CHAPTER 4

HISTOCHEMICAL OBSERVATIONS ON LACTATE DEHYDROGENASE AND Œ GLYCEROPHOSPHATE DEHYDROGENASE IN THE DEVELOPING DEFINITIVE FEATHER OF THE BLUE ROCK PIGEON, COLUMBA LIVIA, DEVELOPING UNDER THREE DIFFERENT CONDITIONS

Significance of various dehydrogenases in the metabolic activities of animal tissues has been well recognised. Lactate dehydrogenase (LDH) and Œ glycerophosphate dehydrogenase (Œ GPDH) which are associated with the Embden-Meyerhoff pathway of glycolysis have evoked considerable interest in view of their significance in reoxidising reduced Nicotinamide adenine dinucleotide (NADH) thereby providing NAD, an essential cofactor in the reaction catalised by glyceraldehyde-3-phosphate dehydrogenase converting glyceraldehyde-3-phosphate into 1,3, diphosphoglycerate. By virtue of this significance it becomes clear that the activities of LDH as well as Œ GPDH are of importance in ensuring an optimal and continued operation of the EMP pathway of glycolysis. A characterisation of the mode of NADH oxidation existing within a particular tissue with glycolytic metabolism is dependent upon the knowledge of the relative activities of Œ GPDH and LDH (Boxer and Shonk, 1960; Chefurka, 1958; 1965). Hence, a comparative information about the two enzymes viz., LDH and Œ GPDH would be of great value in assessing

variations in the metabolic processes of the tissues concerned.

Of these two dehydrogenases, comparatively more is known about LDH; the activity of which has been studied in the metabolic processes of a number of tissues and organs, viz.; ~~avian~~ skeletal muscle (George and Co-workers, 1966; Fritz, 1965), vertebrate nervous system (Nelson & Wakefield, 1973), vertebrate kidney (Beard & Allen, 1966), human skin (Lewis et al., 1967), and mammalian RBC (Duffy and Sanderson, 1971). Further, this enzyme has been studied during development and organogenesis of amphibia (Adams and Finnegan, 1965), chick liver (Conklin, 1965), hatching muscle of chick (Klicka and Kaspar, 1970), avian gizzard (Shah and Panikar, unpublished), mammalian tooth (Fullmer, 1963), kidney (Smith and Kissane, 1963), cerebral cortex (Flexner et al., 1960) and during diverse biological processes like regeneration (Schmidt, 1968; Shah and Ramachandran, 1970; Magon, 1970), wound healing (Asnani, 1971), growth and aging of human cells (Cristofalo et al., 1967) and temperature adaptations in animals (Somero and Hochachka, 1969; Baldwin and Aleksieuk, 1973).

Though comparatively little attention has been paid to CC GPDH, the activity of this enzyme has, however, been

studied in the muscles of both vertebrates as well as invertebrates (Ogata and Mori, 1964; Blanchaer et al., 1963; Sacktor and Cockran, 1957; Chefurka, 1958; Zebe and McShan, 1957; Sacktor et al., 1964; Rosen et al., 1968), various tissues of rat and mouse (Fried et al., 1959), adrenal cortex of mouse (Shivram and Sharma,1966), tumour cells (Boxer and Shonk, 1960), embryonic chick liver (Conklin, 1966), and during development in mammalian tooth (Fullmer, 1963), and also during developmental processes like regeneration, in urodele limb (Schmidt, 1963) and lacertilian tail (Shah and Magon, 1969; Ramachandran, 1972).

In spite of the considerable literature available, there is a general paucity of information about the relative activities of these two enzymes during the developmental process of vertebrate epidermal structures.

The present histochemical investigations have been carried out not only with a view to fathom the extent of involvement of these two enzymes in the developmental process of the definitive feathers of the blue rock pigeon, Columba livia; but also to have a comparative idea thereof between the three different modes of feather development mentioned earlier.

OBSERVATIONS

Normal adult skin: (Figs. 1 & 22)

The concentration of LDH was found to be higher in all the components of adult normal skin as compared to ŒGPDH. A high activity of LDH was discernible in the epithelial cells of the follicular wall and the collar region of the resting germ, as well as the cells of the upper region of the pulp. Smooth muscles and non-feather epidermis were the next in order of enzyme reactivity and depicted a moderate histochemical response for LDH. Connective tissue elements of dermis also depicted a noticeable level of the enzyme activity.

As for ŒGPDH, a moderate level of enzyme reactivity was noticeable in the smooth muscles and epidermis of the follicular wall, while a slightly lower degree of the enzyme reactivity was exhibited by the epithelial and pulp cells of the resting feather germ, non-feather epidermis and dermal components like fibrocytes.

LDH activity during normal post-hatching development: (Figs. 2-7)

A moderate activity of LDH was the feature on the day of hatching, though varied intensities of the histochemical reaction could be discernible in the various components of the skin. The maximal activity was, however, noticed in the stratum germinativum of both non-feather

epidermis and the developing feather as well as the cells of the pulp. Smooth muscles were next in the order of intensity of enzyme reactivity. Fibrocytes registered a low but noticeable activity.

By the 3rd day, though most of the component tissues of the skin retained the same level of LDH reactivity as observed earlier, nevertheless a slight increase was discernible in the epithelial cells of the collar region and the barb ridges which have been organised by now. This condition remained unchanged upto the 5th day. However, keratinization of epithelial cells of the non-feather regions of the skin was clearly discernible at this stage and in these keratinised cells, the enzyme reaction was found to be negative.

An overall gradual increase in LDH response after the 5th day of hatching ultimately culminated in its attainment of a high peak level of activity on the 10th day. In the non-feather region of the skin, cells of the stratum germinativum, fibrocytes and smooth muscles, all registered this peak of enzyme reactivity. Amongst the components of the developing feathers, the highest activity of the enzyme was observed in the epithelial cells of the barb ridges, especially those which are in

proximity to the pulp. A gradual reduction in enzyme concentration, proportional to the degree of keratinisation could be observed in the cells of the barb ridges away from the pulp.

After the peak response period, of the 10th day, a gradual decline in the LDH activity commenced which fell to a moderate level in all the skin components by about the 15th day. Such moderate level of the enzyme activity continued to remain so until the 25th day after which by about the 30th day a slight increase resulted in the attainment of a level observed in the adult normal skin.

LDH activity during induced development: (Figs. 8-15)

About 15 hours after plucking the adult definitive feathers, the concentration of LDH showed a sharp decline in the feathered as well as the non-feather region of the skin, in all the components. However, an upward trend in the enzyme activity could be noticed by about 24 hours after plucking, in almost all the components, viz., epidermis in the non-feather region, fibrocytes, smooth muscles and the feather follicles themselves. In the feather germ, the epithelial cells of the collar region

registered a high enzyme activity. LDH activity was found to be comparatively more in the cells of the pulp towards the apical regions than in those at the basal region. This condition was retained up to the 3rd day after which a peak enzyme reactivity was discernible in the developing germ, especially in the cells of the barb ridges. This peak level of LDH was found to be maintained on the 5th day as well when the cells of the barb ridges proximal to the pulp registered a higher concentration than those away from the pulp. The smooth muscles exhibited a moderate enzyme response.

The peak level of LDH observed on the 5th day continued upto the 10th day only to decline to a moderate level on the 15th day which remained unchanged uptill the 25th day after which, a slight increase by the 30th day resulted in the attainment of a level similar to that observed in the various components of the normal adult skin.

LDH activity during regeneration: (Figs. 16–21)

24 hours after inducing regeneration, the LDH activity was found to be moderate in the collar, pulp cells, follicular wall as well as in the smooth muscles. However, the collar showed a slightly higher concentration

EXPLANATIONS FOR FIGURES

Fig. 1 Part of section of normal adult skin showing the resting feather germ. Note high concentration of LDH in the collar, pulp cells and in the smooth muscles.

NORMAL DEVELOPMENT

Fig. 2 Section of skin with developing feather one day after hatching. Note enzyme activity in the components of feather, non-feather epidermis and the smooth muscles.

Fig. 3 LS of 5 day old feather, showing LDH activity in the barb ridges and pulp cells.

Fig. 4 LS of 10 day old feather depicting peak activity of the enzyme in its components.

Fig. 5 Section of skin showing a decline in the enzyme activity of the developing feather on 15th day post-hatching.

Fig. 6 Section of developing feather on the 20th day. Note LDH activity in epithelial and pulp cells.

Fig. 7 LS of the base of follicle showing enzyme activity in the pulp cells and epithelial covering of the future resting germ being laid. Note enzyme activity in smooth muscles.

INDUCED DEVELOPMENT

Fig. 8 LDH activity in the germ, follicular wall and smooth muscles 24 hours after plucking the adult feather.

Fig. 9 Oblique section of follicle on 3rd day of induced development. Note high activity in the feather epithelium of barb ridges.

Fig. 10 TS of 5 day old inducedly developing feather showing peak enzyme activity in cells of barb ridges proximal to the pulp.

Fig. 11 Section of skin showing TS of developing feather on 7th day, with peak LDH activity.

Fig. 12 Section of skin with TS of 10 day old feather showing high enzyme activity in epithelial cells.

Fig. 13 Oblique section of feather on the 15th day showing moderate LDH response. Keratinised regions appear dark.

Fig. 14 Oblique section at the base of 20 day old feather registering moderate enzyme activity in pulp cells, collar and barb ridges.

Fig. 15 Oblique section passing through the base of 30 day old feather, showing an increased enzyme activity in the dermal papilla, pulp cells and smooth muscles.

REGENERATION

Fig. 16 Section of skin showing LS of one day old regenerate. Note enzyme activity in collar, epithelial covering pulp cells and smooth muscles.

Fig. 17 Section of regenerating feather on the 3rd day showing LDH activity.

Fig. 18 LS of the feather regenerate on the 5th day. Note high LDH activity.

Fig. 19 TS of part of feather on 15th day of regeneration. Note decline in enzyme activity.

Fig. 20 LS of 20 day old regenerate depicting LDH activity.

Fig. 21 Section of the 30 day old regenerate passing through the basal region, showing the enzyme activity in future resting germ. Note LDH activity in smooth muscles.

Fig. 22 Section of feather follicle showing CC GPDH activity in the resting feather germ and smooth muscles.

NORMAL DEVELOPMENT

Fig. 23 Section of skin ffom 3 day old pigeon showing the enzyme activity in developing definitive feathers and other components.

Fig. 24 Section of skin from 5 day old pigeon showing the enzyme activity in developing feather and the smooth muscles.

Fig. 25 Part of Fig. 24 enlarged to show the enzyme activity in barb ridges and pulp cells.

Fig. 26 LS of feather on the 10th day post-hatching showing a decline in the enzyme activity.

Fig. 27 LS of 15 day old feather showing the enzyme activity in various components.

Fig. 28 Oblique section of 25 day old feather showing the enzyme activity of pulp cells and smooth muscles.

Fig. 29 Section of 30 day old definitive feather passing through the basal region, showing the enzyme activity in pulp cells, epithelial covering, follicular wall and smooth muscles.

INDUCED DEVELOPMENT

Fig. 30 LS of feather germ 15 hours after plucking, showing the enzyme activity.

Fig. 31 TS of part of the feather on 5th day of induced development showing enzyme activity in pulp and epithelial cells of barb ridges.

Fig. 32 LS of basal region of 10 day old developing feather showing the enzyme activity in epithelial cells of the collar and the pulp cells.

Fig. 33 Section of part of the feather on 15th day showing the enzyme activity.

Fig. 34 LS passing through the basal region of 20 day old inducedly developing feather showing the enzyme activity.

Fig. 35 LS of feather on 30th day of induced development showing enzyme activity in pulp and epithelial cells of the future resting germ.

REGENERATION

Fig. 36 LS of 5 day old regenerate showing the enzyme activity in pulp cells, epithelial cells and the follicular wall.

Fig. 37 Section of 10 day old regenerate showing high enzyme activity in pulp and barb ridges.

Fig. 38 Portion of Fig. 37 enlarged to show enzyme activity in barb ridges.

Fig. 39 Section of 15 day old regenerate showing peak enzyme activity.

Fig. 40 Section of feather on the 20th day of regeneration showing reduced enzyme reactivity. The dark areas are keratinised regions.

Fig. 41 TS of 25 day old regenerate showing enzyme activity in the pulp cells.

ABBREVIATIONS

AA - Axial artery
AT - Adipose tissue
BR - Barb ridges
C - Collar
DP - Dermal papilla
DDF - Developing definitive feather
FE - Feather epithelium
FW - Follicular wall
FS - Feather sheath
KR - Keratinised region
NFE - Non-feather epidermis
P - Pulp
PC - Pulp cells
R - Rachis
SM - Smooth muscles

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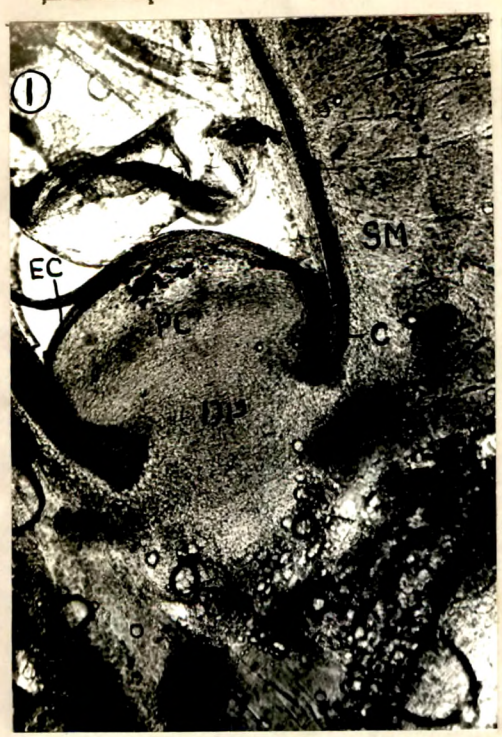

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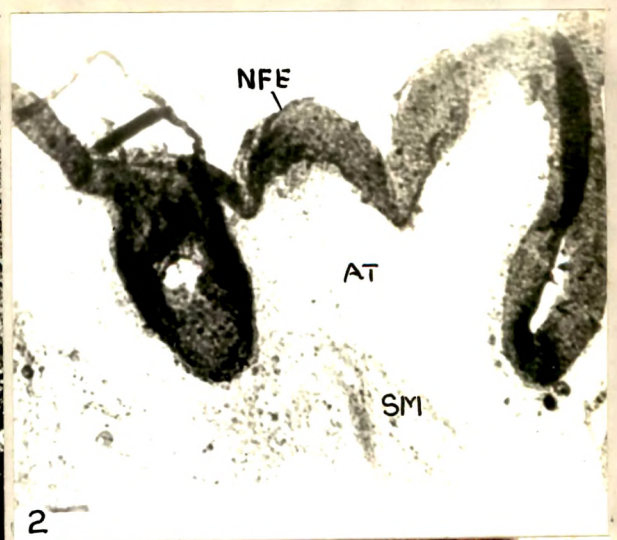

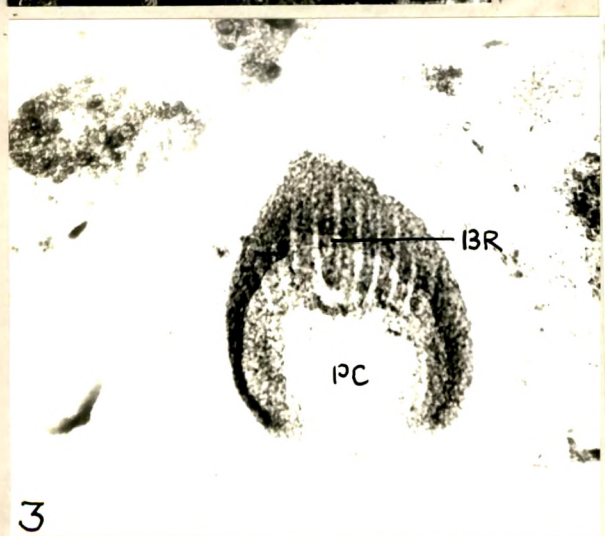

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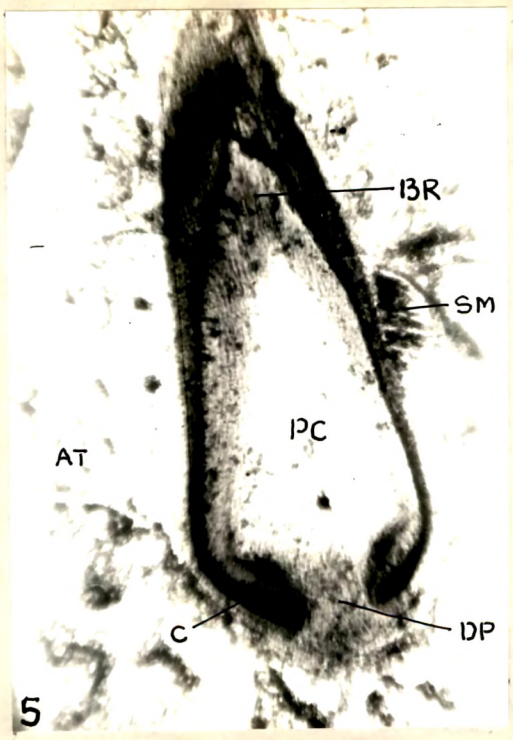

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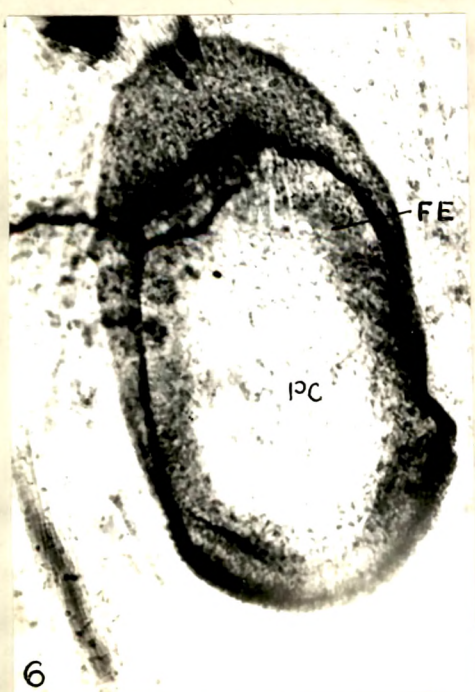

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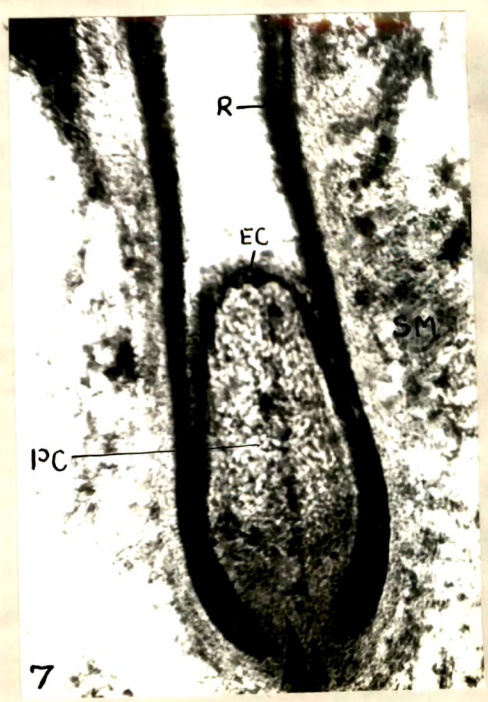

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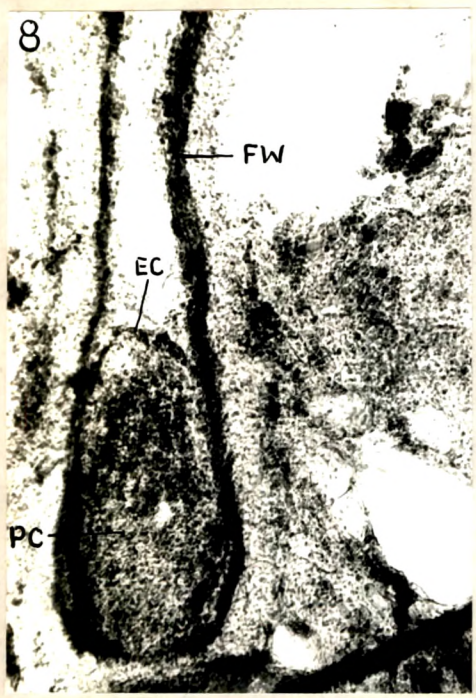

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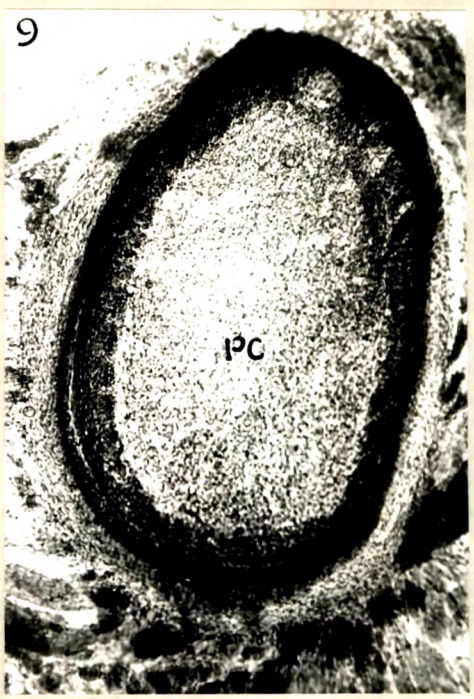

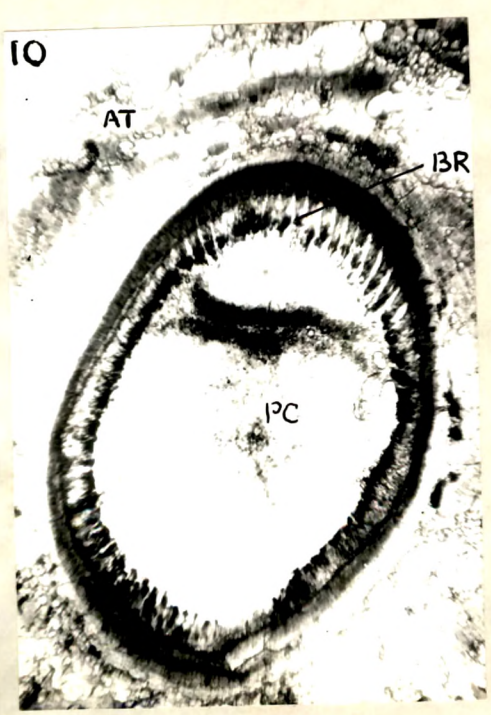

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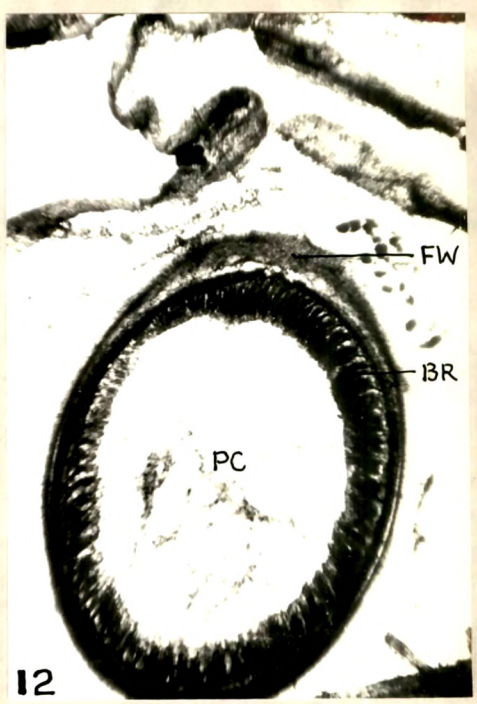
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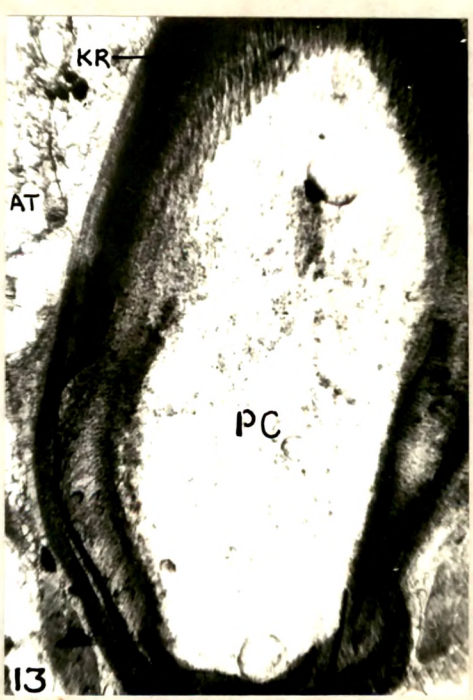
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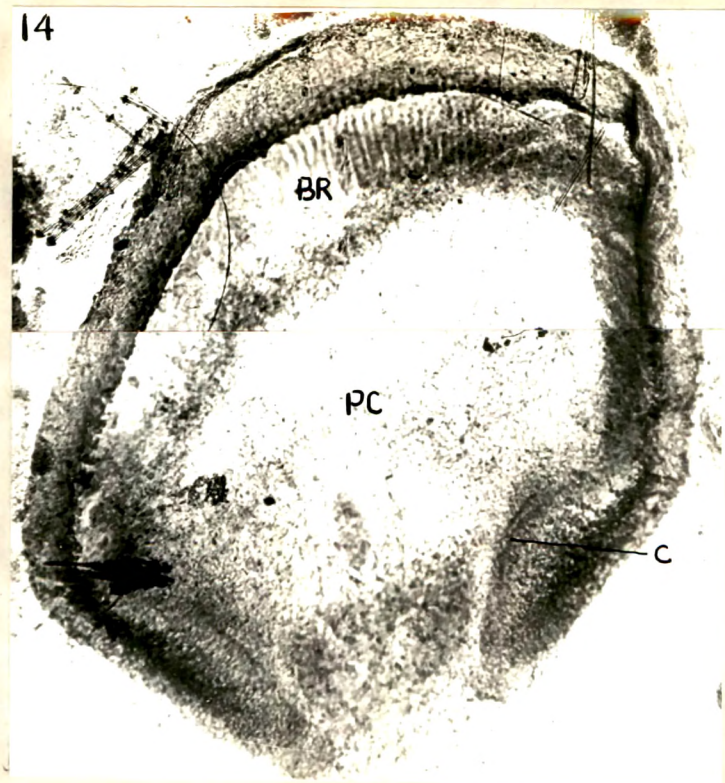

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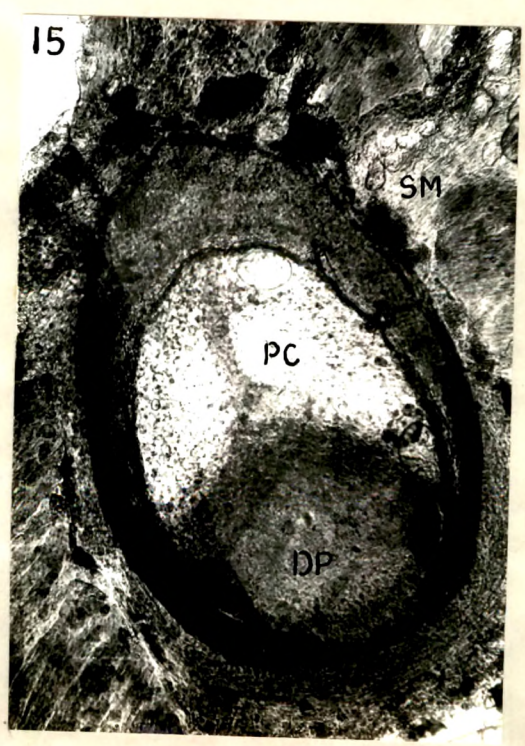

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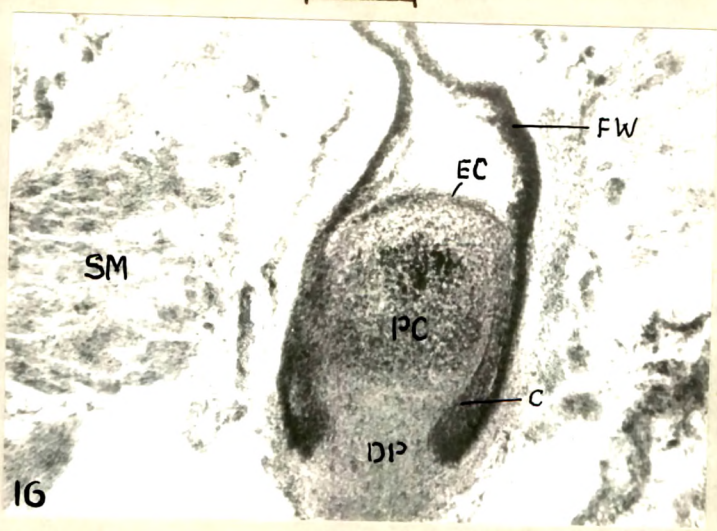

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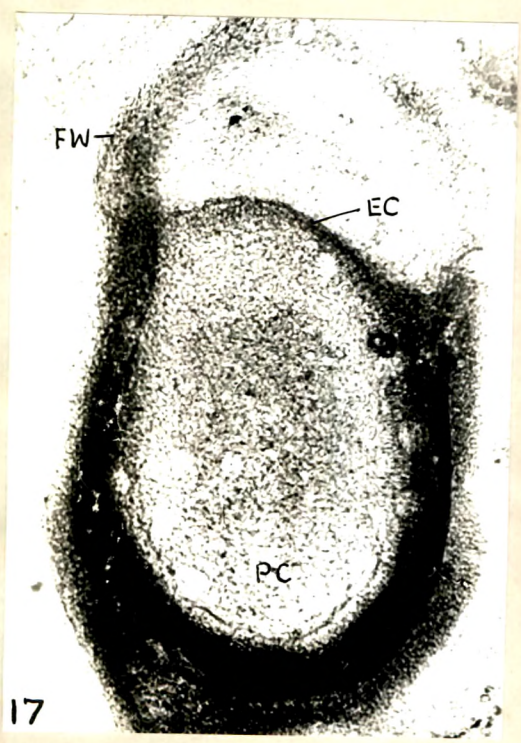

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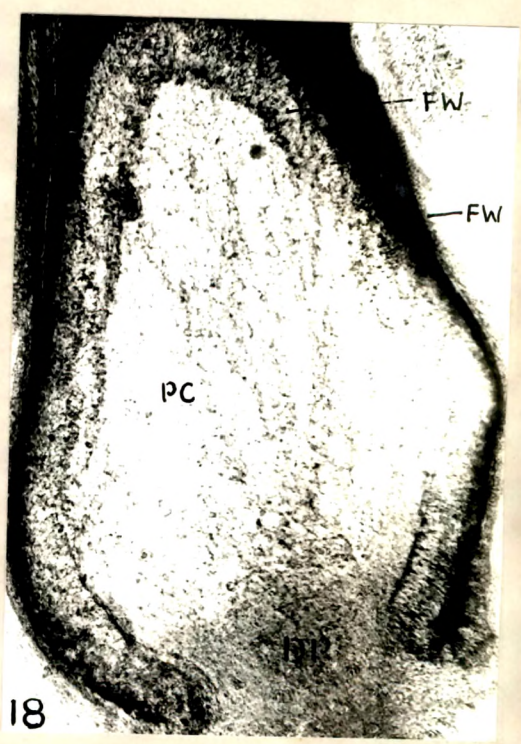

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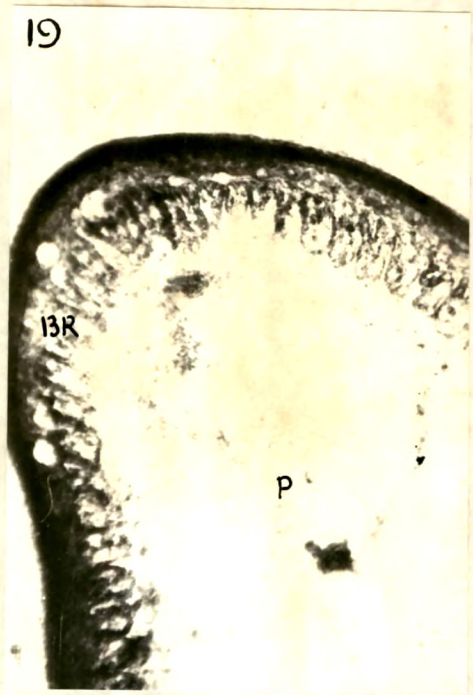

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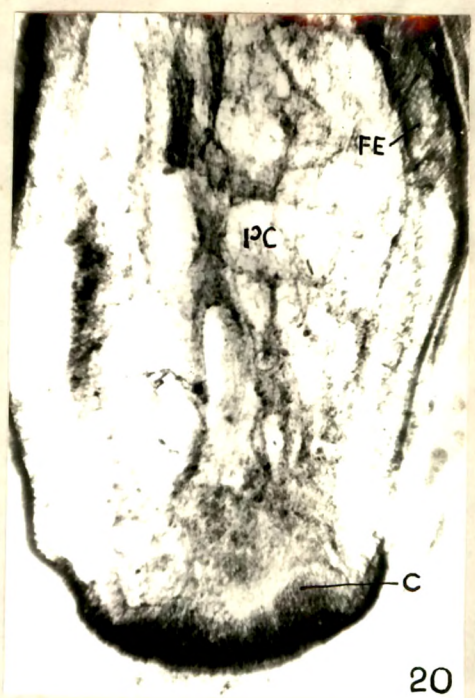

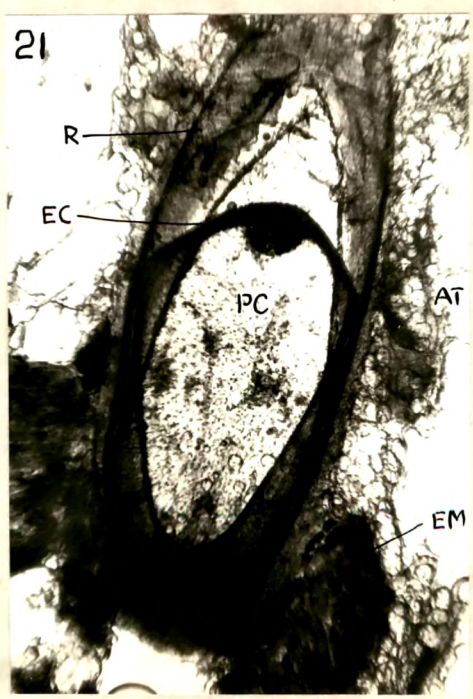

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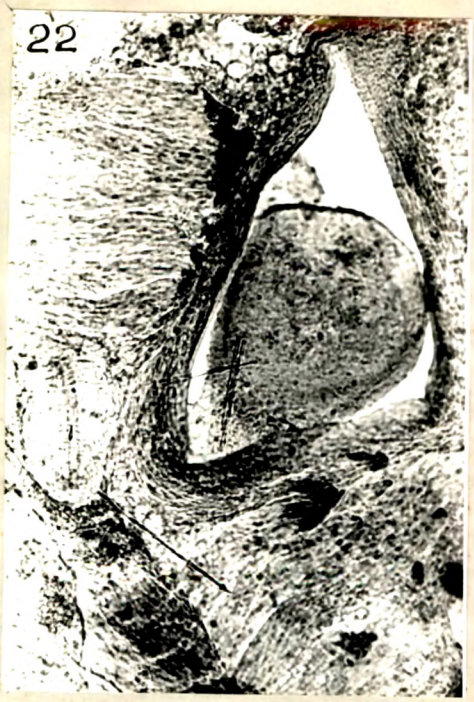

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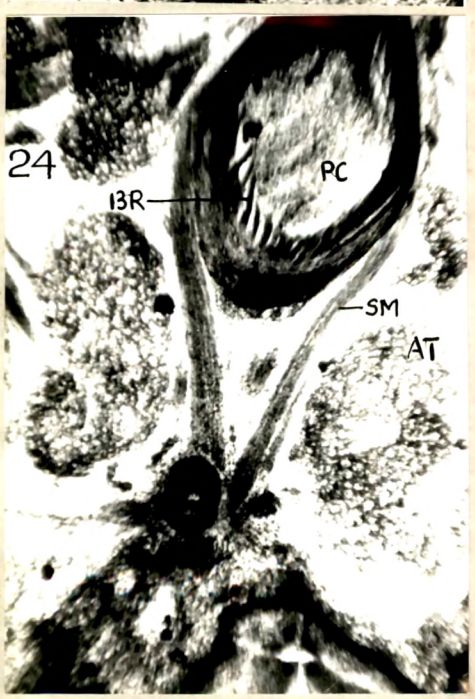

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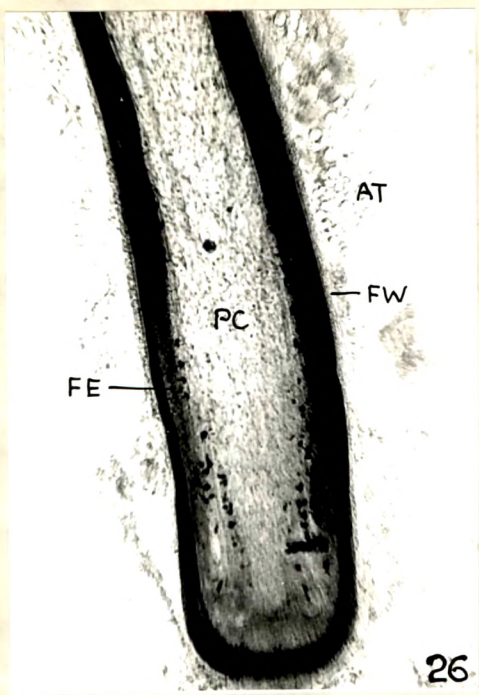

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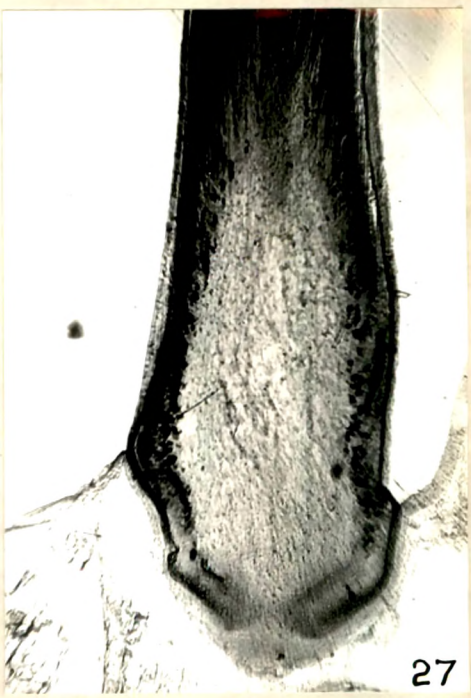

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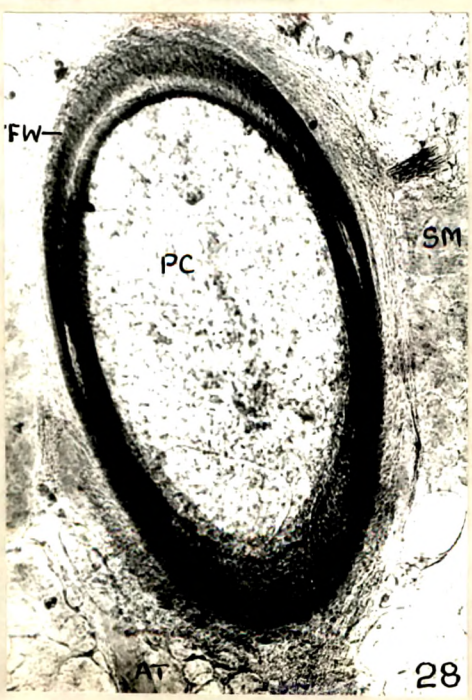

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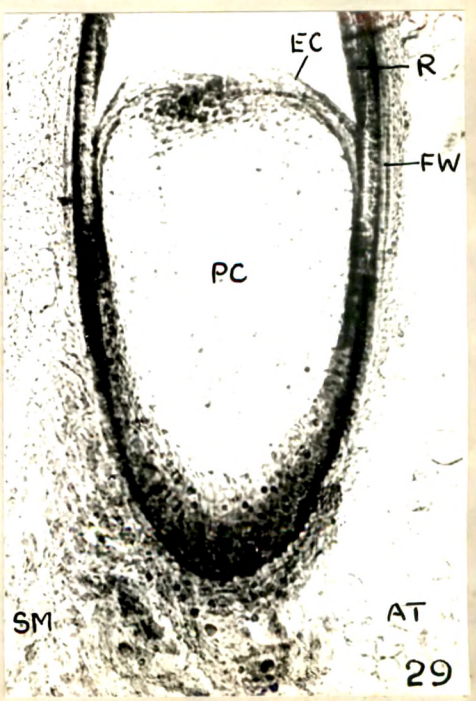

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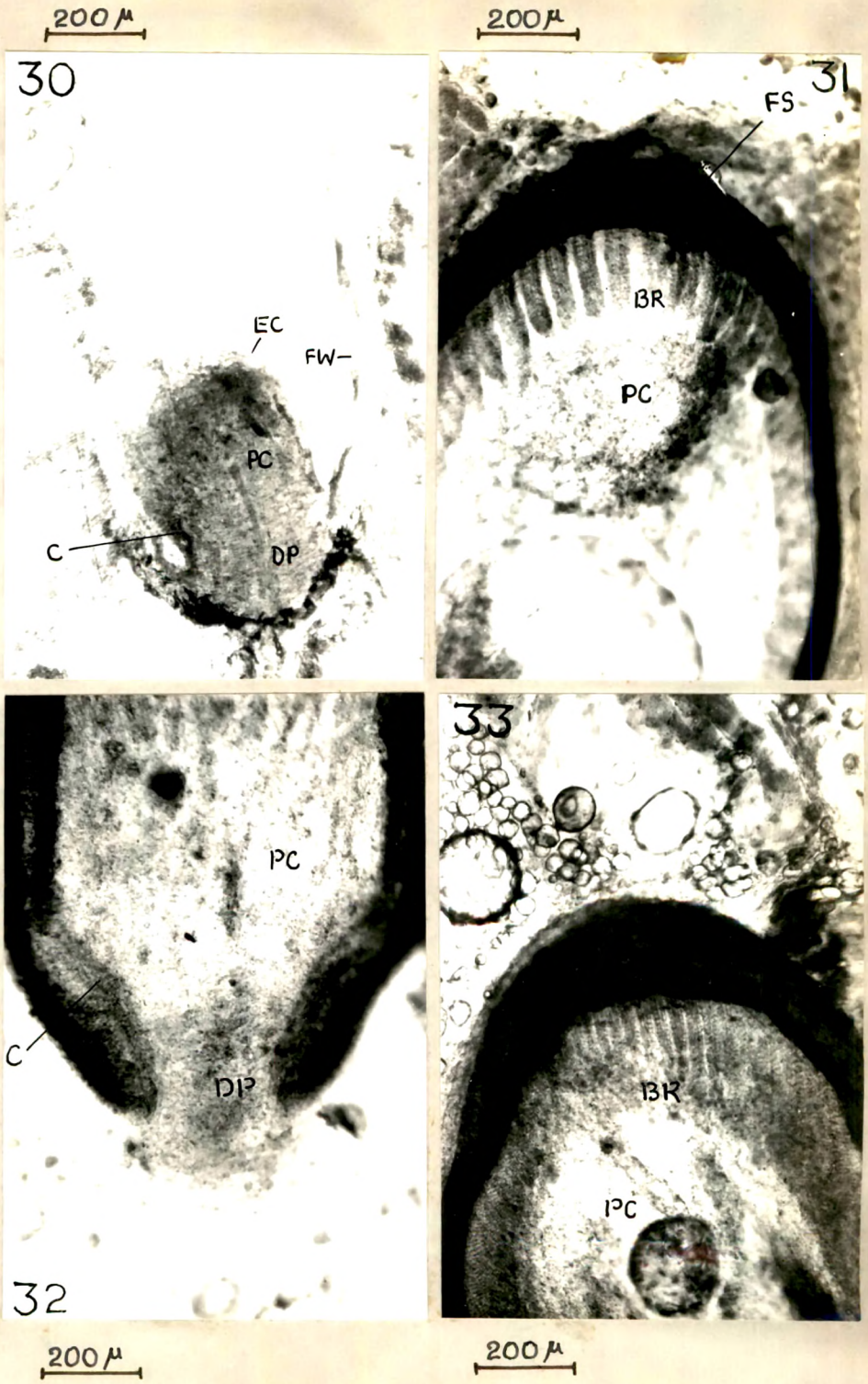
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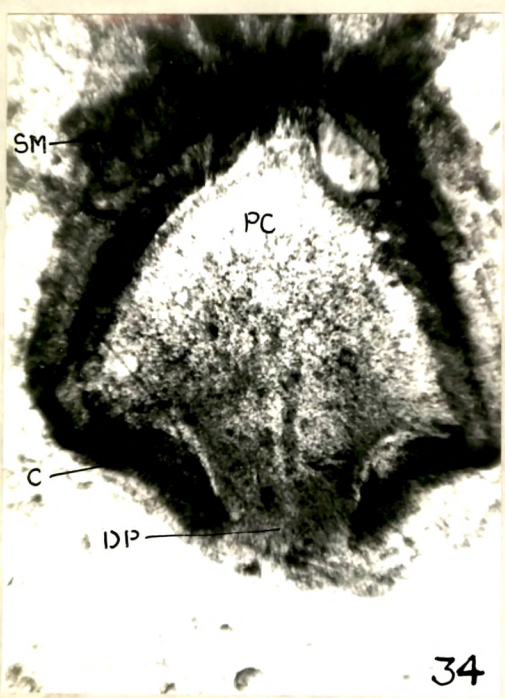

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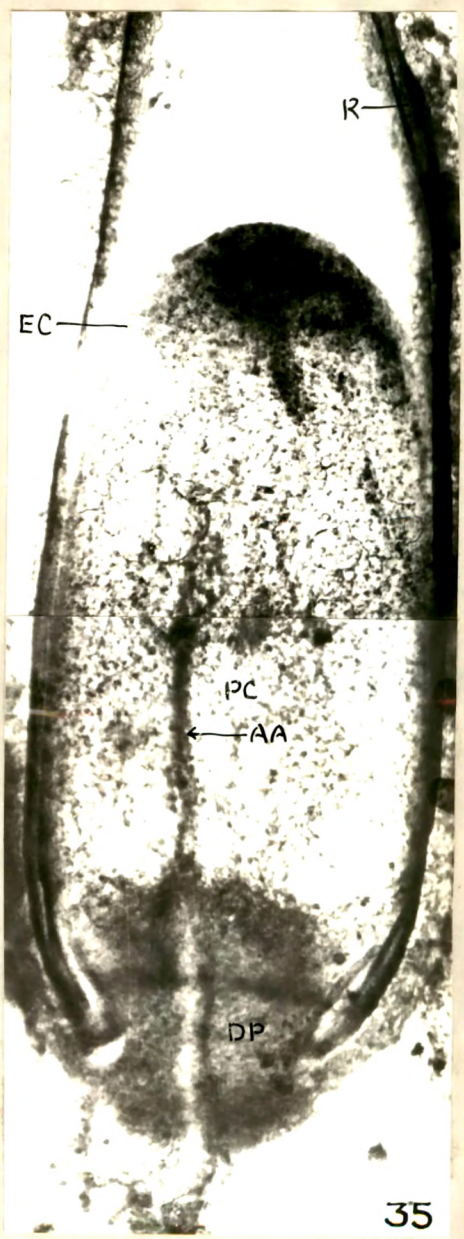

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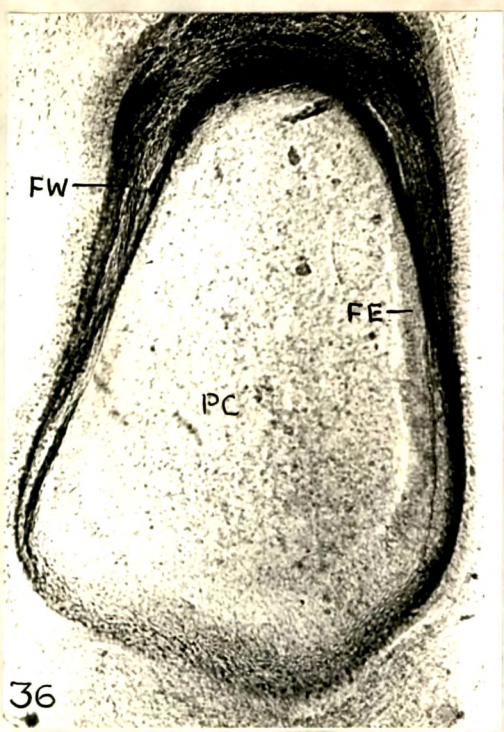

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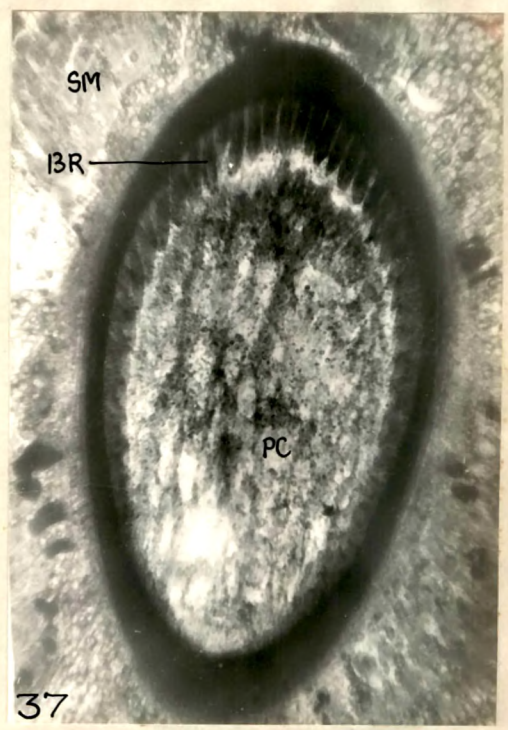

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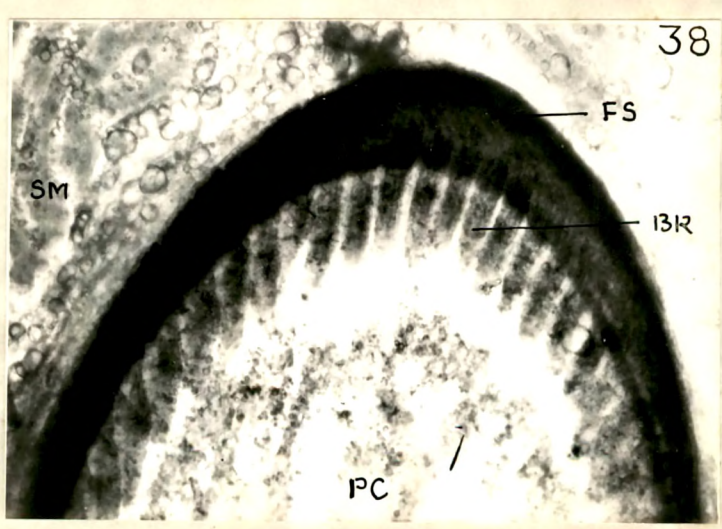

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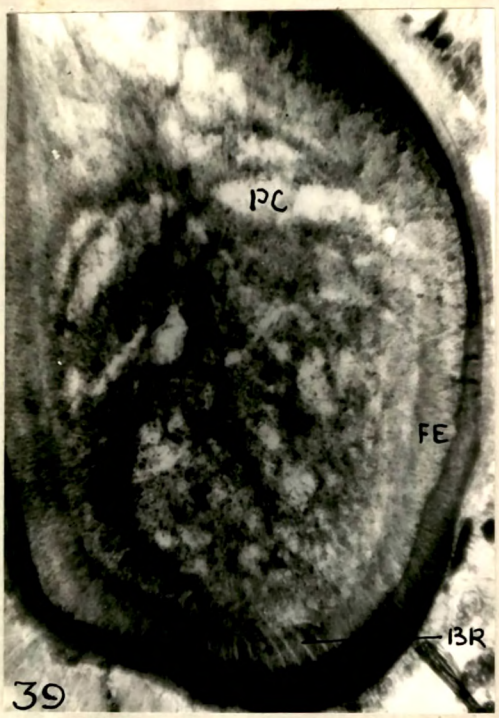

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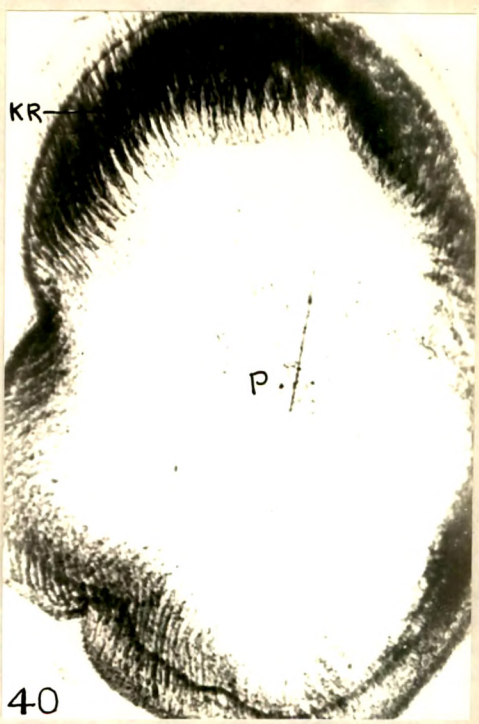

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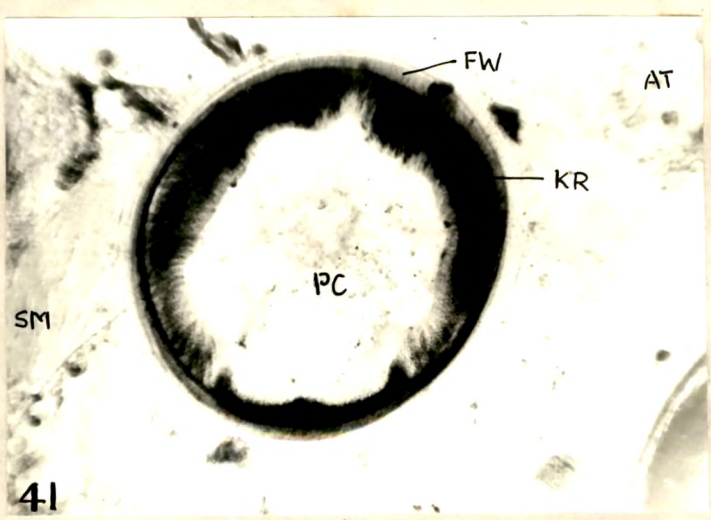

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of the enzyme than the other components. This moderate response of the enzyme was found to be maintained upto the 3rd day.

On the 5th day, a peak level response of LDH was noticed in the epithelial cells, pulp cells and the cells of the follicular wall while a moderate enzyme reactivity was observed in the cells of non-feather epidermis, smooth muscles and the fibrocytes. An overall decrease in the enzyme activity observed on the 7th day, was not, however, well marked in the follicular wall, epithelial cells at the basal region of the barb ridges and those of the collar region. This declined activity remained so upto the 10th day.

After this phase, a peak response was noticed by the 12th day in LDH reactivity, especially marked in the smooth muscles, pulp cells and epithelial cells of the barb ridges in proximity to the pulp. Those cells at the region of the barb ridges distal from the pulp showed a gradual reduction in the enzyme concentration. However, by the 15th day, the enzyme registered a decline in its activity again, only to increase once more to attain the level characteristic of the corresponding components of the normal adult skin, by

about the 20th day of regeneration and remained unchanged thereafter.

<u>α GPDH activity during normal post-hatching development</u>: (Figs. 23-29)

From the day of hatching till the 3rd day, the histochemical reactivity for α GPDH in the various components of the skin was only a faint, negligible one. However, on the 5th day, a peak activity of the enzyme was observed in all the components excepting the adipose tissue, being particularly more so in the smooth muscles and fibrocytes and stratum germinativum of the non-feather area. In the developing feather, this level of the enzyme activity was registered by the epithelial and the pulp cells. By about 10th day after hatching, there was a considerable overall decline in α GPDH activity compared to that observed on the 5th day and was most striking in the non-feather epidermis and fibrocytes. However, in the smooth muscles and the components of the developing feather, this decline was not so prominent or drastic. Amongst the epithelial cells of the barb ridges, those at the proximal region were comparatively more enzyme reactive than those at the distal region. This state of relative enzyme concentration was retained so by the various components until the 25th day, by which time, a slight increase

resulted in the attainment of a level of enzyme activity almost similar to that observed in the corresponding components of adult normal skin. By this time, the formation of the barb ridges was almost completed and the keratinized cells were enzyme negative and this condition was retained so thereafter.

<u>CC GPDH activity during induced development</u>: (Figs. 30-35)

The activity of CC GPDH, in comparison with that of LDH, remained quite low in almost all tissue components of the skin uptill the 5th day (after plucking the fully grown feathers of the adult bird), when a moderate enzyme response was observed. The epithelial and pulp cells of the developing feather were observed to have comparatively maximum enzyme response than the other components. In the dermal components like the smooth muscles and fibrocytes, this enzyme activity though low, was, nevertheless noticeable. Till upto the 15th day, the enzyme activity in the above mentioned components remained unchanged. However, a slight increase in its activity was noticed in the pulp and epithelial cells on the 15th day. The enzyme activity during the induced development reached a peak level in the pulp and

epithelial cells and the smooth muscles, by the 20th day to be maintained so upto the 30th day by which time the feather was fully restored. However, thereafter, a decline was noticed as a result of which the enzyme level reached an almost similar value as that observed in the corresponding components of the normal adult skin.

<u>αGPDH activity during regeneration:</u> (Figs. 36-41)

Only a faint reactivity, almost negligible, could be observed in the various components of the skin on the 1st day of feather regeneration. A slight reactivity, far lower than that observed in the resting germ of a normal feather, was discernible in the epithelial cells of the healed feather and follicular wall on the 5th day of regeneration. Smooth muscles maintained the low level activity as was observed on the 1st day.

A slight increase in αGPDH activity compared to that observed on the 5th day, ~~was~~ discernible in the pulp and epithelial cells of the regenerating feather, was the distinctive feature on the 7th day. Follicular wall also showed a similar intensity of enzyme reactivity. However, even in spite of this increase, in general, the enzyme activity was still low at this stage, after which a gradual

increase culminated in a high level, especially in the barb ridges by the 10th day. On the 15th day of regeneration, peak activity of αGPDH was observed in all the components of the regenerating feather, only to decline to a moderate level by the 20th day and continued to remain so during the rest of the developmental period and thereafter, thus indicating the attainment of a level of activity in the regenerating feathers, similar to the one observed in the normal adult skin.

DISCUSSION

The operation of anaerobic glycolysis during the initial phases (upto the 5th day of post-hatching) of feather development is well indicated by the herein observed intense activity of LDH in the different components of the skin at this time. Though the developing feather is noted to be dependent upon a predominantly active HMP pathway during this period for the various synthetic activities (chapter 2), it may be surmised that glycolysis also assumes an important function probably concerned with the problem of energetics. The presence of LDH at this time is suggestive of lactate production with

simultaneous reoxidation of NADH resulting in the formation of NAD which is important for assuring a continued activity of the EMP pathway of glycolysis. Thus the significance of LDH activity as a regulatory factor for glycolysis in the developing feather becomes rather apparent. Such a regulatory function in glycolysis has been ascribed to LDH by Somero (1973) and Nelson and Wakefield (1973).

In this wake it is interesting to note attainment of the peak activity of ∝GPDH, another dehydrogenase linked with a similar reoxidation of NADH on the 5th day of post-hatching development, a stage when LDH activity remained moderate.

This could be indicative of the fact that the ∝-glycerophosphate-dihydroxy-acetone-phosphate system is probably of more importance for the developing feather at this stage than the pyruvate-lactate system for the generation of NAD. Two types of ∝GPDH have been recognized in animal tissues, one which is NAD linked and found in the soluble fraction of the cell (Baronowsky, 1949; Baldwin, 1952), and the other which reacts through a cytochrome system and is localized in the mitochondrial fractions (Green, 1936; Dianzani,1951;

Kaplan et al., 1951; Tung et al., 1952; Pette, 1966; and Pilström and Kiessling, 1973). Since the presently employed histochemical technique demonstrates both these types, the high incidence of α GPDH at this stage appears to denote the possible involvement of a dominant α GPDH system in the developing feather for a number of reasons. Production of lactate represents one of the rare dead ends in metabolism since, lactic acid can re-enter the main metabolic path only by a reversal of the reactions catalysed by LDH and by which only it was formed in the first place (Boxer and Shonk, 1960). α-glycerophosphate on the other hand, could be utilized in a more versatile manner by what is known as "α-glycerophosphate shuttle" postulated as a mechanism for coupling oxidative reactions of glycolysis with mitochondrial respiratory reactions (Boxer and Delvin, 1961). This shuttle involves both the cytoplasmic NAD linked α GPDH as well as the mitochondrial cytochrome linked one. The former catalyses the conversion of dihydroxy-acetone-phosphate (DHAP) to α-glycerophosphate (α GP), with the reoxidation of NADH to NAD. α GP then could diffuse into mitochondria and could be converted there to DHAP once again by the activity of the non NAD

linked ∝GPDH. During this process, the hydrogen ion, accepted by ∝-glycerophosphate by the oxidation of NADH in the cytoplasm, enters the electron transport system ultimately combining with oxygen, yielding two ATP molecules, or according to Takemori (1959) three molecules. Thus the production of energy needed for various synthetic activities could be one of the major functions of ∝GPDH system in the developing feather at this stage. Significance of this ∝GP system has been well recognized in the energy metabolism of insect muscles (Estabrook and Sacktor,1958) and white fibres of vertebrate skeletal muscles (Wijhe et al., 1963). Apart from energy yield, the production of ∝-glycerophosphate itself could be important for the feather during its development, since this metabolic intermediary is known to be an effective precursor for the synthesis of lipids (Kennedy, 1954) much needed for the laying down of the structural framework and keratinization of the feather (Bell and Thathachari, 1963). The corresponding peak activity of HMP shunt noticed at this stage (Chapter 2) could be expected to yield the necessary triose molecules for conversion to glycerophosphate. The higher activity of ∝GPDH noticed at this stage (5th day) as compared to the earlier days could be considered as indicative of a

greater glycolytic capacity of the tissue as implied by the works of Blanchaer et al. (1963).

However, by the 10th day of development after hatching, when energy demands are also probably higher than during the previous stages mainly due to an active growth of feather, LDH reached a peak histochemical response while CC GPDH registered a decline from a peak to a moderate level. This phenomenon, though seemingly paradoxical, can be explained in the light of a number of factors known about the activities of these two enzymes. One of the factors bringing about this shift could be the pattern of activity of CC GPDH itself. Young and Pace (1958) have shown that the equilibrium of the cytoplasmic CC GPDH favours the reduction of DHAP, while the activity of CC GPDH of the particulate fraction leads to formation of DHAP. Since the histochemical technique employed here demonstrates both the enzymes, it could be possible that at one stage the equilibrium of the particulate enzyme is disposed towards the production of DHAP, which in turn can be visualised to enter the EMP pathway. Such a possibility is reflected in the peak activity of LDH. Moreover, LDH which has been recognized as a regulatory

enzyme of glycolysis (Racker, 1965; Nelson and Wakefield, 1973) is known to be well adapted for channeling the metabolism of pyruvate in directions consistent with the demands imposed by the activities of the tissues (Somero, 1973). Apart from this, the generally accepted contention that within the same tissue or organ enzymes tend to increase or decrease in activity, independently of each other, and that two enzymes, both of which show an increasing trend will rise at different rates and reach their maxima at different times (Moog, 1965) also seems to explain the pattern of activity of these enzymes as noticed during the present investigation.

By the 15th day after hatching, when the differentiative activities like keratinization are in full swing, the slight decline in LDH activity from its peak, with a simultaneous peak response of G6PDH (chapter 2), indicates a reduction in the rate of anaerobic glycolysis and an increased incidence of HMP shunt pathway. At this stage of development, when growth and differentiation, particularly keratinization, occur simultaneously, and lipids are being utilized for the latter process (as evidenced by the high lipase

activity - chapter 5) the operation of an elevated level of HMP shunt pathway is thus understandable. The moderate activity of LDH also suggests that in spite of active HMP shunt, anaerobic glycolysis is still operative to an appreciable extent in the tissues of the developing feather. The gradual attainment by LDH and CC GPDH of a level observed in the adult normal skin bespeaks of the attainment of functional maturity of the skin as a whole and the complete development of the definitive feather in particular. During induced development of the feather, the fall in concentration of these enzymes noted in the germ, 15 hours after plucking the adult feathers, could be due to shock. The increased reactivity of LDH registered on the first day and which gradually attained a peak histochemical response by the 3rd day could be indicative of the activation of the germ. The peak incidence of LDH together with a high activity of G6PDH (chapter 2) upto the 7th day of induced development suggest the importance of both anaerobic glycolysis as well as HMP shunt in the metabolism of the rapidly proliferating and differentiating cells of the inducedly developing feather. However, CC GPDH did not register any peak activity in the early stages of induced feather

development unlike in the normal development of the feather. But the moderate activity of CC GPDH observed on the 5th day of induced development could be considered as a supplementary mechanism to the LDH system for the reoxidation of reduced NAD because of increased energy demands of the feather at this stage marked by high differentiative and proliferative activities.

However, the decline in CC GPDH activity after the 5th day in the wake of the persistent peak LDH response shown by the components of the developing feather imply the importance of LDH over CC GPDH system in the metabolic processes of the inducedly developing definitive feathers at this stage. A gradual increase in the activity of CC GPDH observed from the 10th day concomitant with the decline in LDH activity can be visualised as a compensatory adaptation for the reduced availability of NAD from the LDH system during the later periods of induced development. The attainment of a level similar to that observed in the normal adult skin, by these enzymes by about the 30th day of induced development speaks of a full structural as well as functional restoration of the definitive feather.

During the regenerative development too, the conspicuous LDH activity noticed in the initial stages

(1-3 days) is in agreement with the general contention that anaerobic conditions prevail in the early stages of wound healing and regeneration (Okuneff, 1933). The gradual rise in the concentration of LDH reaching a peak on the 5th day of regeneration again indicates the higher incidence of anaerobic glycolysis at this period. The corresponding low level of Œ GPDH activity persisting up to this stage could be considered as a reflection of the reduced importance of the Œ GPDH system in comparison to that of LDH during the initial days of feather regeneration. Nevertheless, the decreased LDH activity noticed on about the 7th to 10th days of regeneration just prior to the emergence of the regenerate outside the follicle corresponding well with the increased activity of both Œ GPDH and G6PDH (chapter 2) denoting thereby the stepped up operation of the HMP shunt making available the triose molecules and the simultaneous incorporation of these moieties into Œ-glycerophosphate through the mediation of Œ GPDH. Such a pattern of metabolism involving both the HMP shunt and the Œ-glycerophosphate shuttle could be viewed as helpful in providing both the necessary cofactors as well as energy for the anabolic and growth processes.

The second peak response of LDH activity observed soon after this phase together with an unchanged steady

Ⓒ GPDH response and declined activities of both G6PDH and aldolase (chapters 2 & 3) and SDH and MDH (chapter 6) suggest that probably lipids are being utilised at this stage with the glycerol moiety being utilised via the EMP pathway of glycolysis. This high level of LDH concentration reached by about the 20th day, after a slight fall on the 15th day and maintained thereafter; along with a fall in Ⓒ GPDH activity suggest the establishment of anaerobic glycolysis as the chief normal metabolic route in these tissues, along with the completion of the process of maturation and full restoration of the definitive feathers.