

CHAPTER 5

HISTOCHEMICAL DEMONSTRATION OF LIPIDS, LIPASE AND
 β -HYDROXYBUTYRATE DEHYDROGENASE IN THE DEFINITIVE
FEATHERS OF THE BLUE ROCK PIGEON, COLUMBA LIVIA,
DEVELOPING UNDER THREE DIFFERENT CONDITIONS

Significance of lipids as metabolites in various animal tissues has been well recognised (George and Jyoti, 1957, 1958; Fredrikson and Gordon, 1958; Rossiter and Strickland, 1960). By virtue of their high energy yield, which is about twice as much as that of carbohydrates, and the fact that they could be stored in a comparatively water free state in contrast to the latter, make it the choice metabolite for a number of tissues and organs, especially those which are designed for sustained activities for a longer period like the red muscle fibres of vertebrates (George and Berger, 1966). Lipids laid down in adipose tissue in the subcutaneous region of homoeotherms are of considerable significance in thermoregulation. Lipids are reported to be present in the integument of vertebrates including aves and man (Malytoltsy, 1969; Shibaeva, 1970) and have been implicated in the process of keratinisation of epidermis and its derivatives (Bell and Thathachari, 1963; Shibaeva, 1970). Neutral as well as acidic lipids have been demonstrated histochemically in the reptilian epidermis during tail

regeneration where the metabolite and enzymes concerned with its metabolism were shown to exhibit fluctuations in concentration and activities respectively, indicating an active participation of lipid metabolism in the process of development and differentiation of the epidermal derivatives (Chakko, 1967; Radhakrishnan, 1972). However, a detailed investigation of the involvement of lipid metabolism in the developmental processes of vertebrate epidermal derivatives, especially those of birds, has not been reported. It was in this context that the present histochemical investigation was carried out so as to gain an insight into the pattern of lipid utilisation during the different phases of development of the definitive feather of the blue rock pigeon, Columba livia.

Histochemical demonstration of lipase which could indicate lipid utilisation has been carried out in many animal tissues (George and Ambadkar, 1963; George and Iype, 1963; George and Bokdawala, 1964; Chakko, 1967; Radhakrishnan, 1972). β -hydroxybutyrate dehydrogenase, one of the enzymes concerned with the further degradation of the fatty acid produced by the activity of lipase, also has attracted considerable attention (George and Bhakthan, 1963; Magon, 1970; Ramachandran, 1972). It is pertinent here to mention the reported high activities of these enzymes in the epidermal

cells of the regenerating reptilian caudal skin and scales undergoing development and differentiation (Chakko, 1967; Magon, 1970; Ramachandran, 1972; Radhakrishnan, 1972). With this knowledge, it was deemed worthwhile to extend ^{the} histochemical investigations to the development of definitive feathers so as to have a clear understanding about the extent of involvement of these two enzymes in the metabolic processes underlying their development and differentiation.

OBSERVATIONS

NORMAL ADULT SKIN

Lipids (Figs. 1 & 2)

Whereas the highest concentration of neutral lipids was observed chiefly in the intradermal fat depots, that of acidic lipids was localised mainly in the dermal components like fibrocytes and the cells of the resting germ. A higher concentration of neutral lipids in the non-feather epidermis especially in the outermost cornified layers and a moderate concentration in the resting germ and smooth muscles of the feather follicle (where its distribution was of a mosaic pattern) could also be noticed. On the other hand, acidic lipids concentration in smooth muscles was moderate while that in the non-feather epidermis was low.

Lipase (Figs. 35)

Both the epithelial and mesenchymal components of the resting germ of a feather exhibited a moderate intensity of lipase activity with the smooth muscles registering a slightly lower level. The non-feather epidermis also elicited a moderate level of the enzyme activity, that too mainly in the stratum germinativum with its other components remaining negative.

BDH (Fig. 58)

Though the general pattern of enzyme activity resembled that of lipase, BDH was more active in the smooth muscles than in the germ. ~~It was more active in the smooth muscles than in the germ.~~ It was also interesting that the basal region of the pulp and the epithelial cells of the collar region of ^{the} germ were comparatively more enzyme reactive.

NORMAL POST-HATCHING DEVELOPMENT

Lipids (Figs. 3-14^a)

On hatching, lipids, especially neutral lipids, were found to be distributed in a more or less mosaic pattern all over the integument, though their concentration in the non-feather epidermis was comparatively higher. Adipocytes in the dermal region which were clumped together and not

fully organised into adipose tissue were noted to have a high concentration of neutral lipids at this stage itself. The integument in general and the developing feather in particular, however, showed a considerable amount of neutral lipids by the 2nd day. The basal region of the pulp, of ^{the} follicular wall, developing intradermal adipose tissue and muscle fibres (which were getting organised into bundles) were the components which depicted a comparatively higher concentration of neutral lipids. Acidic lipids demonstrated at this stage were of a moderate concentration and localised chiefly in the epithelial cells of the barb ridges and the pulp, near the collar region of the developing feather. By the 10th day, whereas the acidic lipid content decreased further to a lower level, there was a triradial change in the neutral lipid content of the integument, to a moderate level in the barb ridges, a slight decline in the pulp and an unchanged condition in all the other components. However, by the 15th day there was a definite increase in the lipid content in the developing feathers especially in the pulp and epithelial cells and this level was so maintained upto the 25th day, after which there was a decline, bringing the concentration of the metabolite to a level similar to that observed in the resting feather germ.

Lipase and BDH (Figs. 36-42 and 59-67)

On the day of hatching, whereas a moderate activity of lipase was observable in almost all the components of the skin, including adipocytes, BDH activity was more or less negative in all its components excepting for a very low level in the basal parts of the developing definitive feather and an almost negligible one in the non-feather epidermis. By the second day of development, lipase activity was observed only in the developing feathers and the smooth muscles. This pattern which was so retained thereafter changed to a peak level in the developing feathers on the 5th day. At this stage, the enzyme was chiefly located in the epithelial cells and the cells of the pulp at the basal part of the feather, with the non-feather epidermis, smooth muscle and other components depicting a moderate to low level of enzyme activity. This high enzyme activity in the feather components was so retained in the subsequent days when differentiation and keratinization of barb ridges were in progress, and was in evidence even on the 20th day whence the regression of the pulp had commenced. However, by two successive and gradual reductions about the 25th and 30th days respectively ^{the} lipase activity in the feather and other components attained a level characteristic of the normal adult skin.

On the other hand, BDH activity which was low on the day of hatching started slowly to ascend (during the succeeding days) and attained a moderate level by about the 5th day. The activity at this stage was relatively high in the feather epithelial cells, especially those forming the barb ridges, the cells of the pulp and ^{the} follicular wall and also in the epidermis of the non-feather region. Both the smooth muscles and the adipose tissue showed a poor response for the enzyme. During the period between the 5th and 10th days, the enzyme activity was maximal (as compared to other days during development) in the feather components mentioned above, and thereafter the level of BDH showed a slight decline to attain once again a moderate level in the various feather components by the 15th day, with the smooth muscles and fibrocytes depicting a negative response at this period. Following this, there was again a further gradual reduction in BDH activity discernible first only in the barb ridges (by 25th day) and afterwards in the collar region (by 30th day), thereby acquiring a level of enzyme activity almost identical to that observable in the corresponding components of the resting feather germ.

INDUCED DEVELOPMENT

Lipids (Figs. 15-24)

Whereas there was an increase in neutral lipid content only in the distal region of the germ, a day after plucking the adult feathers, there was a high acidic lipid content in the pulp, epithelial cells, follicular wall and smooth muscles. This condition was so retained till the 7th day of induced development with the only interesting point of observation being of a higher concentration of acidic lipids in the barb ridges in comparison to the pulp cells. However, on the 10th day only a moderate concentration of acidic as well as neutral lipids could be observed in the pulp, epithelial cells as well as the smooth muscles. Whereas this concentration was so maintained thereafter in the case of neutral lipids till about ^{the} 15th day, that of acidic lipids reached once again a higher level in all the components. However, by 20th day there was an overall decline of both the types of lipids in all the components. Finally, by about the 25th day whereas the neutral lipid concentration reached a level similar to that observed in the corresponding parts of the adult normal skin, that of the acidic lipid content settled to a level, slightly higher than that observed in the corresponding components of the normal skin.

EXPLANATIONS FOR FIGURES

Fig. 1 Section of skin of adult pigeon, showing neutral lipids in resting germ, smooth muscles and adipose tissue.

Fig. 2 LS of resting germ showing acidic lipids

NORMAL DEVELOPMENT

Fig. 3 Section of skin showing neutral lipids in developing definitive feathers and other components on the day of hatching.

Fig. 4 LS of developing feather on the second day post-hatching. Note neutral lipids in pulp cells.

Fig. 5 Section of skin on 2nd day post-hatching depicting acidic lipid concentration in the components.

Fig. 6 TS of feather on the 3rd day showing neutral lipids.

Fig. 7 LS of feather on the 5th day of post-hatching development. Note distribution of neutral lipids.

Fig. 8 Part of feather on the 10th day of development magnified to show distribution of neutral lipids in barb ridges and pulp cells.

Fig. 9 LS of 5 day old feather showing acidic lipids.

Fig.10 LS of 10 day old feather to show acidic lipids.

Fig.11 TS of 15 day old feather showing concentration of acidic lipids in the pulp cells and barb ridges.

- Fig. 12 LS of 25 day old feather showing acidic lipids.
- Fig. 13 LS of 20 day old feather depicting neutral lipids.
- Fig. 14 LS of 25 day old feather showing neutral lipids in the regressing pulp. Also note neutral lipids in the smooth muscles, ~~also~~.
- Fig. 14a LS of 30 day old feather showing neutral lipids in pulp cells and epithelial covering.

INDUCED DEVELOPMENT

- Fig. 15 LS of feather germ showing neutral lipids one day after plucking the adult feather.
- Fig. 16 LS of feather germ showing acidic lipids one day after plucking the adult feather.
- Fig. 17 LS of 5 day old inducedly developing feather showing neutral lipids.
- Fig. 18 Section showing acidic lipids in feather on the 5th day.
- Fig. 19 Section of feather on 10th day showing neutral lipids.
- Fig. 20 Part of feather follicle on 15th day depicting neutral lipids.
- Fig. 21 Part of section of feather on the 15th day showing acidic lipids.
- Fig. 22 Part of section of feather on the 20th day showing neutral lipids.

- Fig. 23 Oblique section of 25 day old feather showing neutral lipids.
- Fig. 24 LS of 30 day old feather showing acidic lipids.

REGENERATION

- Fig. 25 LS of one day old feather regenerate showing neutral lipids.
- Fig. 26 Two day old feather regenerate showing neutral lipids.
- Fig. 27 Acidic lipids in 3 day old feather regenerate.
- Fig. 28 Section of 5 day old feather showing acidic lipids.
- Fig. 29 LS of 5 day old regenerate. Note the neutral lipids.
- Fig. 30 Neutral lipids in LS of 7 day old feather regenerate.
- Fig. 31 LS of 10 day old regenerate showing neutral lipids.
- Fig. 32 LS of 25 day old regenerate showing acidic lipids.
- Fig. 33 Neutral lipids in 30 day old regenerate.
- Fig. 34 Acidic lipids in the pulp and epithelial cells on the 30th day of regeneration.

- Fig. 35 LS of follicle showing lipase activity in resting feather germ and the smooth muscles.

NORMAL DEVELOPMENT

- Fig. 36 Section of skin from young pigeon on the day of hatching showing the enzyme activity in developing definitive feathers and other components.
- Fig. 37 Section of skin showing lipase activity on the 2nd day of hatching.
- Fig. 38 LS of developing definitive feather (with natal down feather attached to it), showing lipase activity on the 2nd day post-hatching.
- Fig. 39 LS of feather showing lipase activity on the 5th day.
- Fig. 40 Part of base of the feather showing the enzyme activity on the 10th day.
- Fig. 41 Section of follicle showing 15 day old feather and smooth muscles depicting the enzyme activity.
- Fig. 42 Oblique section of the feather on 25th day after hatching. Note lipase activity in the regressing pulp.

INDUCED DEVELOPMENT

- Fig. 43 Section of the follicle one day after plucking the adult feather, showing lipase activity in the germ and smooth muscles.

- Fig. 44 Section of skin showing lipase activity in developing feather and smooth muscles on the 5th day of induced development.
- Fig. 45 Section of feather on the 7th day showing the enzyme activity.
- Fig. 46 LS of feather showing the enzyme activity on the 10th day.
- Fig. 47 LS of feather on the 15th day to show lipase activity.
- Fig. 48 TS of part of a 20 day old feather with smooth muscles, showing the enzyme reactivity.
- Fig. 49 LS of feather on the 20th day showing the enzyme activity.
- Fig. 50 LS of feather on the 30th day of induced development showing the enzyme reactivity.

REGENERATION

- Fig. 51 LS of 1 day old feather regenerate depicting lipase activity.
- Fig. 52 Section of 3 day old regenerate showing the enzyme activity.
- Fig. 53 LS of regenerating feather on the 7th day showing the enzyme activity.
- Fig. 54 Oblique section of the feather on the 10th day of regeneration. Note the enzyme activity in feather epithelium and pulp cells.

- Fig. 55 LS of part of a 15 day old regenerate showing lipase activity.
- Fig. 56 Enlarged part of Fig. 55 to show lipase activity in collar and pulp cells.
- Fig. 57 Section of 25 day old regenerate showing lipase activity in the regressing pulp.
- Fig. 58 LS of follicle from normal adult skin showing BDH activity in components of resting feather germ and smooth muscles.

NORMAL DEVELOPMENT

- Fig. 59 Section of skin from one day old pigeon showing BDH activity in developing definitive feather.
- Fig. 60 Section of skin on the 3rd post-hatching showing the enzyme activity in developing feather and smooth muscles.
- Fig. 61 LS of 5 day old feather. Note the enzyme activity in epithelial cells.
- Fig. 62 Section of skin on the 7th day showing enzyme activity in different components.
- Fig. 63 Oblique section of 7 day old feather showing the enzyme activity in pulp cells and barb ridges.
- Fig. 64 LS of feather on the 10th day after hatching showing BDH activity.

Fig. 65 TS of feather on the 15th day showing enzyme activity.

Fig. 66 LS of base of 25 day old feather showing the enzyme activity.

Fig. 67 BDH activity in the feather follicle on 30th day post-hatching.

INDUCED DEVELOPMENT

Fig. 68 LS of follicle one day after plucking adult feather. Note the enzyme activity in various components.

Fig. 69 Section of inducedly developing feather on the 3rd day, showing the enzyme activity.

Fig. 70 TS of 5 day old feather showing BDH activity.

Fig. 71 LS of feather on 7th day of induced development showing the enzyme activity in its components.

Fig. 72 Section of part of the feather on 15th day of development showing BDH activity in epithelial and pulp cells.

Fig. 73 TS of 20 day old feather showing the enzyme activity of pulp cells.

REGENERATION

Fig. 74 LS of 1 day old regenerate showing BDH activity.

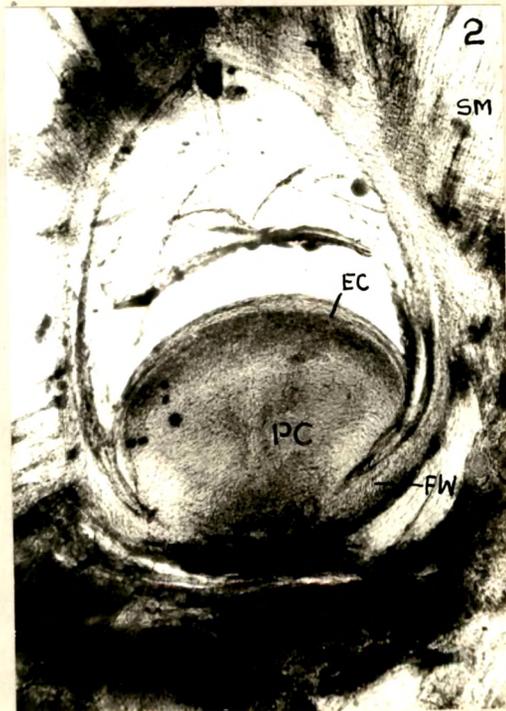
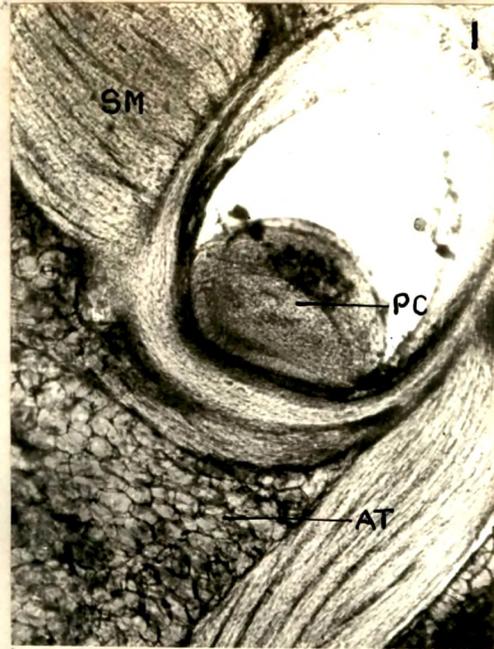
Fig. 75 TS of 5 day old feather regenerate showing the enzyme activity in pulp cells and barb ridges.

- Fig. 76 LS of regenerate on 7th day. Note the enzyme response.
- Fig. 77 Section of regenerate on 7th day to show enzyme activity in the barb ridges.
- Fig. 78 LS of part of the feather on 10th day showing BDH activity.
- Fig. 79 LS at the base of the regenerate on 20th day showing the enzyme activity.
- Fig. 80 Section of skin showing TS of feather on 25th day of regeneration to show the enzyme activity in the regressing pulp.

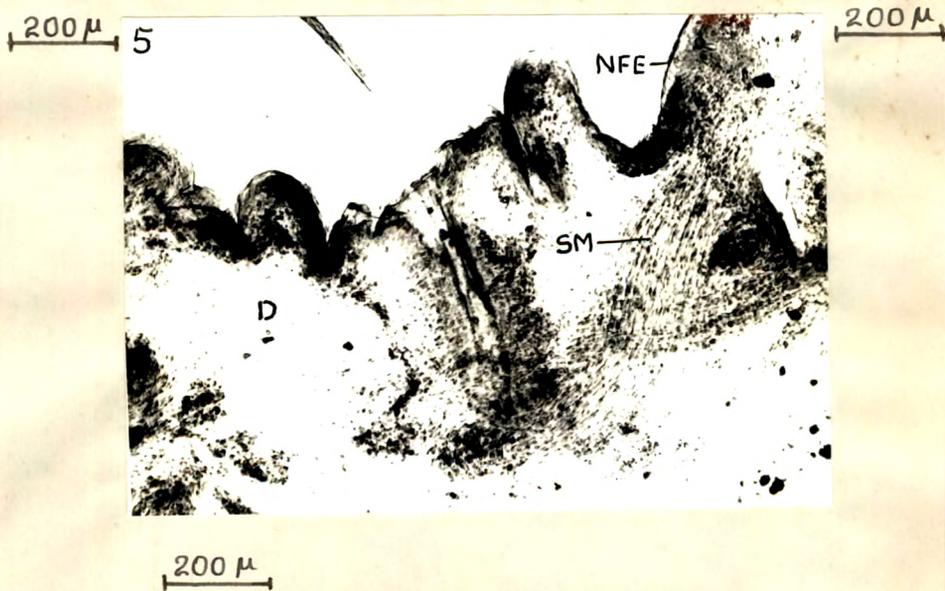
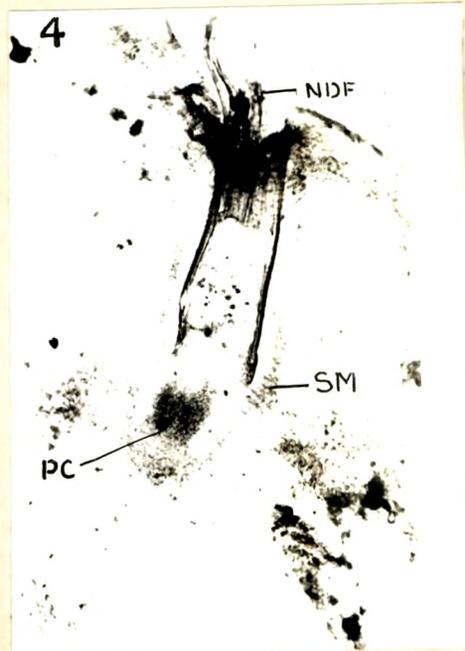
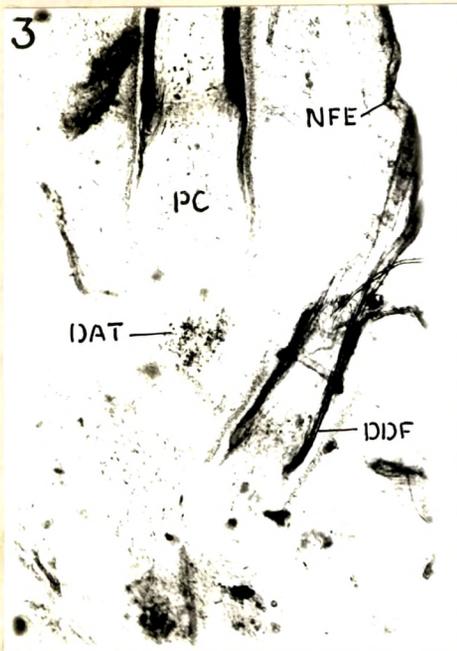
ABBREVIATIONS

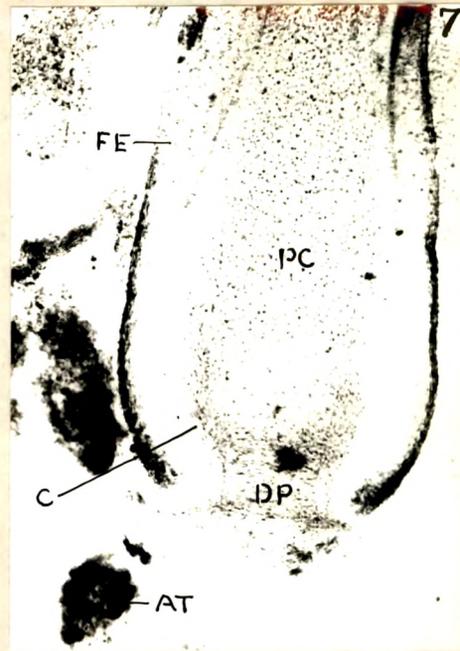
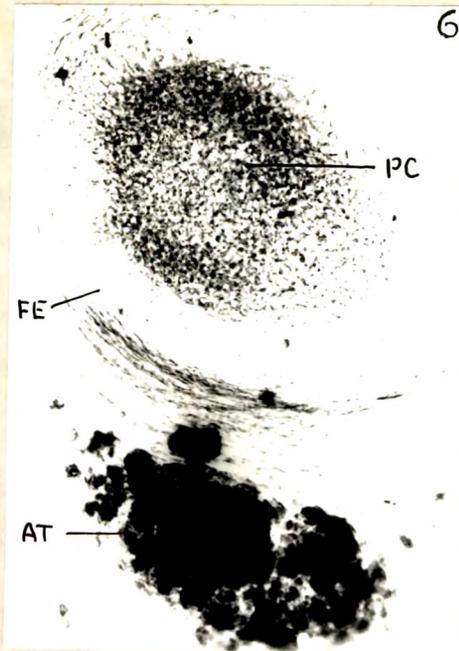
AA	-	Axial artery
AT	-	Adipose tissue
BC	-	Blood clot
BR	-	Barb ridges
C	-	Collar region
DAT	-	Developing adipose tissue
DDF	-	Developing definitive feather
DP	-	Dermal papilla
EC	-	Epithelial covering
FE	-	Feather epithelium
FS	-	Feather sheath
FW	-	Follicular wall
KR	-	Keratinised region
MC	-	Melanocytes
NDF	-	Natal down feather
NFE	-	Non-feather epidermis
PC	-	Pulp cells
R	-	Rachis
RP	-	Regressing pulp
SM	-	Smooth muscles.

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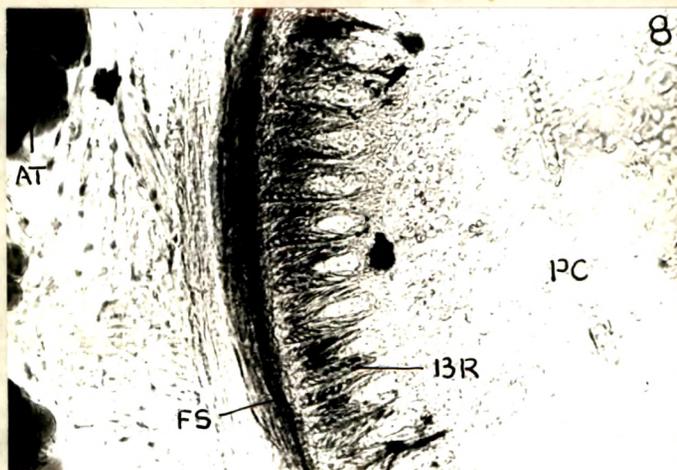


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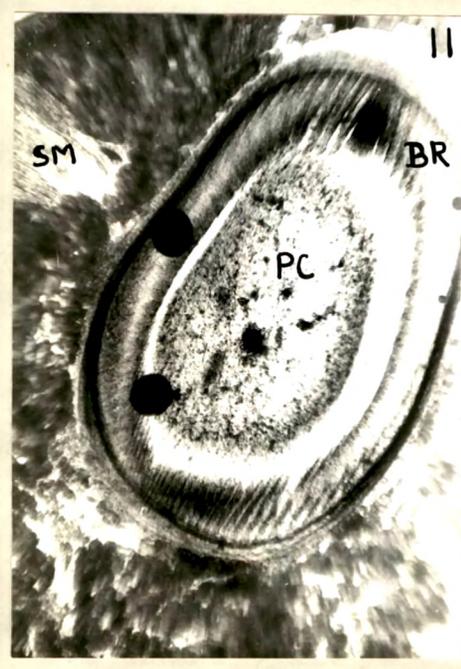
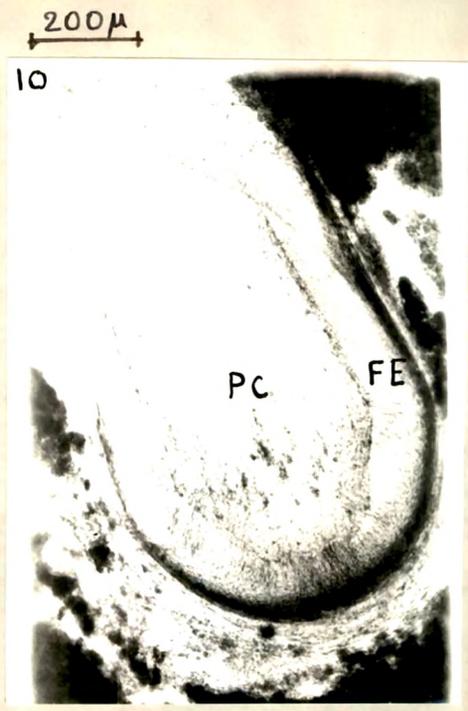
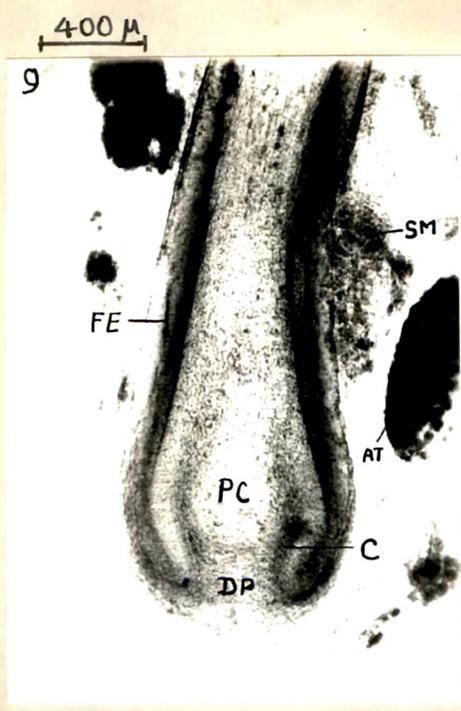
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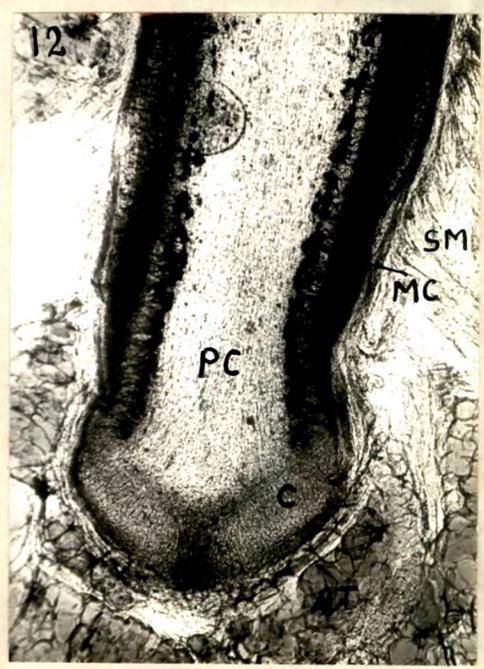
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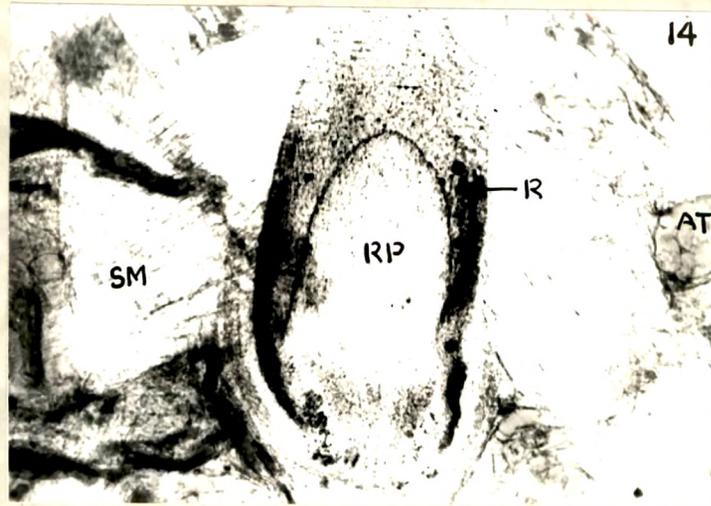
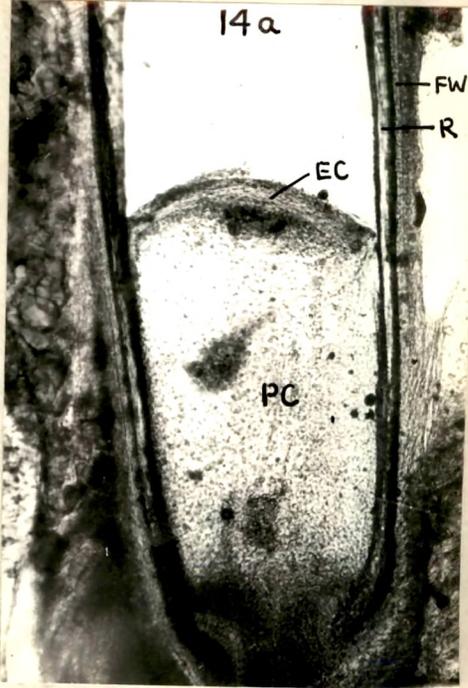
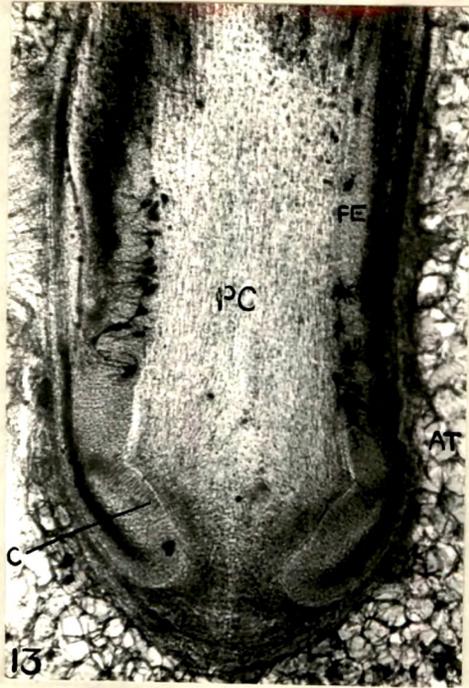
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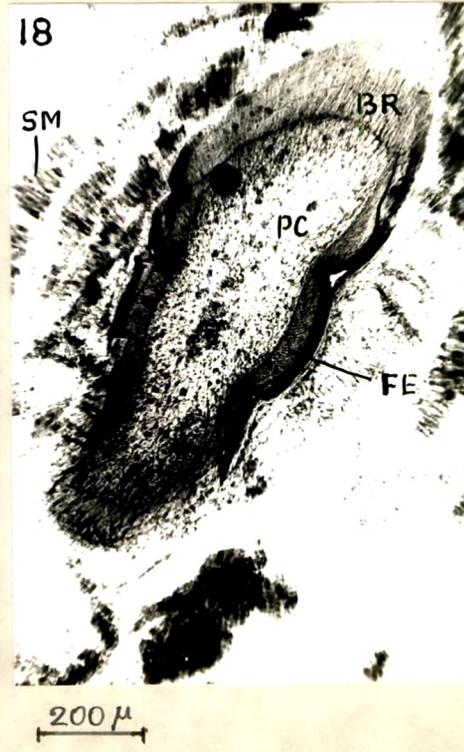
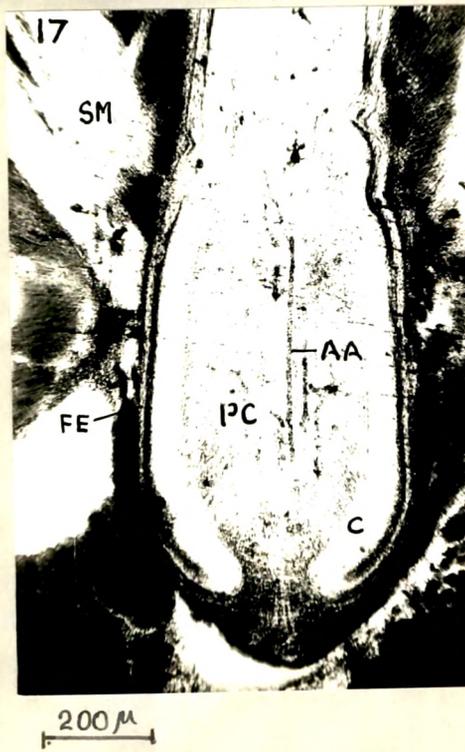
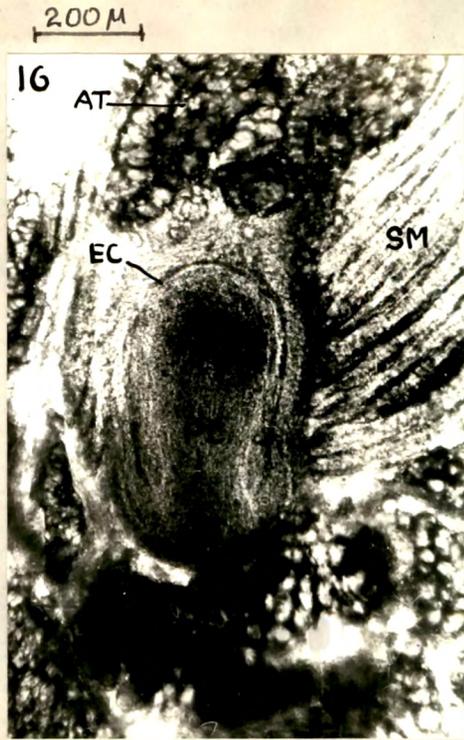
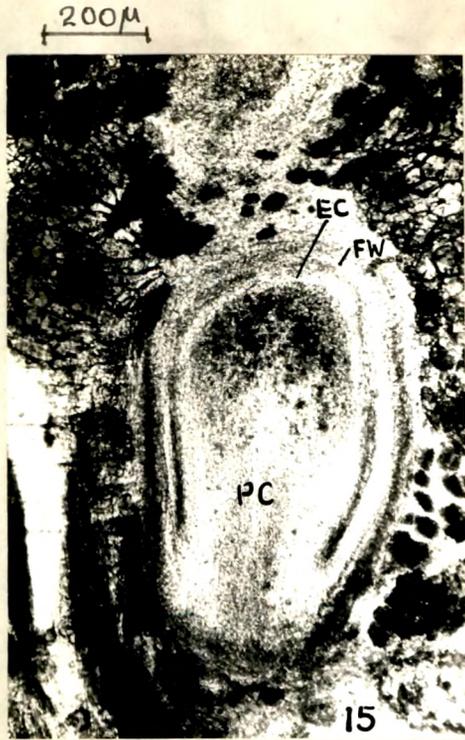


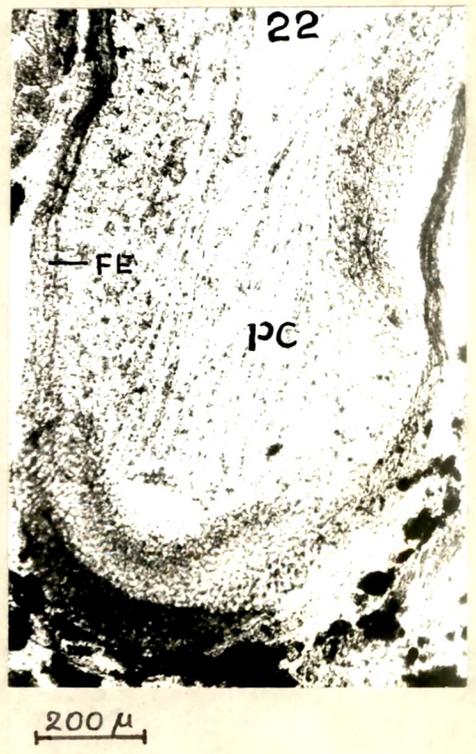
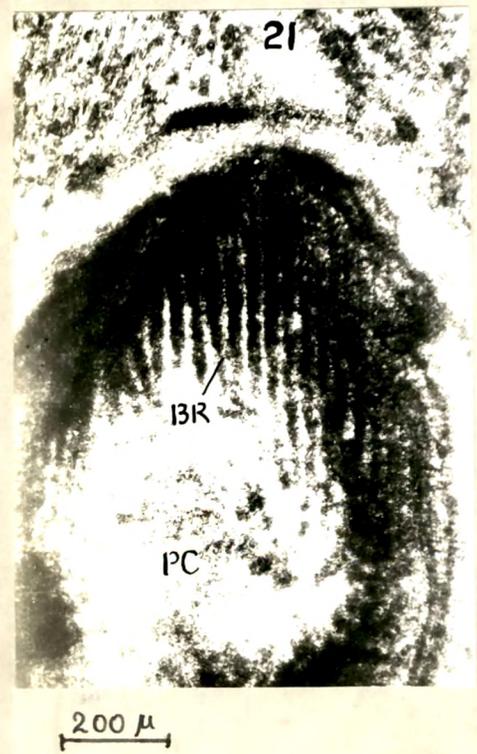
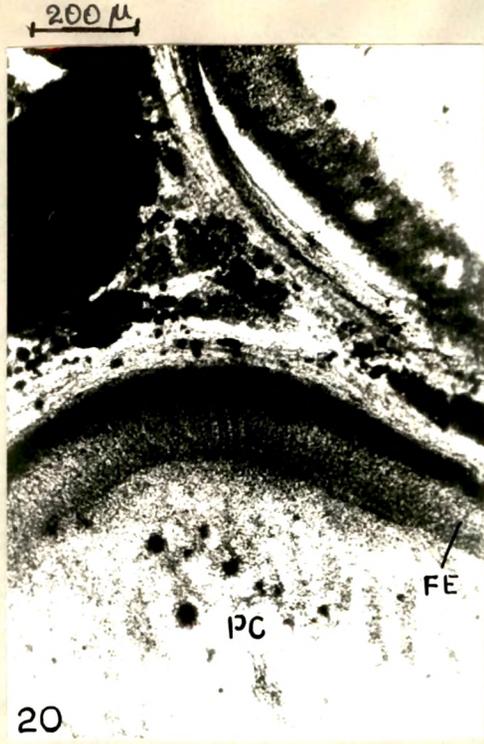
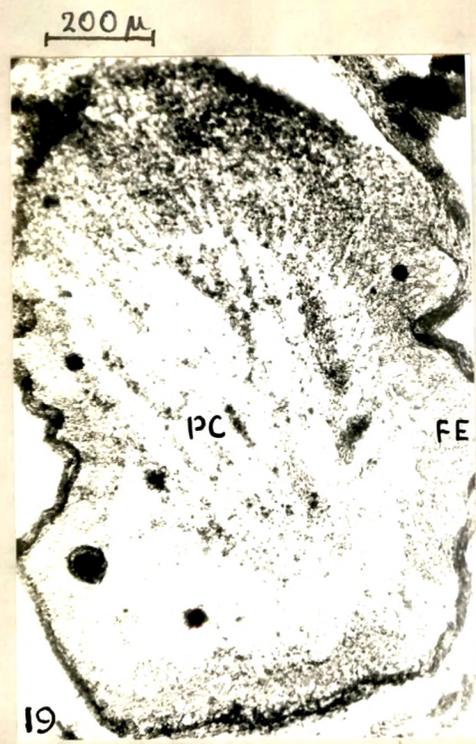
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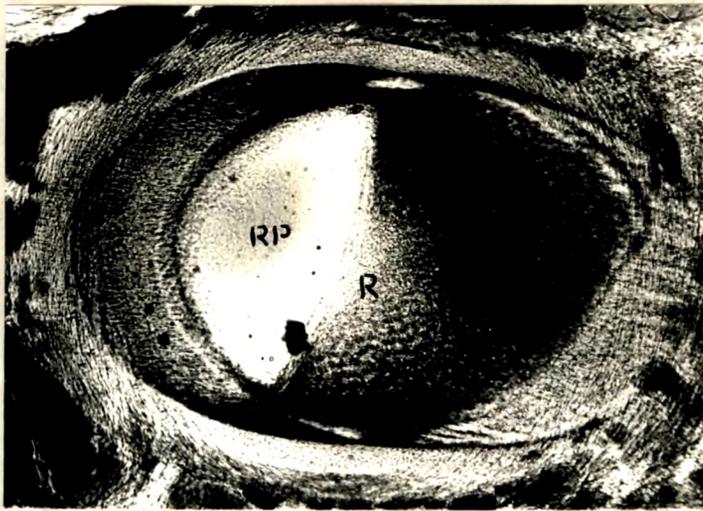
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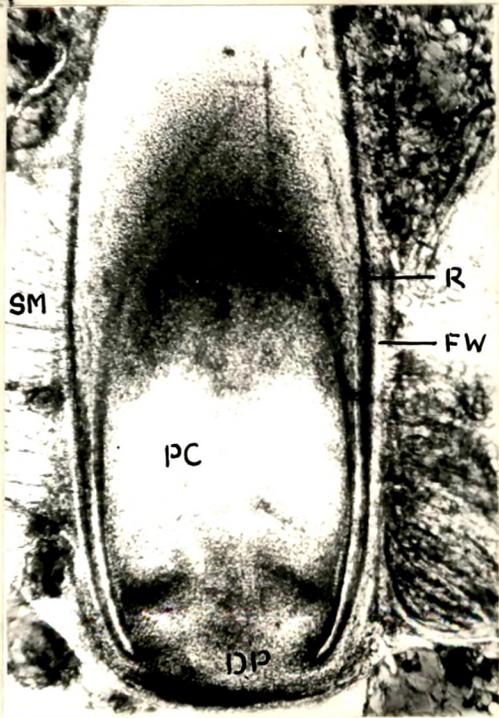


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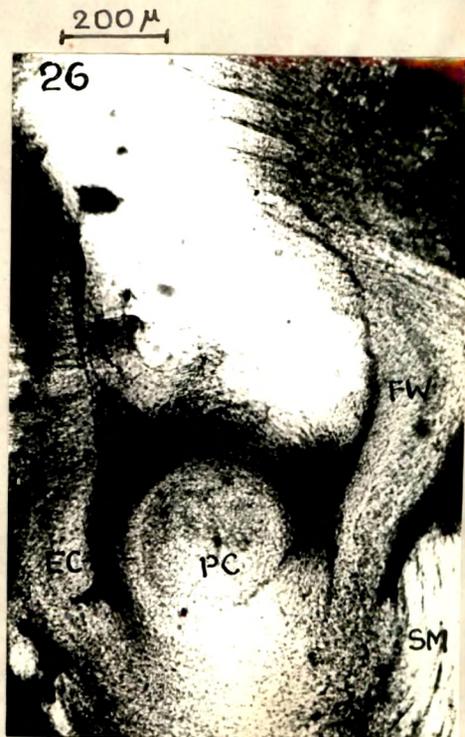
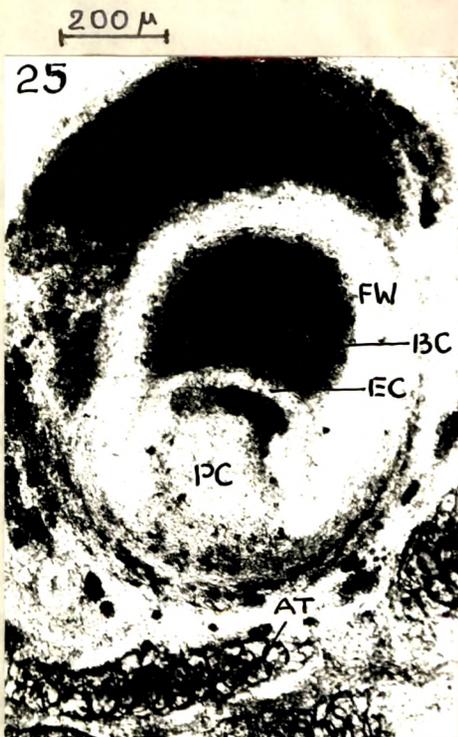


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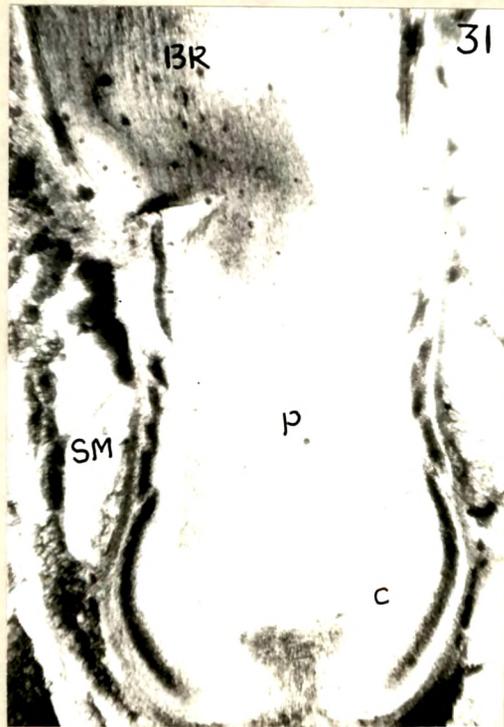
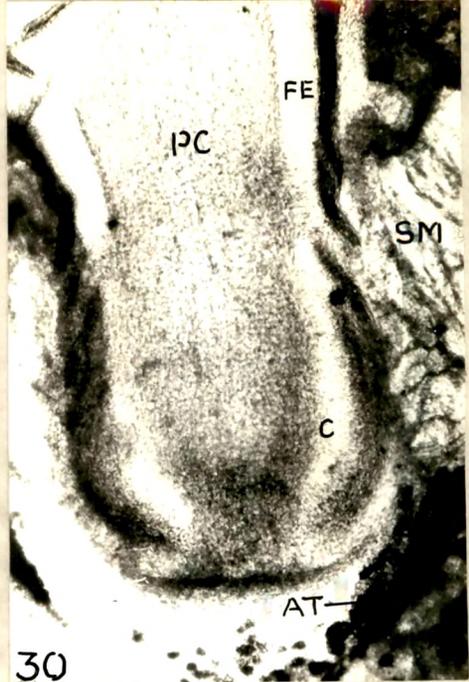
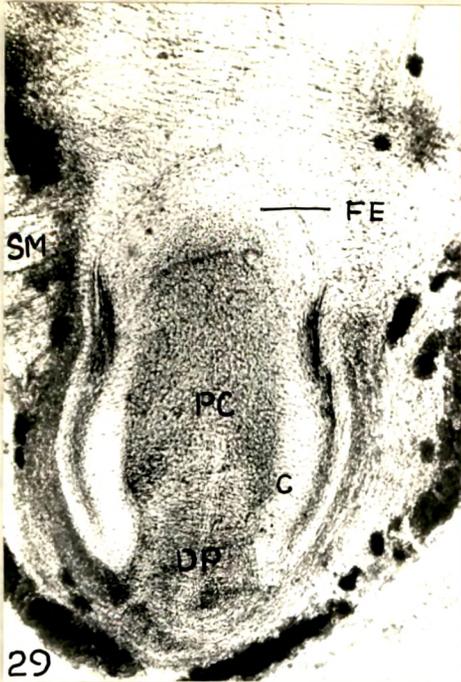


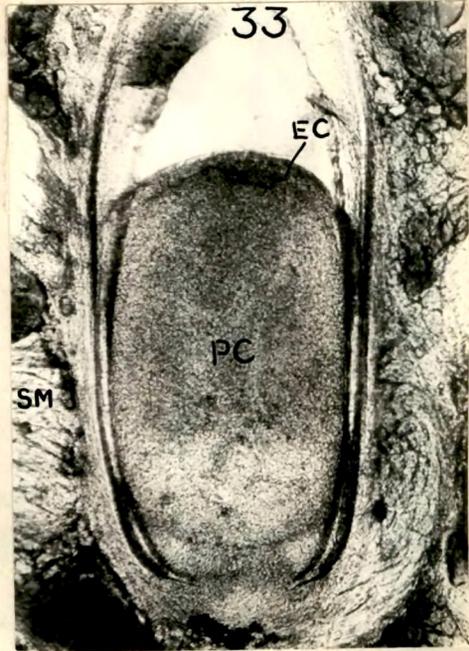
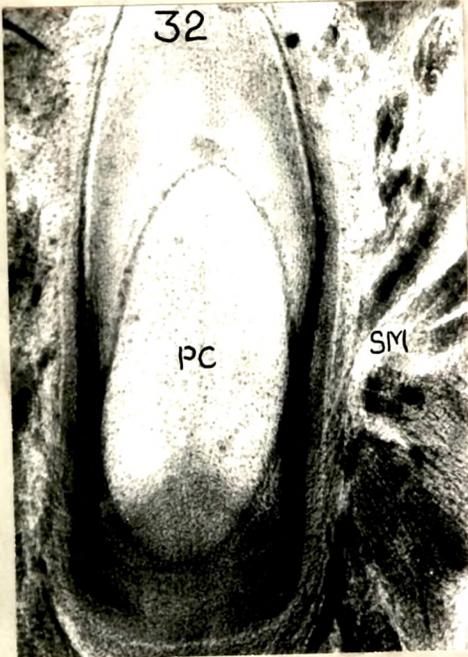
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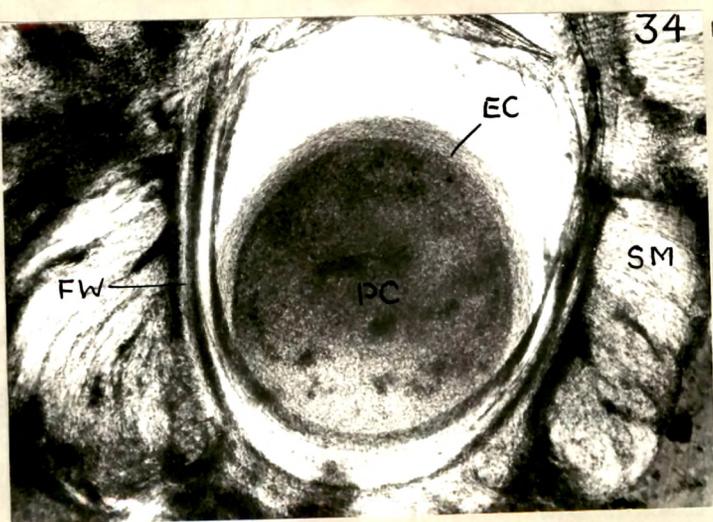
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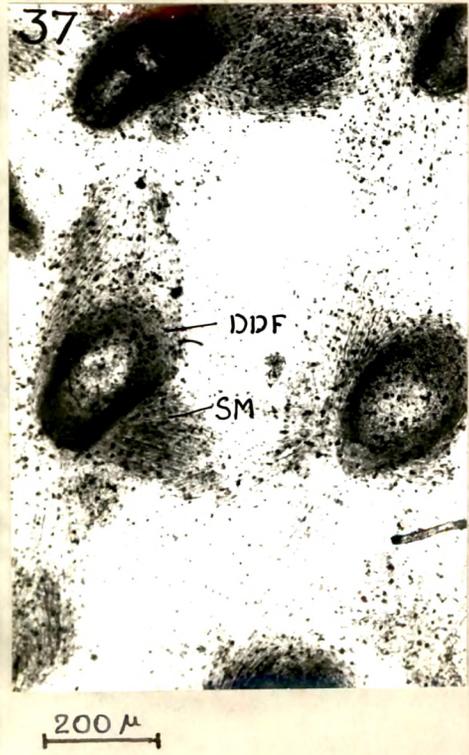
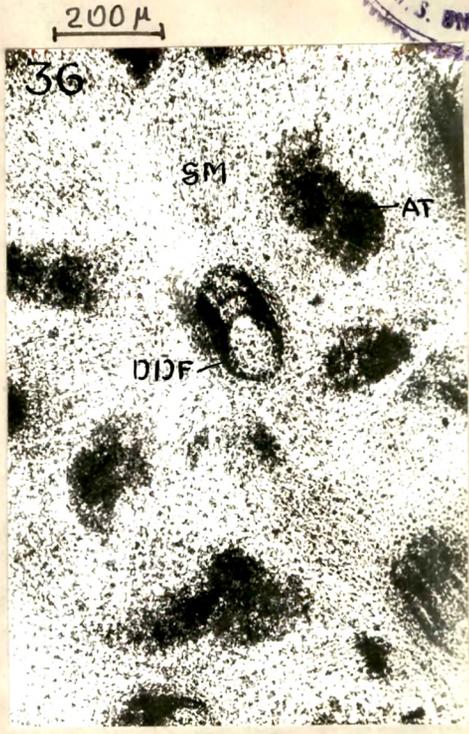
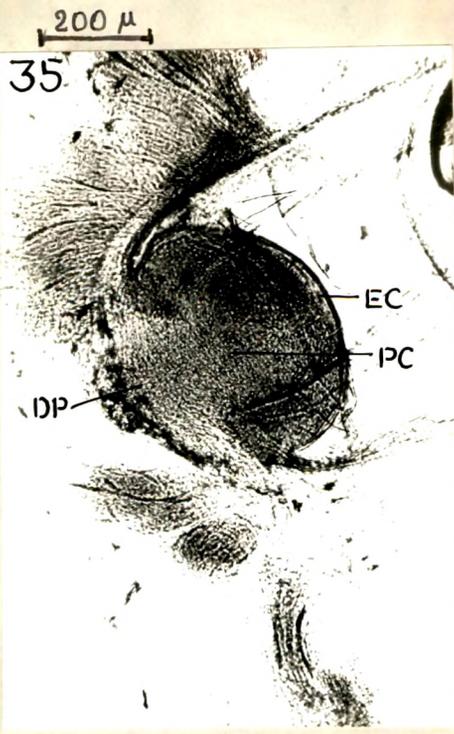


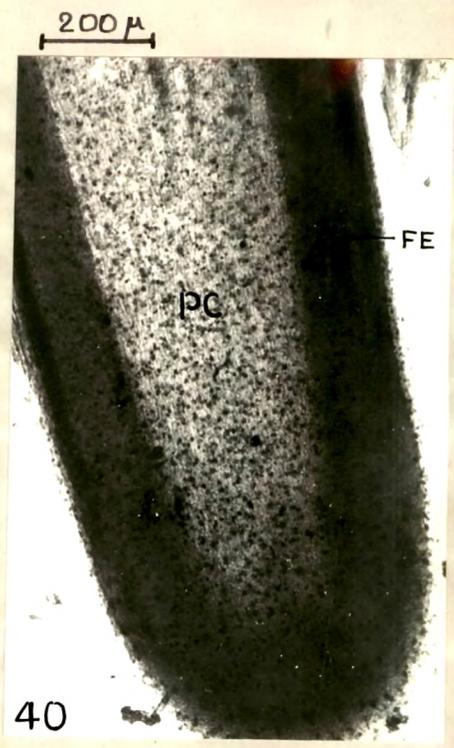
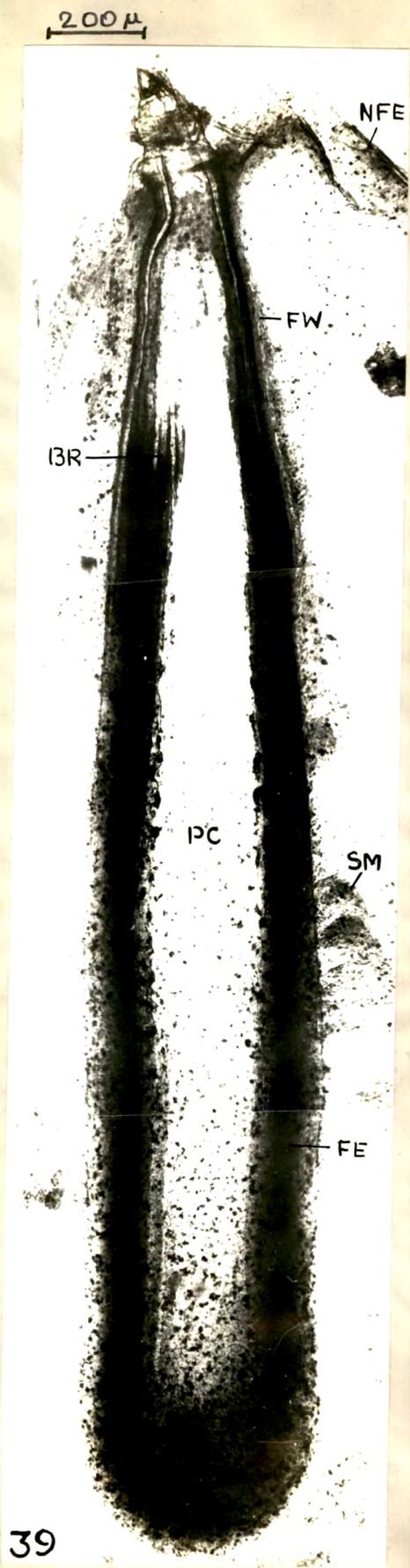
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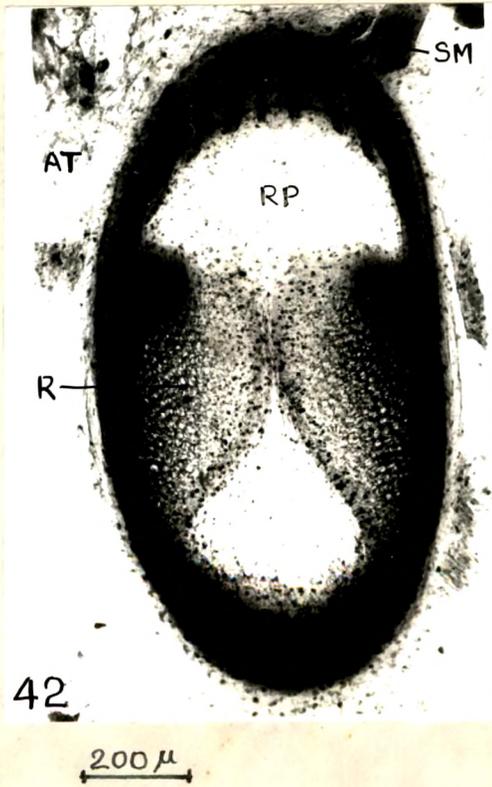
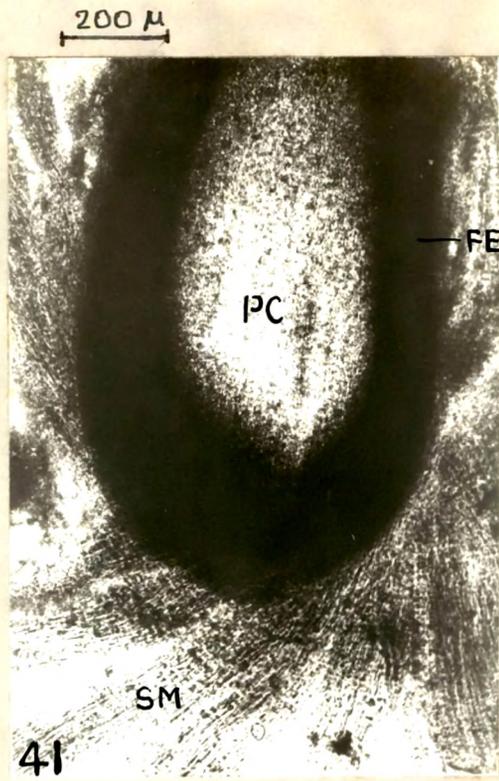


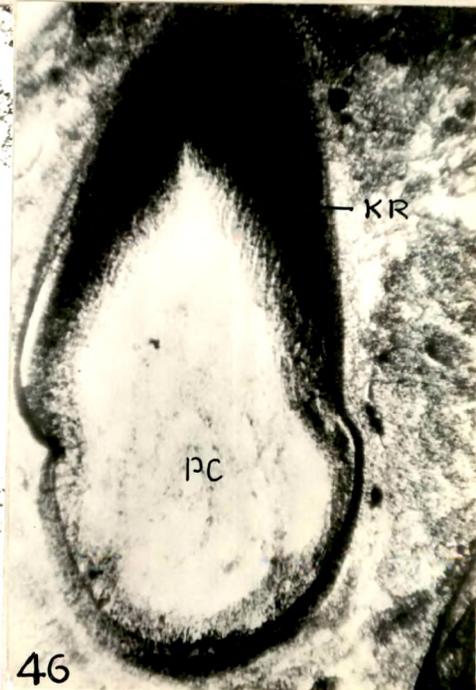
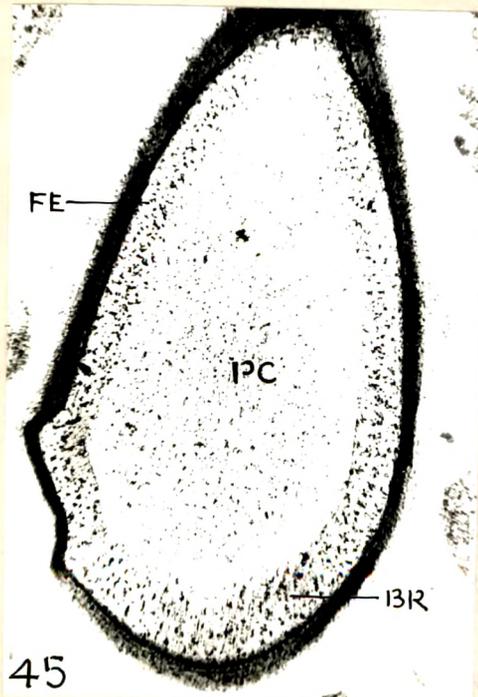
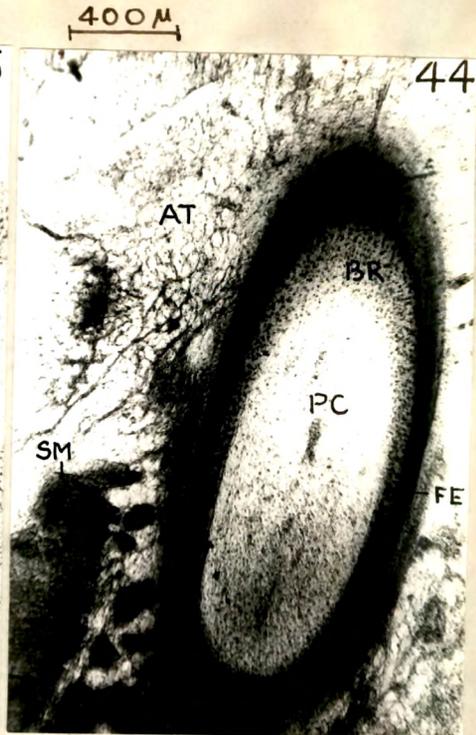
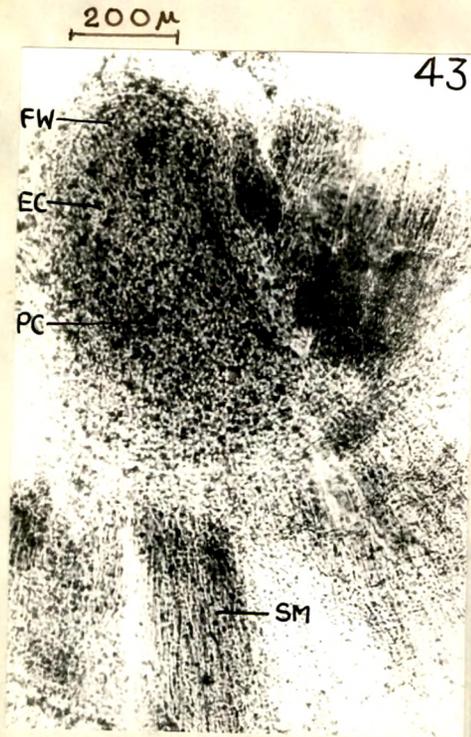
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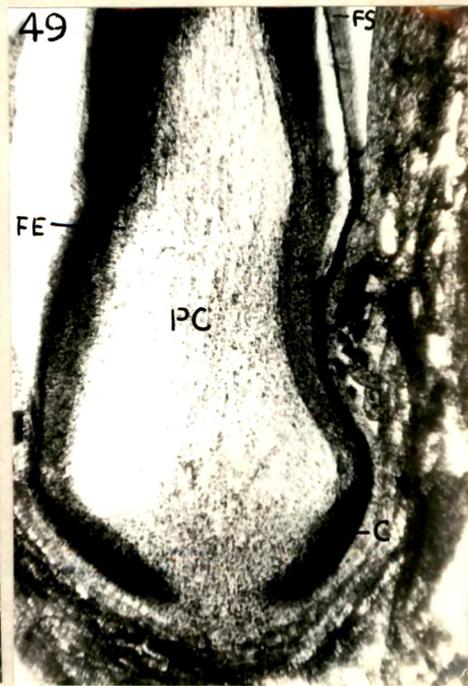
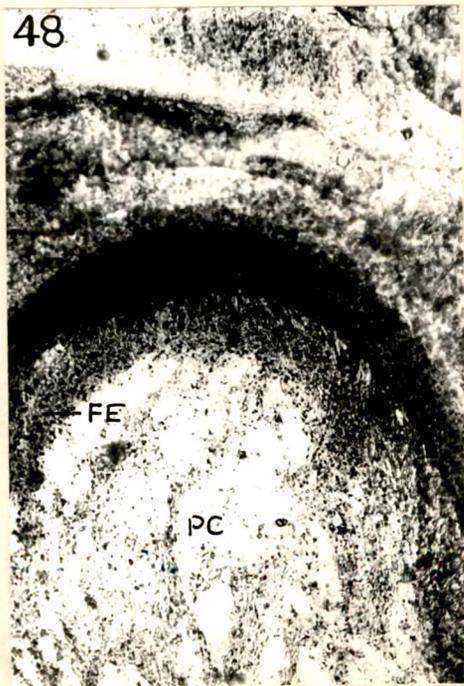
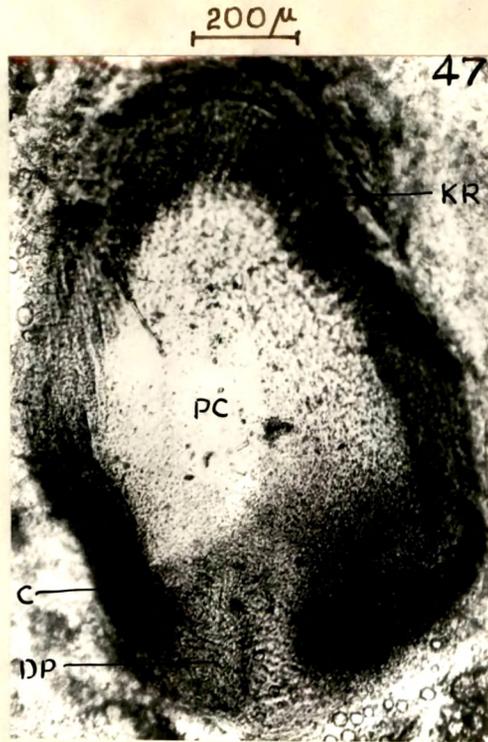
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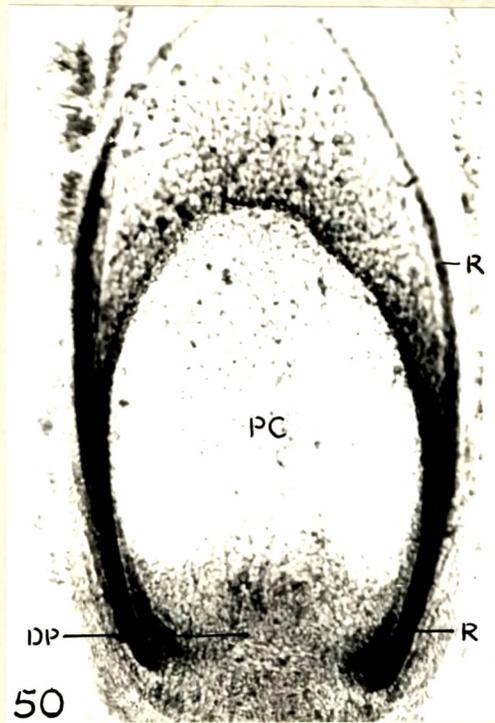




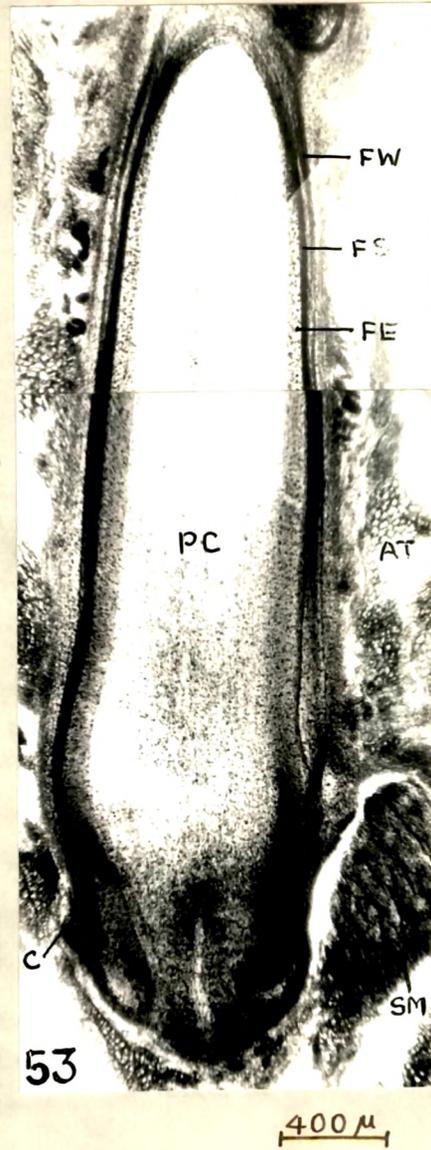
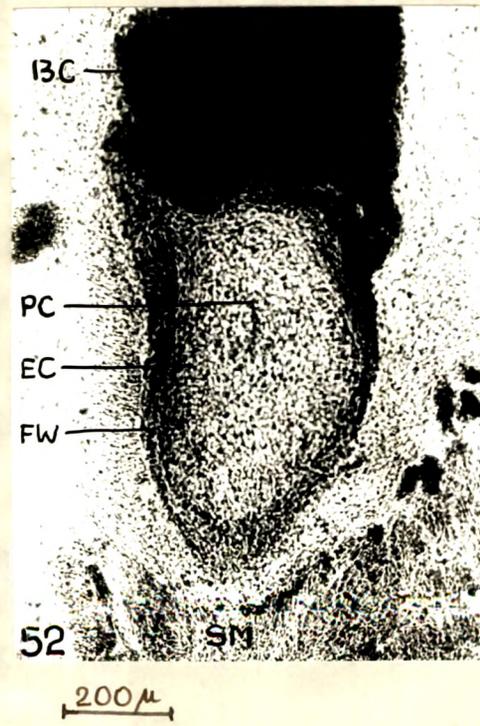
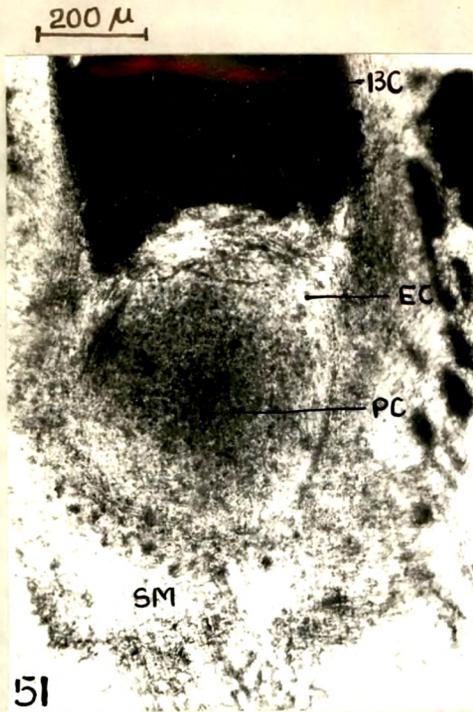


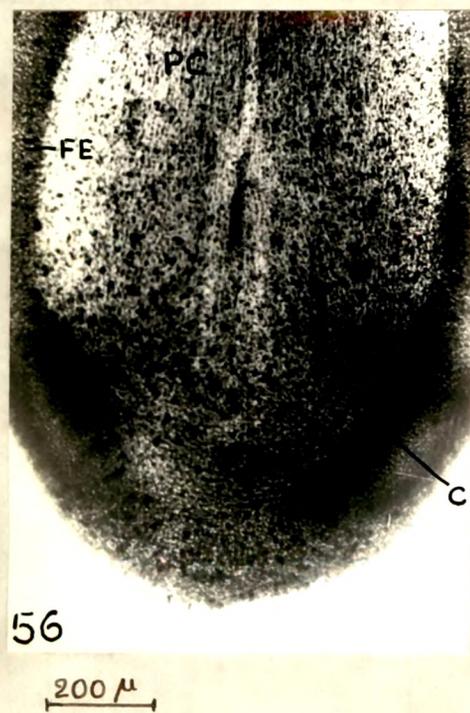
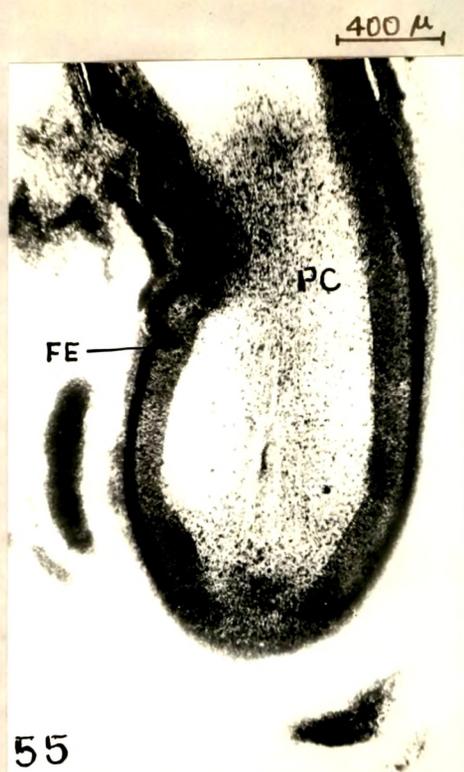
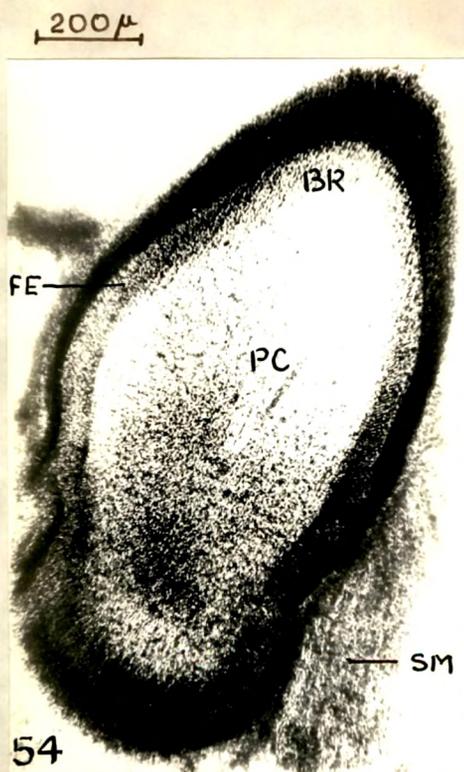
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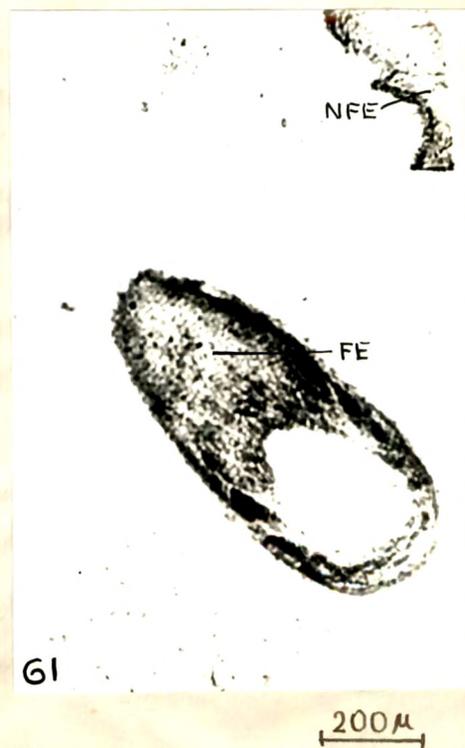
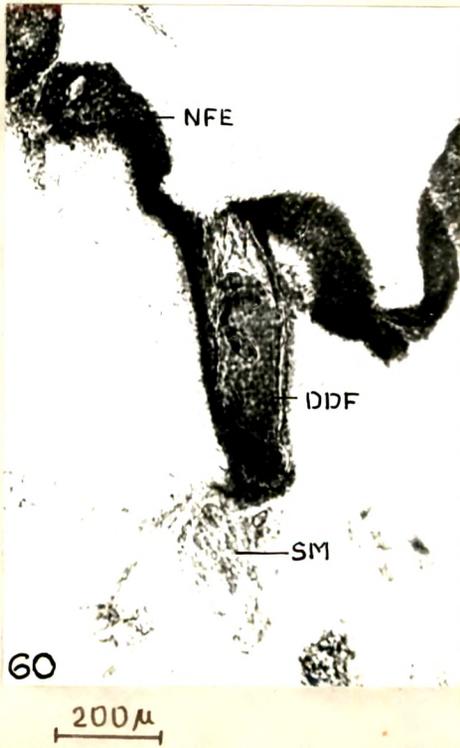
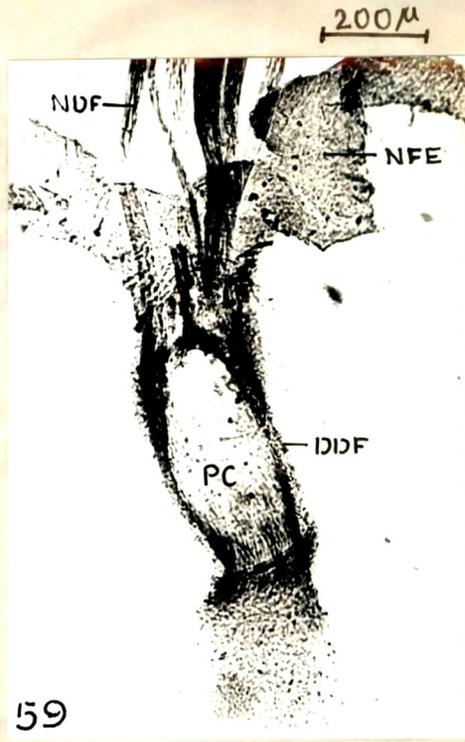
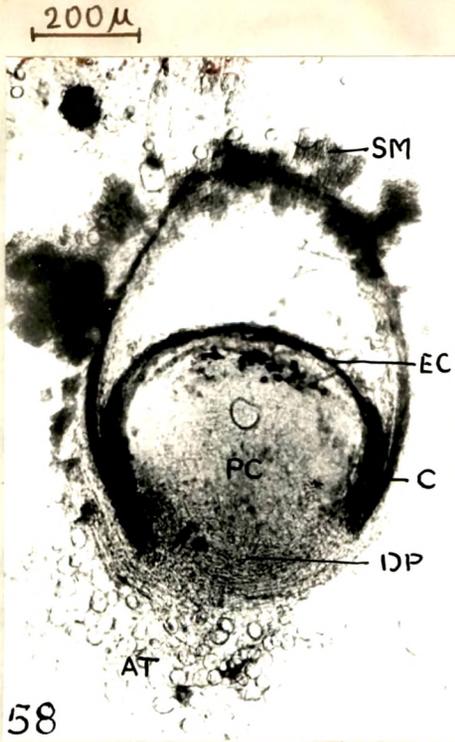
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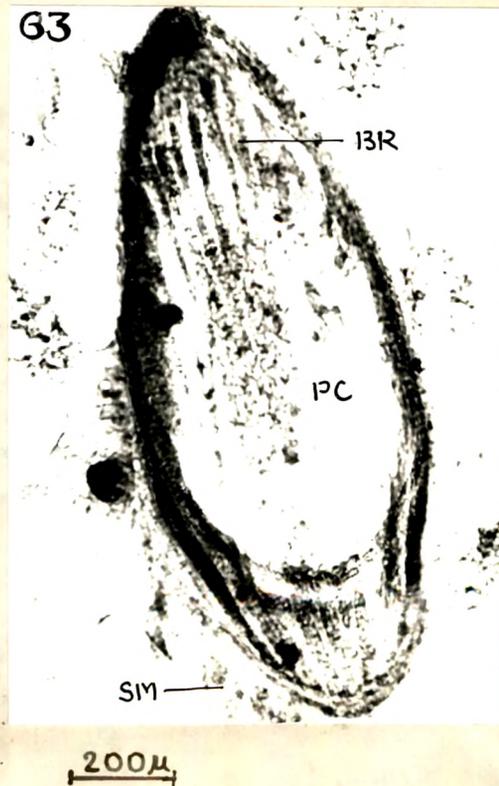
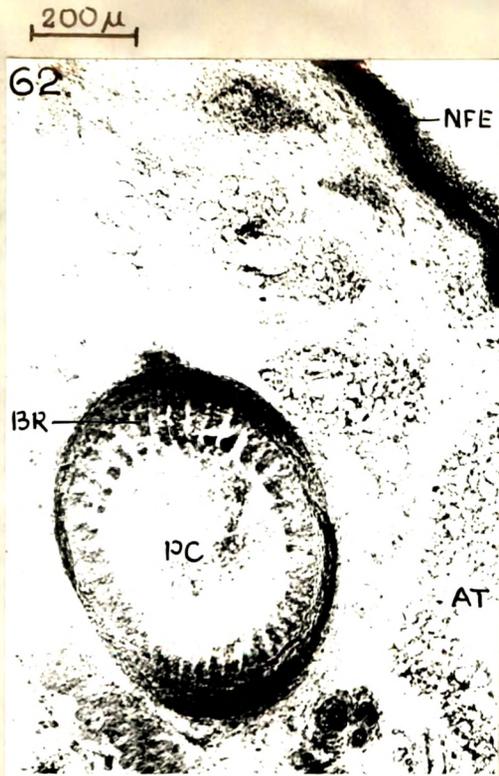


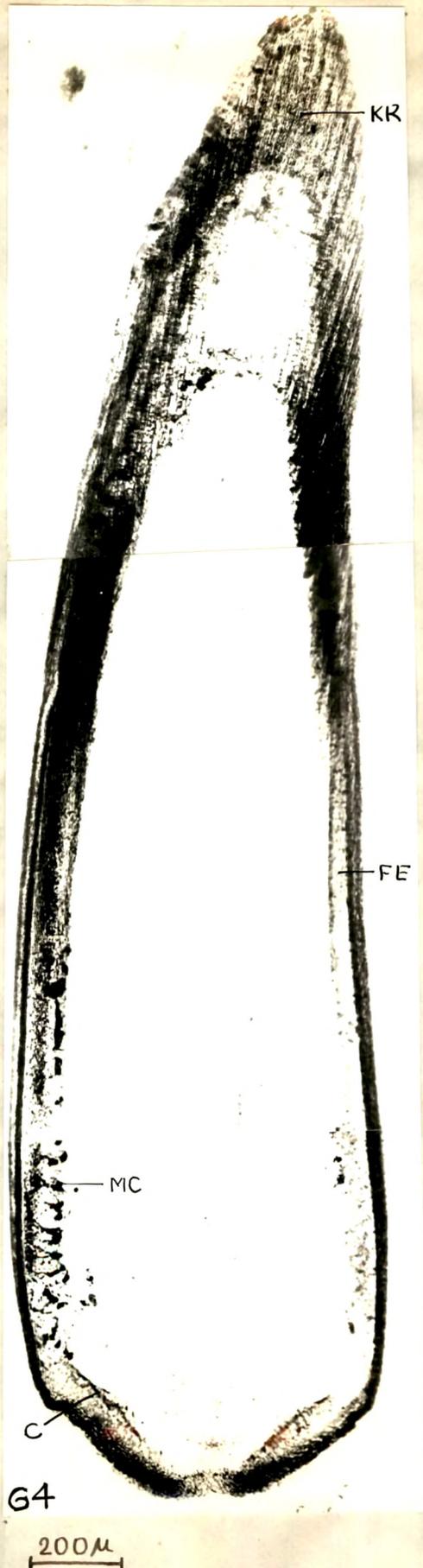
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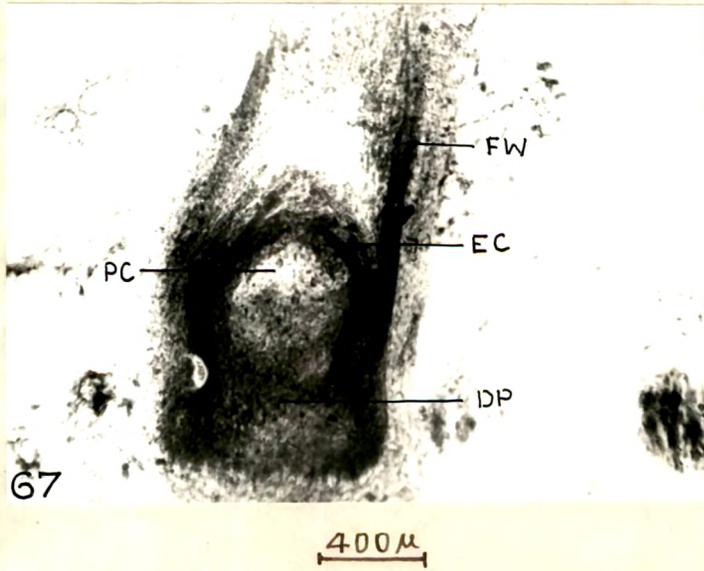
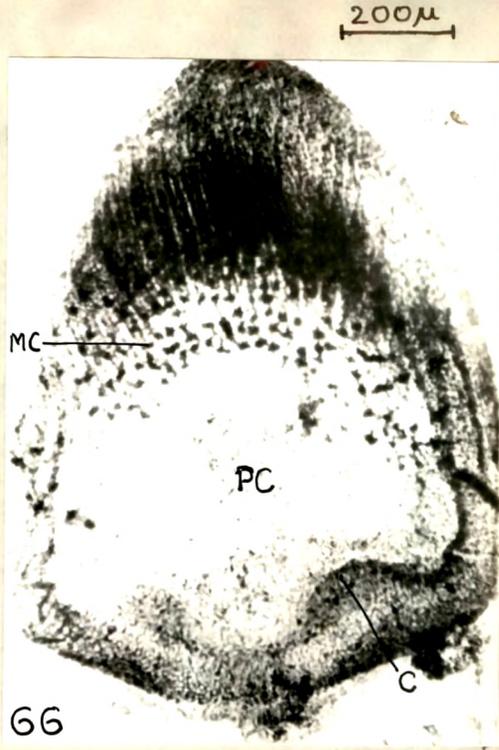
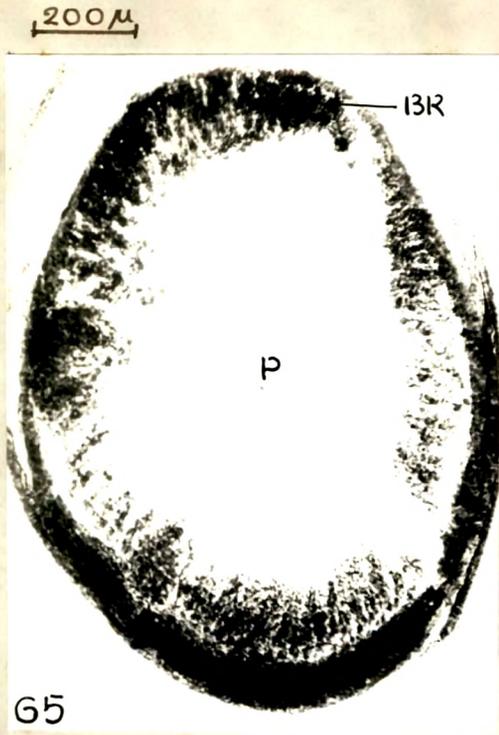


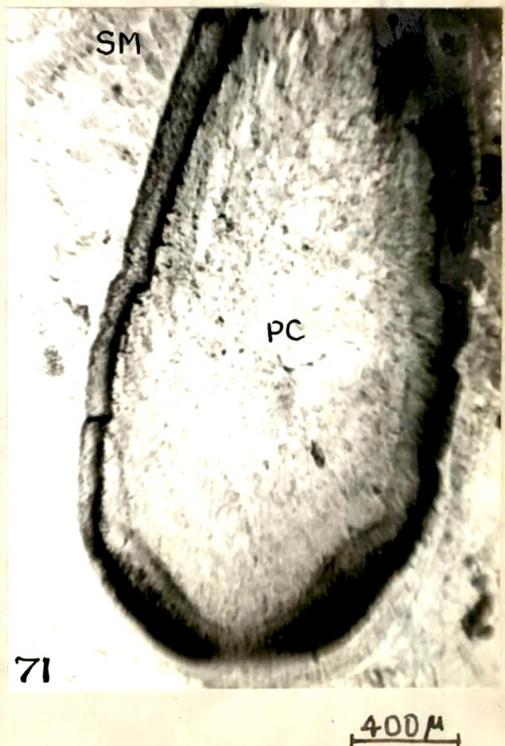
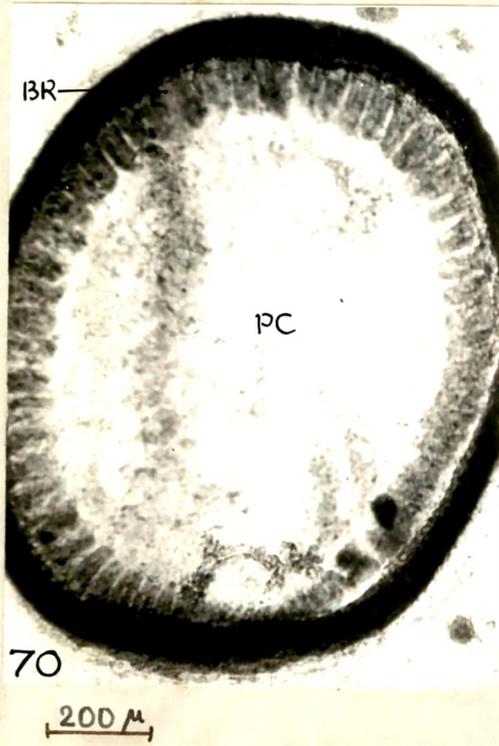
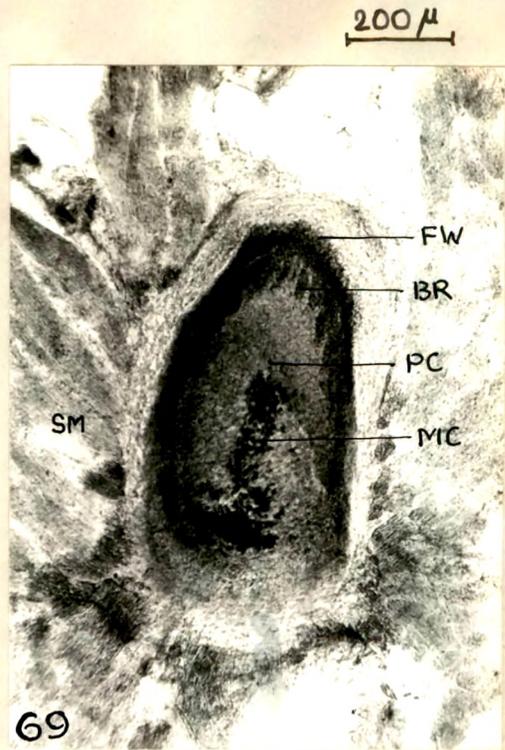
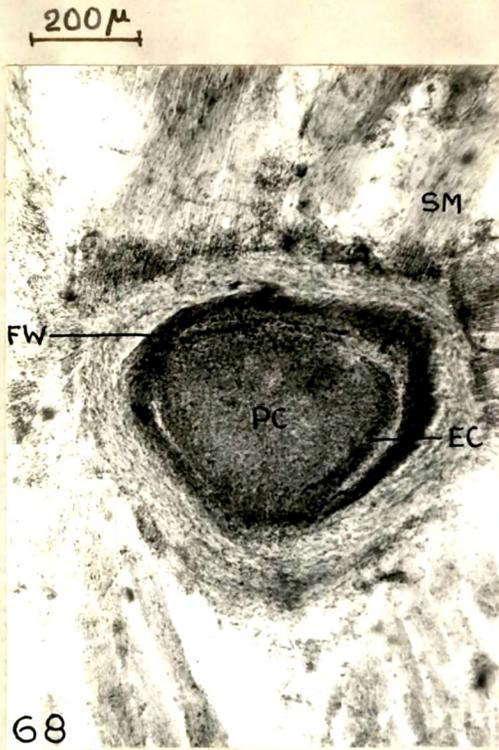


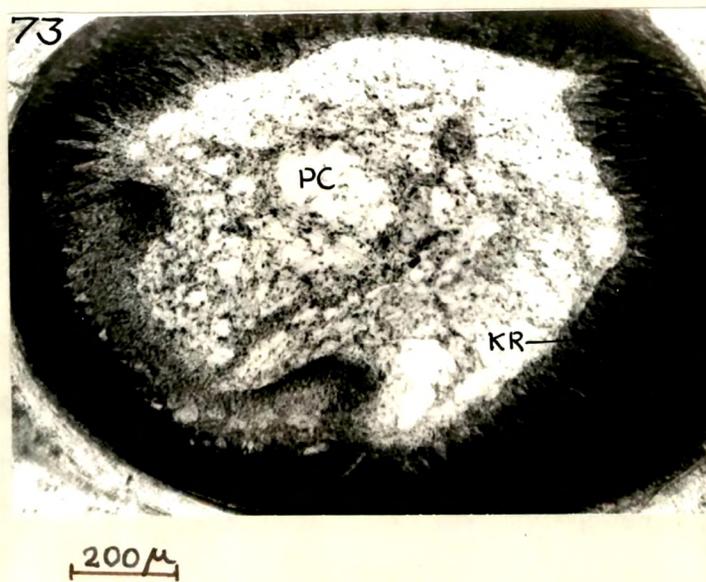
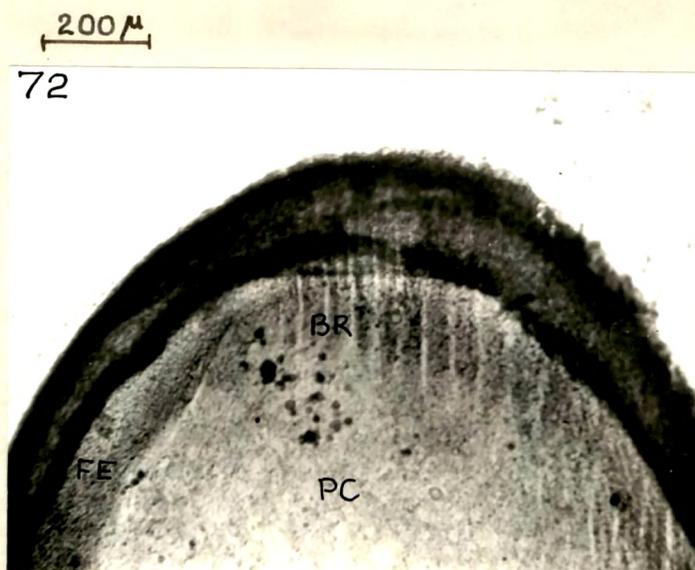


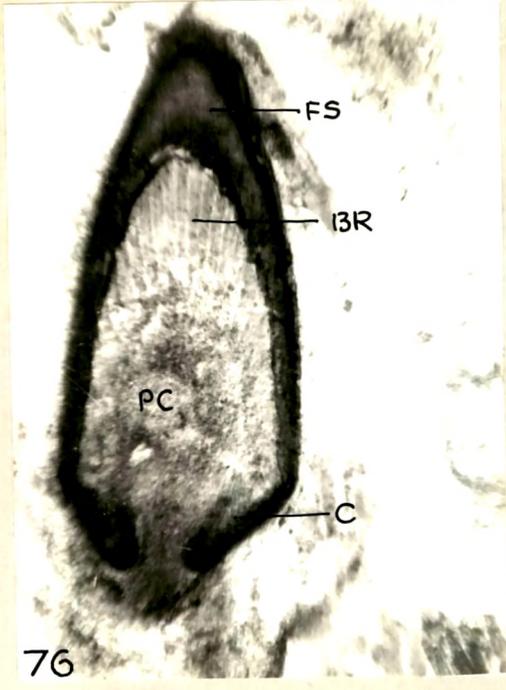
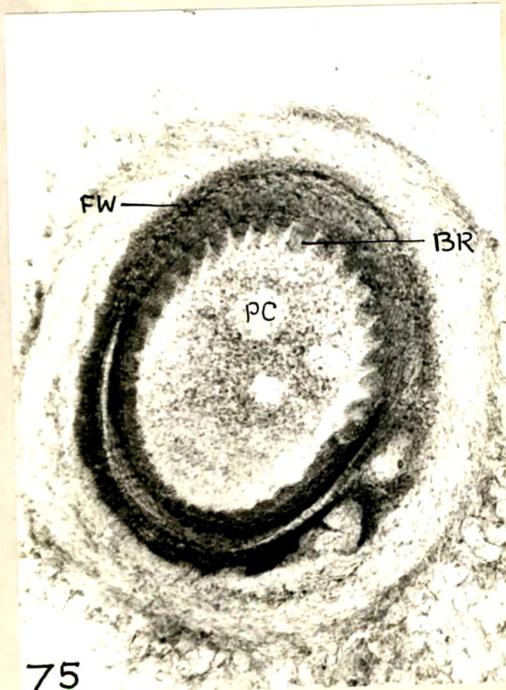


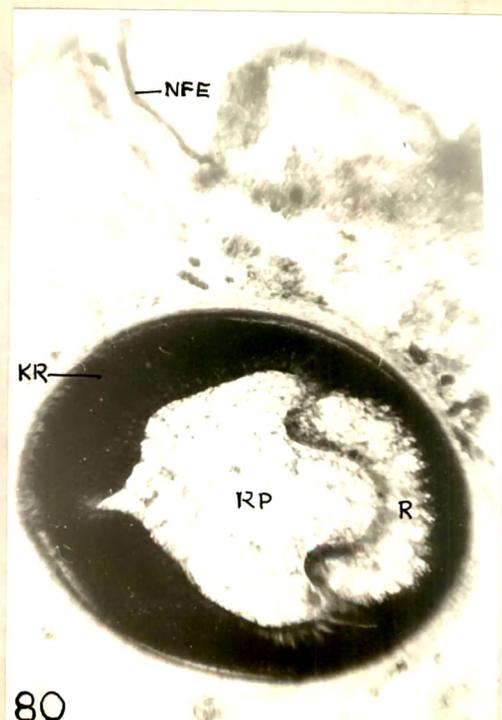
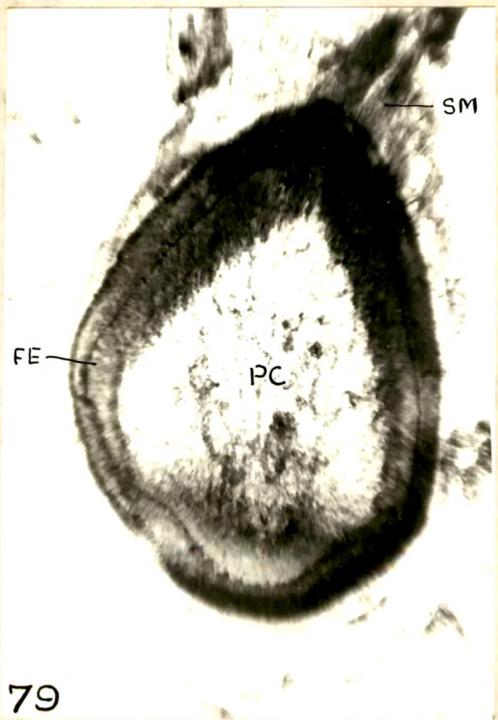
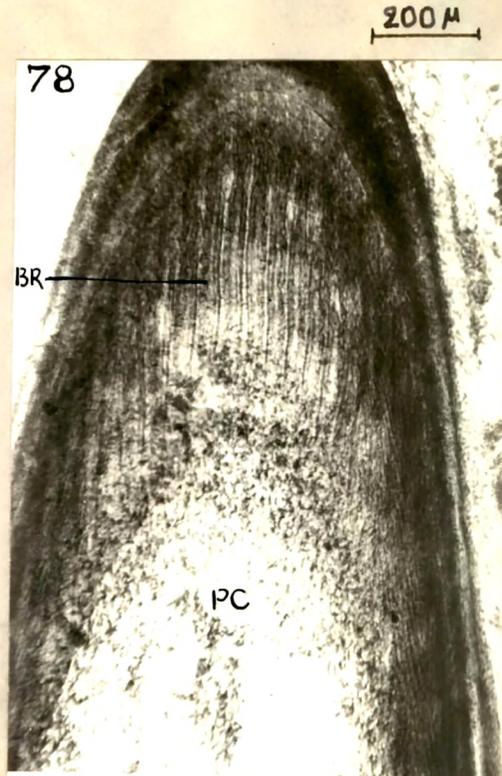
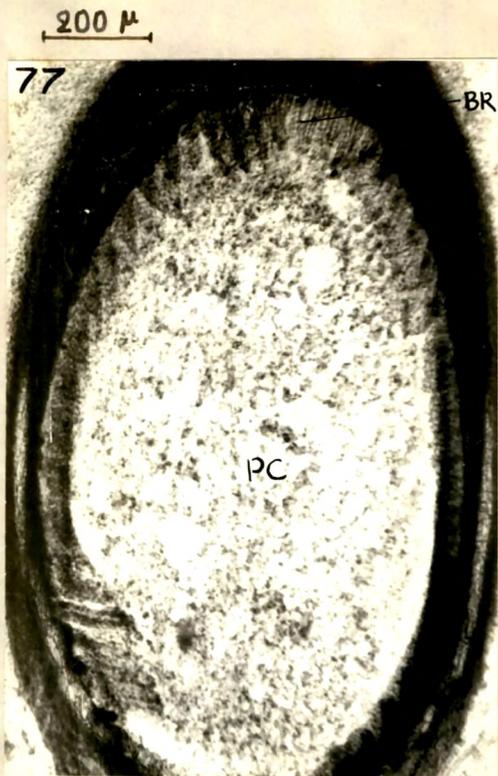












79

400 μ

80

400 μ

Lipase and BDH (Figs. 43-50 and 68-73)

24 hours after plucking adult feathers, whereas lipase activity was noticed to vary from a high to a negligible level in the various components, that of BDH was found to vary only a little. In the case of lipase, a high activity was discernible in the pulp cells and a moderate one in the smooth muscles with a low, though noticeable, activity in the stratum germinativum of non-feather epidermis and a negative one in the adipose tissue and other dermal components. On the other hand, a high BDH activity was discernible in the follicular wall, components of the germ, smooth muscles and the stratum germinativum of the non-feather epidermis, with the fibrocytes and blood vessels showing a comparatively low, though noticeable, activity. The interesting aspects of lipase activity hereafter were a decline to a moderate level on the 5th day in the germ only, and attainment of a peak level in the epithelial cells of the feather on the 7th day, with the pulp cells and smooth muscles registering only a moderate level and other skin components retaining the same level as observed in them earlier. In contrast, on the 5th day, the high BDH activity observed earlier was so retained only in the follicular epithelial cells with the pulp denoting a slight decrease. At the same time, the smooth muscles showed a low concentration of the enzyme and all other

components a negligible one. This pattern of enzyme concentration remained unchanged till the 7th day. From the 10th day onwards, there was a gradual decline of lipase activity which ultimately resulted in the attainment of a moderate level in the epithelial cells and pulp cells at this basal part of the feather on the 15th day. This pattern of lipase activity was more or less retained even on the 20th day of induced development whence the regression of the pulp had already commenced. A further slight decline in enzyme activity noticeable by about the 25th day and retained so thereafter, brought the lipase activity to a level identical to the one observed in the adult normal skin. Unlike lipase, BDH registered a slight decline in the pulp and slight increase in the smooth muscles on the 10th day with no change in the other skin components. An interesting aspect of observation was the proportionate decrease of BDH activity corresponding to the degree of keratinisation of the epithelial cells in the barb ridges. There was no change in the pattern of BDH activity till the 15th day ^{after} ~~since~~ which, the enzyme activity tended to increase, and ultimately attained a peak level on the 20th day in the pulp, epithelial cells of the feather, smooth muscles and the follicular wall. At this stage, ^{the} stratum germinativum of the non-feather epidermis registered a moderate activity and the dermal components a

low one. However, the enzyme activity declined to a low level in all the components by about the 25th day to attain ultimately the characteristic level observed in the adult normal skin.

REGENERATION

Lipids (Figs. 25 - 34)

Upto the second day of regeneration, whereas the neutral lipid concentration was low in all components of the skin, that of acidic lipids was moderate in all excepting for the adipose tissue. Both the lipids were found to increase hereafter till the 5th day whence, the acidic lipids attained a higher concentration and remained so till the 7th day and the neutral lipids continued to increase till the 7th day. At this stage there was a moderate concentration of neutral lipids in the pulp, and epithelial cells of the regenerating feather. This condition was so retained up till the 15th day though there was a slight increase in the acidic lipid contents in the barb ridges. After this, from the 20th day onwards, neutral lipids content started decreasing especially in the pulp and by day 30th the level and pattern of distribution of this metabolite in the various components became almost identical to that observable in the corresponding normal adult skin.

Lipase and BDH (Figs. 51-57 and 74-80)

By the second day after plucking the inducedly developing feather, lipase activity reached a high level in the epithelial and pulp cells of the injured feathers and also in the cells of the follicular wall. However, there was only a moderate activity in the smooth muscles, with other components of the skin exhibiting no enzyme activity at all. The pattern of lipase activity and its distribution remained so till the 5th day, and then by the 7th day a peak level was registered in the cells forming the barb ridges and those at the collar region. The enzyme activity in the pulp and smooth muscles was moderate and in other dermal components negative. The peak lipase activity observed in the regenerating feather continued to remain more or less in the same pattern till ^{fig.} 20th day. However, during this period a slight lipase activity appeared in the adipose tissue (on about the 10th day) and then again disappeared (on about day 15th). Finally, by about the 25th day the enzyme activity started declining to attain the normal adult level. In contrast to the activity of lipase, a peak level of BDH could be observed by 24 hours itself in the injured feathers and smooth muscle. Fibrocytes and adipose tissue too registered an appreciable enzyme activity though the non-feather epidermis depicted only a weak activity. BDH activity declined in the extra follicular tissues on the 5th day.

However, the barb ridges which were getting differentiated, registered a peak response and the pulp cells too had an appreciable enzyme activity. This condition was found to be maintained on ^{the} 7th day as well. Hereafter, on the 10th day all the components of the skin, including the adipose tissue and the blood vessels were found to register a peak BDH activity to be maintained so upto the 20th day of regeneration. Once again, as in the case of lipase, BDH activity too started declining in the various components from the 25th day onwards to finally attain the characteristic adult skin pattern.

DISCUSSION

The high concentration of neutral lipids observed in the subcutaneous and intradermal fat depots of the adult normal skin of the pigeon could be considered to be of significance in thermoregulation; apart from being a reserve fuel store. The negligible amount of acidic lipids observed in the dermal components like fibrocytes, when viewed along with the absence of lipase, seem to point to the fact that acidic lipids represent the structural component of these cells. The noticeable but low amount of neutral lipids and moderate level of acidic lipids in the smooth muscles together with the noticeable activities of lipase and BDH in them indicate the utilization of lipids by these muscles, though

presumably only to a low extent. This inference could be further substantiated with the comparatively higher activities of the dehydrogenases of the EMP pathway observed in these muscles (chapters ~~3~~ & 4). In the mammalian skin also, such high activity of these dehydrogenases have been reported in the arrectores pilorum muscles of the hair follicles (Michael, 1965). Though the smooth muscles in general are known to depend on the oxidation of fatty acids preferentially over carbohydrates, for energy purpose, the follicular muscles of the skin depend on the EMP as well as the HMP pathways to a greater extent than fatty acid metabolism. In the smooth muscle fibres of ^{the} pigeon gizzard, Shah and Panicker (unpublished data) noticed a high incidence of lipid metabolism indicated by high activities of lipase and BDH. This^e observed differences in the enzymological pattern of the smooth muscles from two different locations could be explained in terms of differences in their activities. The muscles of the gizzard are required to put in considerably greater amount of work than those of the feather follicles and hence could be expected to show some differences in the metabolic pattern from the latter, reflected in turn as their enzyme complements. In the case of the resting feather germ, the acidic lipid content appears to be the structural complement while the neutral lipid, together with the noticed lipase and BDH activities could be considered as taking part in the normal metabolic

functioning of these cells. The high neutral lipid content of the non-feather epidermis is noteworthy and when considered along with the moderate lipase and BDH activities in the stratum germinativum seems to be indicative of the normal occurrence of lipid metabolism in this region. A high concentration of lipids has been reported by Ma~~x~~toltsy (1969) in the chick epidermis and Shibaeva (1970) in human epidermis. Ma~~x~~toltsy (1969) considered the possibility of lipids forming a layer over the skin surface (to function akin to sebum spread over the surface of mammalian skin) forming an efficient water barrier. Another possibility is that the high lipid content maintains the pliability of the skin, and this could be considered significant from the fact that avian skin is loosely attached to the body, thereby making it possible for stretching during flight activities. The suggestion of Shibaeva (1970) that lipids participate in the process of keratinisation of the epidermal cells, also seems noteworthy and appropriate from the fact that lipid content of soft keratin is much more ^{higher} than that of hard keratin (Mercer, 1961) and that the non-feather areas contain the former type (Baden and Maderson, 1970).

During the normal post-hatching development of the first generation of definitive feathers, histochemically observable changes in the lipid content and activities of enzymes like lipase and BDH in these epidermal derivatives

point to fluctuations in lipid metabolism occurring there. In the initial phases of post-hatching development, these changes in the extrafollicular tissues of the skin are mainly associated with the development of adipose tissue and smooth muscles. The absence of noticeable change in the lipid content together with the negative histochemical response for lipase in the adipose tissue after they are well formed are suggestive of the lack of any lipid mobilisation from these depots during the normal post-hatching development of the feather. An increase in neutral lipid content of the pulp, a day after hatching upto the 3rd day with a concomitant, slight but gradual, increase in lipase and BDH activities point to the synthesis as well as utilisation of the metabolite by the developing feather. The high HMP shunt activity at this phase (chapter 2) could well aid in lipogenesis. The acidic lipid content of the smooth muscles could be visualised as contributing towards the structural element rather than for energy purposes, since the lipase activity in this tissue is low and no perceptible activity of BDH could be observed. As the development proceeded, the decline noticed in the neutral lipid content of the pulp, attainment of the first peak level of activity of lipase and a moderate reactivity of BDH, noted on the 5th day indicate lipid utilisation as an active process in the metabolic reactions of the developing feather at this stage.

Lipid utilisation for energy supply needed to satisfy the demands of a dynamic proliferative and preparative phase for differentiation as the developing feather is on the 5th day, could be easily envisaged in the light of the known importance of lipids in cellular energetics and also from the high activity of CC GPDH and the TCA cycle at this stage. Histochemically demonstrated activity of CC GPDH is considered to be indicative of glycerol fermentation (Pearse, 1960), and a high rate of operation of the TCA cycle points to increased energy output. A similar pattern of lipid utilization has been found during proliferative activities and differentiation of the epidermal cells of the regenerating lacertilian tail skin (Magon, 1970). Simultaneous synthesis of lipids also seems to be a possibility in the light of the observed high HMP shunt pathway activity (chapter 2). Apart from being metabolised for energy yield, lipids, especially acidic lipids seem to be incorporated as the structural components of the feather forming epithelial cells, as could be evidenced by the observed concentration of acidic lipids in the barb ridges.

The reduction of neutral lipid content of the pulp and its increase in the epithelial cells by the 10th day

post-hatching with a high lipase activity in them is noteworthy and suggestive of lipid uptake and utilisation by the epithelial cells of the developing feather, at a stage when differentiation, i.e., keratinisation, is a major feature of its development along with growth. The reduced reactivity of BDH at this stage might indicate that lipids are not being used for energy production to any great extent. In the light of the suggestion of Bell and Thathachari (1963) and Shibaeva (1970) that lipids participate in keratinisation, it seems tempting to suggest that at this stage, lipids are channeled towards keratin formation. An indirect evidence in favour of such a contention could be derived from the activity of acid phosphatase (chapter 8) which was manifested on the 10th day, for the first time during post-hatching development. Acid phosphatase has been amply implicated in the process of keratinisation and Schmidt (1968) had also correlated the enzyme activity with lipid metabolism connected with keratinisation in the skin of regenerating amphibian limbs. The slight increase in lipid content of the pulp by the 15th day may be due to increased demand for lipid synthesis due to its utilization, as evidenced by moderate BDH activity and high lipase and an increased activity of the HMP shunt pathway. However, a decline from a moderate activity of BDH from the 15th day to the 20th day indicate

a decline in the utilization of lipids for keratinization as well as energy yield at this stage. Such a decline in lipid metabolism could be expected in the later periods of feather development as there is a decline in growth as well as differentiative processes. Thus the near completion of the differentiation would result in decreased demand of this metabolite, due to decreased rate of keratin synthesis, as well as resulting from a drastic reduction in energy demands. Moreover, as the regression of the pulp proceeds, lipids are known to disappear from its cells (Malt & Hartman, 1960). Thus the gradual diminution in the concentration of the metabolite and enzymes concerned with its metabolism in the components of the feather to a level almost similar to that found in the corresponding part of the resting germ, correspond well with the morphological maturation of these epidermal derivatives.

During induced development of feathers, the increase in concentration of lipids in the developing germ and the gradual increase and attainment of peak level of lipase activity by the 5th day and a high BDH activity, point to lipid utilisation. A moderate level of operation of the TCA cycle at this phase of inducedly developing feather also speaks in favour of the fact that lipids are being

utilised though to a moderate extent, for meeting the energy demands of an active process of cellular proliferation and initiation of differentiation i.e., organisation of the epithelial cells into primary barb ridges.

Slight, though noticeable, activity of lipase in the non-feather epidermal region with the observed activity of BDH in these cells as well as the dermal components might be suggestive of an increased rate of lipid metabolism in these tissues of the skin. This, when considered along with the evidence of the operation of TCA cycle in these regions (chapter 6) obtained by the activity of SDH and MDH, clearly indicate the utilisation of lipids for increased energy yield; which could be helpful in compensating to a considerable degree the heat loss from the exposed parts of the skin.

A simultaneous synthesis of lipids in the pulp and epithelial cells of the developing feather also seems to be a possibility in the light of the observed high activity of G6PDH, a key enzyme of the shunt pathway. The moderate level of lipids observed in the feather components from the 7th to the 10th days, considered with the peak lipase activity of its epithelial cells and moderate activity of the enzyme in the pulp and smooth muscles indicate a further

increase in lipid utilisation. The high level of BDH activity on the 7th day is also in favour of such a contention. The declined lipid content and lipase activity by the 10th day along with a high BDH activity in the pulp remaining more or less so upto the 15th day when considered along with the high TCA cycle operation at this stage are indicative of the participation of lipids in the process of keratinisation and growth. The increased concentration of acidic lipids noticed in the barb ridges could be indicative of their participation in the process of keratinisation. The decline in the activities of these enzymes in the various components after this phase to reach a level almost similar to that observed in the corresponding components of adult normal skin, by about the 25th day resembles that observed during the normal development of the feathers.

During the regeneration of feathers, lipids appear to be the chief source of energy during the first half of development. The increase in activity of lipase and BDH to a high level as early as the 2nd day of regeneration is noteworthy. At this phase of development when the wound is healed due to epithelial cell proliferation and the regenerating feather is in a stage comparable to the blastema stage of other regenerating vertebrate appendage, the energy

demand could be expected to be high, Lipids at this phase could be considered as the choice metabolite of the regenerating feather. Such a dependency of the blastemal cells preferentially on lipids have been shown by Magon (1970) and Ramachandran (1972) in the regenerating tail of lacertilians. As the growth of feather regenerate proceeded, the gradual increase in lipid content which became obvious by the 5th day, and the continued high peak levels of lipase and BDH in the tissues of the feather, where a moderate activities of the TCA cycle enzymes were also observed, are all suggestive of the utilization of lipids during this period. An increased acidic lipid content of the regenerating feathers by the 5th day might be due to its incorporation into their structural frame work, while, that in smooth muscles, fibrocytes and non-feather epidermis could be participating in the metabolic processes there; since a high BDH activity was noticed in the former two components and it was moderately active in the latter. Further increase in neutral lipid content of the pulp by the 7th day, when high lipase, BDH and TCA cycle enzyme activities were also observed, could mean a phase of predominant lipid utilisation. This phase of regeneration is a critical one, since the barb ridge differentiation and active growth prior to emergence of feather stubs outside the follicles, are the major features

of development; and resemble that of the 5th day of normal post-hatching development, as well as induced development. An unchanged concentration of lipids maintained in the regenerating feathers upto the 15th day together with the lipase and BDH activities which were in peak concentration in the feathers and the appearance of the same in the adipose tissue also on the 10th day when taken together with the continued high activities of SDH and MDH (chapter 6) indicate, that lipids are probably being mobilised from the intradermal depots and utilised for the purpose of feather development. The continued high activity of lipase and BDH in the feather up till the 20th day though there was a decline in that of the TCA cycle enzymes could imply that lipid utilization during the period of active differentiation is chiefly for keratinization than for the purpose of energy yield. Such a high incidence of lipid utilization has been reported during the differentiation phase of the regenerating reptilian appendages in the differentiating scales (Magon, 1970; Ramachandran, 1972; Radhakrishnan, 1972). The high response of BDH in these scales noticed by Magon (1970) and Ramachandran (1972) are also noteworthy and pertinent here. The moderate concentration of lipid observed after this phase in the regressing pulp, together with a decreasing trend in the activity of lipase and BDH leading to a level similar to that observed in the corresponding components of the adult normal skin indicates the gradual completion of the process of regeneration.