

CHAPTER 9

GENERAL CONSIDERATIONS

Histochemical investigations on a number of enzymes concerned with metabolism have helped to reveal some of the aspects of well balanced and regulated operations of the different metabolic pathways underlying development and growth of the definitive feathers in the domestic pigeon. Proliferation, growth and differentiation of cells are undoubtedly biochemical manifestations translated into effectiveness through the participation of enzymes. In fact, the metabolic configurations of proliferating ^{and} differentiating cells are distinctly oriented to meet the requirements of newer cellular products. A study of metabolism and the collaborating enzymes usually reveal the finer details of multiplication, growth and differentiation. Such studies have been extended to almost all types of tissues and organs during normal development as well as to systems that show adaptive secondary development such as regenerating organs. However, metabolic events are not investigated as far as the post-hatching development of ^{the} definitive feather and its induced as well as regenerative developments are concerned. Therefore, histophysiological investigations on the development of ^{the} definitive feathers of ^{the} domestic pigeon have been chosen as the subject of the present study, so as to add some more information regarding enzymatic adaptational changes during

normal, induced and regenerative developments. Since the purpose of this investigation is to report on certain enzymic and metabolic changes in ^{the} definitive feather developing under three different conditions viz., normal post-hatching, induced and regenerative modes, no attempt is made to explain the control mechanisms that bring about initiation or cessation of the development.

Development is a continuous process and Moog (1965) has aptly stated that the stages in a developing system are clear cut only in the minds of investigators and not in the developing organism. Since, different aspects of developmental processes like growth and differentiation overlap each other in these epidermal derivatives and proceed hand in hand, one is confronted with practical difficulties in defining the different stages during the developmental processes of the feathers which restrict the possibilities of explaining the meaning of fluctuating enzyme activities. However, the differentiation is disto-proximal and the growth is proximo-distal in the developing feather. Since these aspects of development are dependent on various metabolic activities, the simultaneous operation of a number of metabolic routes can be detected in the feather during the course of its development. However, there are certain periods in the developing feather when growth gains priority over differentiation or vice versa

and therefore a corresponding shift in metabolism can be detected. The development is a morphological manifestation, but all the same, metabolic events are in no way less involved. The following account summarises the details of the informations gathered in the present studies on the metabolic pattern during the normal post-hatching development of definitive feathers in comparison with that of induced and regenerative ones.

The dependency of the developing feather on a predominant HMP shunt activity could be visualised by the greater involvement of G6PDH. This is in perfect accordance with the needs of these developing epidermal derivatives as the cellular proliferative activities depend to a great extent on an increased turnover of nucleic acids; the building blocks for which are supplied by an increased activity of the shunt pathway. The ribose sugar moieties are made available for the multiplying cells which require them for synthesising nucleosides, nucleotides and nucleic acids. Moreover, enhanced DNA synthesis is made possible by the provision of NADPH_2 which is used for the direct conversion of ribonucleo-
tides^{into} deoxyribonucleotides, a mechanism associated with rapid cellular proliferations (Reichard, 1959; Moore and Hurlburt, 1962). The high activity of alkaline phosphatase is also significant in that it could be playing a role in

phosphate transfer in DNA metabolism as suggested by Rogers (1960). The shunt pathway would aid in lipogenesis also. This contention is substantiated by the fact that increased concentration of lipids are noticed in the developing feather at this stage.

The considerable activity of aldolase in these initial phases of post-hatching development would, apart from helping in the continued operation of the HMP shunt by synthesising hexose moieties from trioses which are commonly encountered in metabolism, also aid in the operation of the EMP pathway of glycolysis. The energy requirements of the proliferative and synthetic activities of this dynamic state of feather development would thus be satisfied by the operation of the glycolytic pathway, as TCA cycle activity is minimal at this stage. Continued optimal activity of glycolysis has to be ensured and is done by the activity of LDH which would regenerate NAD. Thus in the initial phases of post-hatching development operation of HMP shunt and anaerobic glycolysis to a considerable extent help in maintaining the momentum of metabolic activities at the required pace to provide raw materials, cofactors and energy needed for the proliferative activities.

However, cellular proliferation leads to differentiative activities in the developing feather, which becomes more

obvious on the 5th day when organization of the epithelial cells into barb ridges become evident. Along with increased differentiative activities and cellular proliferation keeping pace, accelerated metabolic activities are only to be expected, and are evident from the attainment of peak G6PDH and LDH activities. The fact that lipid metabolism also registered an increase at this stage points to the enormous energy requirements of a hectic phase of cell proliferation, growth and differentiation. Involvement of lipid metabolism in the form of increased synthesis and simultaneous utilization of the metabolite is evident from the peak activities of G6PDH, α GPDH, lipase and also from the high rate of operation of TCA cycle as inferred from the peak MDH activity. While the peak activity of G6PDH could provide NADPH_2 , an essential cofactor for lipogenesis, α GPDH could be considered as aiding in the formation of α -glycerophosphate, an important intermediary for triglyceride synthesis from carbohydrate sources. Utilisation of lipids at this stage as could be evidenced by the high lipase activity, moderate BDH activity and high rate of TCA cycle operation, would provide an enhanced energy supply needed for the active growth of the developing feather, ~~and~~ as a result of which they ^{feathers} emerge from the follicles on the 7th day after hatching. Acceleration in various metabolic activities leading to a high energy yield, just

prior to the emergence of the feathers from their follicles, could be for satisfying the energy demands of their hectic growth phase. Thus every aspect of metabolism in a growing feather is oriented towards fulfilling the requirements for the energy demands. This becomes evident from the present study which shows delicately balanced activities of the enzymes concerned with the different metabolic routes. The activity of alkaline phosphatase, an enzyme not directly involved in the metabolism concerned with energetics, also showed peak response in the components of the developing feathers at this stage. It is easy to visualise the significance of this enzyme, as its function in the transport of metabolites, especially glucose, across cell membranes is well known. Moreover, with the purported function it plays in DNA metabolism (Rogers, 1960) the peak activity of alkaline phosphatase at a stage when nucleic acid and energy demands are high, seems to be quite understandable and provides another instance of the superbly organised control mechanism of this developing system. It is pertinent here to mention that Hamilton (1965) has stressed the importance of this enzyme in the differentiative processes of the down feather, where inhibitors of this enzyme result in inhibition of differentiative processes of ^{the} feather resulting in the formation of only an epithelial cylinder.

By about the 10th day of feather development, differentiative activities become more pronounced as keratinization of the barbs comes well into focus, though, evidences of initiation of keratinisation are clear even before this stage. This increased rate of differentiative activities overlap an active growth phase, i.e., increase in length and bulk of the feather. At this phase, differences in the intensities of enzyme activities are only to be expected. The process of keratinization is known to involve lipids (Bell and Thathachari, 1963; Shibaeva, 1970) and in the developing feather much of the lipid utilization seems to be for this purpose rather than energetics, during this phase. This contention could be drawn from the reduced BDH activity and the decline in GPDH activity observed at this stage of feather development.

The existence of metabolic regulations and their versatility are clearly indicated by the appearance of acid phosphatase activity, known to participate in the process of keratinization, at this stage (along with a slight decline in that of alkaline phosphatase).

Utilization of lipids for keratinization would impose the necessity of increased dependency of the system

on carbohydrates for energetics as could be visualised from the peak activities of aldolase, LDH and also the TCA cycle enzymes. The increased activity of LDH ~~when~~ ~~also~~ the TCA cycle is optimally operative seems a little puzzling. One feasible explanation seems to be that a declined rate of NAD regeneration due to decline in G6PDH activity, is compensated to some extent by high LDH activity, thereby warranting the optimal operation of the glycolytic pathway. It is pertinent in this connection to consider that LDH has been recognised as a regulatory enzyme by Racker (1965) and Nelson and Wakefield (1973). However, what exactly controls this regulatory enzyme remains speculative at present.

By the 15th day of feather development, peak G6PDH activity aided by a considerable activity of aldolase ensues ^y_x the optimal operation of the HMP shunt, making available the cofactors and ribose sugar required for the high rate of differentiative activities. The fact that TCA cycle enzymes (SDH & MDH) reactivity was of a high intensity indicates increased energy production at this stage. Activity of LDH noticed at this stage to be of only moderate level is indicative of the reduced significance of anaerobic glycolysis in the energetics

of the developing feather. During this phase, both the developmental processes, *i.e.*, growth and differentiation, seem to be gaining equal importance, and progress on a well balanced basis. This fact could be visualised by the high activities of shunt pathway and the TCA cycle which provide the necessary cofactors and energy respectively. The slightly elevated concentration of lipids, along with the noticed activities of lipase and EDH show that lipid synthesis and its utilisation for keratinization as well as energetics at this stage is a possibility. However, from the 20th day onwards, with the slowing down of differentiative activities heralding the oncoming of the functional maturation of the feather, changes in enzyme activities could be detected as only to be expected. As the differentiative activities slowed down, a reduction in G6PDH activity became noticeable. Aldolase and LDH activities remained at a moderately high level similar to that noticed in the adult skin and feather components. The major phenomenon in the maturation process of the feather, in its last phase, is the regression of its pulp which so far has been providing nourishment for the proliferating and differentiating epithelial cells. The enzymological pattern of the regressing pulp seems to fit in two categories. One of them probably involves synthesis

and/or activation of lytic enzymes as is evident from the appearance and increased activity of acid phosphatase - which is known to be participating in many of the degenerative phenomena involved in the normal developmental processes (Brachet et al., 1958; Misch, 1962). Another one seems to be concerning the normal metabolic enzymes, where mitochondrial enzymes like SDH and MDH are probably lost from its cells. ^{the} Regression of ^{the} pulp involves death of its cells starting ^{completely} distally till the germ is formed. There are two schools of thought about the mode of cell death, one considers the involvement of lytic enzymes while the other, ^{the} loss of mitochondrial enzymes. Hammer and Motett (1972) have stressed on the loss of enzymes like SDH as the main cause of the cell death. The regression of ^{the} pulp probably involves both these processes as could be contended by the gradual decline in TCA cycle enzymes and increased activity of acid phosphatase (lytic enzyme). It is also noteworthy that aldolase and LDH are considerably active in the cells of the pulp during its regression. Thus the high activities of these catalytic enzymes (LDH and Aldolase) along with the concomitant increase in the activity of the hydrolytic enzyme (acid phosphatase) aid in the regression of the pulp.

As the regression of the pulp is completed, the new feather germ for the next generation is laid at the base

of the fully developed feather and the concentrations of various enzymes there, are noticed to be resembling that observed in the corresponding parts of the adult normal skin where the resting germs of adult feathers are present. Here a preponderance of anaerobic glycolysis, as is known to be the case with the reptilian and mammalian skin (Ramachandran, 1972; Adachi and Uno, 1968; Novikova, 1970) is evident.

During induced development, the general pattern of enzyme activities resembles that observed during normal post-hatching development. The emergence of the feathers outside the follicles during the induced development was also observed on the 7th day as was the case during normal post-hatching development. However, the definitive feathers were already in a state of development on the day of hatching as opposed to the induced development where the germs are in a "resting condition", physiologically unprepared for development on the day of plucking. It is the plucking of the adult feather that activates the resting germ, and this activation would result in putting the entire enzyme machinery into full gear and relatively at a higher pace in order to have the developing feather emerge from the follicle on the 7th day. The physiological necessity of restoring these epidermal structures of considerable significance in thermoregulation, would

necessitate an enhancement of metabolic processes of the feather forming tissues. Such an accelerated commissioning of the metabolic machinery can be envisaged from the increased activities of many of the enzymes as early as 24 hours after plucking. Thus the enhanced activities of G6PDH, aldolase, LDH, MDH and lipase are observed at a relatively early stage of induced development.

The requirement of (a) co-ordinated and enhanced activities of the various phenomena like cell proliferation, differentiation and growth of the feather can be considered to be satisfied by the pattern of metabolic activities observed herein in the initial phases of induced feather development. The highly active HMP shunt aided by an appreciable aldolase activity is evident from the 2nd day of induced development. The high incidence of glycolysis, together with the involvement of lipid metabolism (as indicated by increased lipid content and lipase and BDH activities) by about the 5th to 7th days and also the moderate activities of TCA cycle enzymes (SDH & MDH) aid in the growth and differentiative activities of the feather germ induced to develop. The high rate of operation of the TCA cycle during the period between the 7th to 10th days points to the high energy demands of the developing feather at this stage, along with the requisition of co-factors.

The declined G6PDH activity by the 10th day when viewed along with increased aldolase and LDH activities points to the fact that during this period of increased growth, the HMP shunt is being regulated in such a way that the carbohydrate moieties are utilised through the EMP pathway of glycolysis to meet the increased energy demands. This demand is further met by the high rate of lipid utilization as evidenced by increased activities of lipase and BDH. This is further substantiated by the peak activity of the TCA cycle enzymes at this stage.

The decline in the activities of enzymes studied, as the development advanced, is seen to be on a similar pattern as that ^{was} observed during the final phase of normal post-hatching development of the feathers.

The regenerative development of the feathers is characterised by a predominant lipid utilising and oxidative mode of metabolism involving a greater participation of the TCA cycle, during the first half of such developmental period. Presently observed deviation in the metabolic pattern from the one observed during early phases of normal and induced development seems to fit well with the functional demand of a faster rate of restoration of the definitive feathers when being

regenerated. When inducedly developing feathers are plucked in order to initiate regeneration, the whole area of the ventral tract is exposed bare for a longer duration. Hence the importance of the feathers in thermoregulation would demand a faster rate of the growth of the regenerates, thus a lipid centered metabolism which can result in increased energy yield is only to be expected. For the other aspect, like provision of co-factors, the noticed activities of G6PDH and aldolase can be considered competent to meet these requirements. Fluctuations in metabolic patterns observed after the first half of regenerative development resemble in general those observed during the similar phase of feather development under the other two conditions.

Another significant point of deviation in the enzymological pattern observed during induced and regenerative modes of developments from that during the normal development of the first generation of definitive feathers concerns the activities of phosphatases. Whereas alkaline phosphatase activity is predominant during the normal development, both acid and alkaline phosphatases are found to be more or less equally active all throughout the developmental processes under induced and regenerative conditions. It is interesting

in this connection to consider the fact that acid phosphatase is predominant in the adult pigeon skin, both in its follicular and extra-follicular components, while alkaline phosphatase is active in the resting feather germ only. Acid phosphatase, hence, could be considered as the normal complement of the adult skin, which continues to participate actively in the developmental processes of epidermal derivatives of the adult skin during induced as well as regenerative developments. The contention of Moog (1965) that acid phosphatase is a constitutive enzyme, appearing in tissues with their maturation, and alkaline phosphatase an inducible one, seems pertinent and noteworthy in this connection. In the case of induced feather development, increased activity of alkaline phosphatase appeared in the feather forming tissues after activation of the resting germ by plucking the adult feather, which could be considered as one of the inducing factors. Such an induction by an increased enzyme activity appears to be necessary to initiate the activating process of the development of the germ into a definitive feather. The existence of some regulatory factor involved in induction of the activity of alkaline phosphatase by its synthesis and/or activation thus becomes apparent. On the other hand, acid phosphatase could be considered as participating in

the developmental processes of the definitive feather induced to develop, as it is considered to be the normal complement of the adult skin, though regulated by some factors according to the need and physiological state of the developing organ. ^{the} Same arguments can be put forth to explain the patterns of activities of these two phosphatases during the regenerative mode of feather development. What remains enigmatic at present is the nature of the factors that regulate the activities of these phosphatases.

The present study, as summarised above, has provided some understanding of the metabolic events that underlie the various phenomena involved in the development of the definitive feather under three different conditions. Existence of provisions for changes in metabolic patterns in order to adjust to a new physiological demand on the organism is indicated by the present study. Further work of experimental and quantitative nature is needed to evaluate various factors involved in the developmental processes of the feathers, viz., those ^{which} influence regulation of the enzymic machinery directly or indirectly through the endocrine glands. Many of the metabolic enzymes known to be regulatory in function, and also the inducible

enzymes offer an excellent system for investigating
the nature of control ^{mechanisms} exerted by the external and
internal environments of an organism. Histochemical
investigations supplemented by quantitative and
experimental analysis, initiated in this laboratory,
are expected to yield certain concrete results in the
near future.