I N T R O D U C T I O N

Feathers are unique amongst vertebrate epidermal derivatives and their development has thus rightfully been the focus of much research since the last century. Though the developing feathers of chick embryo were noted and mentioned for the first time by Coiter (1573), it was only a century later that the facts forming the foundation of our present knowledge were laid by Malpighi (1697). His observations on development and structure of feathers especially in chick and turky, have been later translated and reviewed by Adelmann (1966).

The formation of the radially symmetrical down feather~~s~~ in early embryonic life, its replacement by the bilaterally symmetrical definitive feather~~s~~ arising from the same follicle~~s~~, and the periodic renewal of the adult plumage, are the various aspects that have been investigated along many lines of approach. Much of the work is concerned with the histological structure of the growing and differentiating feathers; and the works of Hosker (1936), Waterson (1942) and Wessels (1965) have essentially completed the description of feather development in embryos at the histological level. Lucas and Stettenheim (1972) have compiled a large amount of data published so far; along with their owm observations, on the various events during the development of down as well as definitive feathers. Research on plumage formation has wider implications in general biology since the plumage is of importance in thermoregulation, protection from mechanical injury and is an adaptation for flight. The seasonal change in plumage, like the one resulting in the nuptial plumage in many birds, is of significance in reproduction too. The convenience in examining and manipulating these epidermal structures for experimental work, have made the feather follicles a favourite object of research for embryologists and developmental physiologists. Apart from this, the full development of feathers can be observed within a short time, and the effects of various factors on the morphology of the feather could be determined with comparative ease.

The feather arises from the feather germ, a composite structure laid down in the early embryonic development, consisting of the epidermis, dermal papilla with its blood vessels, and the melanocytes; while only the epidermis contributes to the structure of a feather. The epidermis of the feather germ produces the feather, that of the feet produces scales and on the beak and claws it gives rise to the keratinous coverings. No special epidermal derivatives are formed in the apteria except for the formation of waxy keratinized layers. Various experimental works by Saunders and Giesseling (1957), Senegal (1958) and Rawles (1963, 1965) have all emphasized the importance of dermal-epidermal interactions in deciding the regional specificities of the epidermal cells.

Within the developing feather, the epidermal cells proliferate, orient themselves in the right pattern and differentiate sequentially in the distoproximal axis. A system of morphogenetic movements and differential rates of growth and gradients in thresholds of reaction, based on the observations of Hosker (1936) and experiments of Lillie and Juhn (1932; 1938) deploying surgical operations on the follicles and/or feather germs, have been reported. Vilter (1934, 1935) also independently formulated similar theories. Further experiments of Lillie and Wang (1941, 1943, 1944) and Wang (1943) revealed that the epidermis of a feather germ is dependent on the underlying dermis for the stimulus to produce a feather and for its orientation. Cohen and 'Espinasse (1961) on the basis of their surgical experiments re-evaluated the manner in which the epidermis of a feather grows during regeneration.

The periodic renewal of birds' plumage has been noticed as early as the time of Aristotle who described the seasonal nature of moult in birds. Severtsov's (1856) excellent survey which brought out the complex dependence of various periodic phenomena including moult in birds, on environmental conditions, led to further work which extended and deepened the knowledge of the laws governing the development and moult of plumages in birds. The extent of involvement of the various neuro-humoral factors in these processes have been investigated and presented by Voitkevich (1960).

However, much of the interest focussed on the developing feather in recent times is mainly due to embryologists and developmental physiologists interested in understanding of the general phenomena of cellular differentiation. Developing feathers offer an excellent system for analysis of various processes of development, viz., cellular proliferation, growth and differentiation. It is quite well known that the morphological differentiation of the feather becomes evident even while it is still in the process of growth and parts of feather begin to become hard and horny while they are still taking shape, which is due to the production of a specific substance, keratin, and not merely due to the drying up of the cells. Though the production of this specific protein i.e., keratin, could be considered more or less synonymous with feather differentiation; it is the orderly manner in which the keratin fibrils are oriented, that gives the feather its final shape. In the words of Prakash (1961) it could be summed up that "development is essentially a sequence of chemical and physical changes exerting a modifying effect on the differentiating cells, and that the differences between differentiating and non-differentiating cellular elements arise gradually and progressively leading to morphological distinctions" ~~Moog (1965) and~~ Brachet (1950) have shown that organogenesis, histogenesis and eventual functional differentiation or the acquisition of functions by a particular organ fit into an orderly pattern characterised by synthesis or loss of enzyme systems. Thus the idea that chemodifferentiation preceded morpho-differentiation has become well accepted. In fact ever since the enunciation of the concept of differential gene activity as the fundamental basis of histodifferentiation (Morgan, 1934) and the acceptance of the fact that protein synthesis is under genetic control, much interest has been focussed on the genetic aspects of differentiation. The significance of enzyme activities and their fluctuations during developmental processes have come to be well recognized. However, it is not possible to have a single generatised pattern of enzymic activities for all tissues of a developing organism. In spite of the vast literature on the subject available (see Moog, 1965), the actual meaning of the alterations of enzyme activity during functional maturation or organogenesis remains obscure. The problem is further complicated with the recognition of multiple forms or isoenzymes. All the available evidences point to the fact that enzyme activity and growth in the developing tissues are controlled by diversity of mechanisms like, the genic complements, hormones, as well as extrinsic and intrinsic factors. Thus measured changes in the activity of any enzyme at any stage of development are generally not directly interpretable in terms of growth and function of the tissue where the enzyme is active. A major factor which has to be considered in trying to understand these phenomena is the fact that differentiation is essentially a morphologic problem involving many critical periods when numerous enzymes are variously or simultaneously affected, according to the nature of the tissues concerned. Once the status of an enzyme at various times of development is understood, the problem of its control would have to be considered - whether it is synthesised, activated or transported through the blood stream. Moog (1965) has clearly shown that the patterns of change for individual enzymes are frequently multiphasic and that enzymes tend to increase or decrease in activity independently of each other. However, many of the parallel changes, wherein two enzymes both of which show an increasing trend could rise at different times and reach their level maxima at different times, are of considerable importance. Significance of the existence and regulation of biochemical pathways, the recognized source of metabolic energy and cofactors required for various synthetic activities during vertebrate development, has been well understood (Papaconstantinou, 1967).

The interpretation of the functional significance of an enzyme depends to a large extent on the knowledge of its localization. Histochemical techniques are of great value in this respect and have been employed in a number of studies in enzymology. Literature on the histochemistry of feather development, except for the pioneering works of Hamilton and his coworkers (see Review by Hamilton, 1965) are scarce. They have investigated a number of enzymes, especially alkaline phosphatase during the development of chick down feather and provided valuable information regarding the chemical regulation of down feather development. The present study comprises histochemical investigations on various enzymes connected with the metabolic pathways, viz., the HMP shunt, EMP pathway of glycolysis and the TCA cycle, along with lipase and phosphatases and the metabolite lipid, during the developmental processes of the definitive feathers of the blue rock pigeon, Columba livia.

In pigeon, the bilaterally symmetrical definitive feathers which replace the radially symmetrical natal downs, appear outside the skin surface several days after hatching and attain morphological and functional maturity by about a month. After completion of growth, the feathers become dead structures and the germs for the next generation of feathers are laid down in the follicles at the base of the existing ones. Birds periodically shed (moult) their feathers and in the natural moult, the germs which have been so far in a quiescent state, start growing and push the older feathers out of their follicles (Watson, 1963; Lucas and Stettenheim, 1972). This growth of the germ can be prematurely induced by plucking the mature feathers which are not yet ready to moult. It is found that the germs are not destroyed when mature feathers which are not to moult in near future are plucked. The growth of the germs after such experimental removal of mature feathers as well as during the normal seasonal replacement, have been termed feather regeneration by some workers (Lillie, 1942; Lucas & Stettenheim, 1972 and also see Voitkevich, 1960). However, Voitkevich considers the terminology as somewhat misleading since this involves only growth of a preexisting and predestined feather germ for the replacement of a feather plucked which even otherwise would have been replaced when that feather is cast off during natural moulting. Hence, such a growth of the feather germ cannot be compared to the reparative regeneration process found in other vertebrates. To avoid any controversial terminology, the term "induced development" is used in the present study to denote the development of the feather from a germ which has been "activated" by plucking the previous generation of mature feathers but which are not to moult in near future. The germ of such a feather prior to its plucking is termed as resting germ(Fig. 1).

In order to study the phenomenon of feather regeneration, immature feathers (developing feathers) have to be plucked at a stage when the germs for the next generation of feathers are not yet laid. In this case, the formation and growth of feathers after such plucking of immature developing feathers are not from a preformed anlage and hence would require to repair the wounded area and form

EXPLANATIONS FOR FIGURES

Fig. 1 Semi-diagrammatic illustration of the structure of a resting feather germ in the feather follicle of the adult pigeon skin, showing various components.

Fig. 2 Semi-diagrammatic illustration of the germ activated to develop after plucking the adult feather (induced development). This illustration depicts elongation of the germ as a result of proliferation of epithelial and pulp cells and desquamation of the epithelial lining of the follicular wall facilitating the emergence of the developing feather.

Fig. 3 Semi-diagrammatic illustration showing the feather follicle on the first day of regeneration, showing the beginning of the healing process of the injured feather as a result of which the upper surface of "dome" is being formed. The blood clot and cellular debris and desquamation of the follicular wall are also shown.

ABBREVIATIONS

AA - Axial artery

AT - Adipose tissue

BC - Blood clot

C - Collar region

DP - Dermal papilla

DSE - Desquamating epithelium

EC - Epithelial covering

FE - Feather epithelium

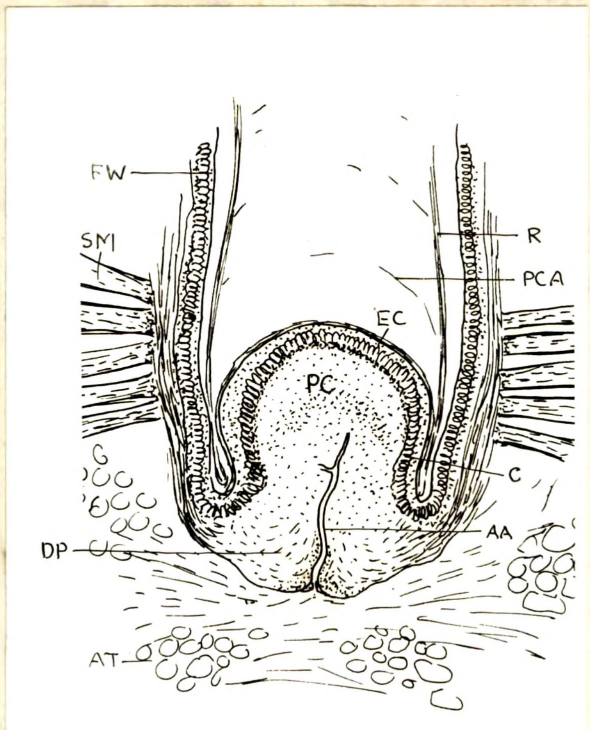
FW - Follicular wall

PCA - Pulp cap

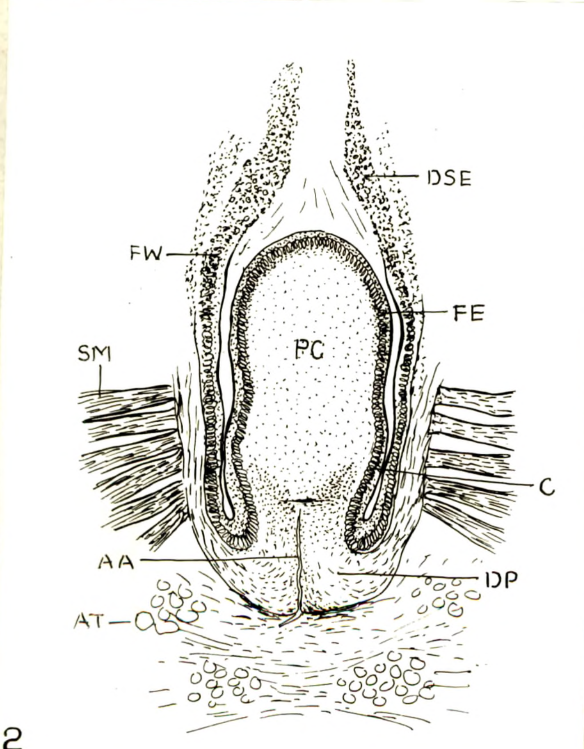
PC - Pulp cells

R - Rachis of mature feather

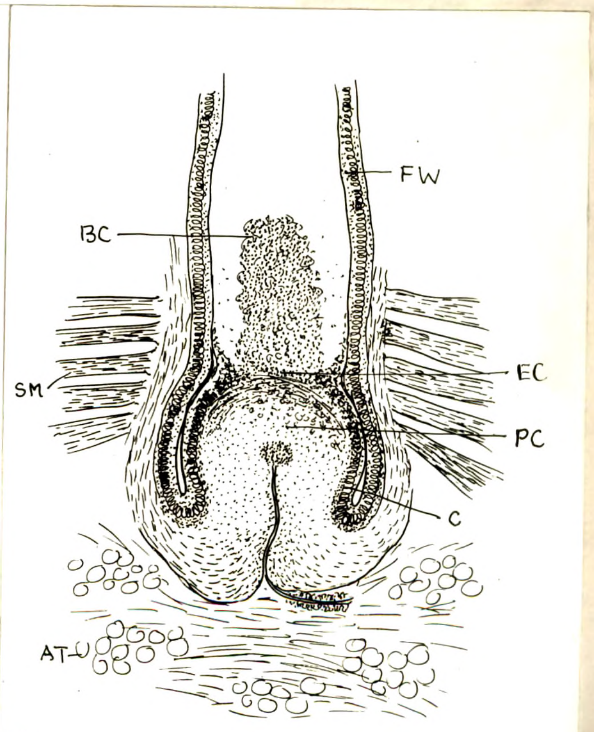
SM - Smooth muscles


FW
SM
R
PCA
EC
PC
C
AA
DP
AT

1


DSE
FW
FE
SM
PC
C
AA
DP
AT

2


FW
BC
EC
SM
PC
C
AT

3

EXPLANATIONS FOR FIGURES

Figs. 4,5 and 6 Diagrammatic representations of events in feather regeneration on the 3rd, 5th and 7th days. Note the regenerate pushing the blood clot and cellular debris out of the follicle. Also note the formation of barb ridges at the distal end of the regenerate.

ABBREVIATIONS

AA - Axial artery

AT - Adipose tissue

BC - Blood clot

BR - Barb ridges

C - Collar region

DP - Dermal papilla

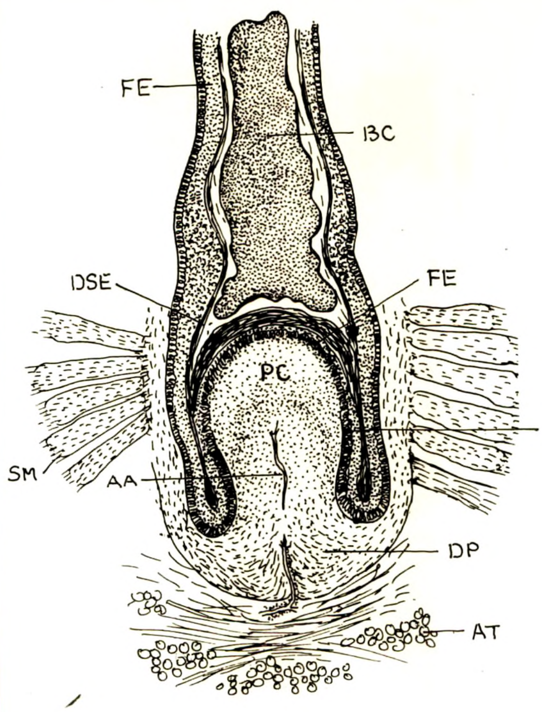
DSE - Desquamating epithelium

FE - Feather epithelium

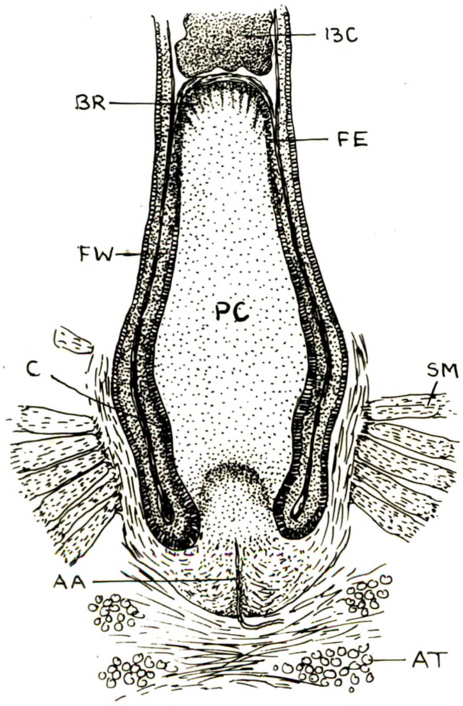
FW - Follicular wall

PC - Pulp cells

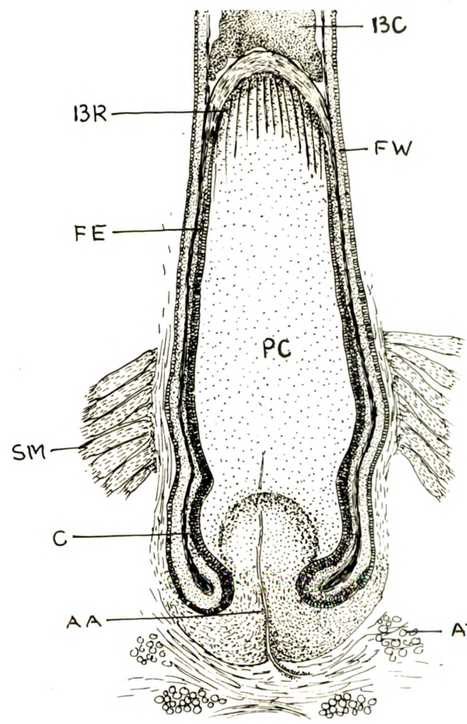
SM - Smooth muscles


FE
BC
DSE
FE
PC
C
SM
AA
DP
AT

4


BC
BR
FE
FW
PC
C
SM
AA
AT

5


BC
BR
FW
FE
PC
SM
C
AA
AT

6

a collar region from where cellular proliferation can proceed. In pigeons, after the first plucking or inducing development, the feathers appeared projecting from the follicles above the skin level, on the seventh day of germ activation. On the 10th day of induced development, the growing feather stubs were still within the feather sheaths (*i.e.*, an immature condition), and this stage was chosen for the second plucking in order to induce regeneration. The growth of feathers following such plucking of immature feathers could be justifiably termed as feather regeneration. On plucking the first generation of definitive mature feathers, the resting germs gave rise to the second generation. However, when the second generation of feathers are plucked while they are still immature, the injured parts heal and grow out of the follicles as a regenerate. Thus what grows out of the follicles is the second generation of feathers only (since the germs for the third generation are not yet established). Thus on a morphological basis, such development of feathers could be considered equivalent to the regeneration of any other vertebrate appendages.

As far as the morphological features are concerned, the differences between the induced and regenerative modes of development are noticeable only in the initial stages. (Figs. 2 & 3).

The growth of the germ (in the ventral tract) during induced development results in its emergence outside the follicle, above the skin surface, on the 7th day; the differentiation of the barb ridges having been initiated as early as the 3rd or 4th day of germ activation. During normal post-hatching development of definitive feathers too, the developing feathers of the ventral tract appear above the skin surface by about the 7th day of hatching. However, during the process of regeneration, the emergence of the feather stubs was observed only on the 10th day of inducing regeneration. In the initial phases, the injured area of the developing feather is covered by the blood clot and cellular debris. However, the process of healing is completed by about 24 hours by the proliferating epithelial cells; thereby forming the "dome" - as it is termed by Cohen (1966) - at the distal end of the papillae. The sides of the dome form the future collar region, from where the epithelial cell proliferation can proceed- resulting in the regeneration of the feather. (Figs. 4-6).

As mentioned earlier, the histological features of feather development are well understood, but the chemical events have hardly been studied. The present study therefore, was conducted to gain an insight into the various metabolic reactions underlying feather development and also to evaluate the similarities and/or dissimilarities inherent therein between the three modes of feather development, i.e., normal, induced and regenerative.

The operation of Hexose Monophosphate shunt (HMP) which has long been recognised as the chief metabolic route in developing organs and tissues, could be inferred by the activity of Glucose-6-phosphate dehydrogenase (G6PDH); an enzyme that channels the carbohydrate moiety into this pathway. Histochemical investigations on this enzyme provided an insight into fluctuations in the operation of HMP shunt and its relation to other metabolic routes corresponding to the different phases of definitive feather development under different conditions (chapter 2).

Demonstration of the activity of aldolase (chapter 3), a key enzyme of the Emden-Meyerhoff pathway of glycolysis, as well as lactate and α-glycerophosphate dehydrogenases (chapter 4) both aiding in providing optimal operational conditions for this metabolic route, helped in establishing the existence and significance of glycolytic pathway in the adult pigeon skin as well as during the developmental processes of the definitive feather and its possible relation to the metabolism of lipids.

The role of lipids in the energetics and its significance in contributing to the structural elements of vertebrate tissues have long been realised. Histochemical studies on lipids, and lipase and β-hydroxybutyrate dehydrogenase, enzymes concerned with its metabolism; helped to reveal the involvement of this metabolite in the development and regeneration of the definitive feathers (chapter 5).

Operation of the tricarboxylic acid (TCA) cycle and its participation in the metabolic processes of feather development were evaluated by following up the activities of Succinate and Malate dehydrogenases (SDH & MDH). These studies helped to reveal adaptations at the biochemical level for controlling and aiding in the process of development as per functional demand, under the three different conditions of feather development (chapter 6).

Phosphatases have attracted considerable attention from developmental physiologists by virtue of the multifarious roles played by them in different tissues and in a tissue at different phases of its development. The significance of alkaline phosphatase in the down feather development has been stressed by Hamilton (1965). However, studies on acid phosphatase have not been carried out in detail. The present investigations on these two categories of phosphatases were conducted in order to have a comparative idea of the possible roles played by them in the various aspects of the process of development of the definitive feather (chapters 7 & 8).

It is realised that some explanation for the word moult used in the title of the thesis is necessary to convey its meaning as conceived by the author. The word moult is used in the present circumstances to mean the development of feathers from pre existing feather germs. However, this development of feathers resembling the moult, is induced by plucking number of feathers from a specific region. This method was adopted for the reason, that normally pigeons do not cast off large numbers of feathers from one region simultaneously during natural moulting, and hence it would become difficult to study the biochemical events of developing feathers under natural moulting conditions. Thus to avoid confusion and to distinguish the feather development resulting from plucking, from the natural moult, the term "induced development" is used instead of moult in the text.