CHAPTER 4

ON THE ACTIVITY AND ROLE OF PHOSPHATASES (ACID AND ALKALINE) IN THE DEVELOPING PIGEON GIZZARD: A HISTOCHEMICAL INVESTIGATION

Phosphatases have drawn considerable attention in recent times due to their purported importance in different aspects of cell metabolism. The association of alkaline phosphatase (Alk Pase) with the formation of fibrous protein has, by now, been a well established fact (Verzár & McDougal, 1936; Moog, 1946). It has also been ascribed the function of helping the passage of metabolites across the cell membrane (Bradfield, 1950; Danielli, 1954). Cori & Cori (1952), Cushworth (1958), Duncun (1959) and Rosenthal et al., (1960) have related the genetic factors with the biosynthesis of phosphates in diseases such as hypophosphatasis and glycogen storage disease which are characterised by phosphatase deficiency. A correspondence between acid phosphatase (Acid Pase) activity and protein synthesis has been suggested by Vorbrodt (1958). Histochemical distribution of phosphatases has been demonstrated in a wide variety of tissues of vertebrates such as amphibians (Schmidt, 1963; Schmidt & Weary, 1963), reptiles (Shah & Chakko, 1966; Radhakrishnan, 1973), birds (George & Pishawikar, 1961; George et al., 1958; Vallyathan & George, 1965; Khan &

George, 1967) and mammals (Moog, 1951,1953,1961; Hugon & Borgers, 1966, 1967, 1968a). The operation of two types of phosphatases in animal tissues is, by now, well established wherein one (Acid Pase) hydrolyses phosphate esters optimally in an acidic environment and the other (Alk Pase) hydrolyses phosphorylated compounds in an alkaline medium. Acid phosphatase has attracted particular attention since its identification with other lysosomal hydrolases (de Duve, 1959; Novikoff, 1961, 1963). The molecular localization of this enzyme has also been brought to light by Wolf et al. (1943) in the muscle tissue, Becket & Bourne (1958) too in their studies on normal and diseased muscle noted a granular enzyme response at the poles of the nuclei. Excepting for the studies of Prakash (1961 on fishes, Moog (1944, 1950), Moog & Richardson (1955) and Hugon & Borgers (1969) on birds and Moog (1951, 1953, 1961); Hugon & Borgers (1966,1967,1968a) on mammals, there is only scanty literature available on the role of these two enzymes in the alimentary canal of vertebrates especially those of aves and particularly those of developing (post-natal) pigeon. It is in this light that it was considered pertinent to undertake the study on the histochemical distribution of acid and alkaline phosphatases in the developing (post-natal) pigeon gizzard so that not only the role of these two enzymes could be understood in

the gizzard, a very important component of the alimentary canal of birds, but also the histochemical and biochemical differences if any between striated and smooth muscle.

MATERIALS AND METHODS

Healthy young pigeons of different age (in days) of post-hatched development and adult pigeons from a well maintained aviary were used for the present study. Both young and adult pigeons were decapitated under mild anaesthesia and the gizzard was immediately separated, blotted well to remove the contents, blood and other tissue fluids and fixed on a chuck of a cryostat microtome maintained at -20°C. Frozen sections of 10-15 μ thickness were cut and fixed either in 10% neutral formalin (Pearse, 1960) for one hour or in cold acetone (Burstone, 1962) for 20-30 minutes. They were then washed thoroughly in several changes of distilled water and incubated for 10-15 hours in the following media as suggested by Burstone (1962).

Ingredients	Acid Pase	Alk Pase
Naphthol AS-BI, Sodium salt (substrate)	1 mg	--
Naphthol AS-MX, Sodium salt (substrate)	--	1 mg
Red Violet LB (Diazonium salt)	6 mg	6 mg

Ingredients	Acid Pase	Alk Pase
Tris Buffer, 0.2 M, pH 9.1	--	5 ml
Acetate Buffer, 0.2 M, pH 5.2	5 ml	--
Distilled water	5 ml	5 ml

After incubation, the sections were washed thoroughly in several changes of distilled water and mounted in glycerine jelly.

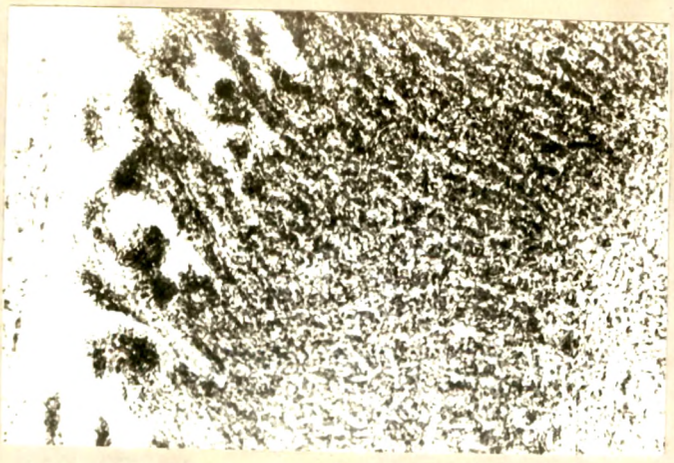
Control:- Sections incubated in a medium devoid of specific substrates served as respective controls. Few sections were heated in water to 80°C for about 5-10 minutes and they were incubated in a medium identical for the samples, also served as controls.

OBSERVATIONS

(Figs. 1 to 11 and 1a to 11a)

During the different periods of post-hatched development of pigeon gizzard, the phosphatases in the acid range showed a recurring fluctuations in their concentration while that in the alkaline range showed no such fluctuation but a gradual decrease in its concentration. On the day of hatching, a period when the squabs were fed with 'pigeon milk', both the phosphatases were in low concentration. But on the

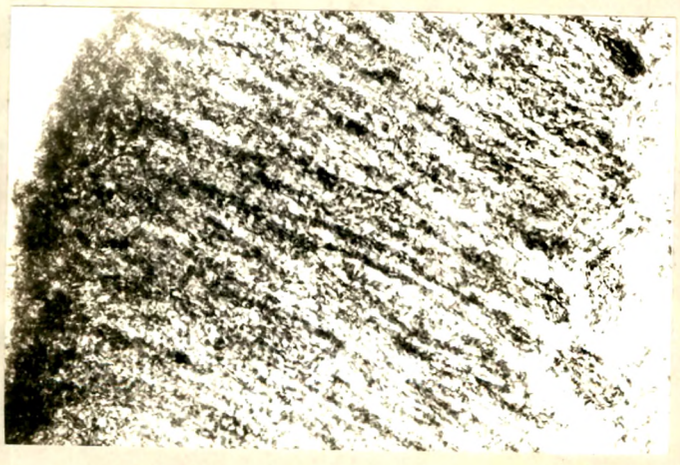
ACID PHOSPHATASE ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

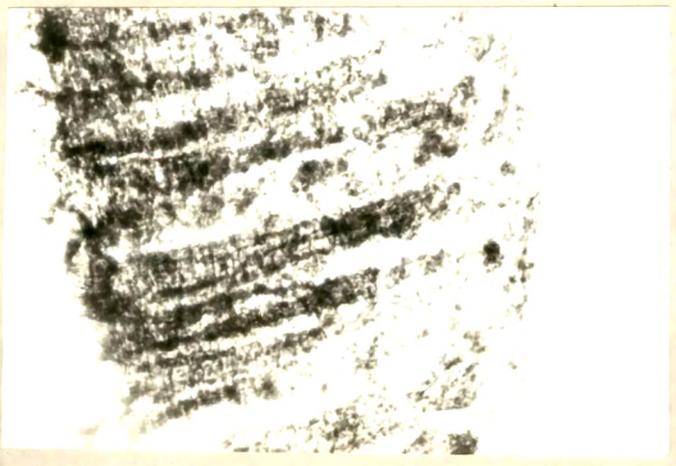
1



100 μ

5 DAY OLD

2

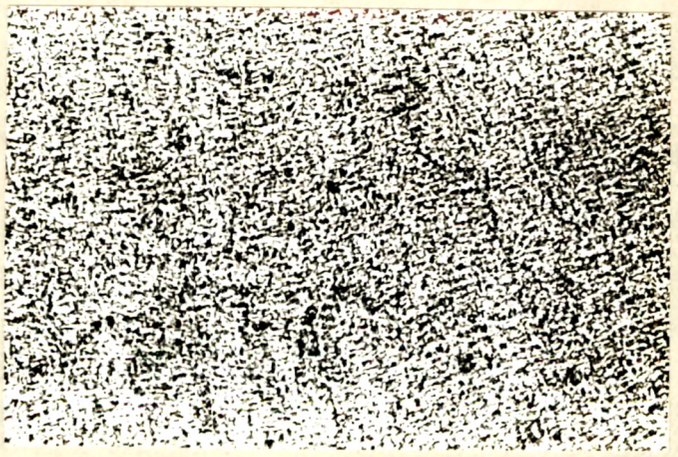


100 μ

10 DAY OLD

3

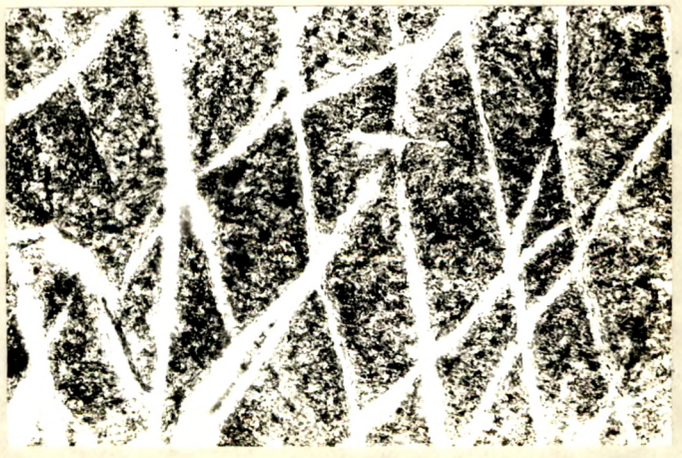
ACID PHOSPHATASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200 μ

1 DAY OLD

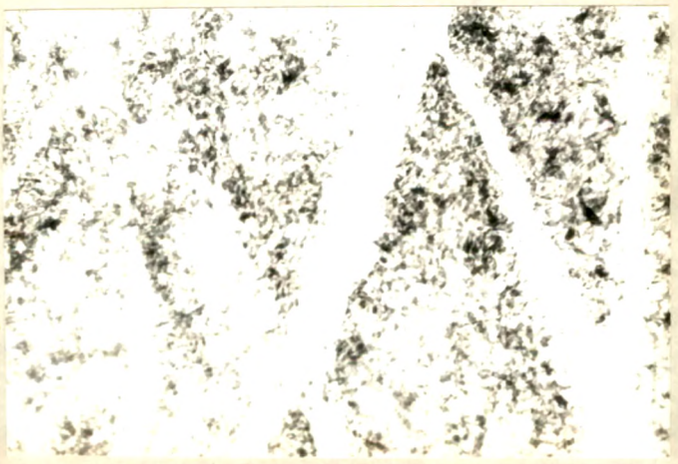
1a



200 μ

5 DAY OLD

2a

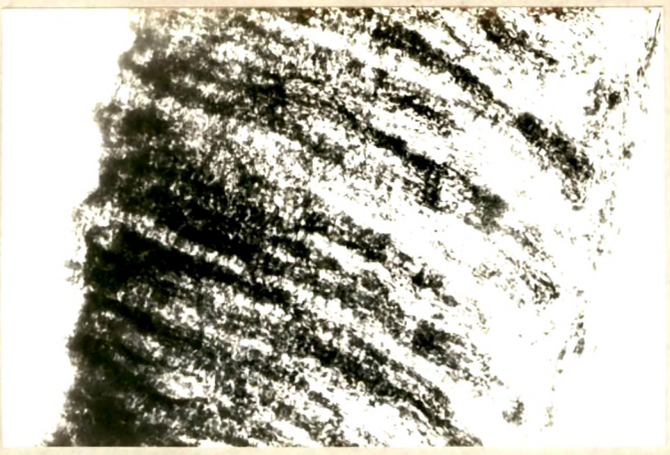


200 μ

10 DAY OLD

3a

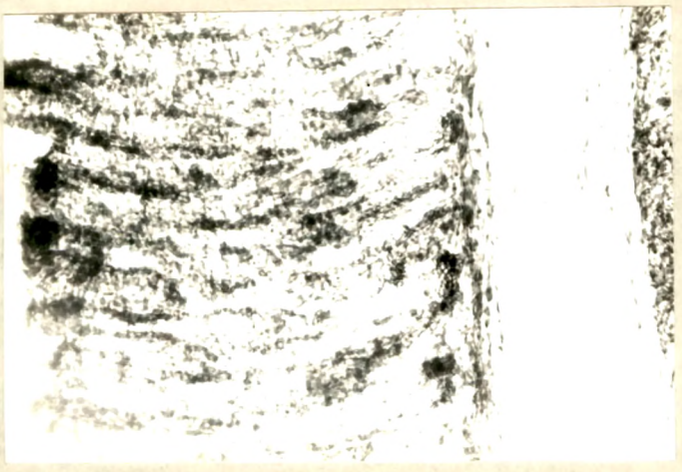
ACID PHOSPHATASE ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

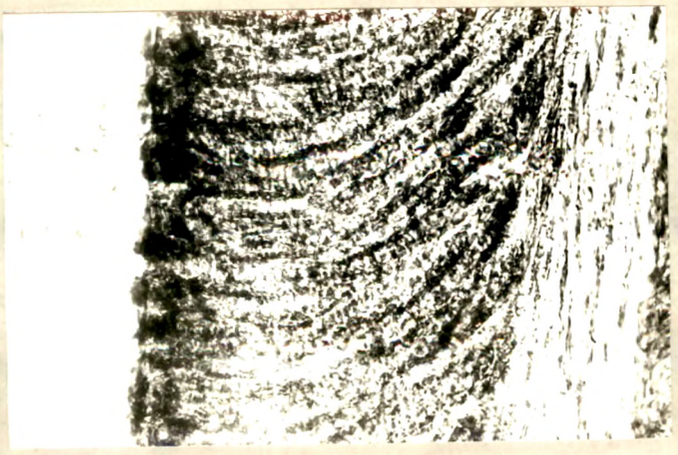
4



100 μ

20 DAY OLD

5

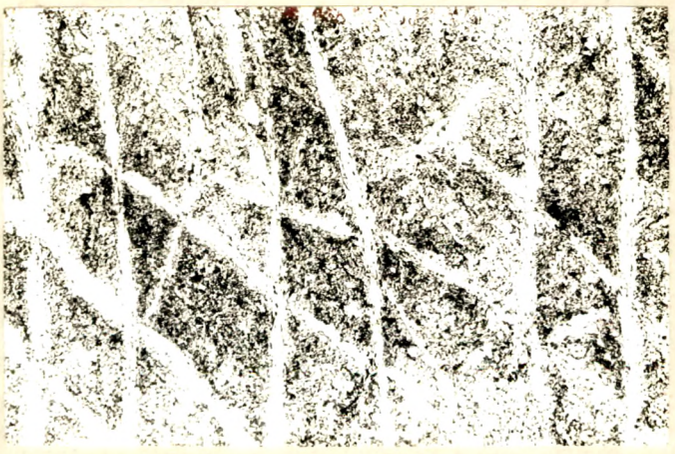


100 μ

25 DAY OLD

6

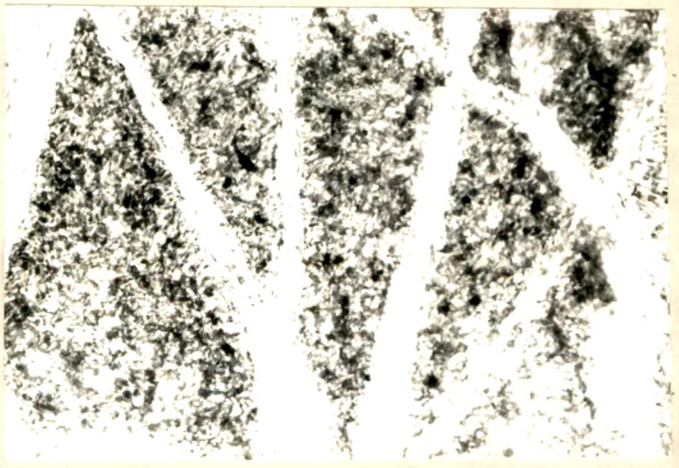
ACID PHOSPHATASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200 μ

15 DAY OLD

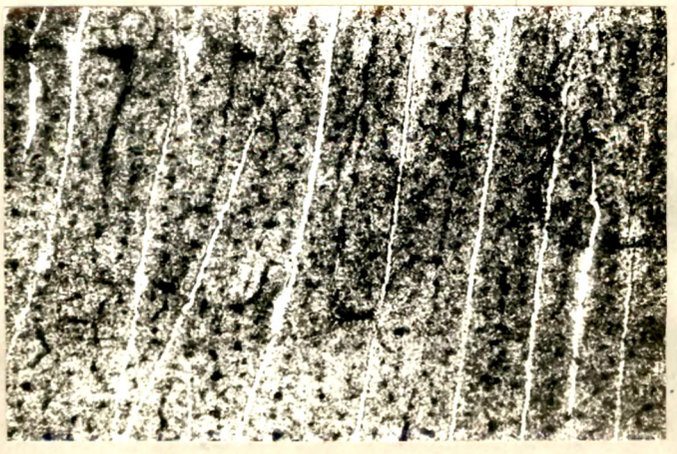
4a



200 μ

20 DAY OLD

5a

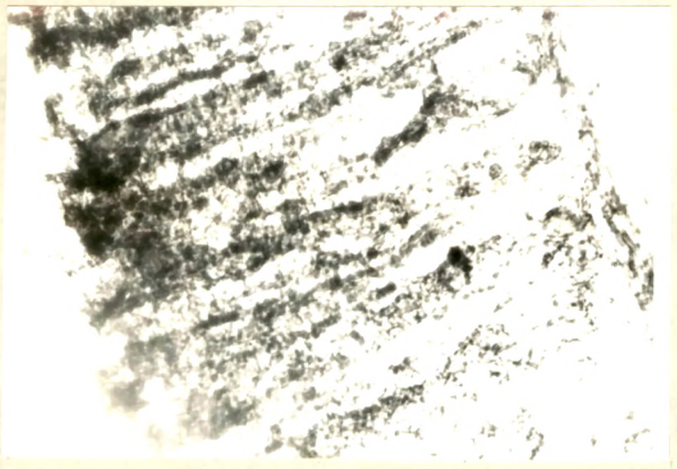


200 μ

25 DAY OLD

6a

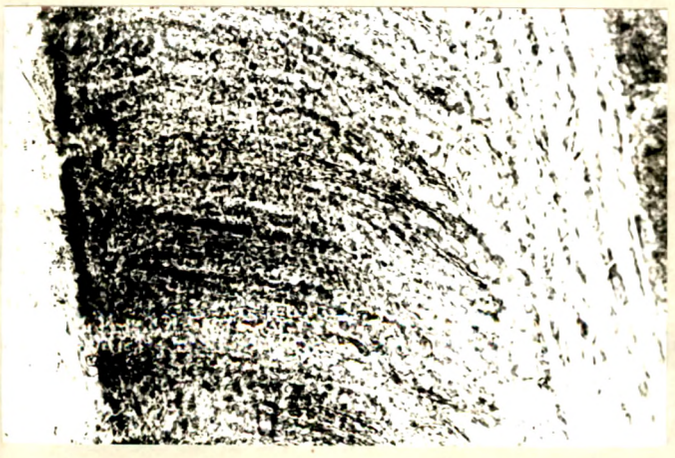
ACID PHOSPHATASE ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

7

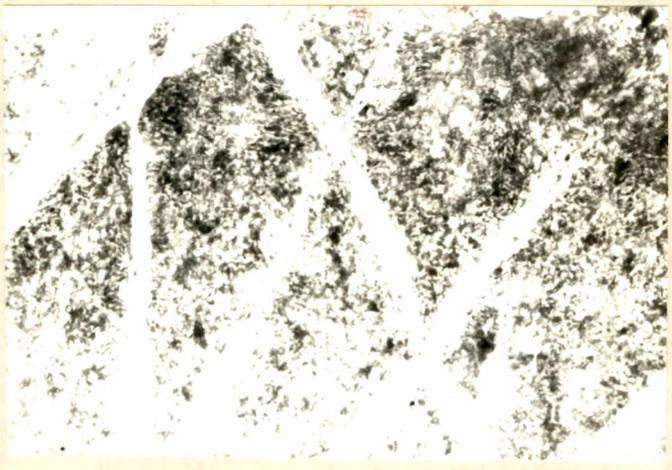


100 μ

ADULT

8

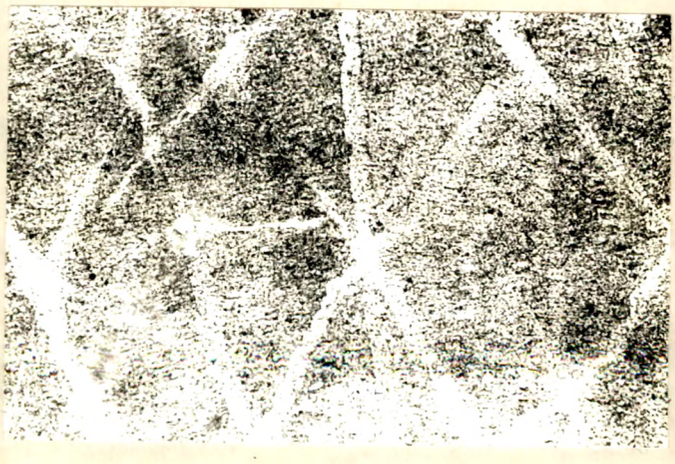
ACID PHOSPHATASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200 μ

30 DAY OLD

7a

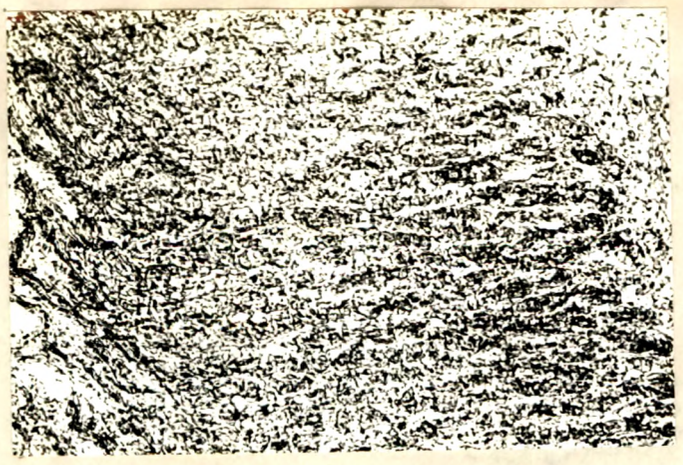


200 μ

ADULT

8a

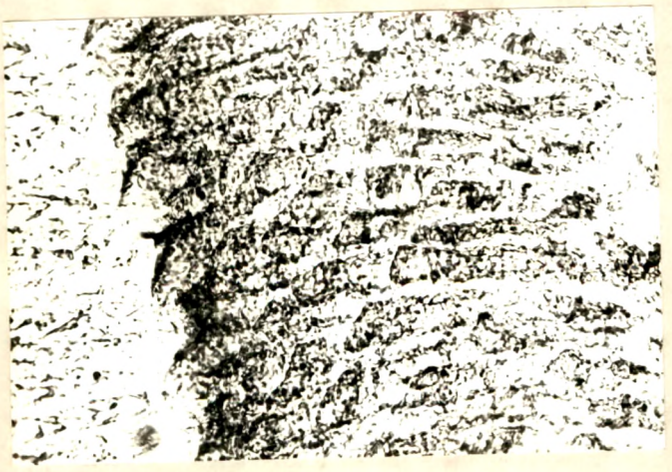
ALKALINE PHOSPHATASE ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

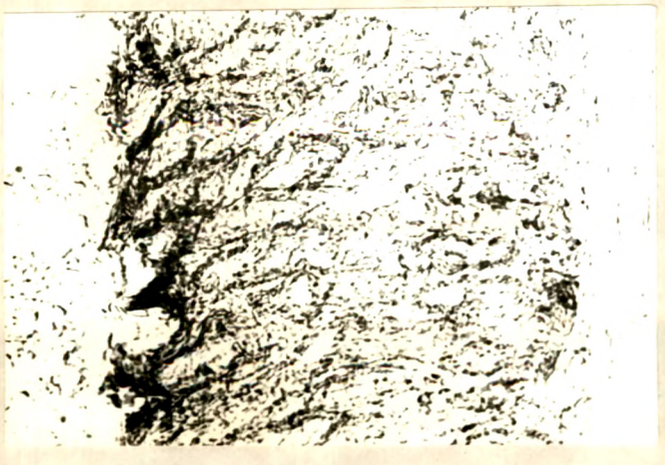
9



100 μ

15 DAY OLD

10

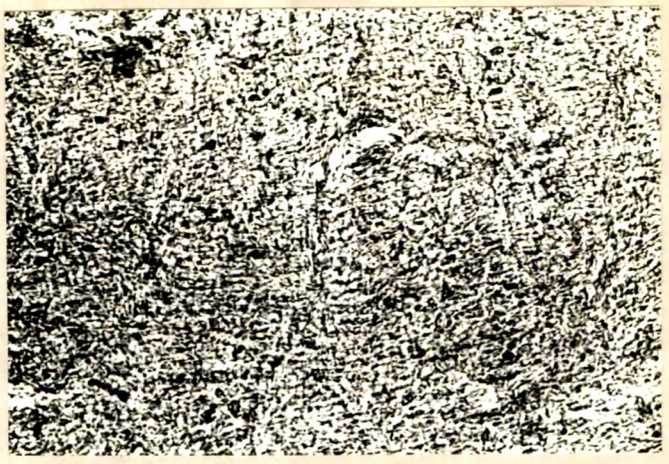


100 μ

ADULT

11

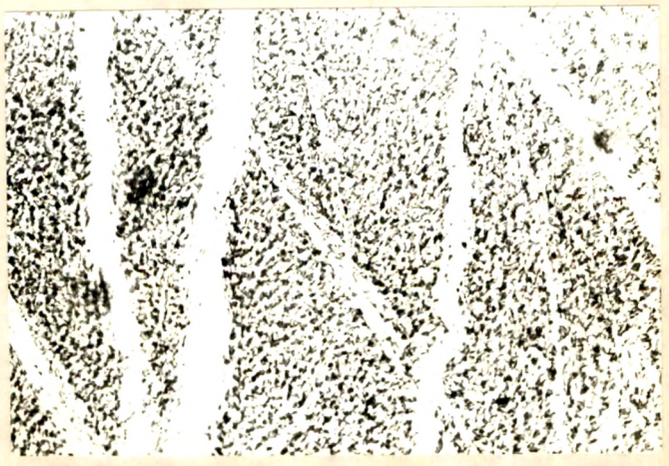
ALKALINE PHOSPHATASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200 μ

1 DAY OLD

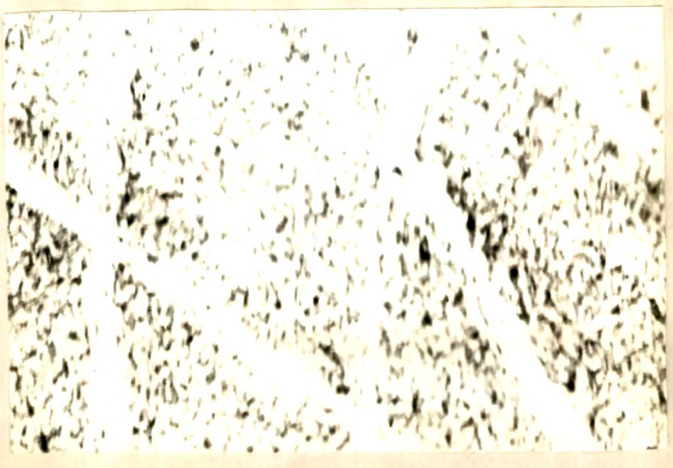
9a



200 μ

15 DAY OLD

10a



200 μ

ADULT

11a

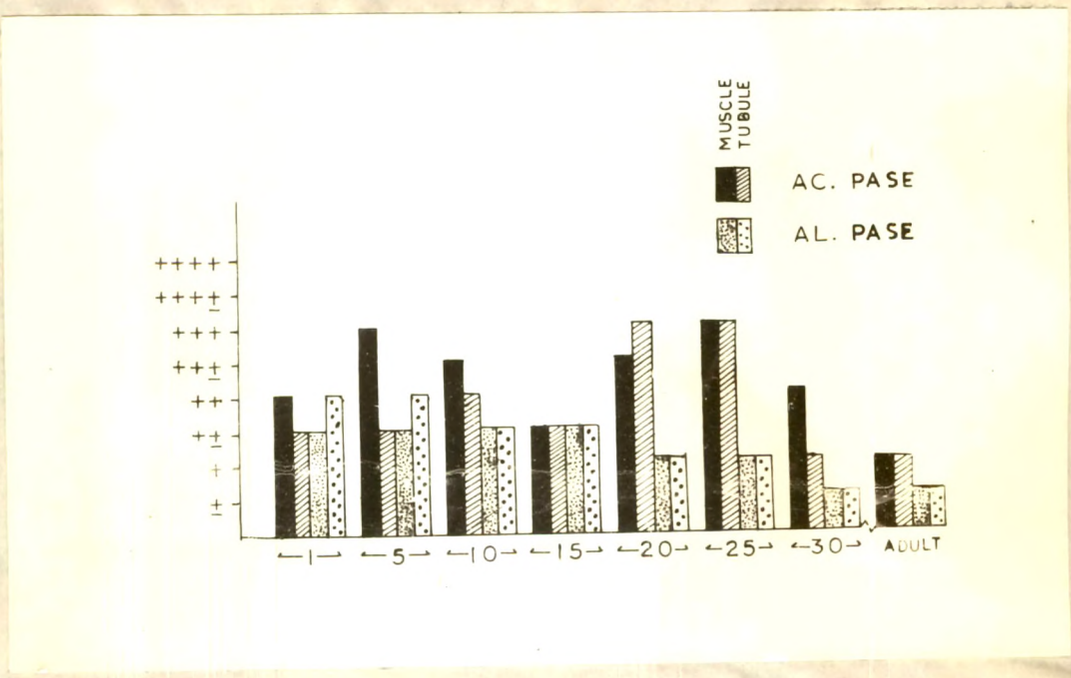


FIG. 12

Graphical representation of the changes in the acid and alkaline phosphatases distribution pattern during the various days of gizzard development

5th day, along with the concomitant change in the diet from 'pigeon milk' to partially digested grains, the concentration of acid phosphatase showed an increase whereas that of alkaline phosphatase remained unchanged, However, in the 15 day old gizzard both the phosphatases showed a reduction in their activities which became first evident by about 10th day itself. In the case of acid phosphatase this was followed by a gradual and steady increase attaining a peak activity on the 25th day whereas in the case of alkaline phosphatase, during the corresponding period the activity tended to remain more or less at the same level. On the 30th day, along with the aquisition of the capacity by the bird to feed by itself, there was a corresponding decrease in the concentrations of both the enzymes and this low level was maintained all throughout the rest of the adult condition.

It is again of significance to note here that the activity of acid phosphatase tended to be perceptibly higher than that of the alkaline phosphatase all throughout the various periods of gizzard development.

The histochemically observed concentrations of acid and alkaline phosphatases during the different

periods of development are represented diagrammatically in figure 12.

DISCUSSION

It is quite significant that the present observations have revealed the pigeon gizzard, during its post-natal development, to be acid phosphatase active. Comparatively alkaline phosphatase appears to be very poor, excepting for the first 10 days after hatching. The activity of alkaline phosphatase during this period though not very prominent, was nevertheless, noticeable. Moreover, the concentration of this enzyme not only maintains the same level from the day of hatching uptil the 10th day but also an identical concentration is maintained in both the mucosal tubules as well as smooth muscle fibres. Interestingly enough, this very period is also characterised by a quick development of the connective tissue around the muscle bundles. It could be suspected, in this light, that alkaline phosphatase might be of some utility in the laying down of connective tissue elements in the developing gizzard. Such a surmise is rather reasonable as this enzyme has been associated with the collagen formation (Fell & Danielli, 1943) as well as fibrous protein formation and collagen differentiation (Marchant, 1949; Junquiera,

1950). Whereas the activity of alkaline phosphatase in the smooth muscle may be correlated with the synthesis of contractile proteins (during these initial days) its activity in the tubular (mucosal) epithelium may be ascribed to a function of imparting an initiating influence to the process of keratinization, as it is between the 5th and the 10th day that the keratin lining makes its appearance in the gizzard. Moreover, the presence of alkaline phosphatase during these initial days could also be correlated with high metabolic incidence at the same period as a number of non-specific functions have been ascribed to this enzyme in cell metabolism (Saev, 1963; Morton, 1968; Vallyathan & George, 1965; Asnani, 1971; Cori & Cori, 1952; Cushworth, 1958; Duncun, 1959; Rosenthal *et al.*, 1960; Verzar & McDougal, 1936; Moog, 1946; Bradfield, 1950; Danielli, 1954; Radhakrishnan, 1972). Excepting for this initial short period of activity, alkaline phosphatase was almost negligible all throughout the rest of the period of gizzard development with an ultimate little or no activity in the adult condition. It is rather interesting that Shah & Menon (unpublished data) have also observed a total lack of alkaline phosphatase in the smooth muscles during post-natal development and also those in the adult pigeon integument.

The activity of acid phosphatase in the two components of the gizzard is more or less identical and parallel. An elevated level of the enzyme activity is the feature between the day of hatching and the 10th day. Correspondingly this is the period characterised by a tremendous pace of morphological and physiological development of that organ too, marked not only by the development of the musculature and the connective tissue but also the progressive increase in the process of keratinization of the lining of the epithelium. The role of this enzyme at this stage could well be for the functional differentiation of muscular tissue and help in the synthesis of contractile proteins in the process, as such fibrous proteins have been associated with smooth muscle too as in the case of striated muscle (Cobb & Bennett, 1969). At the same time, in the tubules, it could be suggested that the acid phosphatase might be aiding in the synthesis and secretion of keratin around the epithelium. This gains validity by the suggestion of Vorbrodt (1958) of a correspondence between acid phosphatase activity and protein synthesis and the correlation between acid phosphatase and keratinization drawn by Shah & Chakko (1966) and Radhakrishnan (1972) in epidermis of the regenerating tails of the lizards,

Hemidactylus flaviviridis and Mabuya carinata respectively. Further Mori (1953) working on the epithelial tumors also observed the localization of this enzyme in the keratinized cells and that too in a degree proportionate to that of keratinization. Thus, it could be said that the pattern of acid phosphatase activity recorded herein appears to fit well with that of the gizzard development.

This is followed by a fall in the enzyme activity by about the 15th day coinciding, probably, with the completion of the process of development and differentiation of the two structural and functional entities of the gizzard viz., the smooth muscle fibres and the mucosal tubules with full thickness of keratin layer. Following this, is a spurt in enzyme activity attaining a maximal value during the 20th to 25th days of development. It is during these days that the pigeon starts its first feeding experimentations with solid grains as observed from the contents of the gizzard. The concomitant spurt in the acid phosphatase activity observed may thus be in good correlation with the attaintment of the full mechanical functioning of the organ wherein the muscles are initiated to contract vigorously when

in use and to crush the grains with the agency of thickly keratinized layer over the tubules.

As the attainment of structural and functional maturity of the gizzard is preceded by a constant process of proliferation and division of the various cellular elements, the presence of acid phosphatase, it could be reasoned, might also be of importance in the removal of the resultant cellular debris thus paving way for the effective functional maturation of the organ. Such activities are termed as post-mitotic autophagosis by Klockars & Wegelius (1969).

With the acquisition of the full functional capacity by the gradual action and working in the following days and the completion of the process of post-natal development and differentiation, the activity of acid phosphatase in the gizzard, from about 30th day onwards, drops down towards the characteristic low adult level.