

## CHAPTER 7

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

Alkaline phosphatase has been associated with a number of activities such as the formation of fibrous proteins and passage of metabolites across cell membranes (Verzar and McDougall, 1936; Moog, 1946; Bradfield, 1950); calcification of bones (Robison, 1923; Moog, 1944; Pritchard, 1952); and phosphate transfer in DNA metabolism (Rogers, 1960). Besides, alkaline phosphatase has also been implicated in the organogenesis of chick and mouse intestine (Moog, 1950, 1951); digestive system of steel head trout (Prakash, 1961); avian brain (Rogers, 1963); lungs of guinea pigs and rats (Sorokin *et al.*, 1959) and oesophagus and trachea of chick (Hinch and Buxbaum, 1965). These reports showed that increased alkaline phosphatase activity preceded the onset of function of the organ in question. Compared to the bulk of information available on alkaline phosphatase activity in the developing visceral organs, that on the vertebrate integument is relatively less. Notable amongst them are those of Glener and Burstone (1958) on the dermis of Necturus maculosus; of Schmidt and

Weary (1962) on the skin of Diemictylus viridescens;  
Fell and Danielli (1962) on the skin wound of mammals  
and of Hamilton (1955 and 1965) on developing down  
feathers in birds, it was thought desirable to investigate  
the role of alkaline phosphatase in a comparative way  
during the development of definitive feathers in pigeon  
under three different conditions as specified earlier.

#### OBSERVATIONS

##### Normal adult skin: (Fig. 1)

Unlike acid phosphatase, alkaline phosphatase was noted to be absent in almost all the components of the non-feather region of pigeon integument. The only component that evoked a positive response in this region of the skin were the walls of blood vessels lying in the dermis. In the resting feather germ, the enzyme was found to be chiefly localized in its mesodermal components. Amongst the epithelial cells, a comparison easily revealed the epithelial cells at the collar region to be relatively more enzyme reactive than those lying below the incipiently keratinized layer.

Normal post-hatching development: (Figs. 2 - 9)

At the time of hatching, the first generation of definitive feathers which were found to be in a state of active growth within the follicles, registered an appreciable histochemical response for the enzyme. This enzyme response could be well evidenced in the mesenchymal pulp cells, with the epithelial cells covering them also depicting a though<sup>low</sup> nevertheless perceptible enzyme activity. Amongst the epithelial cells, those of the collar region were comparatively more alkaline phosphatase reactive. By about 24 hours, there was a gradual increase of alkaline phosphatase activity in all the components of the developing feather attaining ultimately, the first peak level on the 5th day, (almost 3 times the level observed in the resting germ of adult feather) a period when there was a visible differentiation of the barb ridges. However, the enzyme concentration in the non-feather regions of the skin remained almost negligible.

Hereafter with the emerging out of the first generation of definitive feathers from the follicles, pushing out the natal down, there was a gradual diminution in alkaline phosphatase activity which by about the 10th day post hatching in the various components reached a low

## EXPLANATIONS FOR FIGURES

- Fig. 1 LS of a normal adult feather follicle showing alkaline phosphatase activity. Note enzyme reactivity of the pulp.

## NORMAL DEVELOPMENT

- Fig. 2 Section of skin on day of hatching. Note enzyme activity in the developing feather.
- Fig. 3 LS of follicle on the second day, depicting the enzyme activity.
- Fig. 4 TS of developing feather on 10th day post-hatching depicting peak enzyme activity.
- Fig. 5 Oblique section of feather on 10th day post-hatching, Note the decline in enzyme activity.
- Fig. 6 LS of 15 day old feather. Note peak enzyme activity in pulp and epithelial cells of collar and barb ridges.
- Fig. 7 Enzyme activity in LS of 20 day old feather.
- Fig. 8 Oblique section of 25 day old feather. Note enzyme activity in cells of regressing pulp.
- Fig. 9 Alkaline phosphatase activity in 30 day old feather follicle.

## INDUCED DEVELOPMENT

- Fig. 10 Enzyme activity in the feather germ on 1st day after plucking.

- Fig. 11 LS of developing germ on the second day after plucking. Note the enzyme activity in epithelial cells.
- Fig. 12 LS of 5 day old inducedly developing feather showing enzyme activity. Black granules are due to melanin.
- Fig. 13 LS of base of 10 day old feather showing alkaline phosphatase activity in collar and pulp cells.
- Fig. 14 Oblique section of 14 day old feather. Note the decline in the enzyme activity.
- Fig. 15 Oblique section of 25 day old feather. Note enzyme activity in the pulp cells.
- Fig. 16 LS of base of the feather on the 30th day. Note the enzyme activity in dermal papilla and collar region.

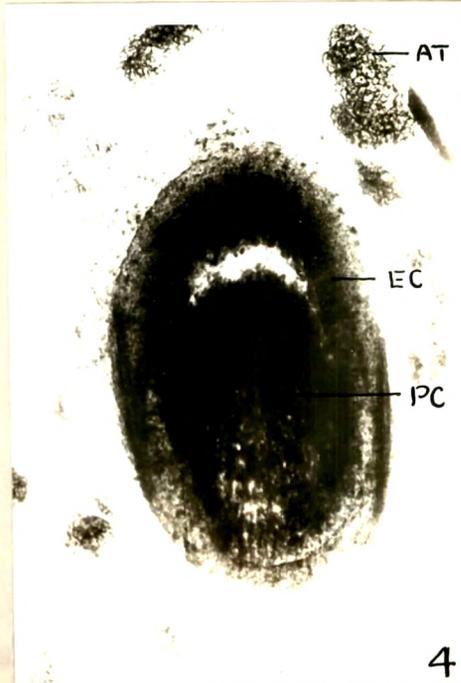
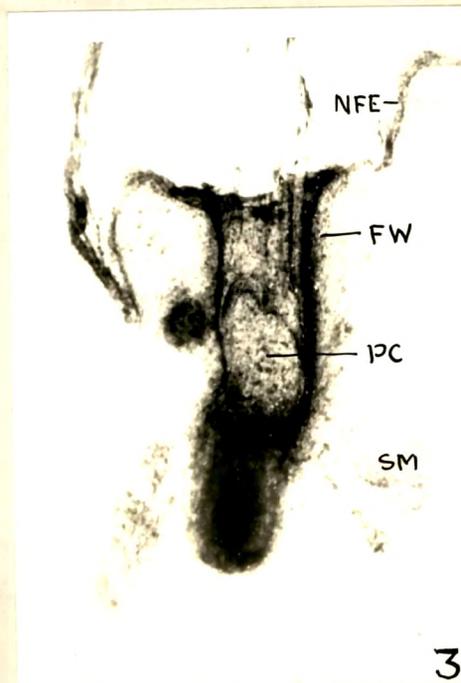
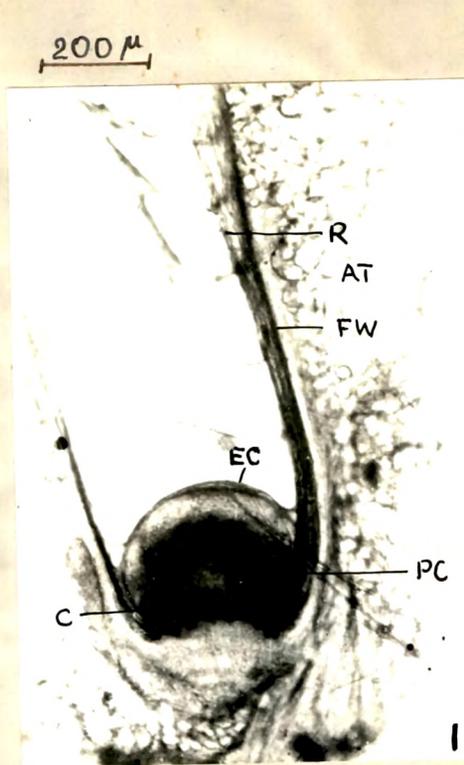
#### REGENERATION

- Fig. 17 LS of follicle on 1st day after inducing regeneration showing the enzyme activity.
- Fig. 18 LS of 3 day old regenerate depicting high enzyme activity in pulp and epithelial cells.
- Fig. 19 Section of 5 day old regenerate. Note the enzyme activity in pulp cells and barb ridges.
- Fig. 20 Section of 12 day old feather regenerate showing declined enzyme activity.

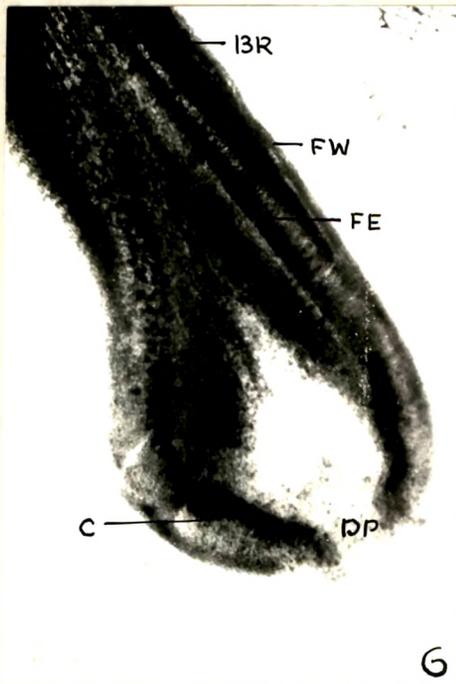
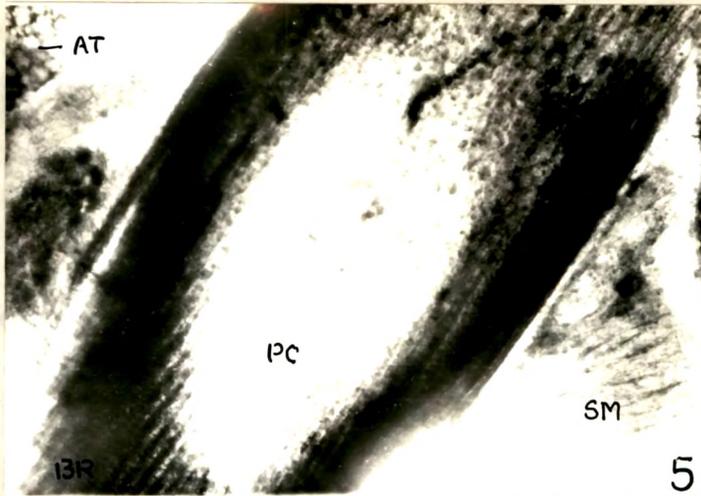
- Fig. 21 Section of feather on the 15th day regeneration.  
Note activity in pulp and feather epithelium.
- Fig. 22 TS of feather on 25th day of regeneration. Note  
enzyme activity in cells of regressing pulp.
- Fig. 23 LS of base of 30 day old feather showing the  
enzyme activity.

## ABBREVIATIONS

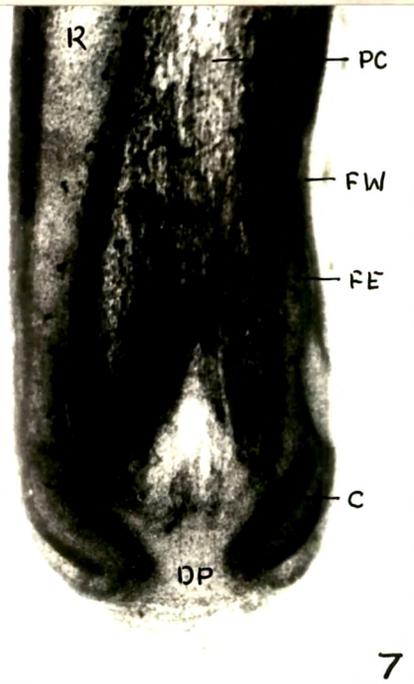
- BC - Blood clot  
BR - Barb ridges  
C - Collar  
DDF - Developing definitive feather  
DP - Dermal papilla  
FE - Feather epithelium  
FS - Feather sheath  
FW - Follicular wall  
MC - Melanocytes  
NFE - Non-feather epidermis  
PC - Pulp cells  
RP - Regressing pulp  
R - Rachis  
SM - Smooth muscles



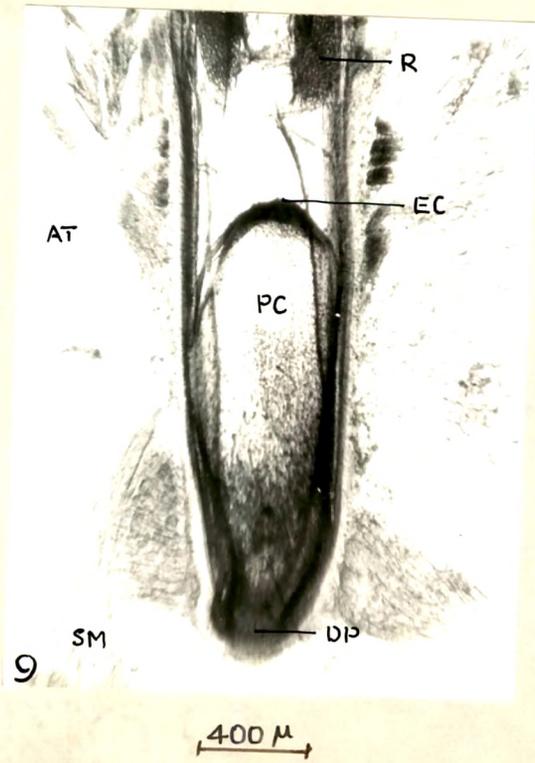
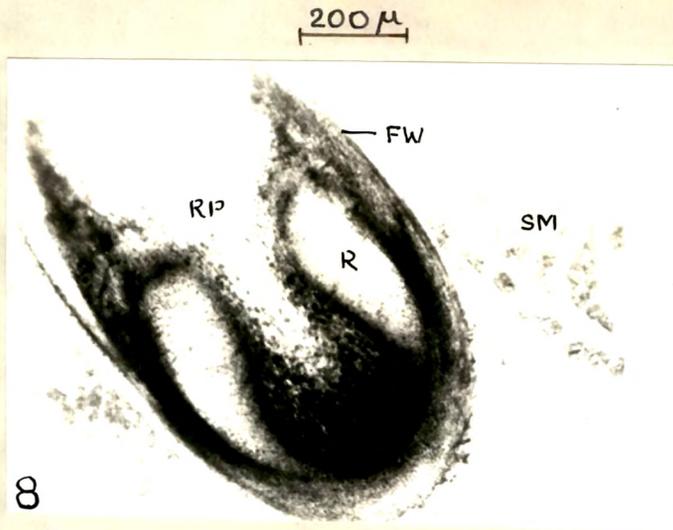
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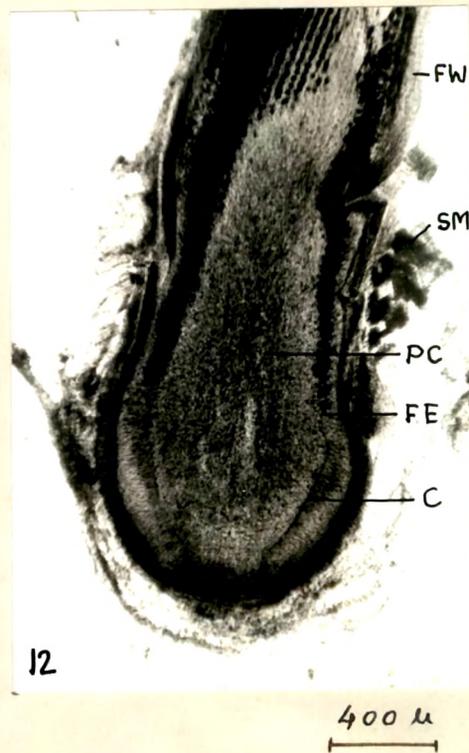
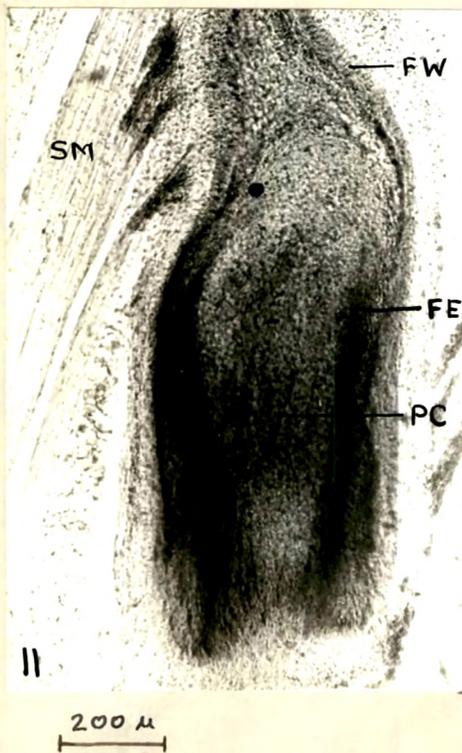
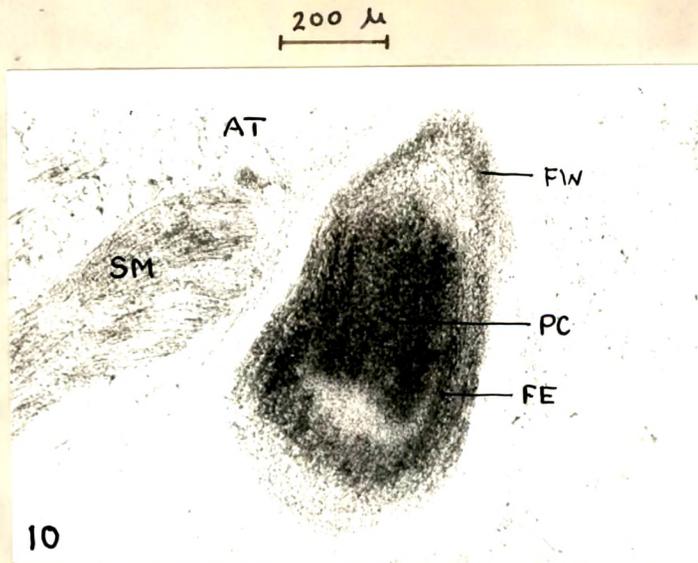


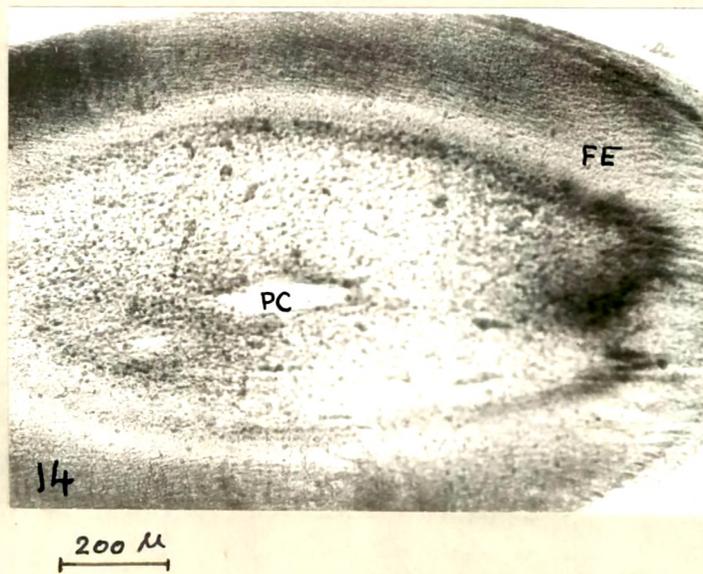
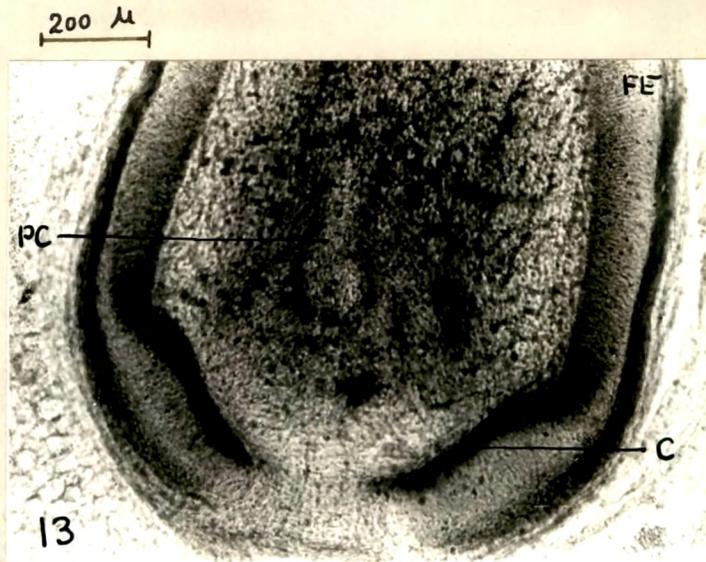
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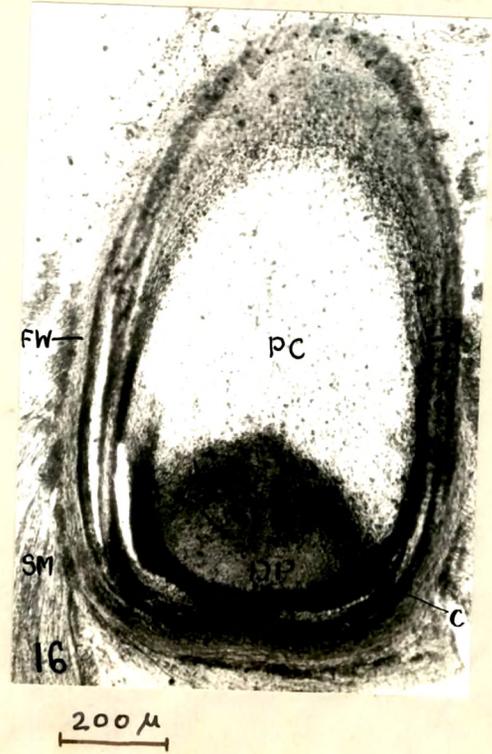
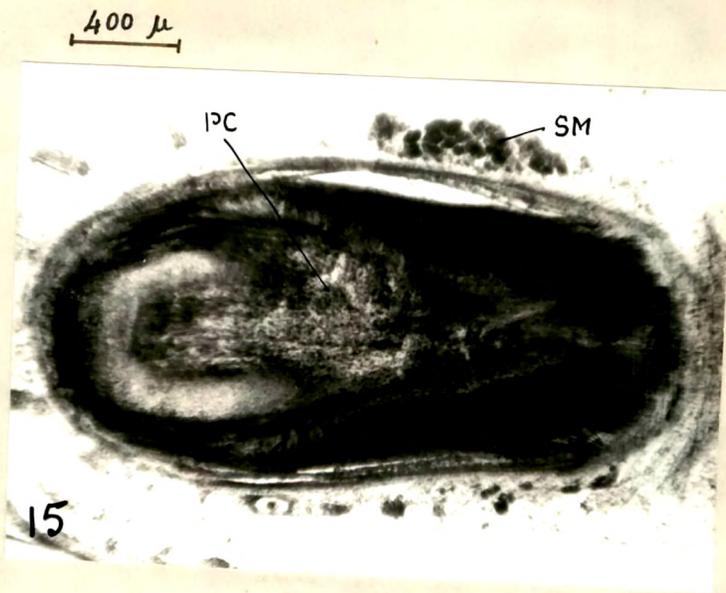


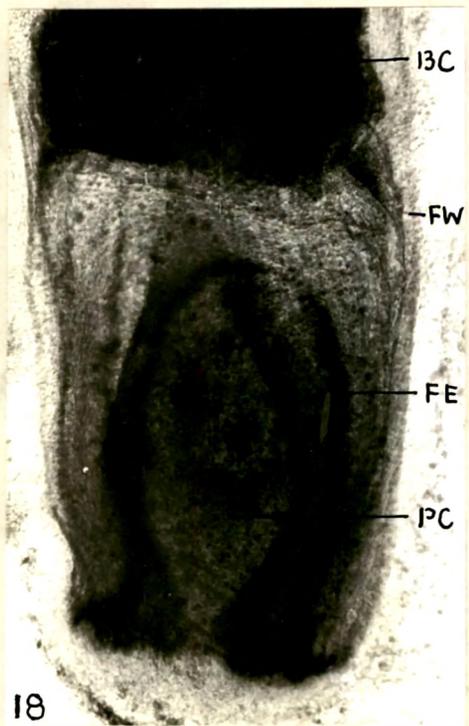
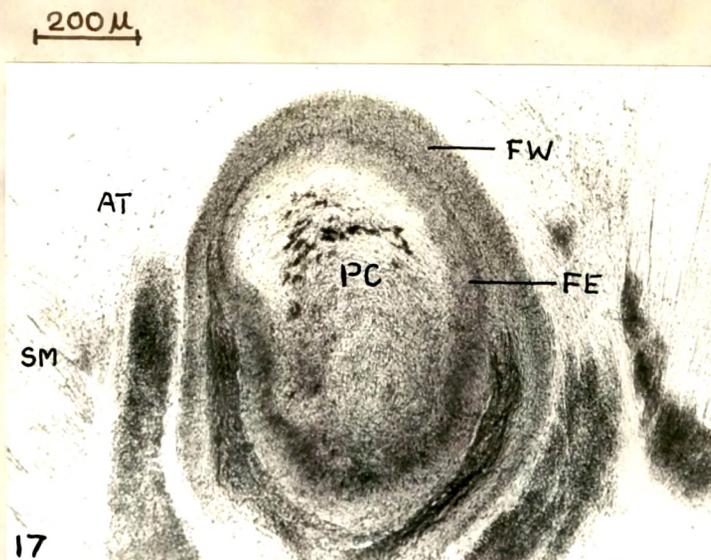
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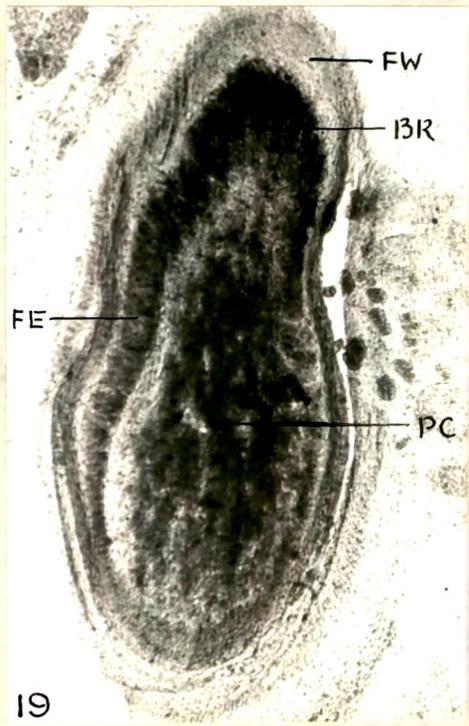




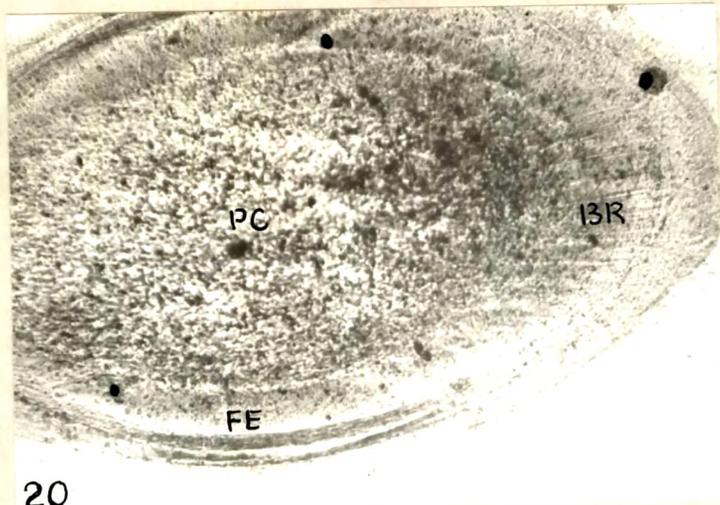




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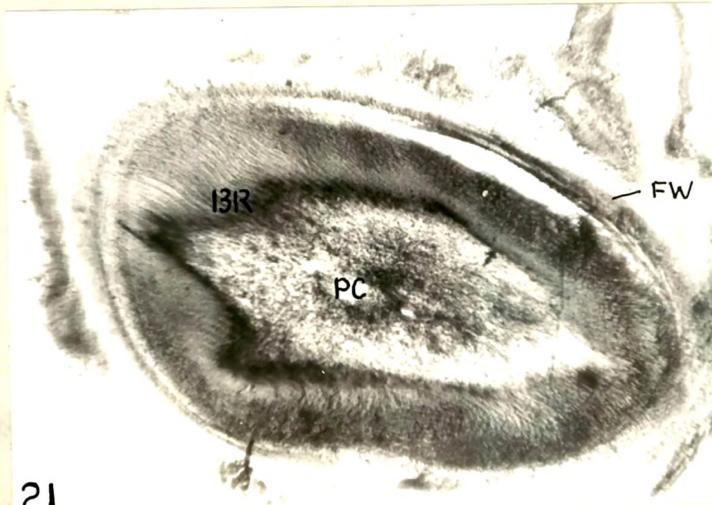


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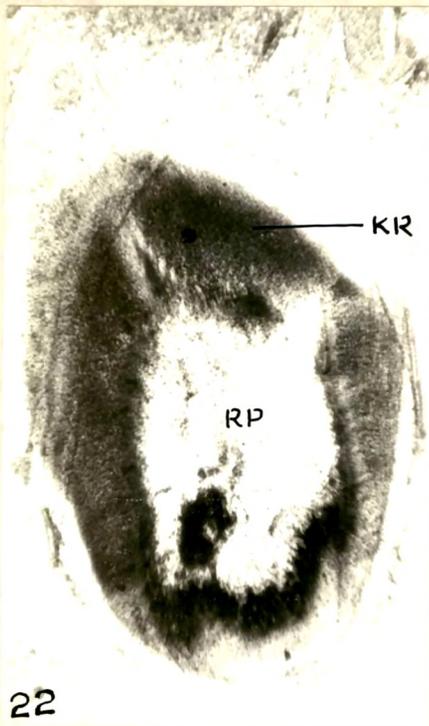
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level characteristic of the cells of the resting germ of the adult feather. The process of keratinization of the barb ridges was evident by this time and keratinized epithelial cells were negative for enzyme reactivity. However, by about <sup>the</sup> 13th day there was again a spurt in alkaline phosphatase activity, culminating once again in a second peak level (as high as was observed during the first peak period) by about <sup>the</sup> 15th day, a stage when active keratinization is the predominant feature, in the various components of the developing feather excepting for the epidermal cells of the follicular wall ~~all~~ where the enzyme activity tended to remain low. This high level of enzyme activity was so retained even up to the 30th day when the regression of pulp is in an advanced stage, to be followed thereafter by a gradual decrease which with the completion of the process of normal feather development on the 35th day settled down to the normal adult level.

Induced development: (Figs. 10-16)

The cells of the feather germ (which under normal conditions would have given rise to the next generation of feathers during normal moulting) recorded a drop in alkaline phosphatase activity, 15 hours after plucking

the adult definitive feathers which were not in any state of moulting. Though there was a slight increase in enzyme response by about 24 hours, a level noticeably above that observed in the resting germ, was attained in the cells of the pulp and epithelial cells only on the 2nd day.

Hereafter, with gradual increase in enzyme activity, the first high peak of alkaline phosphatase activity was reached in the above mentioned components of the feather on the 5th day and thereafter remained at the same level till the 7th day of induced development. During this period a comparison obviously revealed the pulp cells to be more enzyme reactive than the epithelial cells which are already getting organized into barb ridges. However, even amongst the epithelial cells themselves, a distinct gradation in the enzyme concentration could be noted, with the cells at the collar region depicting relatively more enzyme concentration than those at other regions of the feather.

This was followed by a phase of gradual fall in enzyme activity. Whereas this gradual fall in enzyme activity was immediate and became well evident only by about the 14th day. At this stage, (14th day) the

alkaline phosphatase activity in the various components of the developing feather touched the low normal level characteristic of the resting feather germ. But soon after (within 24 hours), there was again a gradual increase in the enzyme activity in the various components of the developing feather attaining the second peak level by about the 20th day which, after lasting for a short while started once again to decline by about the 25th day, a stage when regression of the mesenchymal pulp was evident, ultimately reaching the low normal adult level by the 30th day of development, whence the feather was fully grown and differentiated.

Regeneration: (Figs. 17-23)

24 hours after plucking the 10<sup>th</sup> day old inducedly developing feather stubs, a marked decrease in enzyme activity could be observed in the cells of the injured feather and its follicular wall epithelium. However, a high peak of enzyme activity (three times the level observed in the resting germ) was recorded in all parts of the healed feather blastema by the third day itself, by which time the feather was in the initial phase of regeneration. This high level of enzyme activity lasted uptill the 5th day and thereafter, receded to a low

level towards the 7th day of regenerative development when clear evidence for the organization of epithelial cells to form barb ridges was discernible in the developing feather. But immediately after this there was again a rise in the enzyme activity which by the 10th day rose to a second high peak level (threefold that of the resting germ) only to be followed by a decline to a low level of activity on the 12th day. After remaining so for a few days, there was again a discernible increase in alkaline phosphatase activity which became evident by the 15th day of regeneration. This increased phase of enzyme activity lasted upto 20th day and thereafter it gradually regressed to settle down to the normal adult level by about the 30th day by which time the feather was fully regenerated.

The observations recorded on alkaline phosphatase during three different modes of feather development denoted an interesting disparity by the attainment of a peak level activity thrice during regeneration in contrast to the attainment of such a level twice during the other two modes <sup>of</sup> development.

## DISCUSSION

Koning and Hamilton (1954) reported the presence of several enzymes including alkaline phosphatase in the mesenchymal pulp cells of the chick down feather during its morphogenesis and growth. They had observed a large concentration of RNA and concomitant high activity of alkaline phosphatase in the pulp cells adjacent to the epidermal collar where greatest morphogenetic activity existed. The present study revealed that in the resting feather germs of adult skin, alkaline phosphatase activity is low in comparison to that of acid phosphatase (chapter 8). However, throughout the course of normal post-hatching development of the first generation of definitive feathers, alkaline phosphatase activity was perceptibly higher than that of acid phosphatase. But during induced and regenerative modes of development in general, alkaline phosphatase tended to remain less active in comparison to acid phosphatase. The presently noted fluctuations in the alkaline phosphatase activity in the definitive feathers of pigeon, developing under the three conditions, appear to be in accordance with the findings of Moog (1950) and Prakash (1961) of such a fluctuating pattern of the enzyme activity during development of the alimentary canal of chick and steel-head trout respectively.

In the case of feathers too, alkaline phosphatase showed periods of maximal and submaximal activity corresponding to periods of active growth and differentiation. The high incidence of alkaline phosphatase noticed in the pulp and epithelial cells of the growing feathers during the initial phases (as per observations) in all the three types of development seems to be suggestive of a significant role for this enzyme in the early stages of feather development. The development of integument is known to involve three concomitant processes, viz., growth, differentiation, and organogenesis of its derivatives. According to Jacobson (1966), the third one is dependent upon the former two processes and if either of them is blocked, organogenesis would be inhibited. A similar and closely related suggestion is that of Hamilton (1965) who by his studies, showed that inhibition of alkaline phosphatase at a stage when the growing feather appeared outside the follicle could interrupt down feather development, thus establishing the importance of alkaline phosphatase in growth and differentiation. In this light, the presently observed higher incidence of alkaline phosphatase during the later half of the first week of

definitive feather development <sup>is</sup> in that it coincides with <sup>x</sup> the commencement of differentiation of primary barb ridges and emergence of the feather outside its follicle, which is mainly due to the process of active growth. Growth and differentiation which are interdependent during feather development, and which involve the rapid cell proliferation and synthesis of keratin respectively could be expected to have an increased demand of nucleic acids. It is pertinent in this context to note that Rogers (1960) has hypothesised the possibility of alkaline phosphatase playing a role in phosphate transfer in DNA metabolism. Further, the potential importance of G6PDH and HMP shunt in the generation of NADPH<sub>2</sub> and ribose phosphate essential for nucleic acid synthesis cannot be overlooked. It is in this light that the concurrent high activity of G6PDH (chapter 2) during this period (later half of the first week of development) gains significance and validity. It is again of interest that Reichard (1960 and 1961) has shown an NADPH dependent mechanism for the direct conversion of ribonucleotides to deoxyribonucleotides in chick embryos. In the wake of the above facts and observations, it is tempting to suggest the possibility of the existence of an interrelation of G6PDH and alkaline phosphatase during feather development

in the synthesis-reduction reactions and transfer mechanisms respectively connected with nucleotide and nucleic acid metabolism. The present observations gain further validity by the reported observations of Ehlers (1970) of a simultaneous occurrence of alkaline phosphatase and G6PDH in the cells of stratum germinativum and the superficial layer of <sup>the</sup> cornea in a number of mammals.

Again, the possible involvement of alkaline phosphatase in mucopolysaccharide synthesis, important for the laying down of structural elements of tissues, also appears to be probable during this active phase of feather growth, as such a function has been hinted by Kroon (1952); according to whom mucopolysaccharides could be synthesised from hexoses liberated by the action of phosphatases from hexose phosphate esters reduced during glycolysis. Ten Cate (1956) has also implicated the activity of alkaline phosphatase in the formation of mucopolysaccharides.

Another possible role of alkaline phosphatase that deserves consideration in the light of the observation of its localization in the highly vascularised pulp known to have a nutritive function, is in the transport of metabolites. It may be noted in this connection that

Raekallio (1970) has opined the functional correlation of alkaline phosphatase with transport of metabolites across cell membranes, to be the most acceptable theory to date. However, involvement of alkaline phosphatase in transport of glucose has been pointed out also by Anagnostopoulos and Matsudaira (1958). Taking all these facts into account, it could be surmised that glucose brought in by blood to the pulp cells of the growing feather is phosphorylated through the mediation of alkaline phosphatase present there, enhancing its entry into the epithelial cells. However, since the process of differentiation (keratinisation) also commences before the completion of <sup>the</sup> active growth phase (5th day onwards), a possible involvement of alkaline phosphatase in the initiation of the process of keratinisation also cannot be overlooked. Moreover, an association between the enzyme and keratinisation has been drawn by a number of workers in the regenerating amphibian and reptilian appendages (Schmidt, 1969a; Shah and Chakko, 1966; Radhakrishnan, 1972). Nevertheless, it could be presumed that alkaline phosphatase plays a multifarious role during the initial phase of feather development whence there is a concomitantly high rate of cellular proliferation and growth. This assumption gains

credibility not only by the reduced activity of this enzyme between <sup>the</sup> 10th and <sup>the</sup> 14th days corresponding to a phase of decreased growth and active differentiation, but also by the increased activity of the enzyme noticeable by about the 20th day whence once again there is an active growth of <sup>the</sup> feather. Incidentally, the period of reduced alkaline phosphatase activity was marked by a phase of increased acid phosphatase activity. It is of further interest that reduced alkaline phosphatase activity was also paralleled by decreased lipid content (chapter 5). This observation finds support from the contention of Morton (1954) that alkaline phosphatase of vertebrate integument may be existing as lipoprotein complexes where lipids serve as carrier molecules.

From the observations, it becomes evident that the pattern of duration of enzyme activity in the normally developing feather is somewhat different from those during induced and regenerative development of <sup>the</sup> feather. Further, the time of feather emergence from the follicle varies slightly during regeneration from the other two conditions. These variations corresponded well with the varying duration of the enzyme activity. Just after hatching, on the first day, the developing definitive feathers of the

first generation showed a level of alkaline phosphatase activity which was slightly above that observed in the resting feather germ of adult skin, compared to that of a reduced level on the first day in the germ which is induced to develop. However, such a low level of enzyme activity was not discernible on the first day of regeneration. These observations could be explained on the ground that the germ of the normally developing feather was already in a state of growth; the germ induced to develop by plucking the adult feather was not physiologically prepared for growth, but was activated only on such plucking which resulted in the necessity to have an increased enzyme activity. However, for inducing regeneration, it was the growing feather with an already geared up enzyme machinery which was plucked and hence the enzyme concentration on the first day of regeneration was not as low as that at the same time during induced development. Therefore, it could be surmised that plucking adult feather stimulates increased activity of alkaline phosphatase, which may play a role in inducing further development and growth of the feather. This contention finds support in the work of Voigt<sup>e</sup>kwich (1960) who considered alkaline phosphatase to be an activator substance in the growing feathers.

Whereas the 2nd and 3rd high peak levels of alkaline phosphatase activity noticed during regeneration corresponded well with the 1st and 2nd peaks observed during normal and induced developments of the feather and may be viewed as important for the synthetic reactions that occur prior to and during active cellular proliferation and growth, the first peak <sup>of</sup> enzyme response attained during regeneration (on the 3rd day) fails to have any parallels during the other two modes of development and may be considered to be of significance in the process of wound healing of the injured feather whence there is a high rate of epithelial cell proliferation.

Thus, it could be surmised that though in general, the basic pattern of fluctuations of the enzyme activity is almost similar under all three modes of development of feather, only the duration of peak level of the enzyme activity is considerably shortened during the induced and regenerative modes of development. A logical explanation for this incidence could be obtained from the considerably low acid phosphatase activity noted during normal development as compared to the other two (chapter 8). This might imply that during normal post-hatching development of pigeon

definitive feathers, the various functional events such as synthesis, transport of substances and other metabolic regulations and activities requiring the mediation of phosphatases depend to a great extent on alkaline phosphatase and that during the other two types of development, such functions are jointly mediated by both the phosphatases.

Finally, the continued higher incidence of alkaline phosphatase together with acid phosphatase <sup>(chapter 8)</sup> in the regressing pulp is a little intriguing and thought provoking, and in the light of the known involvement of the enzyme in transfer mechanisms, and the suggestion of Lillie (1940) that during the process of pulp regression there is a process of resorption of materials into the blood stream; it may be presumed that alkaline phosphatase might somehow be functioning as a carrier or transporting medium, in the resorptive mechanics and or, in the disto-proximal transport along the regressing pulp of probable left over materials after feather growth and differentiation, possibly informative biochemical molecules of importance (in the finer and final aspects of growth and differentiation) to be made available to the future resting germ to be formed soon.