Chapter 7

INCUBATION PATCH FORMATION IN HOUSE SPARROW, *PASSER DOMESTICUS* : DISTRIBUTION PATTERN OF PHOSPHOMONOESTERASES

As compared to studies on mammalian integument there is, in general, a paucity of literature on the histoenzymological profile of avian integument though some reports on these aspects are available in the studies of Hamilton and his co-workers (Hamilton, 1965); Spearman (1966) and Shah and Menon (1971, 1972, 1973, 1974a, 1974b, 1975, 1976). Hamilton (1965) investigated histochemically certain chemical aspects of regulation in the development of chick down feathers. Shah and Menon (1971, 1972, 1973, 1974a, 1974b, 1975, 1976) have studied the activity of a number of enzymes *viz*., acid phosphatase, alkaline phosphatase, succinate dehydrogenase, malate dehydrogenase, lactate dehydrogenase, β-hydroxy butyrate dehydrogenase, alpha glycerophosphate dehydrogenase, glucose-6--phosphate dehydrogenase, lipase, aldolase etc., concerned with the metabolism and energetics of the development of definitive feathers, developing under normal, induced as well as regenerative conditions in pigeons. Whereas, all these workers have laid emphasis on feather development and its regulation the histochemical correlates of the events concerned with suppression of feather development as it occurs cyclically, during the formation of a naked patch of skin from previously

feathered areas (i.e. the formation of incubation patch), remain to be elucidated. Despite the present state of our knowledge regarding the histomorphological, experimental and endocrinological aspects of incubation patch formation (Jones, 1971, a review) the studies on suppression of development of feathers are lacking. The present studies are so designed to fill some of the lacunae in our knowledge regarding chemical regulation of formation, maintenance and regression of incubation patch in House Sparrow. The present chapter deals with histochemical studies on the role of acid and alkaline phosphatases during the above stated phases of incubation patch in the House Sparrow, Passer domesticus.

Acid phosphatase (Ac Pase) is known to bring about hydrolysis of esters of phosphoric acid at an acid pH, and also liberation of inorganic phosphates. Like other hydrolytic enzymes, Ac Pase is localized in lysosomes (Duve, 1959; Novikoff, 1961). Considerable attention has been paid in recent times to this enzyme, elucidating its localization and possible roles in physiological and biological activities of a number of tissues. It is interpreted that the enzyme, according to the functional state of the tissue might be involved in a number of activities such as phagocytosis (Klockas and Wegelius, 1969); dissolution of tissue components (Weber and Niehus, 1961); synthetic activities (Sauter, 1967; Mishra and Mohanty, 1967); fat absorption (Barka, 1963);

differentiation (Ghiretti, 1950) and degenerative processes (Brachet et al., 1958; Misch, 1962; Scheib, 1963; 1965; Weiss, 1966; Mills and Lang, 1972). Shah and Menon (1971) have suggested that Ac Pase is a normal constitutive enzyme in the adult pigeon skin, where its uneven distribution in the epidermis (interplumar and feather) is correlated with the asynchronous mode of moulting of epithelial sheets, and also with the differences in the degree of keratinization and formation of different types of keratin in two distinct regions (viz., feather and non-feather). The role of Ac Pase in the physiological and biochemical changes accompanying differentiation, development and growth of feathers in pigeon is also well established (Shah and Menon, 1971).

Alkaline phosphatase (Alk Pase) has been implicated in a number of physiological activities, for example, formation of fibrous proteins (Verzar and McDougall, 1936; Moog, 1946; Bradfield, 1950), calcification of bones (Moog, 1944; Pritchard, 1952) and phosphate transfer in DNA metabolism (Rogers, 1960). Alk Pase has also been associated with organogenesis and functional differentiation of chick and mouse intestine (Moog, 1950, 1951), digestive system of steel head trout (Prakash, 1961), avian brain (Rogers, 1963), lungs of guinea pig and rat (Sorokin et al., 1959) and oesophagus and trachea of chick (Hinsch and Buxbaum, 1965).

Information available on Alk Pase activity in the vertebrate integument is relatively less. Notable amongst those available are the reports of Glenner and Burstone (1958) on the dermis of Necturus maculosus, of Schmidt and Weary (1962) on the skin of Diemictylus viridescens, Fell and Danielli (1943) on the healing skin wound of mammals and the comparative study of Scheen and Winkelmann (1960) on the activity of the enzyme in the integument of a number of vertebrates. Hamilton (1965), and Shah and Menon (1974) emphasised that Alk Pase activity is essential for the proper development of feathers in the chick and pigeon, respectively.

In the present study, histochemical observations on the distribution and localization of Ac Pase and Alk Pase activity were carried out on the ventral skin of female house sparrow, Passer domesticus, prior to and during the breeding cycles so as to understand the fluctuations, if any, in the distribution pattern and activities of these enzymes that can be correlated with various aspects of the incubation patch formation in this bird.

OBSERVATIONS

Acid phosphatase :

All the tissue components viz., epidermis, dermis, and the blood vessels of the ventral skin of the House Sparrow, during non-breeding (Fig. 1) and early nest building phases showed high concentration of the enzyme. Amongst all these

EXPLANATION FOR FIGURES

Figs. 1 to 6 : Photomicrographs of V.S. of ventral skin, showing histochemical distribution pattern of acid phosphatase activity in various phases of incubation formation in female House Sparrow. All 76 X.

Fig.1 Non-breeding phase.

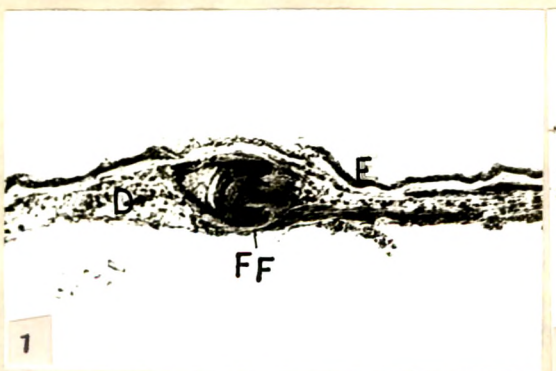
Fig.2 Nest building phase.

Fig.3 Defeathered and Vascularized phase.

Fig.4 Fully formed patch.

Fig.5 Regressing phase.

Fig.6 Refeathering phase.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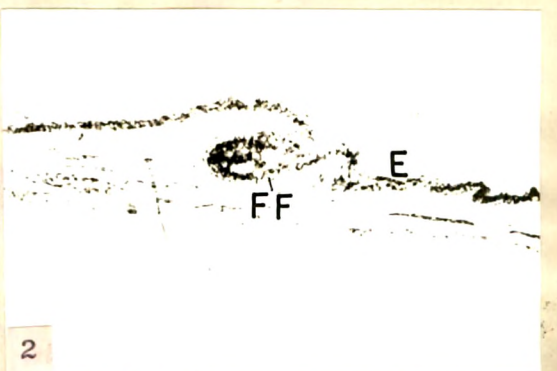
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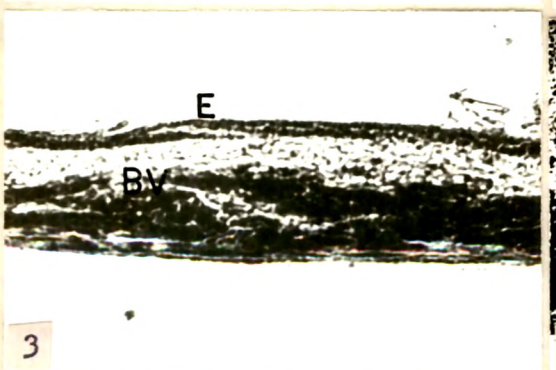
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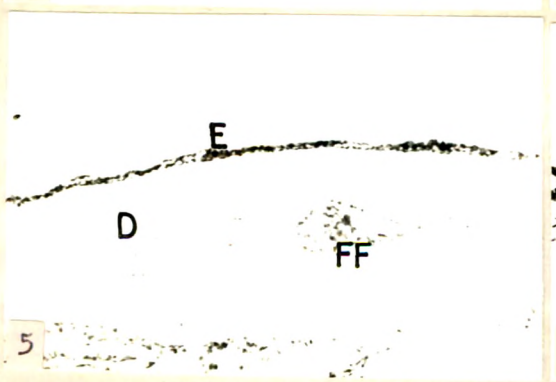
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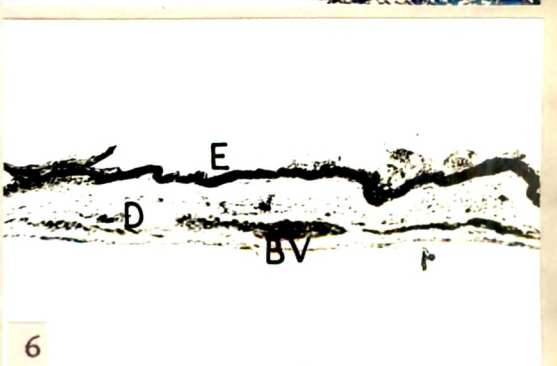
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components of the skin, the highest activity of acid phosphatase (Ac Pase) was elicited in the feather germs.

During the late nest building and defeathering phases, the ventral skin registered a relatively low enzyme response in all the components (Fig. 2). But, thereafter, in the completely defeathered phase whence high vascularization of the incubation patch was evident (Fig. 3), there was again a rise in the enzyme activity in all the components of the skin, except for the feather germs, reaching a level higher than that observed in the previous phase. The peak Ac Pase activity in the fully formed patch was evident in all the above referred components of the skin (Fig. 4). At this time the dormant feather germs presented a low level of the enzyme reactivity.

During the regression phase of the incubation patch (Fig. 5), there was a general decline in the intensity of the enzyme activity, particularly evident in the structural components of the dermis and stratum germinativum of the epidermis. However, the cornium layer of the epidermis and the feather germs registered a slightly higher reactivity of the enzyme than observed during the previous phase.

When the refeathering began, Ac Pase activity enhanced in the interplumar epidermis and feather germs, whereas the dermal components of the skin registered only a moderate enzyme response (Fig.6). At the late refeathering phase, the intensity and distribution pattern of the enzyme was found to be almost

similar to that observed in the corresponding tissues of the skin of the adult non-breeding female sparrow.

Alkaline phosphatase :

Among the components of the skin of sparrow during non-breeding (Fig. 7) as well as early breeding periods (nest building phase), a positive histochemical response for Alk Pase was observed only in the feather germs and the epidermis.

During defeathering of the ventral skin, discernible enzyme response was noted only in the epidermis. Rest of the skin components registered an almost nil to negligible activity of the enzyme.

When vascularization of the nude patch skin began, the stratum germinativum and the dermal connective tissue (which were in a state of proliferation) and also the blood vessels showed fairly high enzyme reactivity (Fig. 8). In the completely defeathered and highly vascularized incubation patch (fully formed) the strength and localization of the enzyme reactivity remained same as that was observed in the earlier phase (Fig.9). However, the feather germs which remained dormant all throughout these phases showed almost negligible activity of the enzyme.

A gradual reduction in the enzyme response could be observed in the dermal components and stratum germinativum, during the regressing phase (Fig. 10) of the incubation patch; i.e., a few days after hatching of the eggs. However, feather germs showed slightly increased response for the enzyme.

EXPLANATION FOR FIGURES

Figs. 7 to 11 : Photomicrographs of V.S. of ventral skin : showing histochemical distribution pattern of alkaline phosphatase activity in various phases of incubation patch formation in female House Sparrow. All 76 X.

Fig.7 Non-breeding phase

Fig.8 Defeathered and Vascularized phase.

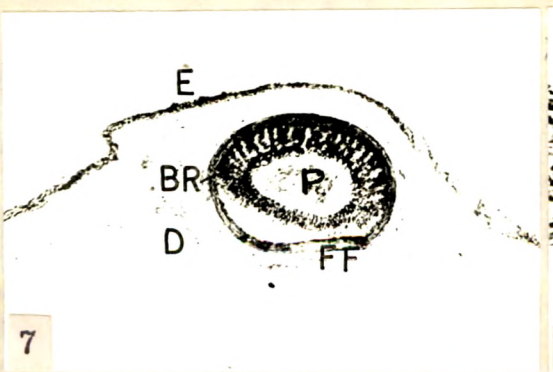
Fig.9 Fully formed patch.

Fig.10 Regressing phase.

Fig.11 Refeathering phase.

ABBREVIATIONS

BV- Blood Vessels ; D- Dermis, DF- Down feather; E-Epidermis; FF- Feather follicle; FG- Feather germ, FP- Feather popillae; FW- Follicular wall; SG- Stratum germination, SM- Smooth muscles.



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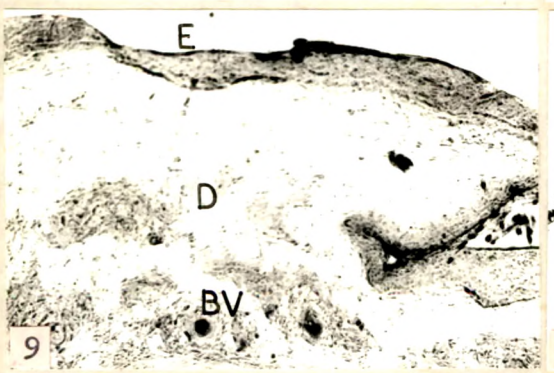

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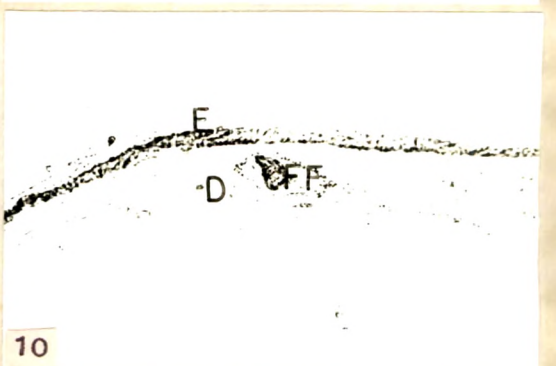

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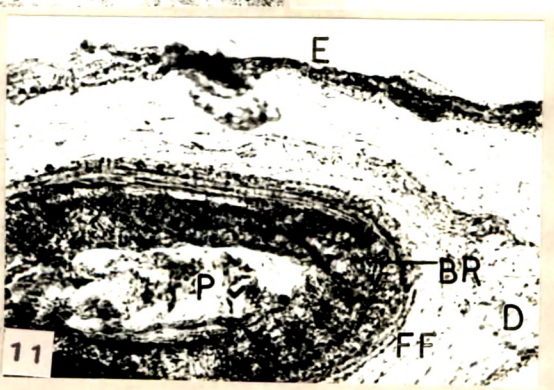

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During refeathering phase (Fig. 11) all the components of the patch skin maintained the same response for the enzyme as that was seen during the regressing phase, but the only change that was noticeable was in the feather germs where it increased to a moderate (normal) level.

DISCUSSION

Presence of higher concentration of Ac Pase as compared to Alk Pase in all the components of the skin of sparrow during its non-breeding as well as early breeding periods seems to be a normal feature. This observation finds support in the work of Montagna (1962) who also observed more or less similar relations for the activities of these two phosphatases in the integument of other vertebrates where Ac Pase predominated. Shah and Menon (1971, 1974) also have recorded similar observations in the pigeon skin. These findings, in general, favour the suggestion of Moog (1965), that Ac Pase is a constitutive enzyme whereas Alk Pase is an adaptive one. Positive Alk Pase activity, found in the feather germs, which are the sites of morphogenetic activity, is only to be expected, considering the known involvement of the enzyme in regulation and initiation of feather development. Besides, some authors consider Alk Pase as an inducer substance.

In the female sparrows during late nest building and defeathering phases, when loss of feathers from the ventrum is initiated and the skin finally becomes completely bare,

the feather germs enter into a phase of dormancy. At this stage the metabolic activities of the feather germs are at a very low level. Thus the observed decline in the activities of Ac Pase and Alk Pase in the feather germs seem to support the above contention. Relatively high response for both the enzymes in the walls of blood vessels, stratum germinativum and dermis, during the next stages, (corresponding to hyperplastic activities of the epidermal and dermal components resulting in a high vascularization and the achieving of an edematous state of the patch) could be correlated to the active specific functional state of the incubation patch. Coincidentally, the lipid content of the epidermis and dermis in this region of the patch skin increases during these phases (Chapter 9), which is indicative of increased synthesis necessary for the process of active cellular proliferation. The increased lipid content of the skin could also be correlated to the loss of feathers. Lack of insulation of the patch skin with feather loss is possibly compensated to some extent by the increased lipoid secretion by the epidermis. Role of epidermal lipids in curtailment of cutaneous water loss from the edematous patch skin is quite possible. The involvement of Ac Pase in lipid metabolism seems to be a possibility and this gains support from the report of Schmidt (1963), who found a positive correlation between lipid metabolism and Ac Pase localization in the limb tissues of the adult newt. It is interesting to note in this

connection that Lasman (1967) has assigned a significant role for phosphatases in various synthetic and metabolic activities in amoeba, Mayorella palastinensis. Further, Ac Pase has been implicated not only in protein synthesis (Eranko, 1951; Pearse, 1968; Novikoff, 1961; Sood and Tewari, 1969), but also in the synthesis and transport of carbohydrates (Sauter, 1967; Mishra and Mohanty, 1969). These functions could easily be attributed to the enzyme in the sparrow skin during formation and maintenance of the incubation patch.

Presence of Alk Pase in the blood capillary walls during defeathered and vasularized state as well as in the fully formed patch, might be for helping in the transport of substances across the blood capilary walls as suggested in case of mammals by Romanual and Bannister (1962). According to Kroon (1952) mucopolysaccharides could be synthesised from hexoses liberated by the action of phosphatases from hexose phosphate esters, produced during glycolysis. Ten Cate (1959) has suggested involvement of this enzymes in the formation of mucopolysaccharides. Thus in the present case the enzyme activity can be well correlated with the increase in the glycosaminoglycan content of the fully formed patch. More significantly, occurrence of non-sulphated (PAS positive) GAG components in the superficial parts of the dermis (subjacent to the epidermis) has been noticed which is correlated to the epidermal hyperplasia, since such mucopolysaccharides are

known to have a growth promoting influence (Chapter 6). Moreover, functional correlation of Alk Pase with transport of metabolites across cell membranes has been pointed out by Anagnostopoulos and Matsudaira (1958) and Raekallio (1970).

The decrease in the activities of Ac and Alk Pases noticed in the skin components during regression of the patch coincided with a decrease in the concentration of glycosaminoglycan content of the skin (Chapter 6). Thus it becomes quite clear that Alk Pase is well involved in the reactions concerning metabolism of glycosaminoglycans (GAG) during the incubation patch formation and also its regression. Enhanced activity of Alk Pase was noticed in the components of the definitive feather, during the course of normal post-hatching, induced, and regenerative modes of its development by Shah and Menon (1974). Hamilton (1956) stressed the importance of Alk Pase and showed that inhibition of this enzyme interrupted the proper development and differentiation of chick down feathers. Voitkevich (1966) has also considered the possibility of Alk Pase being an activator substance in initiation of development and growth of feathers. It is suggested that Ac Pase is also involved in the process of differentiation and keratinization during refeathering. Similar enzyme response is reported by Shah and Menon (1971) in the developing feathers of pigeon.

Gradual and steady increase in the activities of both

the phosphatases in the feather germs seem to coincide well with the resumption of proliferative and differentiative activities of the feather germs during patch regressing and refeathering phases.

Wessels (1965) and Carinci et al., (1976) have suggested importance of acid mucopolysacharides in induction of epithelio-mesenchymal interactions during developmental processes. Extrapolating this fact we can presume that during the refeathering stage of the patch skin, whence the dormant feather germs are induced to develop, the increase in the activity of the phosphatases in these structures can be well correlated to the increase in the GAG concentration there (Chapter 6).