (1976).. GAG are also implicated in metabolic and/or enzymic regulation (Bentley, 1970). The changes observed in the activities of a number of enzymes in the different components of incubation patch (Chapters, 7,8,9) support such a contention.

The gradual decline in alcianophilia as well as PAS positive staining in the incubation patch and the adjacent feathered skin during regression of the patch and attainment of the near normal concentrations of GAG components in these areas during the refeathering of the patch indicate gradual return to normalization of the integumentary functions. It is pertinent to note that by this stage, broodiness of the birds also terminates.The reduced GAG concentration in the skin, a condition that does not favour water retention in the tissues, results in loss of edema which is a feature of patch regression. The noted changes in the distribution pattern and intensity of GAG concentration in the target areas (patch) in the ventral skin of the House Sparrow during patch formation, its maintenance, and regression (which are effected through mediation of some

hormones) throw light on the possible mechanisms that induce epithelial cell proliferation of the interplumar regions and suppression of the same in the dormant feather follicles.