

Chapter 9

INCUBATION PATCH FORMATION IN HOUSE SPARROW, PASSER DOMESTICUS :
HISTOCHEMICAL OBSERVATIONS ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE,
 α -GLYCEROPHOSPHATE DEHYDROGENASE, β -HYDROXY BUTYRATE DEHYDRO-
GENASE AND LIPIDS

Hexose monophosphate (HMP) pathway of carbohydrate metabolism is one of the major metabolic routes operative in the avian integument (Shah and Menon, 1976). Production of ribose sugar needed for nucleic acid synthesis appears to be one of the chief functions of the reactions of HMP pathway (Langdon, 1957; Cohen, 1959). Presence of the activity of glucose-6-phosphate dehydrogenase (G-6-PDH), a key enzyme of the HMP pathway, could be used as an index of operation of this metabolic route. In an organ of heterogenous nature as the avian skin, wherein the epidermal cells are known to be active in lipoidal secretion, reactions of the HMP pathway are of significance. α -glycerophosphate dehydrogenase (α -GPDH) associated with the EMP pathway of glycolysis is an oxido-reductase that is concerned not only with the synthesis of lipids from carbohydrate intermediaries, but also involved in the utilization of glycerol. Activity of this enzyme has been studied in the integument of pigeon during feather development by Shah and Menon (1974) wherein, they have implicated its activity in the epidermis with lipid metabolism. Lipids

are metabolites of considerable significance in the integument of vertebrates including reptiles, birds and man (Chakko, 1967; Radhakrishnan, 1972; Matoltsy, 1969; Lucas and Stettenheim, 1972; Shibaeva, 1970) where, they are implicated in the process of keratinisation of epidermal cells (Shibaeva, 1970; Flaxman, 1972), as well as their derivatives (Bell and Thathachari, 1963). Lipids are also known to be secreted by the interplumar epidermis of birds, so much so that Lucas and Stettenheim (1972) consider the whole of the avian epidermis as a holocrine gland. Exaggerated secretory activity by the epidermis after the caputal skin becomes secondarily apteric in the case of Painted Storks is discussed in Chapters 2 and 3. With these facts in view it was thought desirable to find out the fluctuations in degree of secretory activity of the ventral skin of female House Sparrow where there is a periodical suppression of feather production (during incubation patch formation). Patterns of activity of the enzymes that are directly or indirectly involved with lipid metabolism in the incubation patch of House Sparrow were also studied. For this, histochemical investigations on distribution and localization of G-6-PDH, αC-GPDH, BDH and Lipids in the ventral skin of female House Sparrow during the non-breeding period, various stages of incubation patch formation, its maintenance, regression and refeathering, were carried out.

EXPLANATION FOR FIGURES

Figs. 1 to 6 : Photomicrographs of V.S. of ventrals skin, showing histochemical distribution pattern of G-6-PDH activity in various phases of incubation patch formation in female House Sparrow. All 76 X.

Fig.1 Non-breeding phase.

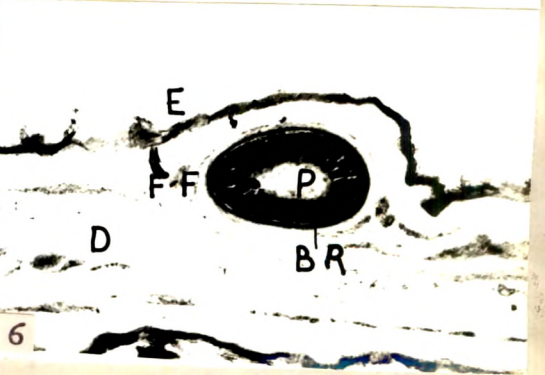
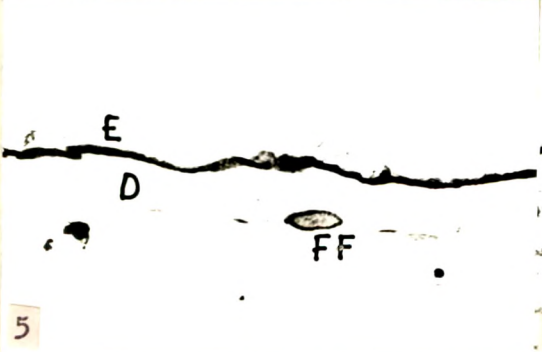
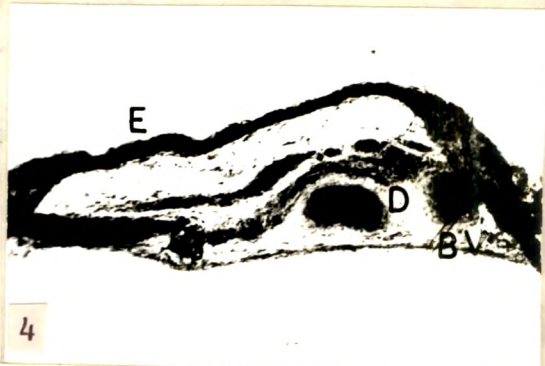
Fig.2 Nest building phase.

Fig.3 Defeathering phase.

Fig.4 Fully formed patch.

Fig.5 Regressing phase.

Fig.6 Reifeathering phase.



OBSERVATIONS

G-6-PDH : (Figs. 1 to 6)

Low histochemical activity of G-6-PDH was seen in the ventral skin during the non-breeding period (Fig. 1) as well as the early nest building phase of the breeding period (Fig. 2). However, at this stage feather germs exhibited a relatively little more response for the enzyme. Gradual increase in enzyme reactivity was evident in the epidermis and dermis in general, during early defeathering (Fig. 3), completely defeathered and vascularized phases of incubation patch formation. Maximum G-6-PDH activity was noticed in the stratum germinativum, walls of the blood vessels and the dermis of the fully formed patch (Fig. 4). During the regressing (Fig. 5) and refeathering phases (Fig. 6), a gradual decline in the enzyme reactivity was evident in all the components of the patch skin. However, the feather germs exhibited relatively enhanced enzyme response which was almost of the same level as was seen in the corresponding parts of the ventral skin during the non-breeding state of the bird.

αC-GPDH : (Figs. 7 to 11)

The histochemical activity of αC-GPDH in all the components of the ventral skin was low but discernible during the non-breeding phase (Fig. 7). From the nest building phase onwards, when the incubation patch was being formed (Fig. 8) there was a gradual increase in αC-GPDH activity in all parts of the

EXPLANATION FOR FIGURES

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

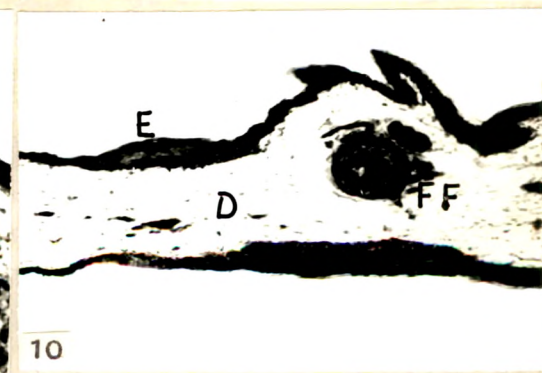
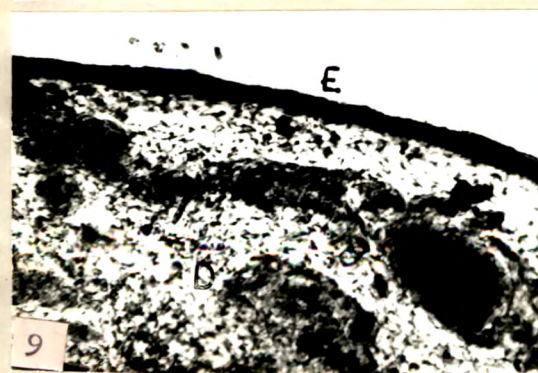
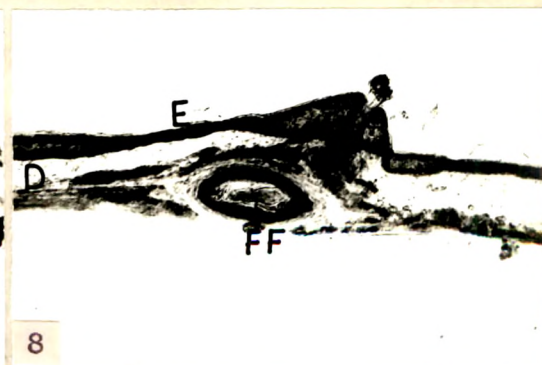
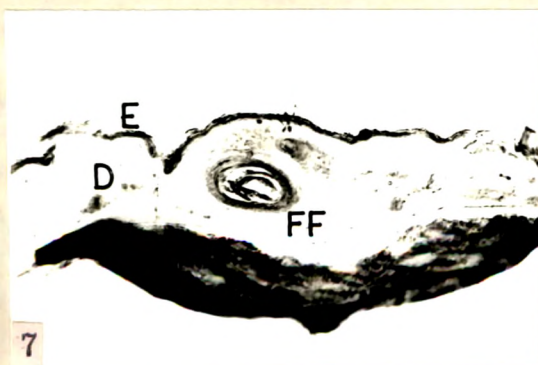
Fig.7 Non-breeding phase

Fig.8 Defeathering phase.

Fig.9 Fully formed patch.

Fig.10 Regressing phase.

Fig.11 Refeathering phase.



EXPLANATION FOR FIGURES

Figs. 12 to 16 : Photomicrographs of V.S. of ventral skin, showing histochemical distribution pattern of BDH activity in various phases of incubation patch formation in female House Sparrow. All 76 X.

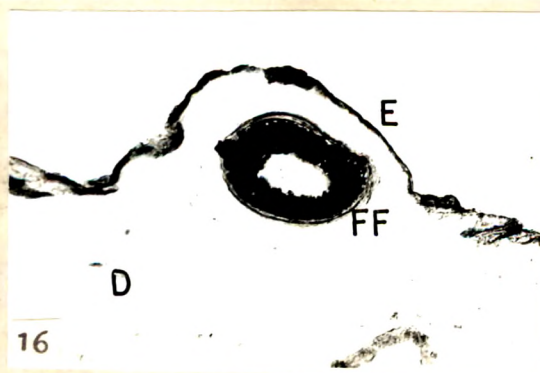
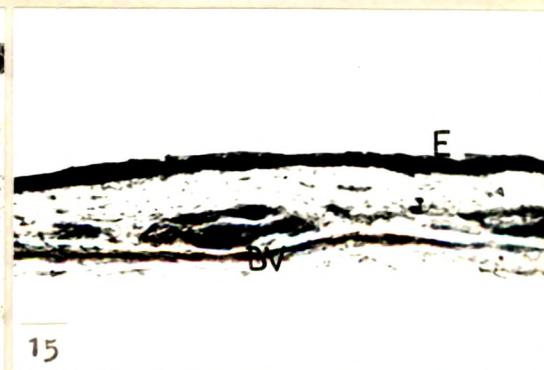
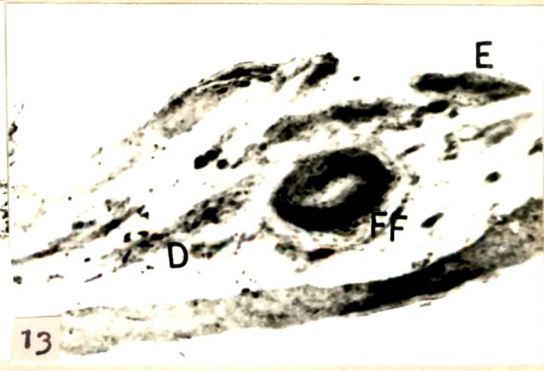
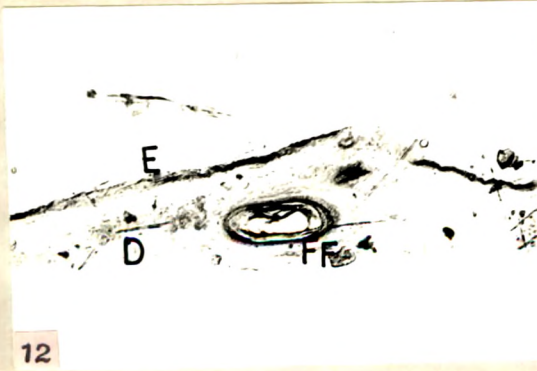
Fig.12 Non-breeding phase.

Fig.13 Defeathered and Vascularized phase.

Fig.14 Fully formed patch.

Fig.15 Regressing phase.

Fig.16 Refeathering phase.



ventral skin of the bird. The highest peak of enzyme activity was seen during the later phases of incubation patch formation, and remained so in the fully formed patch skin (Fig. 9). During the regressing (Fig. 16) and refeathering phases (Fig. 11), decline in α -GPDH reactivity was noted in the dermal regions and blood vessels; but in the epidermis, this decline was not so obvious and the feather germs exhibited moderate enzyme reactivity. Thereafter during late refeathering period, further decline in the enzyme reactivity was noted in the feather follicles and epidermis reaching ultimately in different components of skin, the characteristic levels of the non-breeding state.

BDH : (Figs. 12 to 16)

BDH reactivity was low in the ventral skin during the non-breeding period (Fig. 12). The enzyme reactivity slowly ascended and attained a peak level during defeathering, as well as in the defeathered and vascularized (Fig. 13), and fully formed (Fig. 14) phases of patch formation. It was highest in the epidermal cells, dermis in general (except for the feather germs), and walls of blood vessels. However, during the regression phase (Fig. 15) feather germs showed increased BDH reactivity, whereas the other skin components maintained the same high level as was seen during the early phases. During refeathering phase (Fig. 16) enzyme reactivity gradually began to decline ^{and} finally resulted in the attainment

EXPLANATION FOR FIGURES

Figs. 17 to 21 : Photomicrographs of V.S. of ventral skin, showing histochemical localization of neutral lipids in various phases of incubation patch formation in female House Sparrow, fattest 7 B.

Fig.17 Non-breeding phase. 76 X.

Fig.18 Defeathering phase. 192 X.

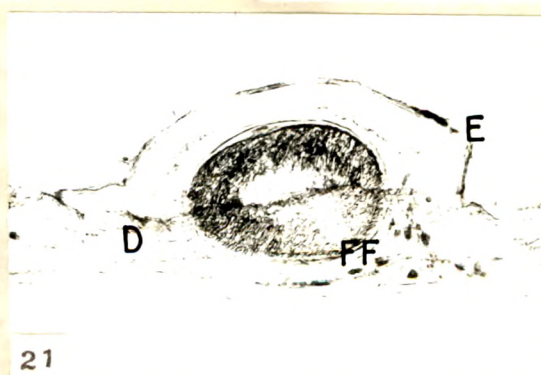
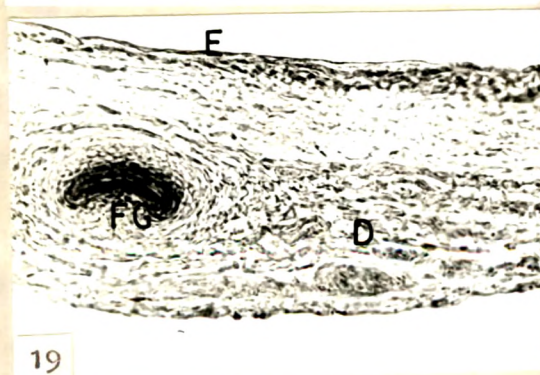
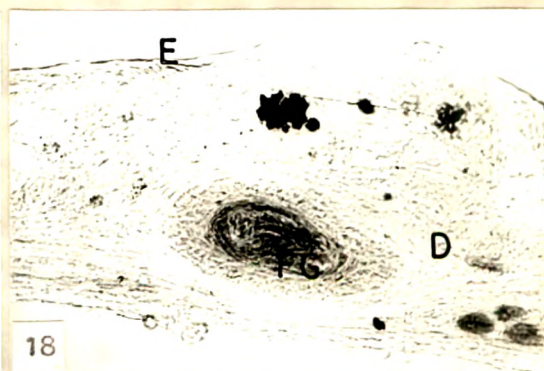
Fig.19 Fully formed patch. 192 X.

Fig.20 Regressing phase. 192 X.

Fig.21 Refeathering phase. 76 X.

ABBREVIATIONS

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



of a level, almost identical to that observed in the skin components during non-breeding phase.

LIPIDS : (Figs. 17 to 21)

Some amount of neutral and acidic lipids was observed in epidermis, dermis and feather follicles during the non-breeding (Fig. 17) and nest building phases. Dermal adipose tissue cells were noticed to have a high content of neutral lipids. Considerable increase in the amount of neutral and acidic lipids was discernible in the skin in general and epidermis in particular during defeathering (Fig. 18) and defeathered and vascularized phases of patch formation. Highest concentration of lipids was observed in the epidermis and subepidermal region of the dermis in the fully formed incubation patch (Fig. 19). During regressing (Fig. 20) and refeathering (Fig. 21) phases, the level of lipids gradually declined in all the components of the skin. However, at these stages in the feather germs, neutral as well as acidic lipids in higher concentration were observed which declined gradually during late refeathering phase to attain a level similar to that noted in the non-breeding phase.

DISCUSSION

The G-6-PDH activity in the feather germs and other components of the ventral skin during non-breeding, nest

building and late refeathering phases of incubation patch formation, could be considered as an indication that HMP shunt is operative as is the case with normal adult pigeon skin (Shah and Menon, 1976) and hair follicles of the mammalian skin (Adachi and Uno 1968).

The low levels of α -GPDH and BDH in the ventral skin components of sparrow conform to a similar state seen in the pigeon skin (Shah and Menon, 1972, 1974) and are suggestive of low lipid catabolism.

The normally occurring epidermal and dermal lipids of the avian integument could be considered to be of significance from the point of view of thermoregulation, in addition to being a reserve fuel store. Matoltsy (1969) suggested that lipids form a layer over the avian skin surface forming an efficient water barrier similar to sebum on mammalian skin. Evidences for active secretion of lipids by the avian epidermis have been provided by Lucas and Stenttenheim (1972), Jacob (1978), Jacob and Girmmer (1975), Shah and Menon, (1972 and 1974).

The gradual increase of G-6-PDH activity in the stratum germinativum, connective tissue of the dermis, and walls of the blood vessels indicating the enhanced operation of the shunt pathway could aid in yielding the ribose sugars needed for cellular proliferation associated with hyperplasia of epidermis and connective tissue during the progressive stages

of incubation patch formation. NADPH_2 generated as a result of the enzyme activity could contribute to the synthesis of epidermal lipids. Enhanced secretion of lipid substances by the epidermis in the fully formed patch of House Sparrow, supports the above stated involvement of the enzyme. The higher epidermal lipid content in the fully formed patch is probably of protective value to prevent dessication and cracking of the exposed skin. The increased lipid content of the epidermis also supports the proposed hypothesis (Chapter 2) that the loss of one function (production of feather) results in an enhancement of the other functional activity of the avian skin, (viz., holocrine secretion) as has been suggested in the case of Painted stork, Ibis leucocephalus (Chapter 2). Simultaneous increased $\alpha\text{C-GPDH}$ and BDH reactivities in the patch skin point to the occurrence of increased lipid metabolism during formation as well as its fully formed state.

Gradual increase in G-6-PDH and $\alpha\text{C-GPDH}$ activities and lipid content in the feather germs in the regressing phase of the patch, herald the initiation of feather development from the hitherto dormant feather germs.

This increasing trend of metabolic activities in the feather germs, well concides with proliferation and growth associated with the re-emergence of feathers in the patch skin. Significance of these enzymes in metabolic reactions

underlying the development of feathers has been discussed by Shah and Menon (1974 and 1975). A decreased BDH reactivity in the developing feathers at this stage could be pointing to a low degree of lipid utilization. This fact, when considered along with the observed higher reactivities of G-6-PDH and oC-GPDH as well as the concentrations of neutral lipids in the developing feathers could be taken as a circumstantial evidence for lipid synthesis from carbohydrate intermediaries. Such an increased synthesis of lipids at this stage is only to be expected, since lipids are essential for laying down the structural framework of feathers (Bell and Thathachari, 1963).