

CHAPTER 6

HISTOCHEMICAL OBSERVATIONS ON THE ACTIVITY OF SUCCINATE
AND MALATE DEHYDROGENASES IN THE DEFINITIVE FEATHERS OF
THE BLUE ROCK PIGEON, COLUMBA LIVIA, DEVELOPING UNDER
THREE DIFFERENT CONDITIONS

Succinate dehydrogenase (SDH) and malate dehydrogenase (MDH), two important enzymes of the TCA cycle have attracted considerable attention in the recent past as the histochemically demonstrated presence of these two enzymes is considered as indicative of the operation of TCA cycle in the tissues concerned. Of the two enzymes, comparatively more is known about SDH, a constituent of all living cells, known to be localised in the mitochondria (Siekevitz & Watson, 1956; Novikoff, 1961), especially, in the inner mitochondrial membrane (Sottocasa et al., 1967; Brdiczka et al., 1968). It serves to catalyze the intracellular conversion of succinate to fumarate during the oxidation of metabolites via the TCA cycle ultimately yielding energy in the form of ATP. The role of SDH in the normal metabolic processes of animal tissues has been well investigated (vertebrate adipose tissue by George and Eapen, 1959; vertebrate skeletal muscle by George and Talesara, 1961; Ogata and Mori, 1964; Bokdawala, 1965; Nene, 1966; Buno

and Germino, 1958; corneal epithelium of mammals by Ehlers, 1970; vertebrate nervous system by Shanta and Manocha, 1968; pacinian corpuscles of cat by Chouchkov, 1969; gastric glands of rat by Ishimura and Fujie, 1970; vertebrate liver by Asnani et al., 1972; and mammalian integumentary system by Montagna and Formisano, 1955; Mojumdar et al., 1973). Further, the enzyme has been investigated during development of avian skeletal muscle (Germino et al., 1965; Dubale and Muralleedharan, 1970), down feather of chick (Koning and Hamilton, 1954), mammalian tooth (Fullmer, 1963) and also during cold acclimatisation of fish (Hazel, 1972), regeneration of urodele limb (Schmidt, 1968), lacertilian tail (Shah and Chakko, 1969; Ramachandran, 1970), amphibian metamorphosis (Yamamoto and Suzuki, 1957; Paik and Cohen, 1960), cellular necrosis during embryonic development (Hammar and Mottet, 1971) and human myocardial infarction (Nachlas and Shnitka, 1963; Morales and Fine, 1966).

On the other hand, Malate dehydrogenase, the NAD linked enzyme converting malate to oxaloacetate during the terminal part of TCA cycle oxidation, has attracted comparatively less attention. It is known to be present in both the mitochondrial as well as the cytoplasmic

fractions of a cell (Englard & Breigerl, 1962; Kitto & Lewis, 1967). Its activity has been demonstrated in invertebrate and vertebrate muscles (Ogata & Mori, 1964; George and Berger, 1966), human foetal tissues (Wiggert & Vिलlee, 1962), during development of sea urchin (Billiar et al., 1966; Gustafson, 1954; Osaki and Whitely, 1970), chick liver (Solomon, 1959; Greenfield & Boell, 1970), mammalian tooth (Fullmer, 1963), hatching muscle of chick (Klicka & Kaspar, 1970), gastric glands of rat (Ishimura & Fujie, 1970), regenerating urodele limb (Schmidt, 1968), lacertilian tail (Shah & Ramachandran, 1970) and also during the temperature adaptation of animals (Baldwin & Aleksuk, 1973).

Though well studied in many animal tissues, employing both histochemical and biochemical techniques, information about the activities of these two enzymes during the developmental processes of avian epidermal derivatives, except for the report on the presence of SDH in the developing down feathers of chick by Koning and Hamilton (1954), is meager. Hence, in this light, the present histochemical investigation has been undertaken in the blue rock pigeon, Columba livia, with a view to study the role of these two enzymes and the extent of involvement

of TCA cycle in the metabolic processes associated with the different aspects of definitive feather development.

OBSERVATIONS

Normal Adult Skin: (Figs. 1 & 22)

The distribution pattern and intensity of activity of SDH and MDH in various components of adult normal skin of pigeons are observed to be equal, but low in comparison to the other dehydrogenases of the HMP and EMP pathways. However, a comparatively maximum enzyme response was given by the errector muscles fibres and the resting germ. In the resting germ, it was the mesenchymal pulp cells which showed a relatively higher reactivity of these two enzymes than the epithelial cells. Further, amongst the epithelial cells, those at the collar region tended to show slightly more enzyme concentration than the remaining ones. The stratum germinativum in the non-feather regions of the skin as well as the walls of blood vessels in the dermis were also noted to be though low, nevertheless, enzyme active.

SDH activity during normal post-hatching development: (Figs. 2-8)

On hatching, SDH reactivity, though present in all the components of the skin, was noticeably low. Amongst

the various components, smooth muscles and the developing feather, especially its epithelial cells, registered a higher level of the enzyme activity. Epidermis of the non-feather region was the next in the order of intensity of the enzyme reactivity. Fibrocytes and adipose tissue cells exhibited only a low activity of the enzyme. This low general level of the enzyme concentration in the skin remained so upto the 3rd day, but by the 5th day, an increase to a moderate level in all the above mentioned components of the skin was observed.

The first high peak of the enzyme activity was observed only in the epithelial cells of the feather and the smooth muscle fibres of the skin on the 10th day of development after hatching. Amongst the epithelial cells of the barb ridges, those nearer to the pulp showed a relatively higher enzyme concentration than those which are away from it. Rest of the components of the skin maintained a moderate level of enzyme concentration as was noticed on the 5th day. However, a slight reduction in the enzyme activity was observed on the 10th day in the cells of the adipose tissue. As the development progressed, a gradual increase in the enzyme activity in the pulp cells was noticed which became quite obvious by the

15th day of development. Till this day, the enzyme concentration in other skin components remained as high as that observed on the 10th day. When the skin became 20 day old, all its components except the pulp region of the feather registered the lowest level of enzyme concentration, to be observed during the entire period of post-hatching development, excepting the first three days. Of the pulp cells, only the basal ones were moderately enzyme reactive while the apical ones were almost devoid of the enzyme activity. After this phase a gradual increase in SDH activity in different parts of the skin except in pulp cells occurred which became obvious on about ^{the} 25th day in the form of a moderate level of its activity. This level was so retained thereafter as it ^{was} the normal adult skin level of the enzyme.

SDH activity during induced development: (Figs. 9-14)

With the plucking of adult definitive feathers to bring about induced development, there was an overall fall in SDH activity towards a negligible level by about 15 hours in almost all the components excepting for the smooth muscles where the enzyme activity though low, was nevertheless, noticeable. But within 24 hours after plucking, there was a slight but discernible increase in

enzyme activity in the pulp, feather epithelial cells, non-feather epidermis and fibrocytes. However, the smooth muscle fibres at this stage not only failed to show this increase but in fact appeared to show a still further decline in its activity. This condition was retained upto the 5th day though in the by now developing feather, the epithelial cells had a relatively higher concentration of SDH than the pulp cells with the smooth muscles registering the ^{lowest} least concentration of the enzyme. The level of SDH activity increased to a moderate level by about ^{the} 7th day and then further rose gradually to a peak level by about the 10th day in the smooth muscles as well as the epithelial and pulp cells of the developing feather. At this stage, the pattern of SDH reactivity in the barb ridges was quite similar to the one observed during normal development.

A gradual reduction in SDH activity was noticeable after the 10th day which became most discernible in the smooth muscles, and pulp cells of the 15 day old feather. At this stage a low but noticeable activity of SDH was observed in the cells of the barb ridges which are proximal to the pulp. This condition of the enzyme reactivity was

retained till the 20th day of induced development only to register a slight increase again on the 25th day, in the epithelial cells, pulp and smooth muscles, thus attaining a level almost similar and characteristic of corresponding components of the normal skin which was retained hereafter.

SDH activity during regeneration:(Figs. 15-21)

On the 1st day of regeneration, the SDH activity was noticed to drop a little below the normal level in both the smooth muscles as well as injured feather, but by ^{the} 3rd day a slight increase in the enzyme activity was noticed in the injured feather which had its wound healed, this being most obvious in the epithelial cells and the basal parts of the pulp and the smooth muscles. Other components of the skin, however continued to exhibit a low level of the enzyme response. An increase in SDH activity to a moderate level in the epithelial cells, especially in the barb ridges, was observed by the 5th day. No noticeable change could be observed in the other regions of the skin.

There was again a fall in the SDH activity in the epithelial cells of the regenerating feather by the

EXPLANATIONS FOR FIGURES

Fig. 1 LS of follicle with resting feather germ. Note the low level of SDH reactivity in the dermal papilla and the pulp cells. A low activity of the enzyme is discernible in the smooth muscles also.

NORMAL DEVELOPMENT

- Fig. 2 Section of skin on the 1st day of hatching, showing feather follicles and smooth muscles with very low enzyme activity.
- Fig. 3 Section of skin showing SDH activity in the cells of developing feather on the 3rd day of post-hatching. Note the enzyme reactivity of the non-feather epidermis.
- Fig. 4 LS of developing feather on the 5th day post-hatching showing moderate SDH activity. Note activity in the smooth muscles also.
- Fig. 5 Peak enzyme reactivity in the epithelial cells of the feather and smooth muscles on the 10th day of post-hatching development.
- Fig. 6 LS of 15 day old feather showing increased activity of SDH in the pulp.
- Fig. 7 Negligible SDH reactivity in the distal parts of the pulp on the 25th day of post-hatching development.

- Fig. 8 LS of follicle on the 30th day of post-hatching development showing SDH activity of the pulp cells.

INDUCED DEVELOPMENT

- Fig. 9 LS of feather follicle showing SDH activity in the germ, 24 hours after plucking the normal adult feather. Melanocytes in the pulp are also seen.
- Fig. 10 TS of a 5 day old inducedly developing feather. Note the enzyme activity in pulp and epithelial cells of the barb ridges.
- Fig. 11 LS of 10 day old feather. High activity of the
& 12 enzyme is discernible in barb ridges and collar region. Note activity of SDH in smooth muscles also.
- Fig. 13 LS of 15 day old feather with declined enzyme activity.
- Fig. 14 25 day old feather cut longitudinally. Note the low enzyme level in pulp cells and epithelial cells except those at the collar region.

REGENERATION

- Fig. 15 LS of follicle on 1st day of regeneration. Note low SDH activity in the epithelial and pulp cells of the healed feather.
- Fig. 16 LS of 3 day old regenerate showing a slight increase in the enzyme activity.

- Fig. 17 TS of 5 day old regenerate exhibiting SDH activity in the barb ridges. Note activity of the enzyme in the smooth muscles also.
- Fig. 18 TS of feather on the 7th day. Note the low reactivity for the enzyme.
- Fig. 19 LS of part of a 12 day old regenerating feather exhibiting peak SDH activity in cells of the pulp and epithelial cells of barb ridges.
- Fig. 20 TS of 20 day old regenerate with declined enzyme activity, and an advanced stage of keratinisation.
- Fig. 21 LS of follicle on the 30th day of regeneration. Note the enzyme activity in the germ for the next generation and the smooth muscles.
- Fig. 22 LS of normal adult feather follicle showing the enzyme^(MDH) activity in the pulp and collar cells of the resting germ.

NORMAL DEVELOPMENT

- Fig. 23 Section of skin on the 3rd day post-hatching. Note the low enzyme activity in the developing definitive feather and the smooth muscles.
- Fig. 24 Section of skin on the 5th day post-hatching showing non-feather epidermis and the developing feather.

- Fig. 25 LS of developing feather enlarged to show peak MDH activity in the pulp and epithelial cells of the barb ridges.
- Fig. 26 LS of feather on the 10th day exhibiting peak enzyme activity in the various components.
- Fig. 27 LS of part of the feather on the 20th day showing a low enzyme activity.
- Fig. 28 LS of the definitive feather on 25th day post-hatching showing enzyme activity in regressing pulp.
- Fig. 29 LS of feather on 30th day. Note MDH activity in the pulp and epithelial cells.

INDUCED DEVELOPMENT

- Fig. 30 The feather germ 15 hours after plucking the normal adult feather, showing increased activity of the enzyme.
- Fig. 31 LS of developing germ on the 3rd day. Note the MDH activity of the epithelial and pulp cells.
- Fig. 32 LS of 5 day old inducedly developing feathers exhibiting MDH activity in the barb ridges.
- Fig. 33 LS of part of developing feather on 7th day of induced development. Note the enzyme activity in the feather ensheathed in the feather sheath.

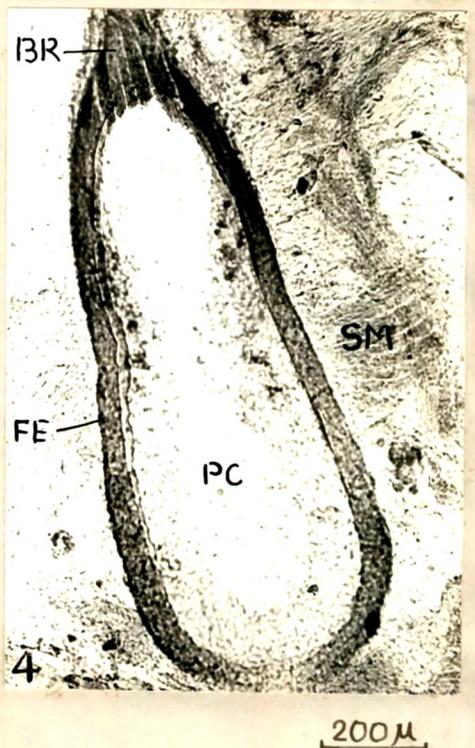
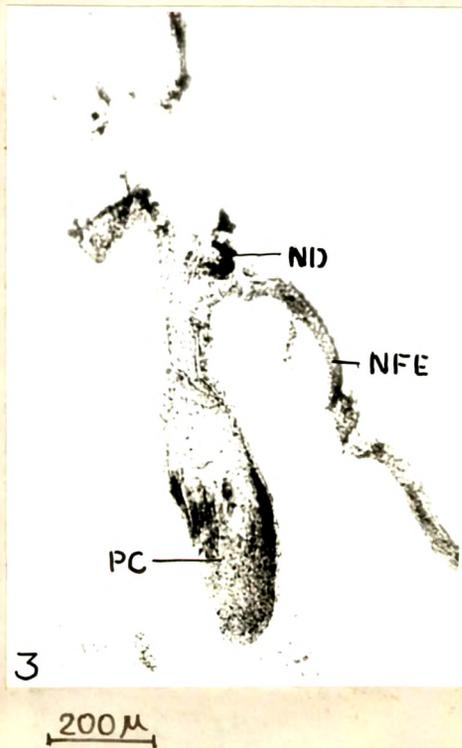
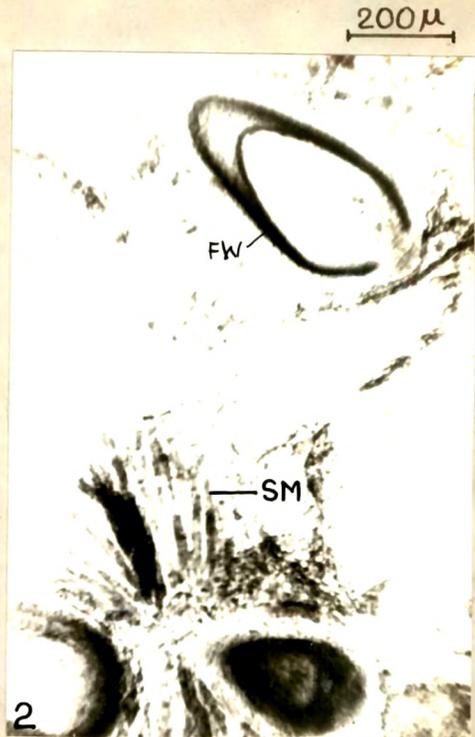
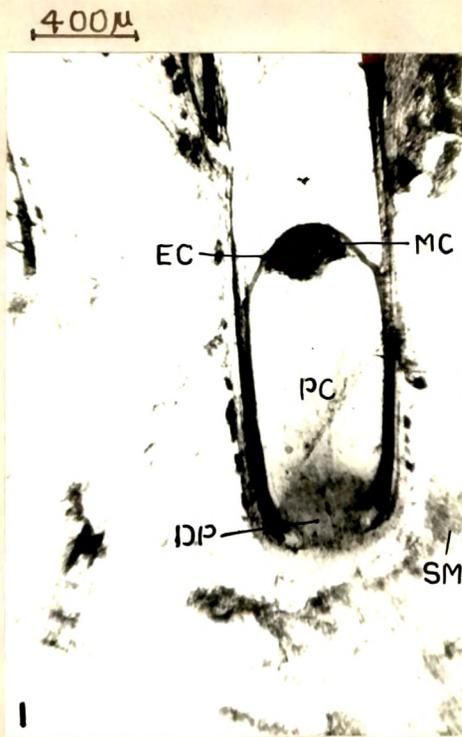
- Fig. 34 TS of part of the feather on the 10th day. The declined activity of MDH is discernible in the pulp and epithelial cells.
- Fig. 35 TS of feather on the 15th day, showing high MDH activity in the barb ridges.
- Fig. 36 Oblique section of the feather on 25th day, showing enzyme reactivity.

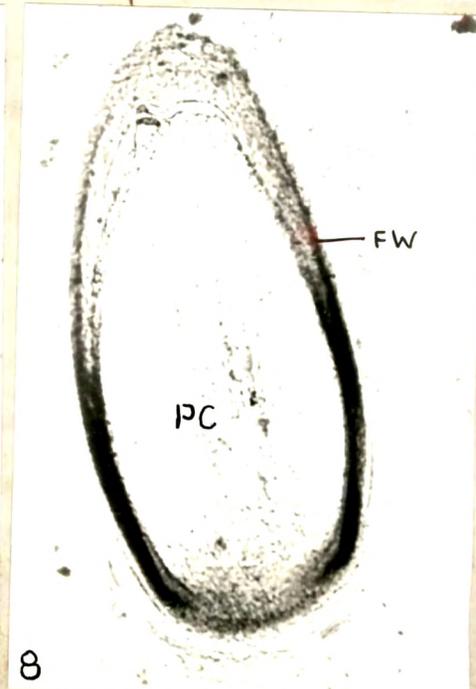
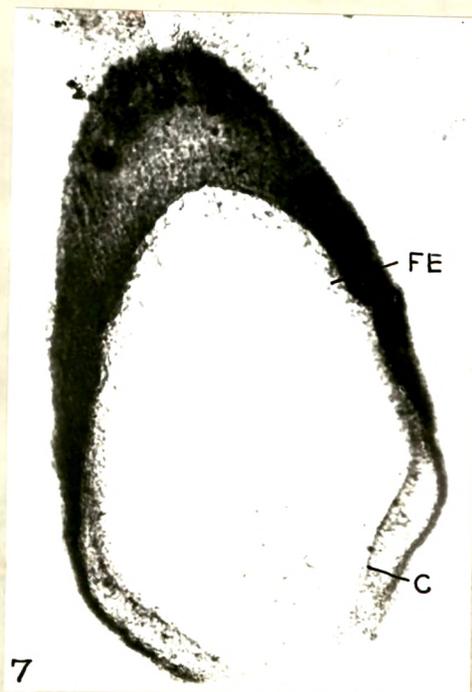
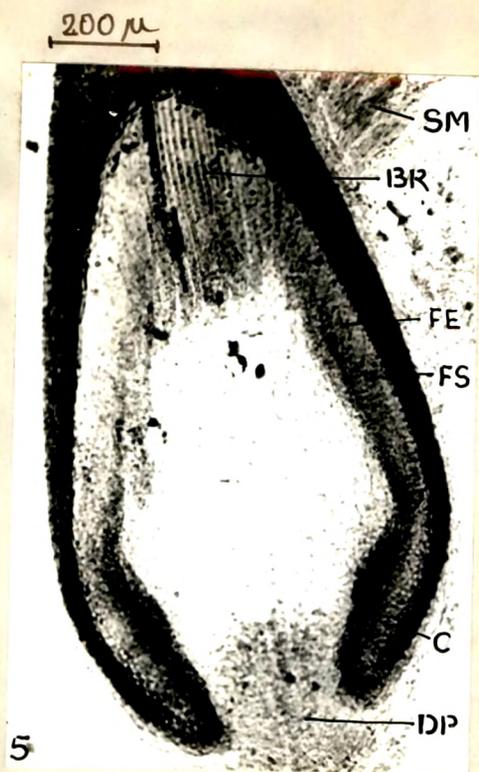
REGENERATION

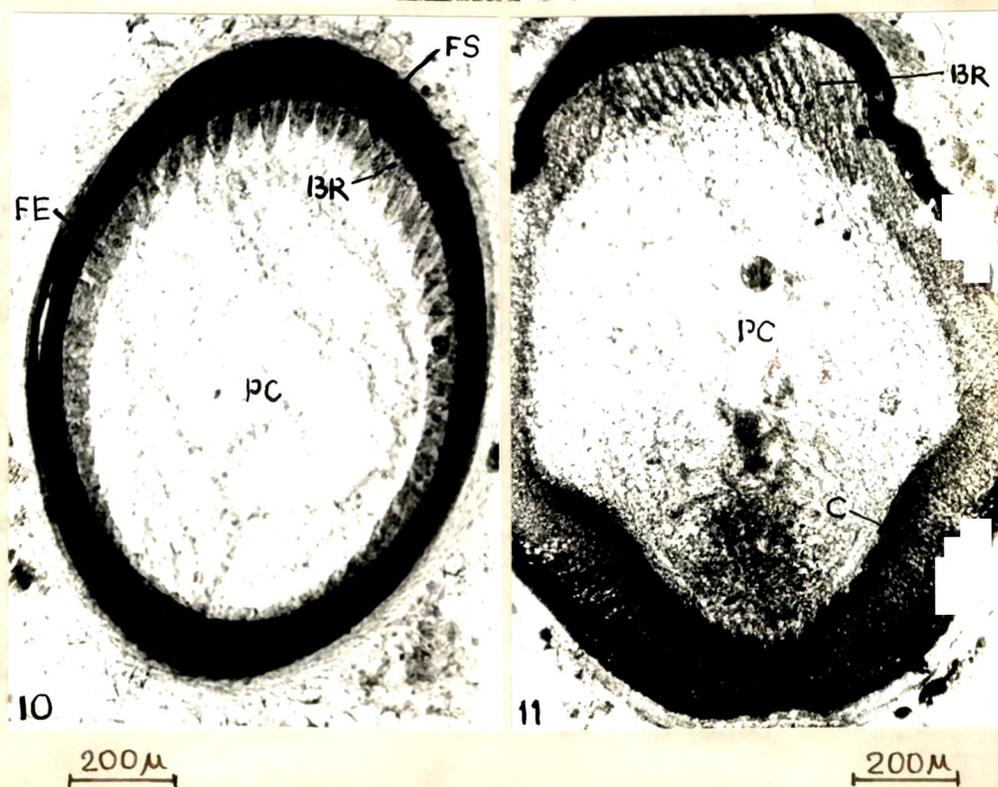
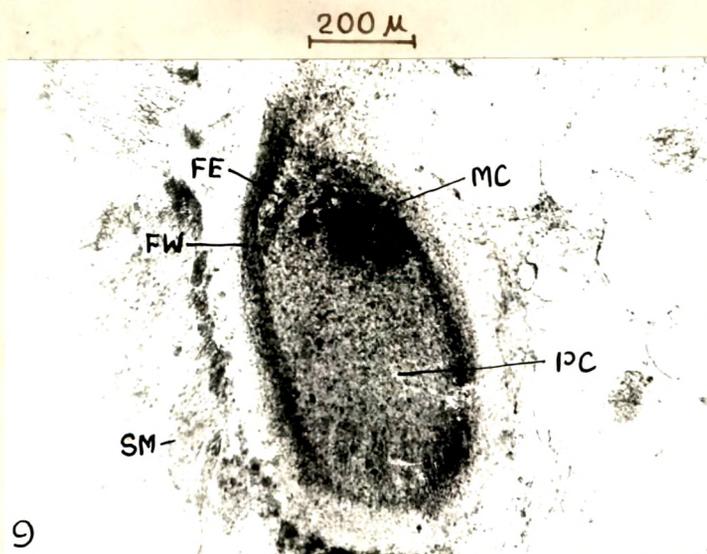
- Fig. 37 LS of follicle on the first day of regeneration showing a moderate MDH response in the pulp, epithelial cells and follicular wall.
- Fig. 38 LS of feather follicle on the 3rd day showing enzyme activity in various components.
- Fig. 39 TS of follicle exhibiting a low MDH reactivity in its components on the 5th day of feather regeneration.
- Fig. 40 Section of feather follicle on the 7th day of regeneration. Note the peak MDH activity.
- Fig. 41 TS of 15 day old regenerating feather. Peak MDH activity is registered by the barb ridges.
- Fig. 42 LS of 15 day old feather regenerate. The enzyme activity is discernible in the basal regions.
- Fig. 43 Oblique section of the regenerate on the 25th day showing keratinised regions and the decline in MDH activity in various components.

ABBREVIATIONS

AA - Axial artery
AT - Adipose tissue
BC - Blood clot
BR - Barb ridges
C - Collar
DP - Dermal papilla
EC - Epithelial covering
FE - Feather epithelium
FW - Follicular wall
FS - Feather sheath
KR - Keratinised region
MC - Melanocytes
NDF- Natal down feather
NFE- Non-feather epidermis
R - R^achis
RP - Regressing pulp
SM - Smooth muscle

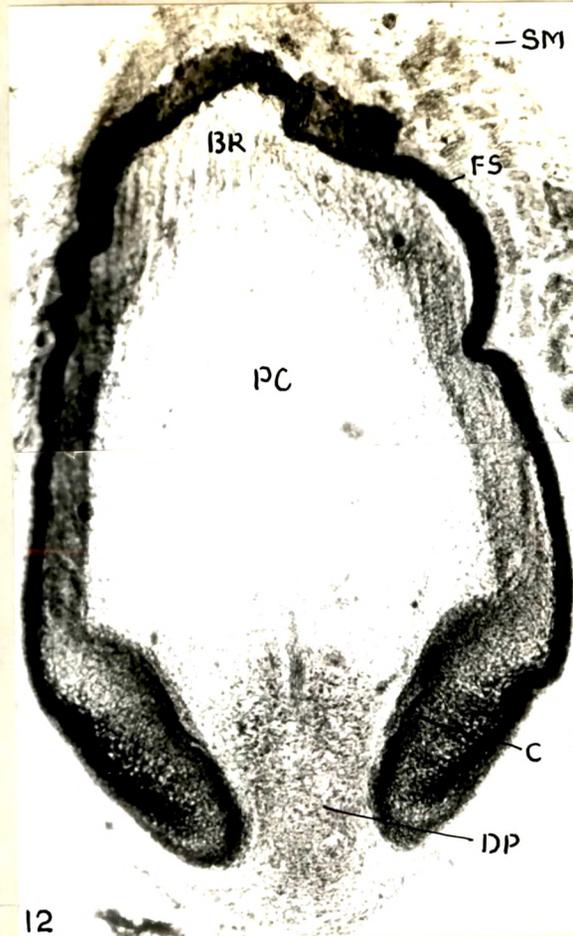




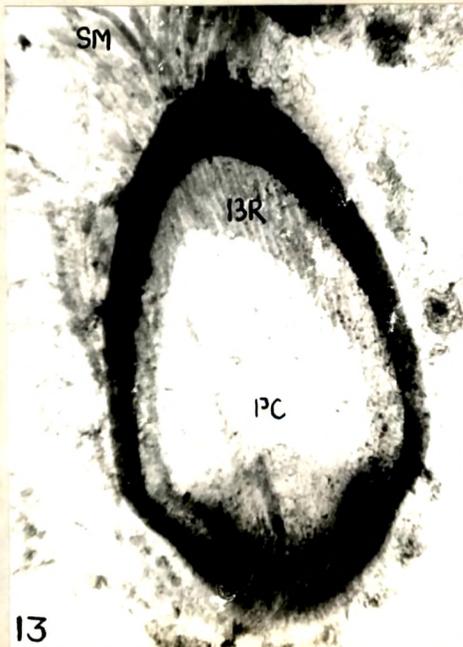


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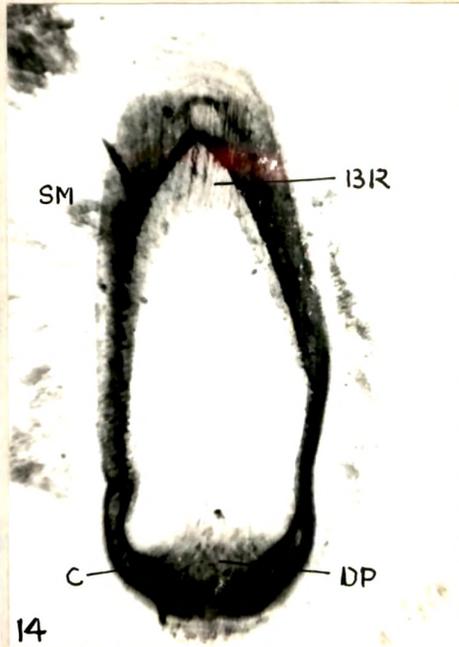


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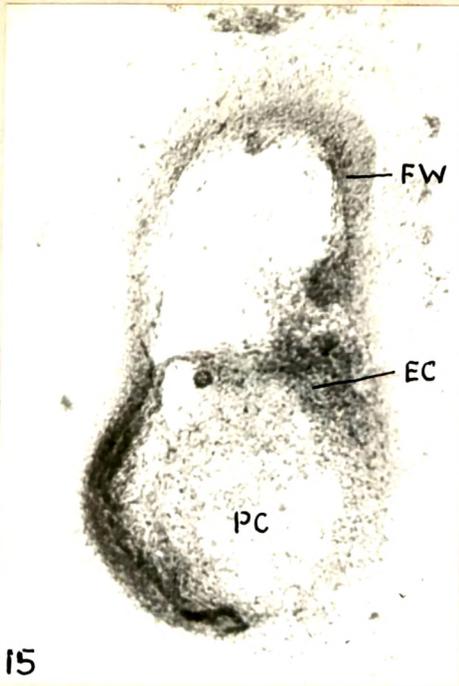
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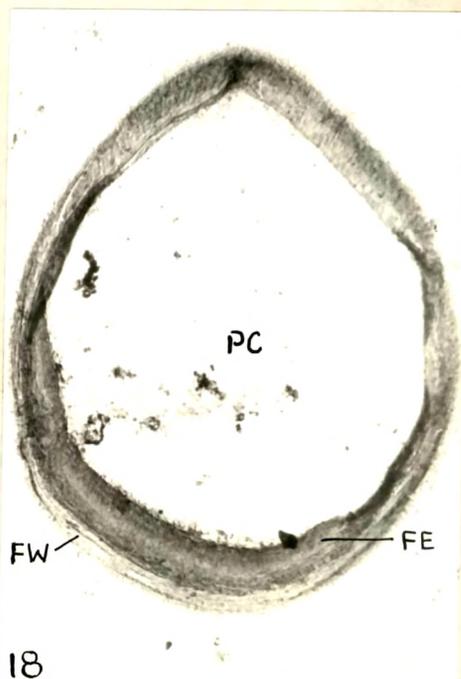
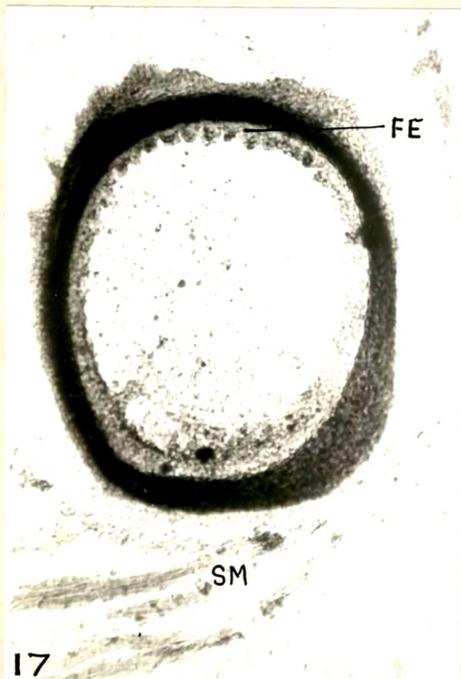
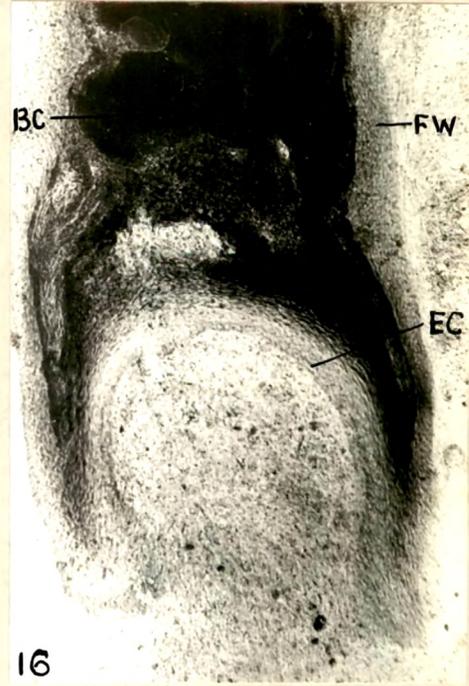
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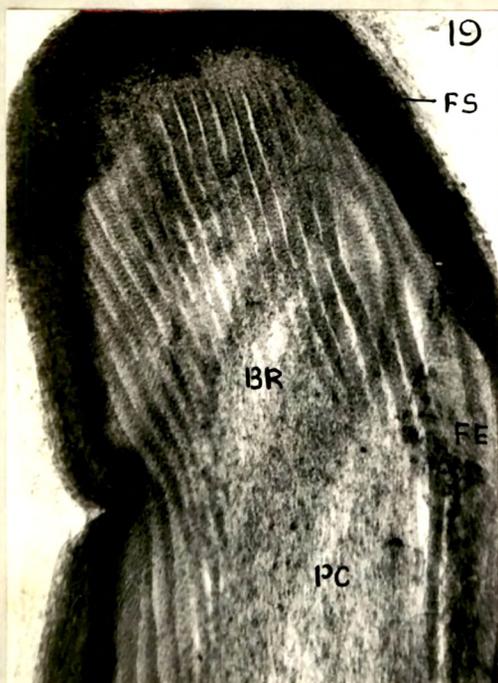
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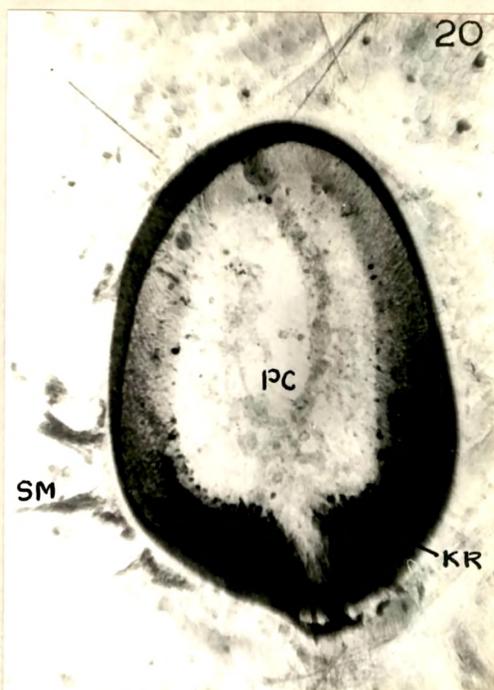


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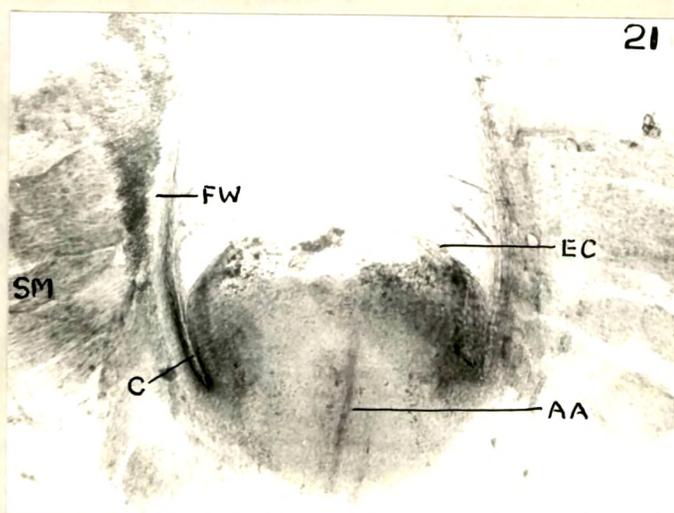




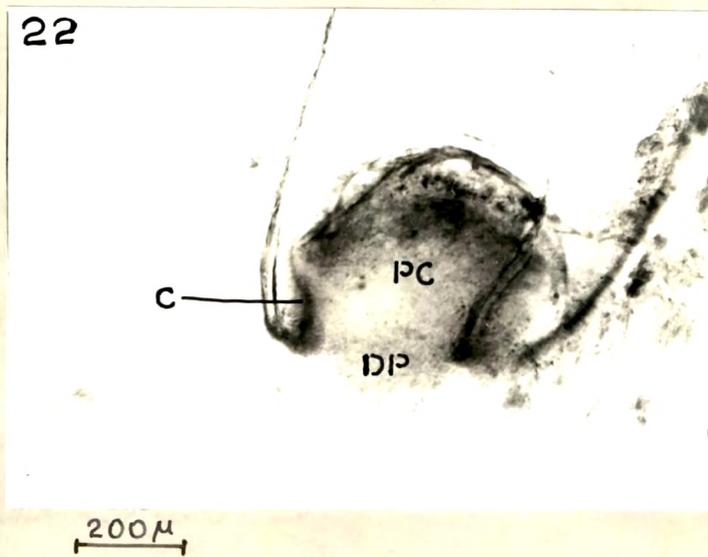
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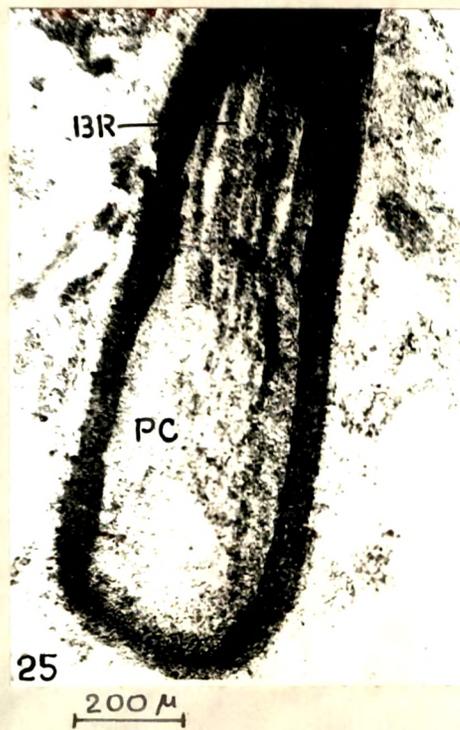
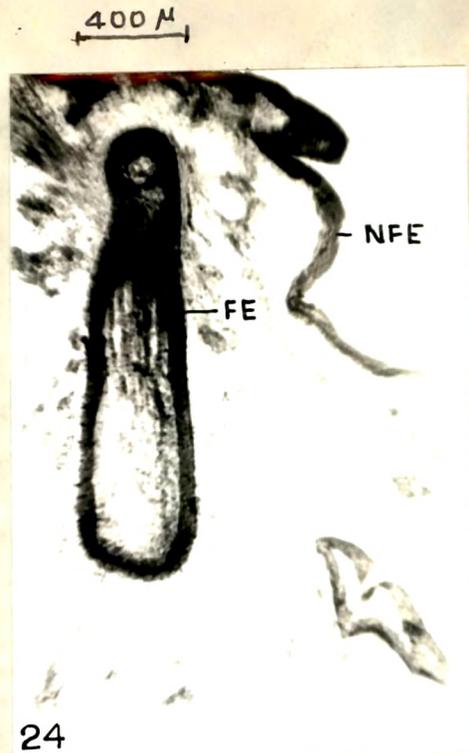
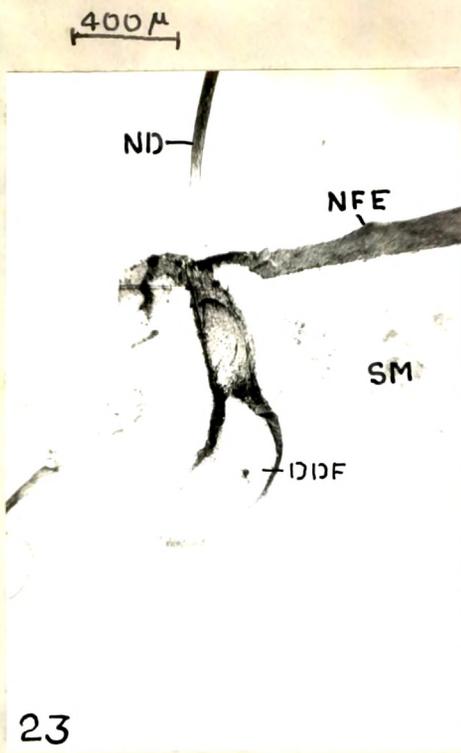


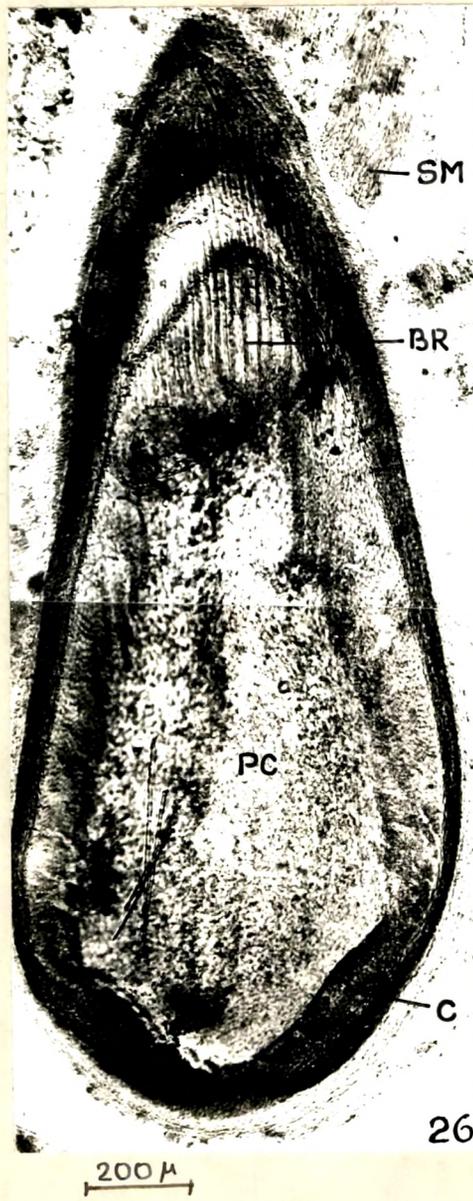
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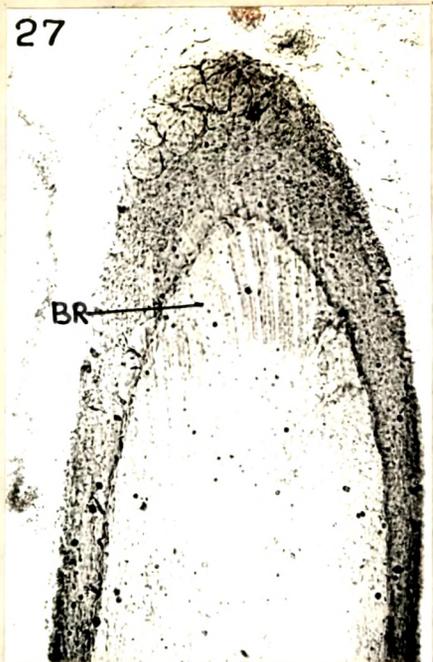




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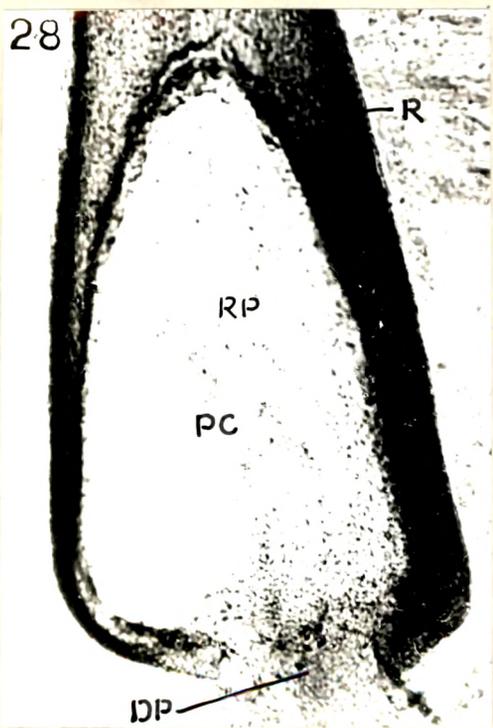
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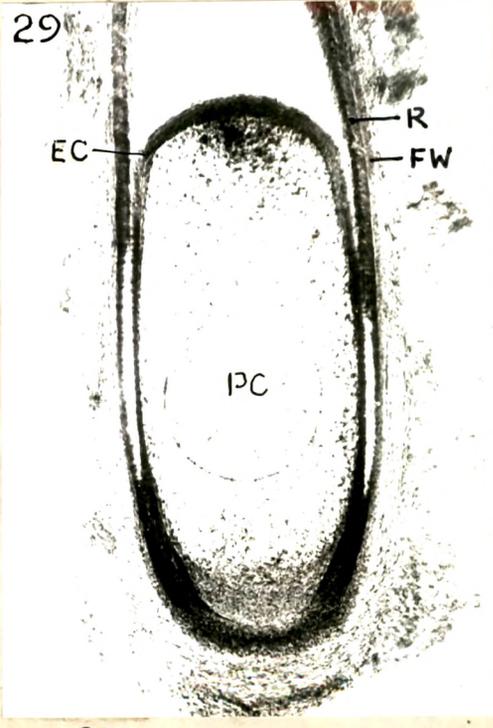
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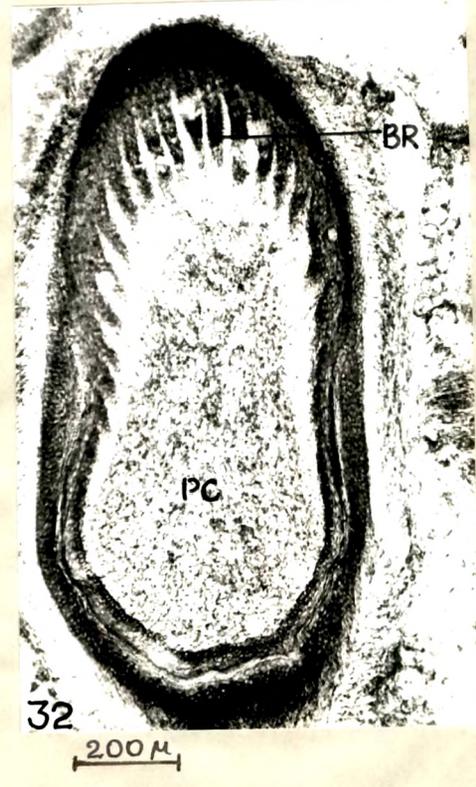
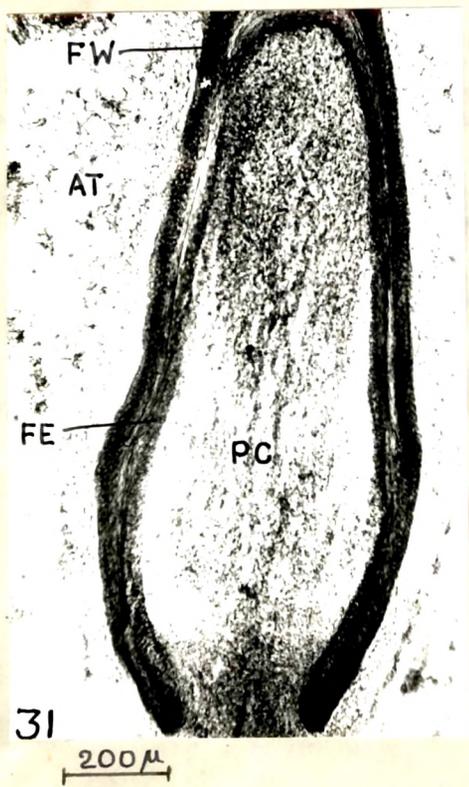
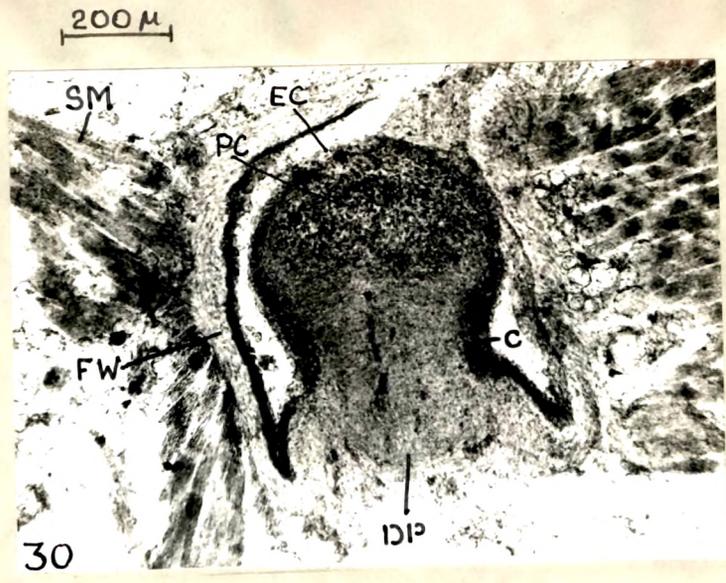
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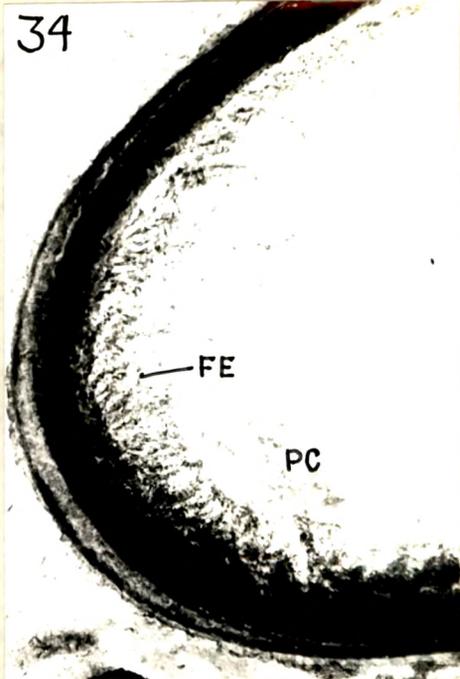




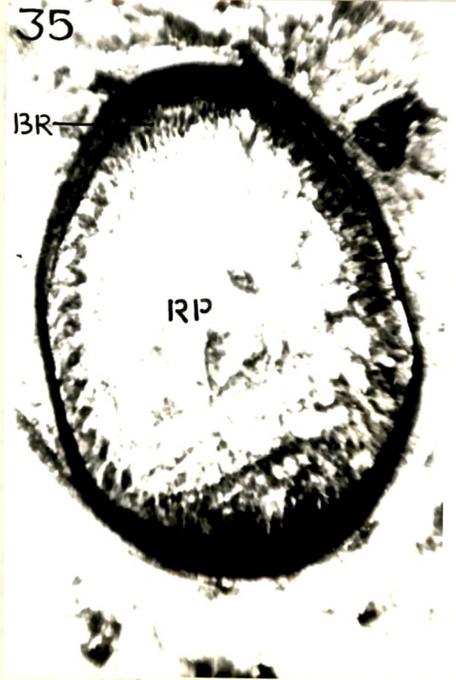
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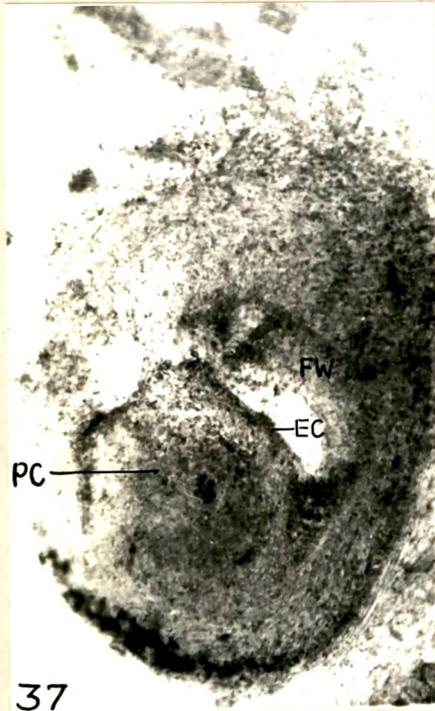


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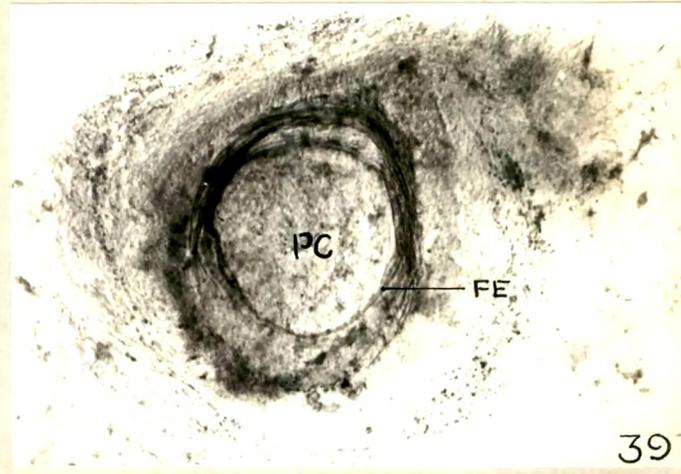
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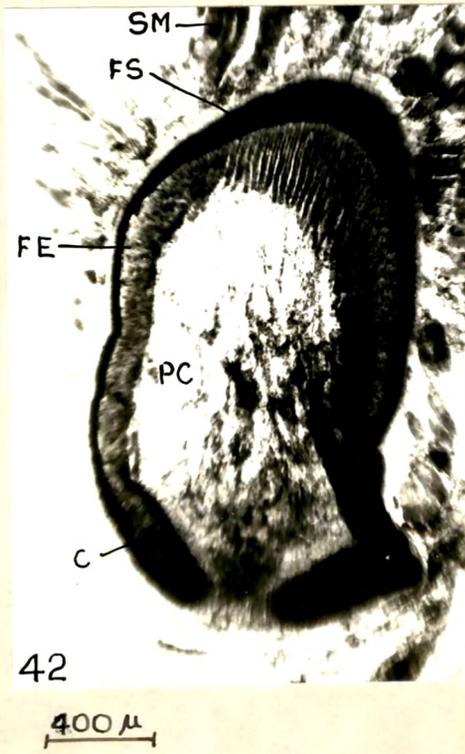
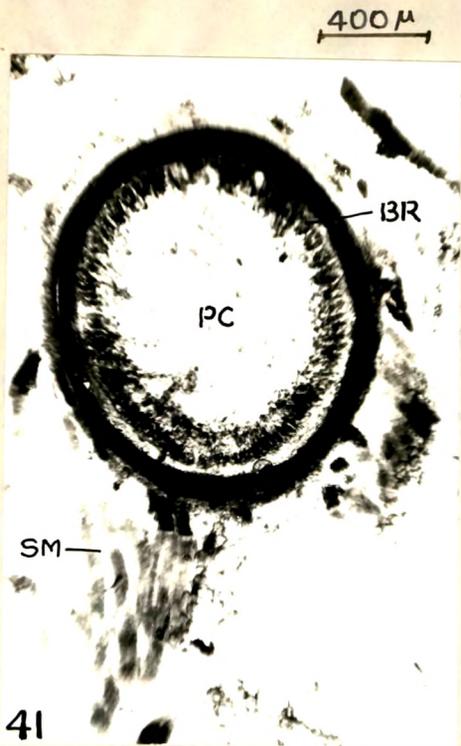
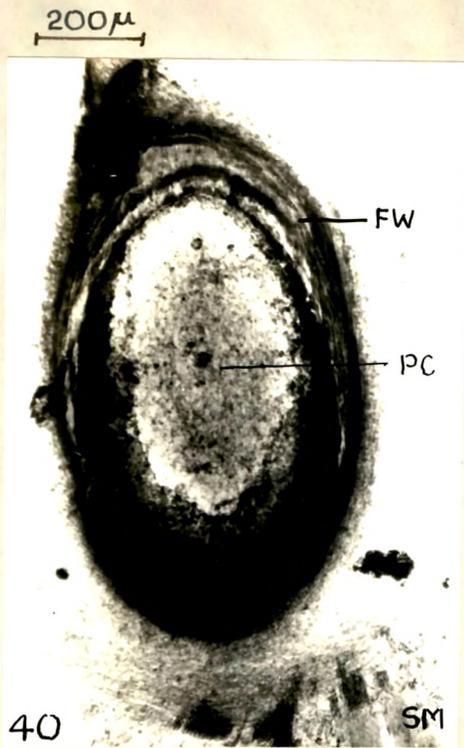
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7th day, only to register a gradual increase in the barb ridges by the 10th day soon after which an overall peak enzyme response was noticed in the 12 day old feather. This peak response of SDH was retained till about the 15th day. However, after this, there was a gradual decline in the enzyme activity in the regenerating feather components till ^{the} 25th day. By about this time, the concentration of the enzyme in the different components of skin was found to be similar to that noticed in the normal adult skin.

MDH activity during normal post-hatching development: (Figs. 23-29)

MDH activity was found to be almost negligible in all the components of ^{the} skin on hatching, but soon registered a slight increase by the 3rd day in the epithelial cells of the developing feather, and the smooth muscles. Nevertheless, this increase was not evident in other components of the skin at this stage. By the 5th day of post-hatching development, all the components of the skin exhibited peak histochemical response for the enzyme, this being most evident in the epithelial and the pulp cells of the developing feathers. This pattern of enzyme intensity and distribution remained so upto the 10th day. In the epithelial cells, its distribution pattern was noticed to be similar to that of SDH, being highest in the cells of the barb ridges proximal

to the pulp and gradually declining in concentration, proportional to the degree of keratinization in those cells away from the pulp. Hereonwards, as the development progressed, a gradual but definite decline in the enzyme concentration occurred, which reached the lowest level, on the 20th day. However, this decline was not so sharp in the pulp cells of the developing feathers. After this, a gradual but little increase of the enzyme activity was observed in the skin components which attained the normal adult level by the 25th day of development and remained so thereafter.

MDH activity during induced feather development: (Figs.30-36)

MDH, unlike SDH, was found to register an increase in activity rather than decrease by 15 hours after plucking, in the various components of the skin, there being a moderate activity in the epithelial cells of the feather germ and the smooth muscles fibres. But, the pulp cells, non-feather epidermis and other dermal components exhibited a comparatively lower level of the enzyme concentration. This elevated MDH activity was found to be so retained in all the components until the 7th day of induced development.

A slight reduction in MDH reactivity except in the smooth muscles was observed by the 10th day resulting in a level characteristic of the normal skin. The enzyme activity was found to increase in all the components of the developing feather and other parts of the non feather skin but a peak histochemical response was observed only in the epithelial cells on the 15th day. This was soon followed by a gradual decline in the MDH activity in the various components reaching on the 25th day, a level almost similar to that observed in the normal skin components and which was retained thereafter.

MDH activity during feather regeneration: (Figs. 37-43)

MDH activity, on the first day of regeneration was found to be of a moderate level in the various components of the skin, viz., stratum germinativum of the follicular wall, the injured feather and its pulp cells, smooth muscles, fibrocytes and non-feather epidermis. Such an enzyme response was found to persist till the 3rd day of regeneration. An overall reduction in MDH activity was observed, hereafter, which on the 5th day touched the lowest level in the components of the skin mentioned above.

But on the 7th day of regeneration the enzyme climbed up again and a peak histochemical response was discernible

in the various components, especially in the developing feather and continued to remain so ^{uptill} the 15th day after which there was a gradual reduction till the enzyme activity reached the normal level similar to that observed in the various components of the normal adult skin by about the 25th day.

DISCUSSION

A low level of activity of SDH and MDH in comparison to the dehydrogenases of other metabolic routes like ^{the} EMP pathway of glycolysis and the ^{the} HMP shunt, suggest that ^{the} TCA cycle plays a comparatively less significant role in the metabolic processes of the component tissues of the adult normal skin of ^{the} pigeon. Such a predominance of glycolytic metabolism in the tissues of human skin has been recognised by Novikova (1970) and in the reptilian skin by Shah and Ramachandran (1972). The quiescent hair follicles of ^{the} mouse are also reported to be low in SDH reactivity (Argyris, 1956).

However, a high incidence of SDH and MDH observed herein in many of the components, especially in the developing feather during certain stages of its development, clearly indicates that the energy yielding reactions of

the TCA cycle are, nevertheless, of importance in the metabolic processes underlying development and maturation of epidermal derivatives.

The presently observed low level activity of both the enzymes in the various components of the skin on the day of hatching seems to imply the nondependence of the skin and the developing feathers on oxidative metabolism at this phase. However, it is interesting that during the development of ^{the} chick down feather, Koning and Hamilton (1954) found SDH to be active in all the components of the skin including the developing feathers, having a general distribution. They did not assign any particular role for the enzyme in developmental processes since they contended that, SDH being concerned with basic ~~meta~~ metabolism, such ^{as} a distribution pattern in all the tissues is only to be expected. Presence of SDH, a mitochondrial enzyme, in all the components of the skin at the time of hatching thus seems to be understandable from this point of view. However, a low level of activity of SDH and MDH, both dehydrogenases of the TCA cycle, along with a high reactivity of enzymes of other metabolic pathways, seems to be significant from the metabolic point of view. It may be surmised that due to the priority of synthetic

activities over energy demands, the production of precursor molecules and co-factors takes precedence during the initial stages and as such appears to be well reflected by the predominance of the activities of HMP shunt and EMP pathways (as discussed in chapters 2 and 3). The slight increase in the MDH activity by the 3rd day after hatching and of SDH reactivity by the 5th day in the epithelial cells of developing feathers seems to indicate a gradual increase of the TCA cycle activity in anticipation of the increase in energy demands during the ensuing active proliferative and differentiative phases. Such an accumulation of enzymes prior to the onset of reactions catalysed by them is known to be a normal feature during development (Moog, 1965).

The peak MDH response attained on the 5th day in the components of the skin, especially the epithelial and pulp cells, along with a moderate activity of SDH, could be considered as indicative of increased energy production at this stage, which when reflected on to the fact that the developing feather at this stage is in an active proliferative and differentiative phase, seems to gain validity. However, the peak activity of MDH noted in the

developing feather on the 5th day was not accompanied by a similar peak activity of SDH though the latter maintained a moderately high level of its activity. A similar difference in the histochemical reactivity of these two enzymes in the activated gastric glands of rat has been noticed by Ishimura and Fujie (1970). They have postulated that an increase in the activity of MDH alone, could be taken as an index of increased TCA cycle operation. Another interesting aspect highlighted by Moog (1965) regarding the general pattern of enzyme activities during development, ^{that} within the same tissue or organ, enzymes tend to increase or decrease in activity independently of each other, also seems to ^{fall in line with} explain the difference in attainment of peak level of activity by SDH and MDH during the early period of definitive feather development. This is also suggestive of the possible differences in substrate utilisation by mitochondria, as has been observed in kidney and heart mitochondria by Fronius and Hajos (1970). They have further suggested that such a variation does not interfere with the ability of mitochondria to provide energy for cellular functions. The significant level of activities of these enzymes at this stage in smooth muscles, could

be attributed to their preparative phase for functional differentiation, as these structures become functionally significant only after the definitive feathers are well formed.

The attainment of the peak level by these two enzymes of the TCA cycle by the 10th day of hatching in the components of the developing feather is understandable in the light of the expected high energy demands imposed by the actively growing feather. The prominent activities of SDH and MDH ^{lipid} coupled with the enzymes of the EMP pathway of glycolysis in the epithelial cells of the basal region of the barb ridges in comparison to those at the distal region seems to indicate a higher incidence of metabolic processes there and which may be considered as aiding in the proliferative activities there. Further, these cells of the barb ridges being nearer to the pulp, have a comparatively higher accessibility to blood glucose which could be metabolised completely for energy yield through the glycolytic and TCA cycle oxidations. Similar observations of high activity of enzymes of glycolytic pathway and TCA cycle in the basal layers, as opposed to the upper layers, has been reported by Michael (1965) in the skin of the Rhesus monkey. It is

also of interest that in the human epidermis, Montagna and Formissano (1955) also observed a higher activity of SDH in the matrix cells of the growing hair and have correlated this finding with the proliferative activities of these cells.

The peak SDH response maintained upto the 15th day post-hatching in the smooth muscles and components of the developing feather bespeaks of a high incidence of the TCA cycle reactions in these tissues. This phase marked by active differentiative processes like keratinization and growth could hence be expected as a period of increased energy requirements. A decline in the activity of both SDH and MDH noticed in the various components of the definitive feathers by the 20th day, could be indicative of a reduced level of energy necessities as the maturation and functional differentiation of ^{the} feather has by now reached an advanced stage. A slight increase by the 25th day, of these enzymes, to a level almost similar to that observed in the corresponding parts of the adult normal skin appears to mark the near completion of the developmental processes. In the pulp, the lower level of activity registered by the cells in the apical region as compared to those at the basal one could be due to the regression of the pulp, in

progress from the apical regions as a result of cellular necrosis. Cellular necrosis during developmental processes in many cases is known to be due to the failure of mitochondrial phosphorylation (Firth and Hicks, 1972). Loss of SDH, an important enzyme of the energy yielding TCA cycle, during necrosis of the cells of inter digital area in the developing chick has been shown by Mottet and Hammer (1972) also. In this light the presently observed declined SDH activity in the cells of the pulp undergoing necrosis seems to be thus quite understandable.

During induced development of the feather, the low level of activity of SDH upto the 5th day and a moderately high activity of MDH appear to suggest a moderate level of TCA cycle operation at this phase. In the non-feather area the TCA cycle activity could be helpful in compensating for the loss of heat due to the removal of feathers. Observed differences between SDH and MDH reactivities could be possibly due to differences in substrate utilization by mitochondria. The gradual increase in SDH and MDH activities reaching ultimately a peak response by the 10th and 15th days respectively of induced development might be taken in favour of a high incidence of TCA cycle oxidations. The course of induced feather development from 5th day onwards

appears to characterise itself into two distinct periods
 > as per the intensity of SDH and MDH activities, one between ^{the} 5th and ^{the} 15th days and other between ^{the} 15th and ^{the} 25th days.

Whereas, the former period was marked by a gradual increase of SDH and MDH activities attaining ultimately the peak levels of these enzymes on the 10th and 15th days respectively, the latter period (15th to 25th day) was marked by a gradual decline and the attainment by the 25th day (by a slight rise in activity from that of the 20th day) in both the, by now, fully developed feather as well as the skin of a level characteristic of the corresponding elements of the normal adult integument, thus marking the completion of the process of development and maturation. These observations thus tend to indicate a phase of increased metabolic activities marked by the elevated level of TCA cycle catabolism (between 5th and 15th days) corresponding well with the growth of ^{the} feather by increased rate of cellular proliferation and the accompanying differentiative process and (compared to normal development), a later period of decreased level of energy necessities (between 15th and 25th days) correlating well with the differentiative and maturation^{al} activities predominant during this stage. It is worthwhile to note in this connection the observations of Mojumdar et al. (1973)

and Montagna (1962) of a high SDH activity during the proliferative period of hair growth and again of Montagna (1962) of a reduced SDH reactivity during the advanced phase of differentiative activities in hair growth.

A drop in SDH activity (from the normal level) during the earlier phases of feather regeneration gains confirmity when viewed in the light of the known prevalence of anaerobiosis during the early stages of wound healing. However, the gradual increase in its activity reaching a moderate level by the 5th day could be suggestive of the importance of this enzyme in the energy yielding reactions of the healed feather stub. Nevertheless, significance of the presently observed fluctuations in the level of the enzyme activities in the regenerating feather uptill the 7th day remains speculative and unascertainable at this juncture. The first peak response of MDH along with an increasing trend in SDH activity, noticed on the 7th day, a stage much earlier than that observed during induced development, could be pointing to an acceleration of metabolic processes in order to have a faster rate of development of the

regenerating definitive feather. A similarity in the pattern of activities of these two enzymes during all the three modes of development is a decline after the 15th day. This could be suggestive of the fact that by ^{the} 15th day, the developing feather (whether normal, induced or regenerating) is similar both morphologically as well as biochemically, in spite of the initial ^cslakened growth due to reparative activities during regenerative development. Thus it appears that the time lost in repairing the "dome" during the initial phases is well compensated by an acceleration of the machinery of ^{the} developmental process, during the middle phase of regeneration. Such a possibility could well be visualised in the light of the prolonged period of peak TCA cycle activity in the feather during the same phase of regeneration, as indicated by the high activity of these two key enzymes of the TCA cycle, which could well aid the regenerating feathers by an enhanced energy supply over a longer period, than during normal and induced development (as discussed in chapter 9).

The gradual attainment by these enzymes of a level similar to that observed in the corresponding normal components of the skin, by the 25th day seems to be suggestive of the near completion of the regenerative process and acquisition of a fully formed and grown regenerate.