CHAPTER 8

HISTOCHEMICAL OBSERVATIONS OF THE ACID PHOSPHATASE ACTIVITY IN THE DEVELOPING DEFINITIVE FEATHERS OF THE BLUE ROCK PIGEON, COLUMBA LIVIA, DEVELOPING UNDER THREE DIFFERENT CONDITIONS

Acid phosphatase is known to bring about the hydrolysis of esters of phosphoric acid in an acidic pH, liberating inorganic phosphates. A suitable histochemical technique for this enzyme was developed for the first time by Gomori (1941). Since then, the presence of acid phosphatase has been demonstrated in a number of animal tissues (vertebrate skin, Kobayashi et al., 1955; Moretti and Mescon, 1956; Reiner et al., 1960; Carranza and Cabrini, 1962; Corneal epithelium of mammals - Ehlers, 1970; Kidney - Maggi and Cling, 1966; vertebrate skeletal muscle - Khan and George, 1967). Ever since the identification of acid phosphatase in the lysosomes with other hydrolytic enzymes (Duve, 1959; Novikoff, 1960), considerable attention has been paid to this enzyme as to its localization and possible role(s) in various physiological and biochemical activities of tissues. It is being increasingly interpreted that the enzyme according to the functional state of the tissues concerned might be involved in a number of activities such as phagocytosis (Klockars and Wegelius,1969), dissolution of tissue components (Weber and Niehus, 1961), synthetic activities (Sauter, 1967; Mishra and Mohanty,1969),

fat absorption in the intestine (Barka, 1963) and differentiation (Ghiretti, 1950). Acid phosphatase activity has been noticed in normal and wound healing skin of many vertebrates (primates- Moretti and Mescon, 1956; Reiner et al., 1957; Braun-Falco and Rupec, 1967; rat- Carranza and Cabrini, 1962; Pigeon- Kobayashi et al., 1955).

Besides, this enzyme has also been investigated with reference to regeneration of appendages of both amphibians (Ghiretti, 1950; Schmidt, 1963) and Reptiles (Shah and Chakko, 1966; Radhakrishnan, 1972). The enzyme is known to participate in some of the degenerative processes during both normal embryonal development and metamorphosis of many species of animals (Brachet et al., 1958; Misch, 1962; Scheib, 1963, 1965; ~~Clever, 1965~~; Weiss, 1966; Mills and Lang, 1972). Koning & Hamilton (1954) have shown that during certain stages of down feather development in the chick, activity of acid phosphatase becomes discernible but this he reports as an artefact and does not attribute any significant role to this enzyme in the development of the feather. It was in this light that an investigation in the pigeon skin during definitive feather development using histochemical techniques adopting Burstone's method for localization and distribution

of acid phosphatase was deemed appropriate so as not only to understand the role of this enzyme during normal feather development (postnatal) in birds, but also, to bring out the similarities and or dissimilarities involved therein between the processes of normal, induced and regenerative development of definitive feather.

OBSERVATIONS

Adult normal skin: (Fig. 1)

All the tissue components of the normal adult pigeon skin except the smooth muscles and the adipose tissue showed appreciable though varied concentration of the enzyme under investigation. The highest enzyme activity elicited in the non-feather regions of the skin were in the cells of the stratum germinativum of the epidermis, fibrocytes in the dermis, blood vessel walls and to a certain extent in the epimysium of the smooth muscle bundles associated with feathers. With regard to the feathered regions of the skin, it is interesting to note that the cells of the stratum germinativum of the feather follicles depicted an enzyme concentration much higher than that observed in the corresponding cells in the non-feather regions. The different parts of the resting germ of a mature definitive feather

showed varying intensities of acid phosphatase activity. The basal mesenchymal pulp cells were more enzyme reactive than the papilla of the germ as well as the epithelial cells covering the germ. Even amongst the epithelial cells, those at the collar region demonstrated relatively higher enzyme concentration than the ones which were incipiently keratinized and formed a protective covering over the germ.

Post-hatching development of the definitive feather: (Figs. 2-4)

From the day of hatching till about the 10th day, there was no detectable acid phosphatase activity in any part of the skin including the developing feather components. The enzyme made its noticeable appearance for the first time in both the feathered and non-feathered regions of the skin on about the 10th day of post-hatching development. However, a comparison at this stage revealed, that the pulp cells and the epithelial cells of the collar region of the developing feathers were more enzyme reactive than the epithelial cells in the non-feather regions of the skin. In the latter region, the only enzyme reactive components were the fibrocytes and walls of blood vessels in the dermis. This phase of enzyme acquisition was followed by a general decline registering a very low level, an almost negligible one, on the 14th day.

EXPLANATIONS FOR FIGURES

Fig. 1 LS of feather follicle showing acid phosphatase activity in the resting feather germ.

NORMAL DEVELOPMENT

Fig. 2 Section of skin showing the enzyme activity in developing definitive feather on the 10th day post-hatching.

Fig. 3 Section of feather showing the enzyme activity in pulp on 20th day post-hatching.

Fig. 4 LS of 25 day old feather. Note acid phosphatase activity in cells of pulp.

INDUCED DEVELOPMENT

Fig. 5 LS of follicle one day after plucking the adult feather showing the enzyme activity in cells of the germ.

Fig. 6 LS of 5 day old inducedly developing feather depicting the enzyme activity.

Fig. 7 Oblique section of 5 day old feather showing the enzyme activity.

Fig. 8 LS of part of feather on 7th day of induced development, showing enzyme activity in various components.

Fig. 9 LS of base of 7 day old feather. Note enzyme activity in the pulp cells.

Fig. 10 LS of feather on the 10th day depicting the enzyme activity.

Fig. 11 Oblique section of 20 day old feather to show the enzyme reactivity of the pulp cells.

Fig. 12 Section of feather on 25th day of induced development showing acid phosphatase activity.

REGENERATION

Fig. 13 LS of feather follicle showing one day old regenerating feather. Note the enzyme activity.

Fig. 14 LS of 3 day old feather regenerate depicting acid phosphatase activity in various components.

Fig. 15 LS of 7 day old regenerate showing the enzyme activity.

Fig. 16 Part of 7 day old regenerate to show the enzyme activity in the pulp cells and barb ridges.

Fig. 17 Section of feather on the 10th day of regeneration. Note the enzyme activity in the pulp cells and barb ridges.

Fig. 18 Section of regenerating feather on the 15th day showing the enzyme activity.

Fig. 19 TS of 30 day old regenerate at the base of the follicle showing acid phosphatase activity.

ABBREVIATIONS

AT - Adipose tissue
BC - Blood clot
BR - Barb ridges
C - Collar
DP - Dermal papilla
EC - Epithelial covering
FE - Feather epithelium
FS - Feather sheath
FW - Follicular wall
KR - Keratinised region
PC - Pulp cells
R - Rachis
SM - Smooth muscles

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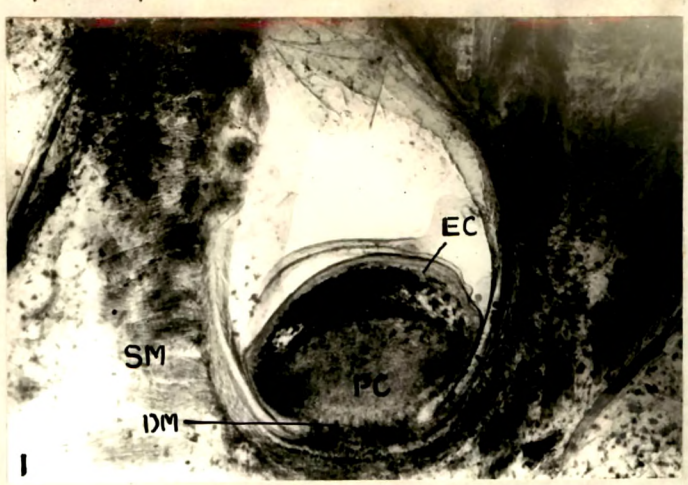

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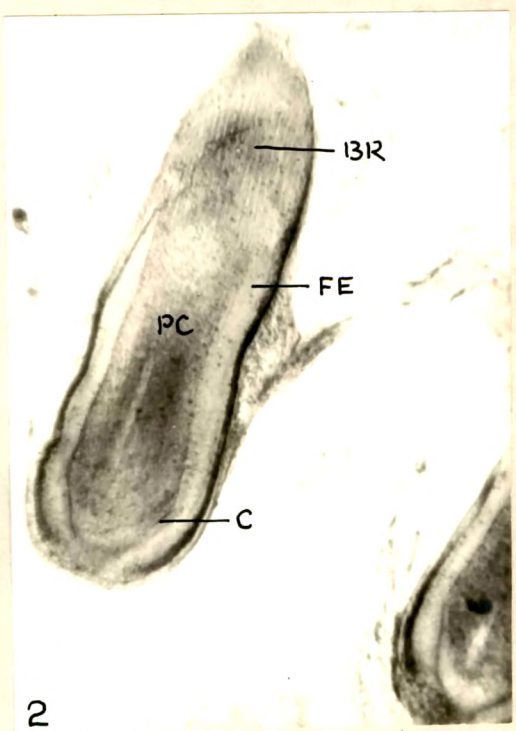

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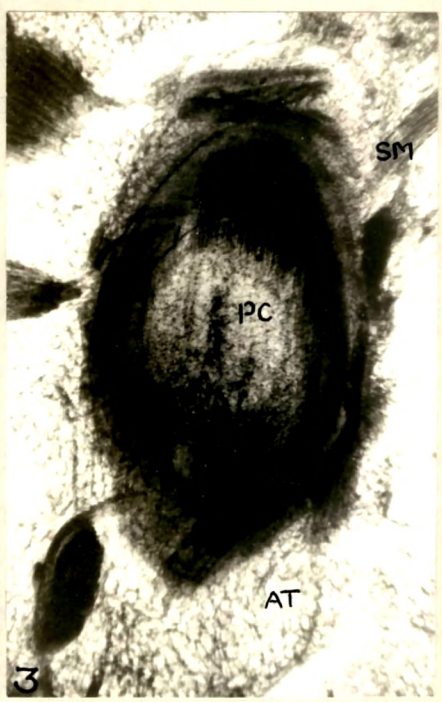

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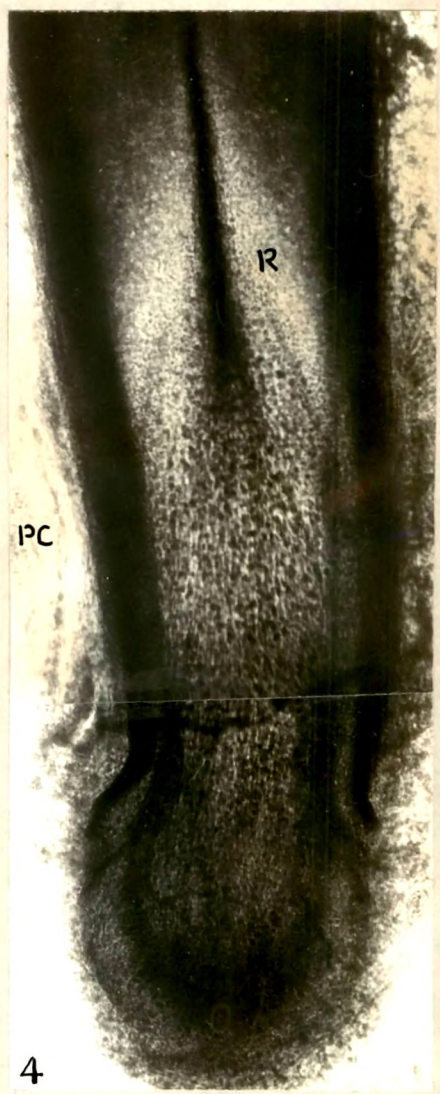

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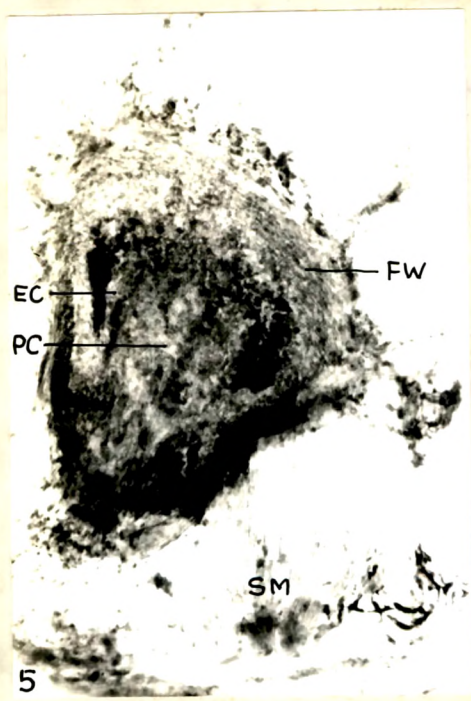

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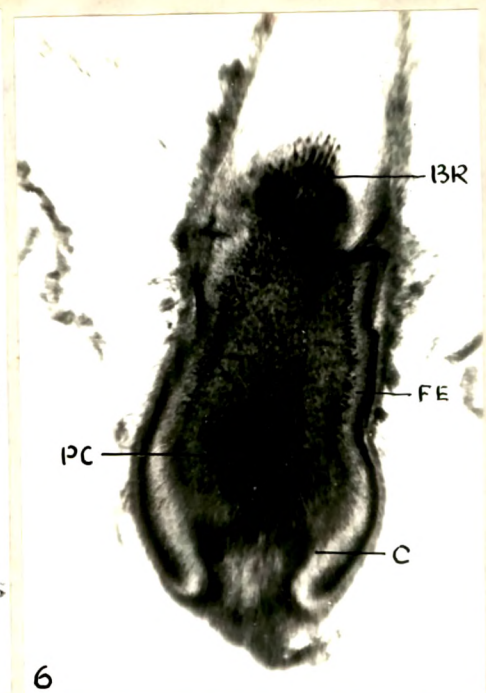

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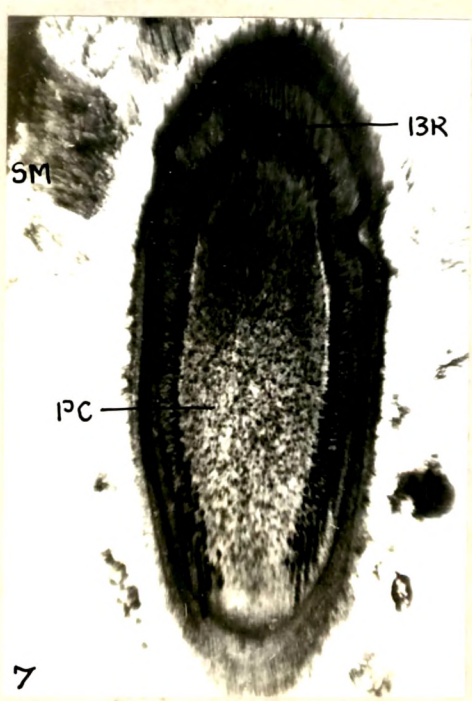

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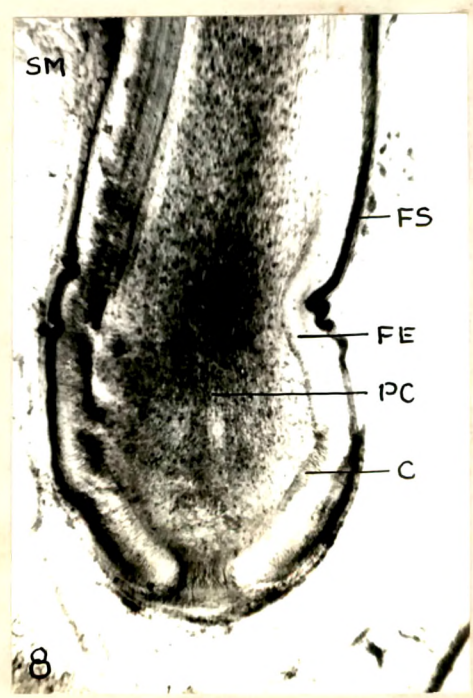

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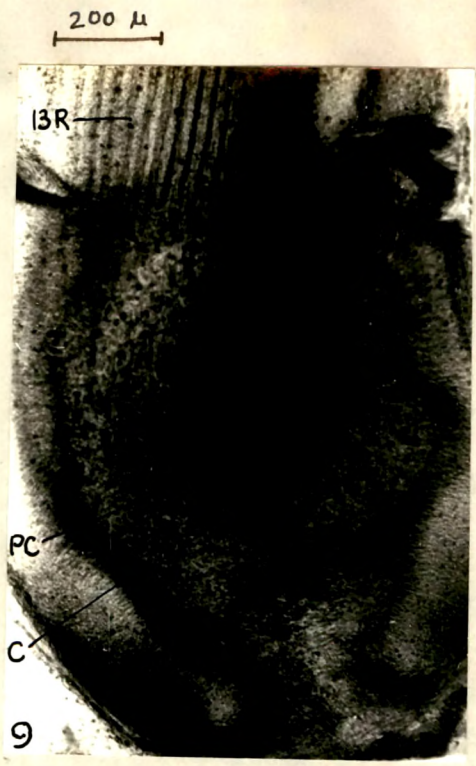
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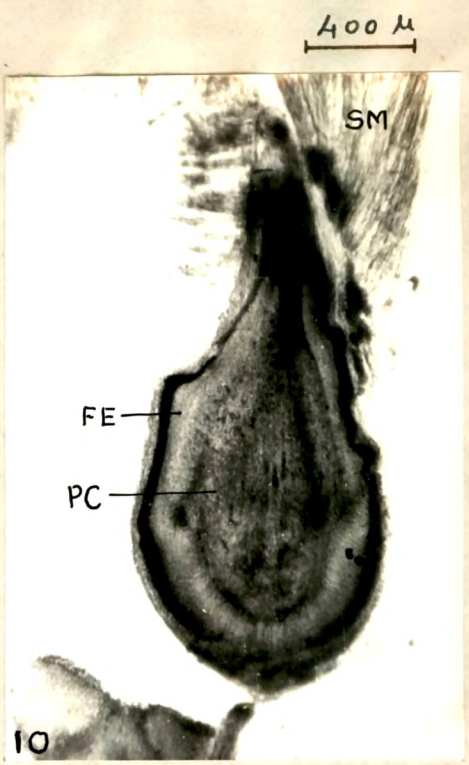
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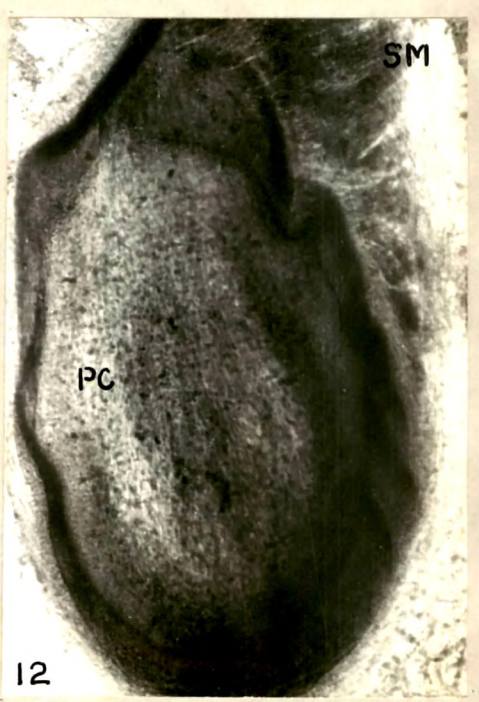
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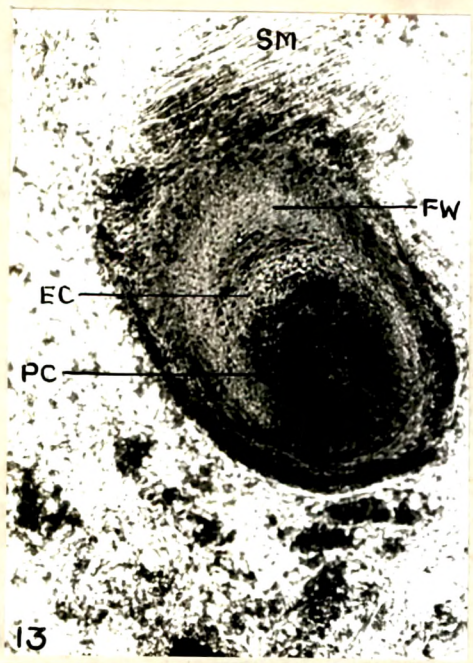

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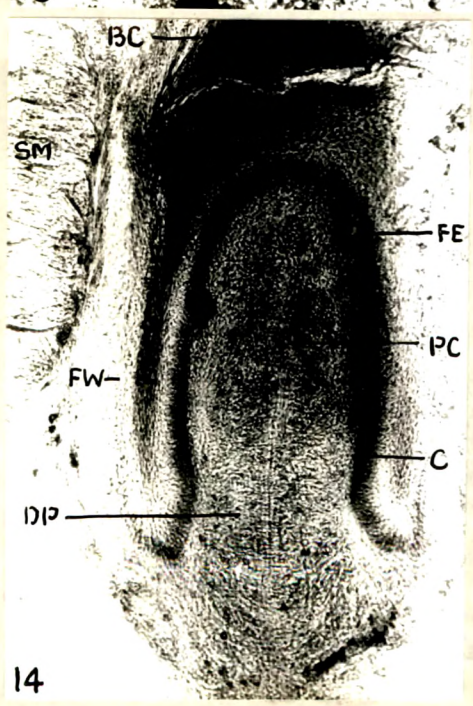

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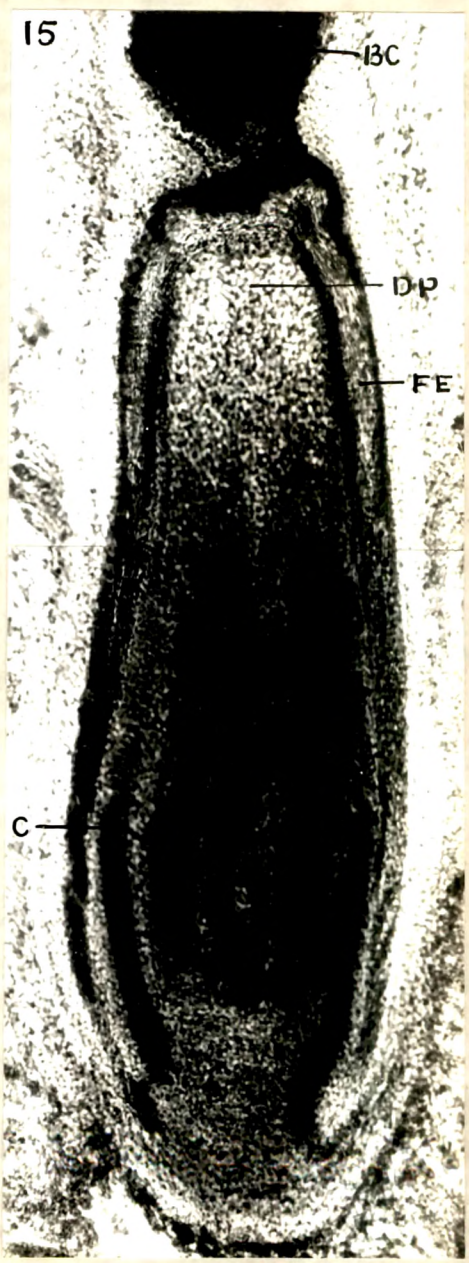

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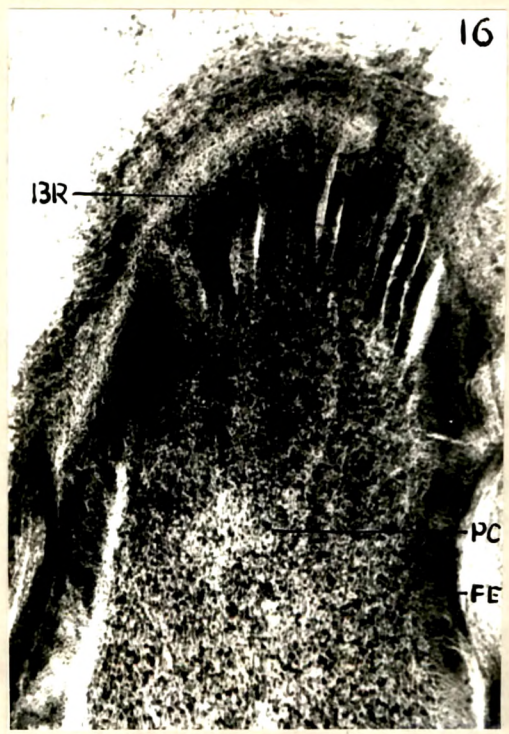

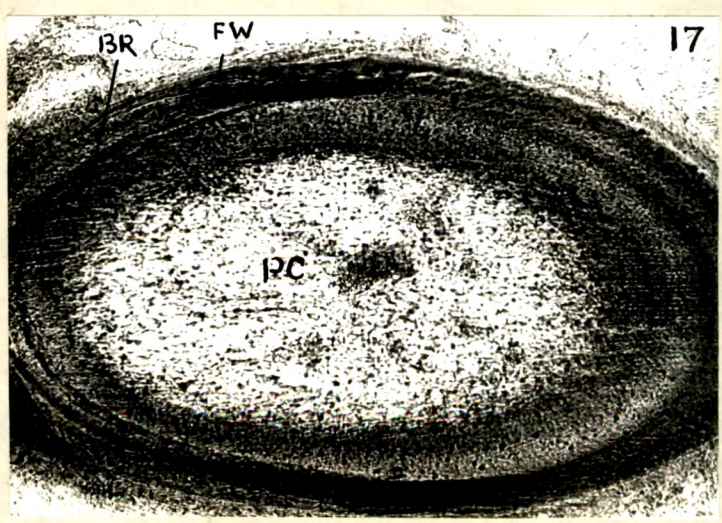

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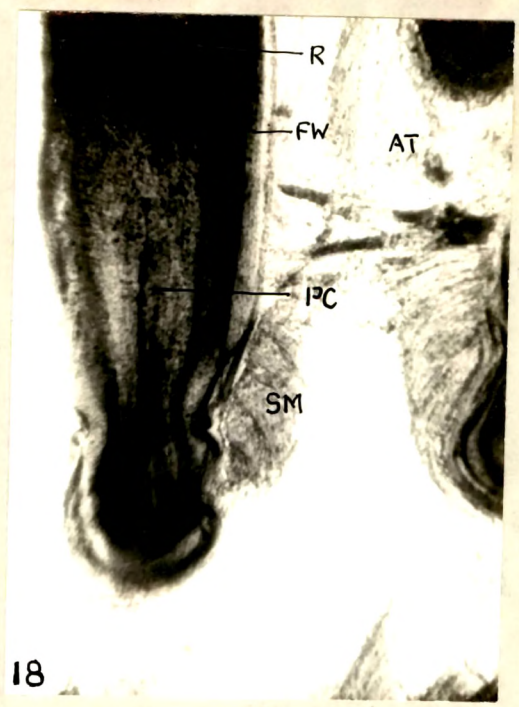

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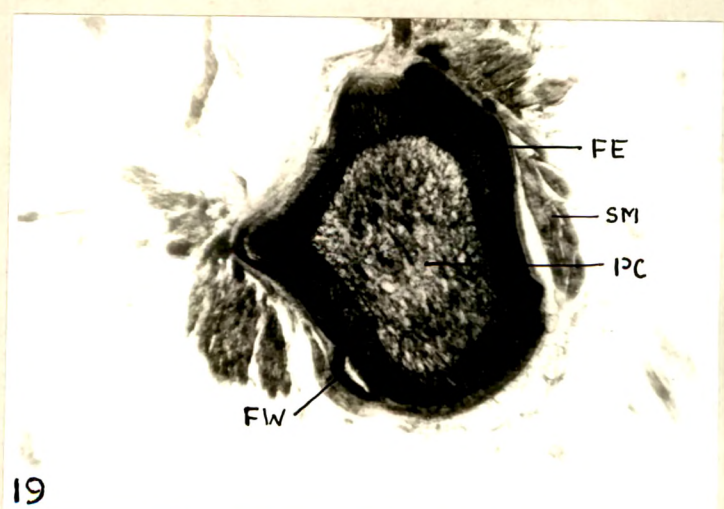

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But thereafter, there was again a rise in the enzyme activity in all the components of the fast developing and differentiating feather, reaching ultimately on the 25th day, a level which was higher than that observed on the 10th day. The appreciably high acid phosphatase activity that could be noted at this period of feather development was in the fast regressing pulp of the feather. This elevated level of enzyme activity was maintained in both the pulp as well as epithelial cells of the basal barb ridges of the differentiating feather till the completion of the process of keratinization of the feather and regression of the pulp on about the 35th day. The activity of acid phosphatase in the resting germ for the next generation of feathers which was organized by this time, was also of the same intensity as observed earlier in the resting germ of the definitive feather of the adult bird.

Induced development of definitive feathers: (Figs. 5-12)

A total loss of acid phosphatase activity in the germ within 15 hours was the immediate effect of plucking of fully grown feathers in an adult pigeon. This loss of enzyme activity was also observable in the epidermis and

dermis of neighbouring non-feather regions. But within the next 24 hours (of plucking) this fall in the enzyme activity was soon reversed by a gradual rise in acid phosphatase concentration in parts of the skin where the enzyme was normally found to occur. However, its activity in the germ was very high, being at its peak concentration. Such an enhanced level of enzyme activity remained till the 5th day when relatively its concentration was higher in the cells of the collar region; the differentiating barb ridges of the developing feathers recorded a diminishing activity while the pulp cells continued to present an unchanged high enzyme reactivity. This contrasting picture became most obvious by about the 7th day of induced development. But within the next three days there was a considerable overall reduction in the enzyme activity in all the components of the skin, eventually on the 10th day a level much lower than that observed in the corresponding parts of the normal adult feather was noticed. At this stage, the dermal components were almost devoid of acid phosphatase activity except for the pulpa of the developing feather which exhibited an unchanged moderate reactivity for the enzyme. The reduced level of the enzyme concentration was maintained till the 14th day whereafter there was again a gradual rise in the

enzyme concentration culminating ultimately in a high peak level, on 20th day. During this maximal enzyme activity period, the pulp region showed a relatively greater enzyme activity than the basal barb ridges. Such a high incidence of the enzyme lasted only for a shorter duration in the regions other than the base of pulp as indicated by a gradual drop in its activity in these regions by the 21st day and attained a low level between the 25th and 30th days. After this there was an increase in the enzyme activity, which, by 35th day reached to a level in the various components characteristic of the corresponding normal adult skin components, as by now the feather was fully grown and differentiated.

Regeneration: (Figs. 13–19)

As stated earlier (introduction), the regeneration of feather was induced by plucking 10 day old inducedly developing feathers. After plucking, there was a low but definite and noticeable increase in the acid phosphatase activity in the injured regions as compared to the feather parts before plucking. The fibrocytes in the dermis, the epithelial cells in the collar region of the injured feather, the pulp cells near the basal region and the phagocytes near

the blood clot formed at the tip of the injured feather stump were the components of the skin which showed the increase in the enzyme activity as mentioned above. However, the undisturbed basal region of the pulp maintained the same activity as was at the preplucking period.

The post injury increase in the enzyme concentration continued until it reached a short-lived peak value on the 7th day of feather regeneration, equalling almost three times the strength noticed in the resting feather germ. Connective tissue elements all over dermis and non-feather epidermis registered a high and moderate level of enzyme activity respectively. By this time, the differentiation of the barb ridges having commenced, the high enzyme activity was localized chiefly in the pulp cells and the barb ridges. This phase of high enzyme activity was soon followed by a gradual declining one, as a result of which on about the 10th to 12th day, the acid phosphatase level touched the low minimal level being slightly lower than that observed in resting germ cells. At this phase, among the extra follicular tissue components of the skin, only the fibrocytes of the dermal region registered a low but perceptible reactivity for acid phosphatase, all others remaining almost

negative for the enzyme response. With the progress of development of the regenerating feather, once again the acid phosphatase rose to a second high peak level in the cells of the pulp and barb ridges on the 15th day ~~equal and~~ identical to the one observed earlier on the 7th day, with the fibrocytes in the dermis also depicting a high enzyme reactivity. This time also the high peak level was short lived and the enzyme concentration tended to go down and reached a level characteristic of the resting feather germ and the corresponding parts of the normal adult skin, in the components of the feather and extra follicular tissues respectively, by the 20th day. Similar ~~values~~ levels of enzyme activity were observed even on the 30th and 35th day whence the feather had developed into a fully grown regenerate and a new resting germ had ~~been~~ organized.

DISCUSSION

Of the two phosphatases, viz., acid and alkaline, the acid phosphatase appears to be the normal complement of the adult pigeon skin from the presently observed higher activity of acid phosphatase in comparison to that of alkaline phosphatase (chapter 7). The suggestion of Moog (1965) that acid phosphatase is a constitutive enzyme

whereas alkaline phosphatase is an adaptive one seems to gain support not only by the above observation in the normal skin of the pigeon but also by the observations during the feather development wherein acid phosphatase appeared to be ~~the~~ more persistent and dominating, than alkaline phosphatase. In wake of the reported presence of acid phosphatase in the keratinized layers of the skin of amphibians, lizards, birds and mammals (Jarrett and Spearman, 1964) and its involvement in the process of keratinization (Novikoff, 1961; Mishima, 1964; Palade and Farquhar, 1965), the presently observed incidence of acid phosphatase in the normal adult pigeon skin becomes self explanatory. Though the enzyme has been found to be uniformly distributed in the skin of reptiles (Shah and Chakko, 1966; Radhakrishnan, 1972), in the case of pigeon skin it has been found to be unevenly distributed with the feather region showing a slightly higher activity than the nonfeather region. A probable explanation for such a difference in the pattern of enzyme distribution might lie in the asynchronous mode of moulting characteristic of avian integument wherein keratinized flakes of epithelium are cast off asynchronously from the nonfeather areas unlike some lacertilians or ophidians where the entire

body synchronously loses its keratinized epithelial covering in the form of a more or less single unit (Maderson, 1970). Another important aspect that merits consideration in this respect is the difference in the degree of keratinization and the type of keratin in the two distinct regions of the pigeon skin (viz., feather and nonfeather region). The very fact that there are two types of keratin identifiable in the pigeon skin i.e., the soft alpha variety and the hard beta variety in the nonfeather and feather region respectively (Baden and Maderson, 1970) appears to be in perfect accordance with the presently observed uneven distribution of acid phosphatase in the two above mentioned regions of pigeon skin.

During the course of all the three types of feather development investigated (i.e., normal, induced and regenerative) the activity of both the phosphatases (acid and alkaline) was found to follow a diametrically opposite pattern. Accordingly there were distinctly separate two periods during which acid phosphatase dominated and two periods whence alkaline phosphatase dominated. Coincidently, the periods between days 7th and 14th and 20th and 30th, when there was a higher incidence of acid phosphatase, corresponded

with the process of feather differentiation marked by differentiation of barb ridges and a high rate of keratinization. Similarly, the periods when alkaline phosphatase reactivity was high between the days 2nd and 10th and 15th and 25th corresponded with the active phase of growth during which there was a higher rate of cellular proliferations and a marked elongation of the feather. In this wake it may be assumed that alkaline phosphatase is involved in the process of cellular proliferation and growth while acid phosphatase is involved in the ultimate process of differentiation and keratinization. This assumption of the association of acid phosphatase with keratinization gains supports from the report of Braun-Falco and Rupec (1967) who found a positive correlation between keratinization and localization of acid phosphatase in the normal and psoriatic cornification of the human skin. Further, Shah and Chakko (1966) and Radhakrishnan (1972) have also observed a good correlation between this enzyme and the process of keratinization in the regenerating tail epidermis of the lizard, Hemidactylus flaviviridis and Mabuya carinata respectively. Moreover, the concomitant depletion of lipid contents corresponding to a high acid phosphatase activity when viewed in the

light of the reported role of lipids as prekeratin substances (Bell and Thathachari, 1963) or its involvement in the process of keratinization (Shibaeva, 1970) and the suggestion of the possible involvement of this enzyme in lipid metabolism (Schmidt, 1963) are highly noteworthy and indicative. However, certain other significant roles also could be attributed to acid phosphatase during feather development, such as synthesis of some specific proteins for its development and differentiation and a possible involvement in carbohydrate metabolism. It is interesting to note in this connection that Gasman (1967) has assigned a significant role for this enzyme in various synthetic and metabolic activities of the amoeba, Mayorella palestinensis. Further, acid phosphatase has been indicated not only in protein synthesis (Eränko, 1951; Pearse, 1960; Novikoff, 1961; Sood and Tiwari, 1969), but also in the synthesis and transport of carbohydrates (Sauter, 1967; Mishra and Mohanty, 1969).

The presence of acid phosphatase activity and its persistance between 20th and 30th days of feather development (post-hatching) in the mesenchymal pulp could be possibly correlated with its involvement in necrosis, as a simultaneous regression of the pulp was in evidence

during this period. Tissue regression as a normal phase in embryonic development has been well recognized, and increased acid phosphatase activity during this phase in a number of developing systems have been reported i.e., in the apical ectodermal ridge cells of the chick embryo (Jurand, 1964), in the larvae of the flesh fly, Sarcophaga during metamorphosis (Misch, 1962), during regression of mullerian duct in chick embryos (Brachet et al.,1968; Scheib, 1963; 1965), during metamorphosis of amphibian tadpole (Weber, 1963; Eckhout, 1965) and during insect metamorphosis (Rasch and Gawlik, 1964; Lockshin and Williams, 1964, 1965a & b; Mills and Lang, 1972). In the case of feathers, the mesodermal pulp which subserves a nutritive function during development, is known to undergo necrosis, once the feathers are fully grown (Voitękwiźch, 1960). Hence the presence of acid phosphatase in high concentration in the pulp towards the final stages of feather development, when there is a corresponding regression of the pulp, could be correlated with the degenerative phenomena of the pulp, which forms part of the totality of feather development. Though, as reported earlier, both acid and alkaline phosphatases are found to alternate in activity during the course of feather development, however, during this final phase, both are

found to be equally active in the mesodermal pulp. A possible explanation in this connection may be drawn from the hypothesis of Cristofalo et al. (1967) that an increase in phosphatase activity lowers the intracellular concentration of metabolically important phosphate esters, thereby gradually shifting the equilibrium in the cell from anabolism to catabolism. However, an alternate possibility that might be suggested (in the wake of the simultaneous presence of both the phosphatases) is that, whereas acid phosphatase is concerned with the lytic processes in the pulp, alkaline phosphatase is possibly involved in the disto-proximal transport of the left over biochemical molecules through the blood stream after feather development and differentiation is over. This assumption finds support in the report of Lillie (1940) about the beginning of events (regression of pulp) wherein he states that "..... a preliminary leucocytosis, but the first action may be autolytic. Presumably the next step is an extensive phagocytosis followed by a restoration of products of resorption to the blood stream". A factor of obvious importance in this connection is the observation that a new resting germ is not formed till the pulp is ~~not~~ regressed.

Finally, though the initial drop in acid phosphatase activity noted within 15 hours after feather removal in both induced as well as regenerative development, could be attributed to a mechanical and physiological shock, the increased level of the enzyme activity between 24 and 48 hours post plucking might well be connected with the processes of germ activation and repair as well as enhanced metabolic activities attributable thereto, respectively.

The overall pattern of acid phosphatase activity during the development of definitive feathers under the three different conditions, does not manifest any striking differences. However, an earlier attainment of peak acid phosphatase activity and its prolonged sustenance during induced and regenerative modes of development of the feather, cannot be overlooked. This might be looked upon as biochemical adaptations at cellular level to help complete the process of feather development in the same period as that for normal development, keeping allowances for the possible loss of time in the process of activation of inactive and unprepared germ in the case of induced development and or the reparative processes in the case of regeneration.