CHAPTER 8

ON THE ACTIVITY AND ROLE OF ACID AND ALKALINE PHOSPHATASES IN THE GIZZARDS OF CERTAIN ADULT REPRESENTATIVE BIRDS: A COMPARATIVE STUDY

Studies on the developing and adult pigeon gizzards have revealed the possible participation of both acid as well as alkaline phosphatases in the initiation of morphological and functional differentiation of that organ (chapter 4). It is being increasingly realized that the phosphatase operating in an acid environment (acid phosphatase) might be aiding in the synthesis of contractile protein (Vorbrodt, 1958; Cobb & Bennett, 1969). A correlation between acid phosphatase activity and keratinization has also been drawn by Shah & Chakko (1966) and later by Radhakrishnan (1972) in the regenerating reptilian tail. The other phosphatase operating in an alkaline environment (alkaline phosphatase) has been associated with collagen formation (Fell & Danielli, 1943) as well as fibrous protein synthesis and collagen differentiation (Marchant, 1949; Junquiera, 1950). A number of non-specific functions, too, have been attributed to this enzyme in cell metabolism (Bradfield, 1950; Danielli, 1954; Cori & Cori, 1954; Cushworth, 1958; Duncun, 1959;

Ros$enthal *et al*., 1960). Eventhough there have been a number of studies dealing with the presence and localization of these two enzymes in various tissues of different vertebrates such as amphibians (Schmidt,1963; Schmidt & Weary,1963), reptiles (Shah & Chakko, 1966; Radhakrishnan, 1972), birds (Vallyathan & George, 1965; Khan & George, 1967; Pilo *et al*., 1972) and Mammals (Moog, 1951, 1953, 1961; Hugon & Borgers, 1966, 1967 and 1968a),such studies are scanty on the smooth muscles of vertebrates as well as on the avian gizzards. The available literature to date dealing with the localization of phosphatases in the organs of alimentary canal are of Prakash (1961) on fishes, Moog & Richardson (1955) on birds, and Moog (1951, 1953), Pelichova *et al*. (1967), Etzler & Moog (1968), Hugon & Borgers (1969), Williams (1970), Goldstein *et al*. (1971), Saini & Dove (1972), Chang & Moog (1972) on mammals. Since the smooth muscle predominates the general histological structure of the gizzard (Calhoun, 1954), which is an important part of the avian alimentary canal, functioning as a grinding mill, this organ becomes a suitable material for the study of the smooth muscle physiology as well. A comparative account of the enzymatic adaptations of the gizzard would be more useful because the gizzards of different birds show anatomical and morphological differences according to differences in their food and feeding habits. It is with this background in mind that a detailed comparative study has been undertaken on the presence and distribution of acid and alkaline phosphatases in the avian gizzards so as not only to farther our knowledge regarding the role of these two enzymes in the smooth muscle physiology but also to correlate the enzyme responses with various grades of the gizzard activity observed in birds based on their food and feeding habits.

MATERIALS AND METHODS

All the birds studied herein were shot from the University campus in the morning hours by means of an air rifle. They were grouped as given in chapter 6.

Gizzards from these birds were immediately separated, blotted well to remove their contents, blood and tissue fluids and fixed on a chuck of a cryostat microtome maintained at -20°C. Sections of 12 μ thickness were cut and processed in the respective incubation media as described in chapter 4 for the histochemical observations on acid and alkaline phosphatases.

OBSERVATIONS

(Figs. 1 to 20; 1a to 20a)

Of the different groups of birds used in the present study the gizzards of grain eaters registered the least response towards phosphatase reaction. Acid phosphatase activity was slightly more than alkaline phosphatase activity in both the components viz., smooth muscle fibres as well as mucosal tubules of the gizzard. In the frugivores, acid phosphatase activity was perceptibly higher than that noted in the granivore whereas alkaline phosphatase showed a negative reaction in the latter. The group of flesh eaters (carnivores) also registered an almost identical concentration of acid phosphatase as noted in parakeet (frugivore) but the alkaline phosphatase activity was more pronounced in their gizzards than that of pigeon and parakeet. Amongst insectivores, drongo, crow pheasant, red-vented bulbul. and koel registered a strong response towards acid phosphatase staining while the rest of the birds recorded an enzyme concentration identical to that of frugivore, though the concentration was slightly less in the former than the latter. The alkaline phosphatase activity was moderate in the gizzards of these birds except for drongo and red-vented bulbul where a negative response for this

EXPLANATIONS FOR FIGURES

(Figures 1 - 12 Acid Phosphatase activity in the mucosal tubules of gizzards of various birds)

and

(Figures 1a - 12a Acid Phosphatase activity in the smooth muscle fasciculi of gizzards of various birds)

Figs. 1 & 1a - Shrike

Figs. 2 & 2a - Kite

Figs. 3 & 3a - Crow

Figs. 4 & 4a - Fowl

Figs. 5 & 5a - Bee-eater

Figs. 6 & 6a - Drongo

Figs. 7 & 7a - Crow Pheasant

Figs. 8 & 8a - Bulbul

Figs. 9 & 9a - Babbler

Figs. 10 & 10a-- Koel

Figs. 11 & 11a - Parakeet

Figs. 12 & 12a - Sunbird

EXPLANATIONS FOR FIGURES

(Figures 13 - 20 Alkaline phosphatase activity in the mucosal tubules of gizzards of various birds) and

(Figures 13a - 20a Alkaline phosphatase activity in the smooth muscle fasciculi of gizzards of various birds)

Figs. 13 & 13a - Shrike

Figs. 14 & 14a - Kite

Figs. 15 & 15a - Fowl

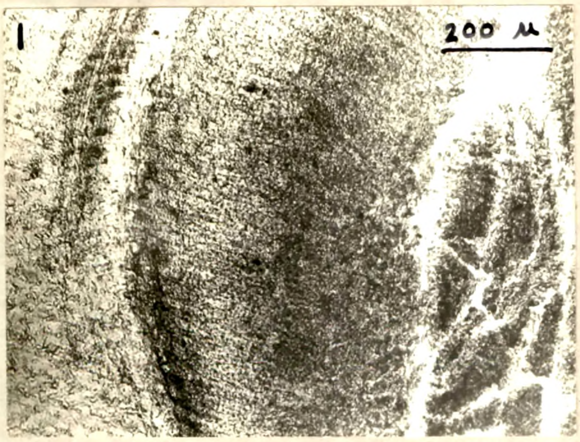
Figs. 16 & 16a - Bee-eater

Figs. 17 & 17a - Babbler

Figs. 18 & 18a - Koel

Figs. 19 & 19a - Parakeet

Figs. 20 & 20a - Sunbird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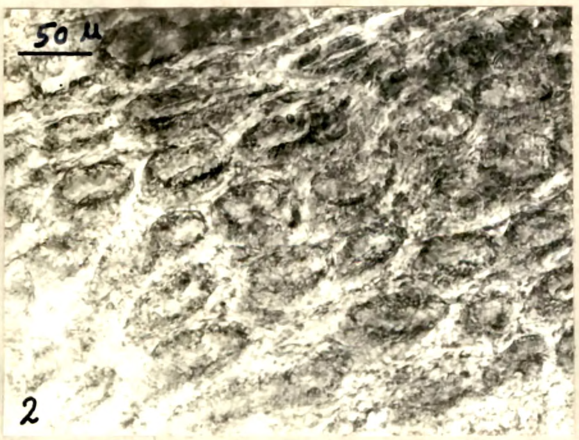
1a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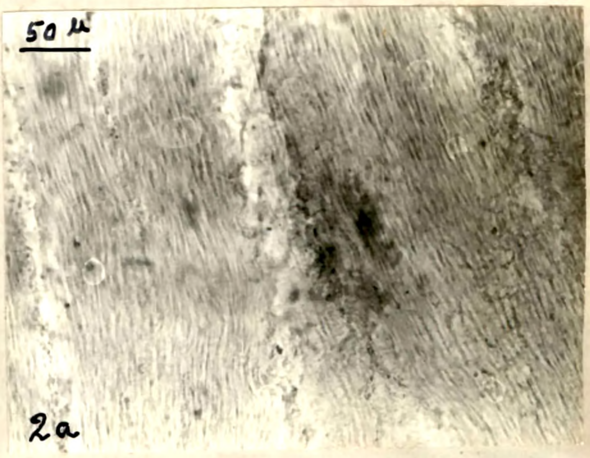
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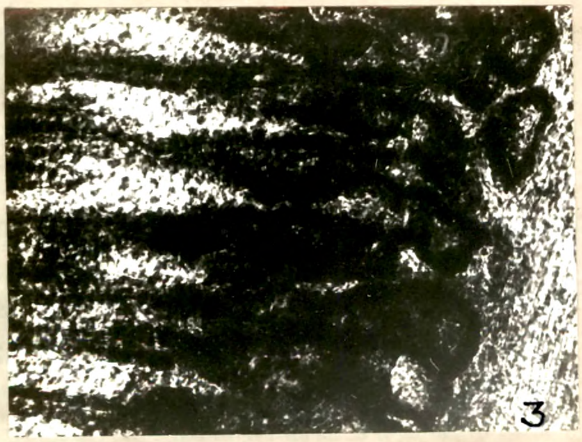

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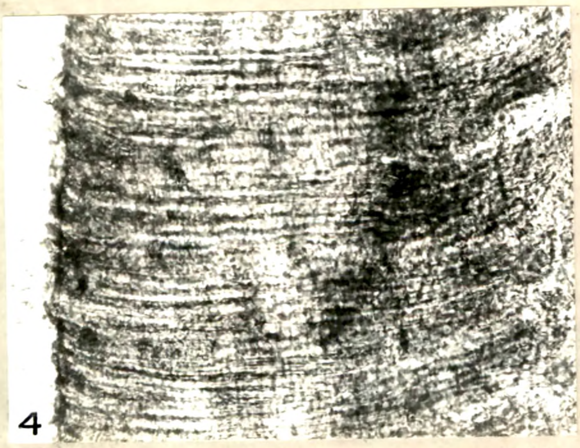

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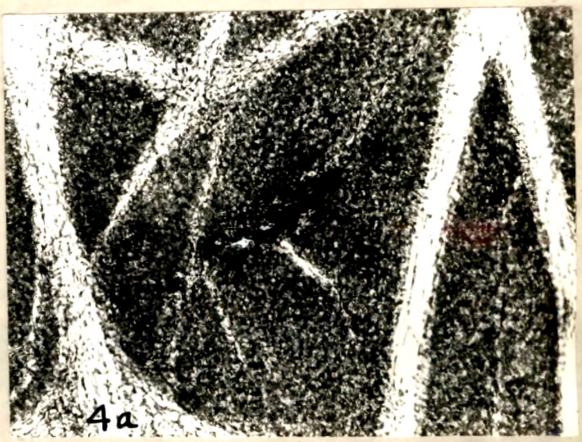

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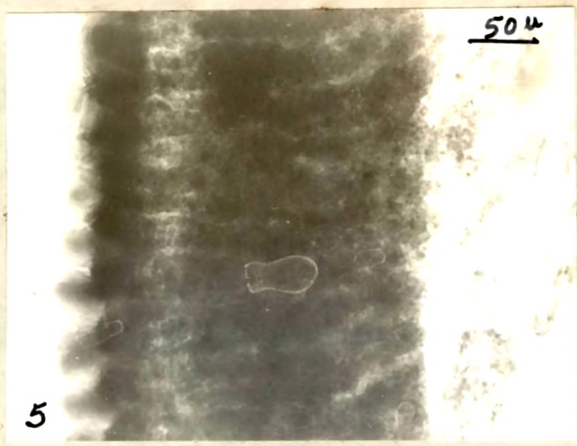
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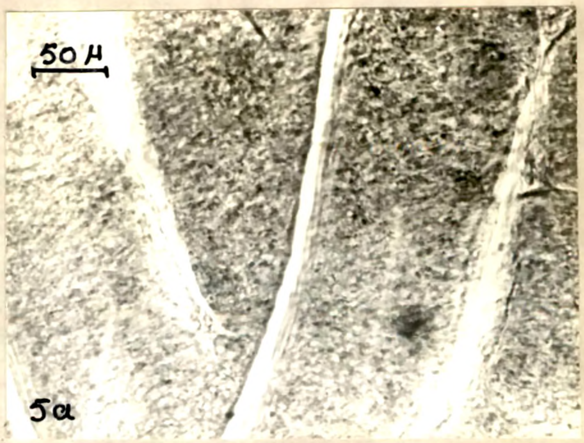
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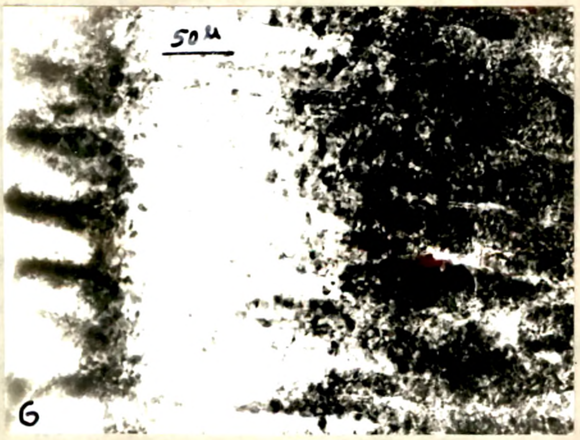
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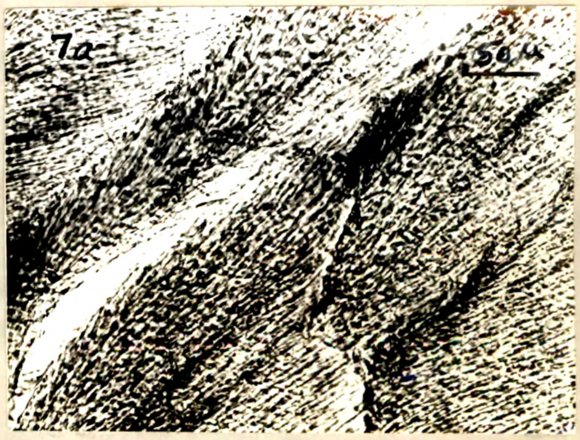
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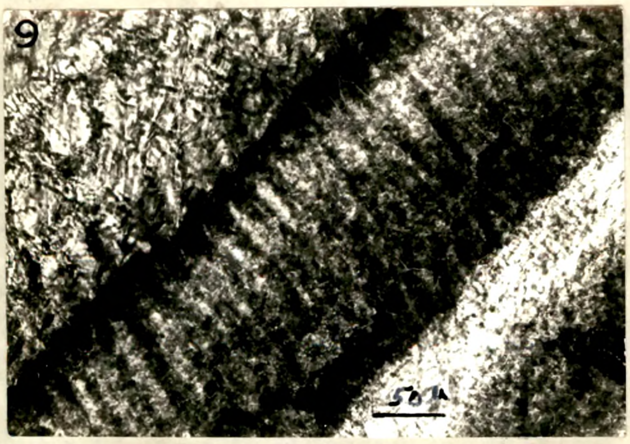
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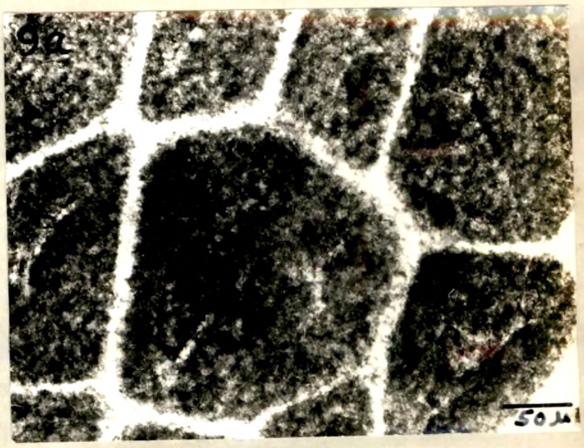
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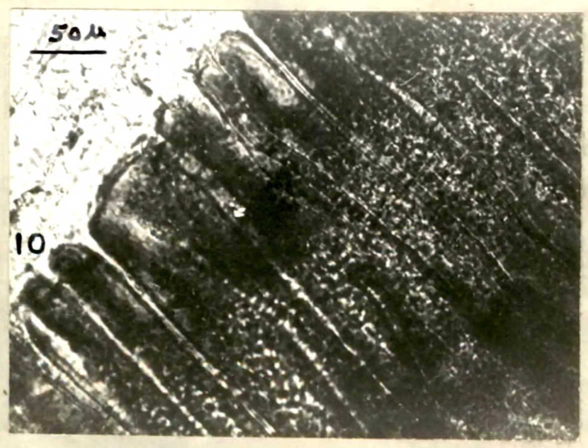
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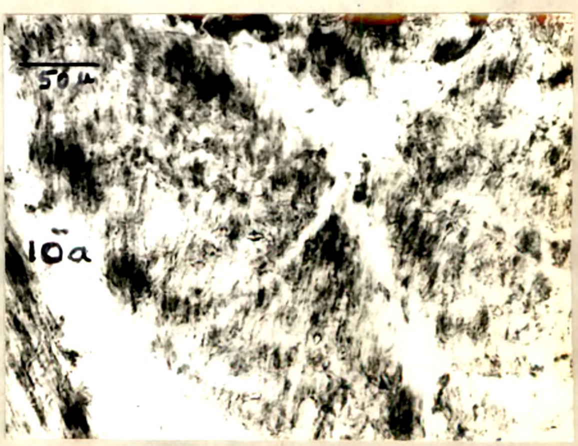
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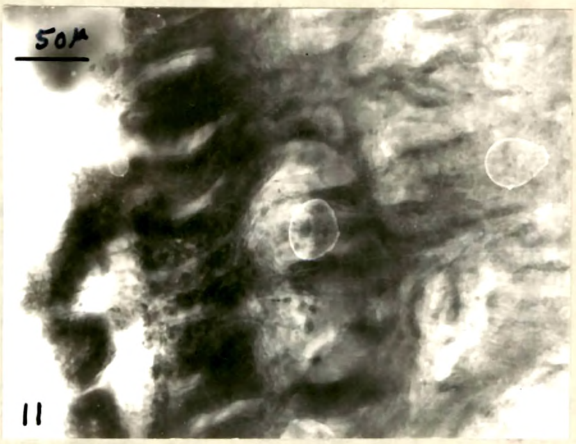
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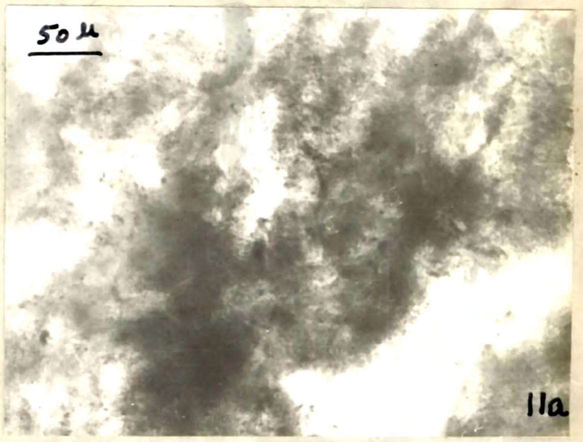
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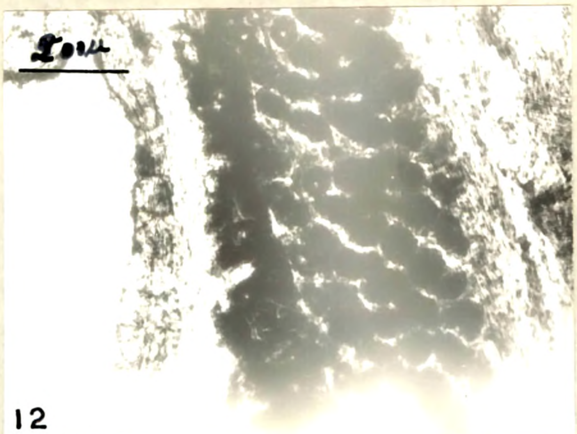
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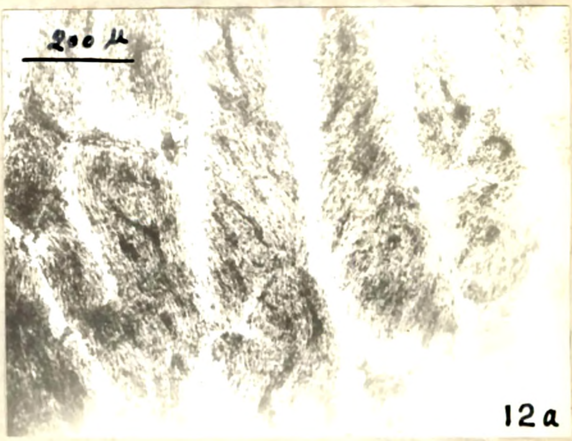
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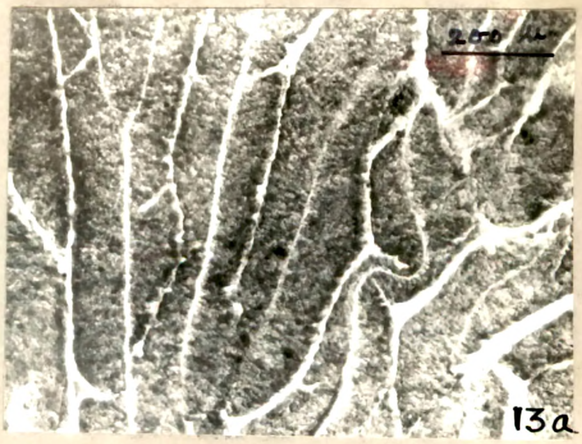
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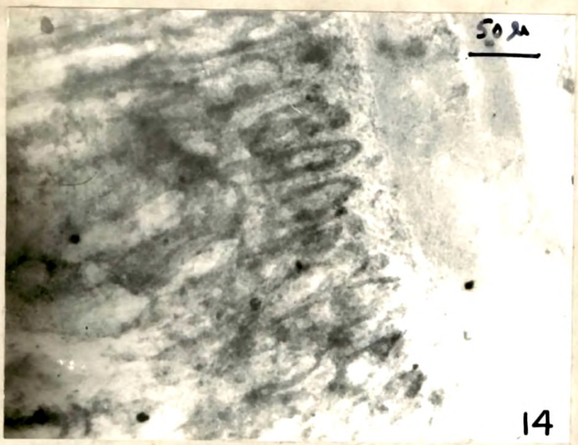
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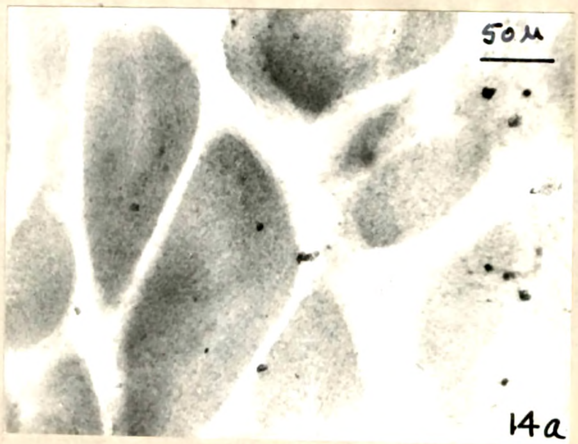
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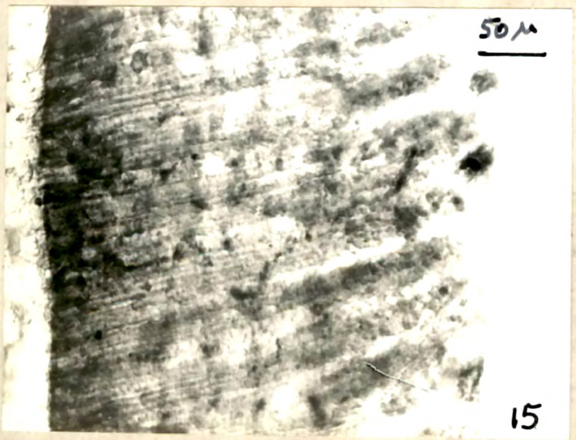
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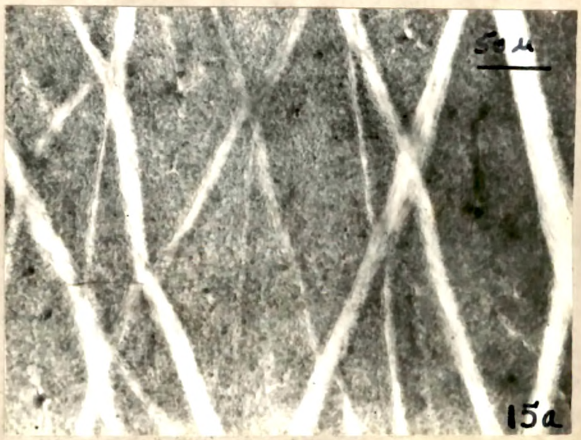
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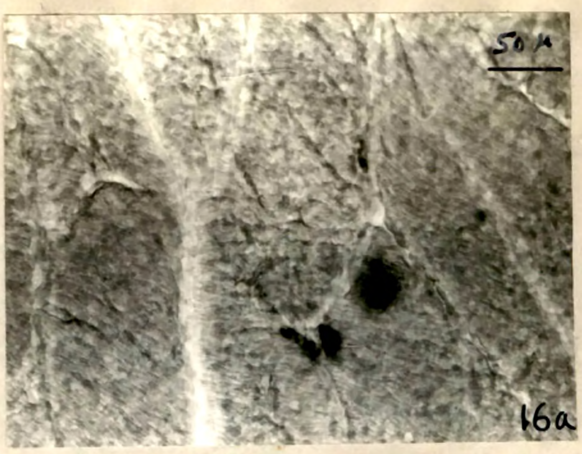
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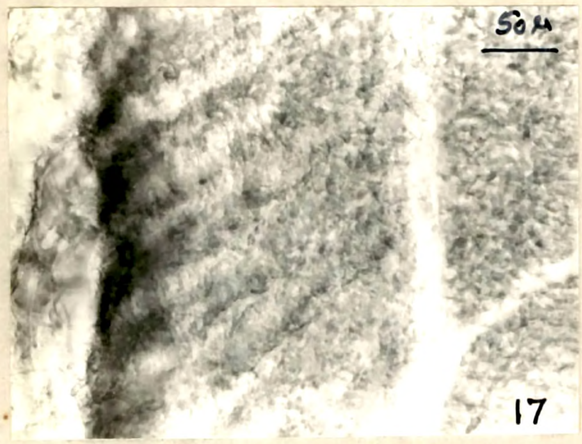
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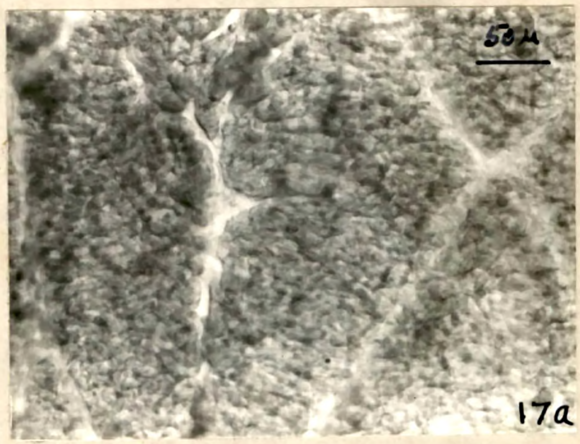
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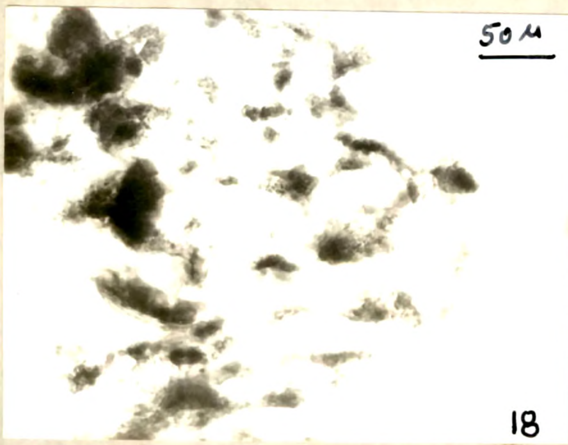
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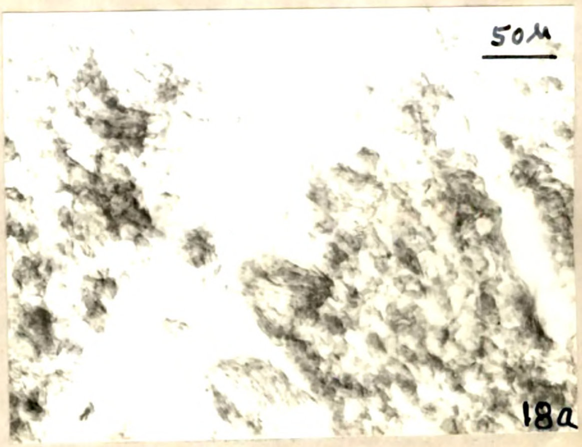
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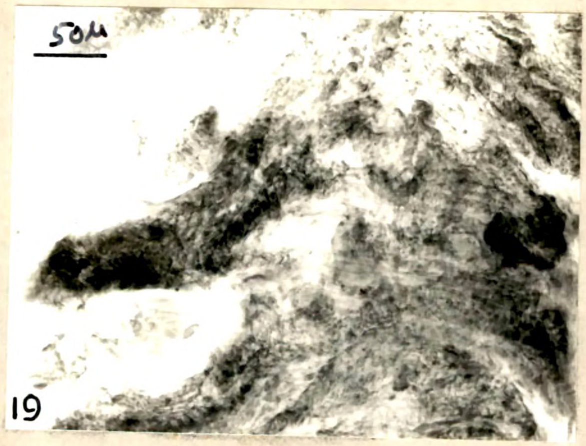
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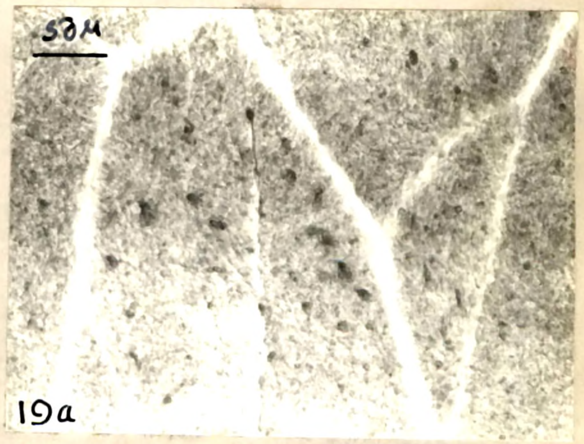
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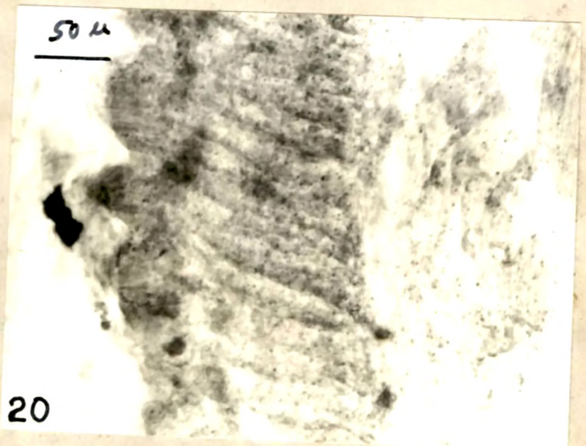
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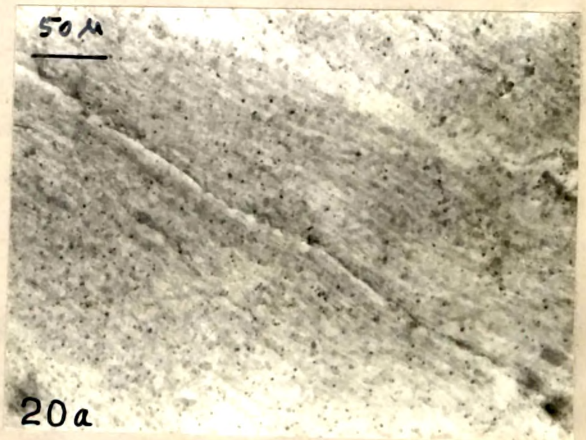
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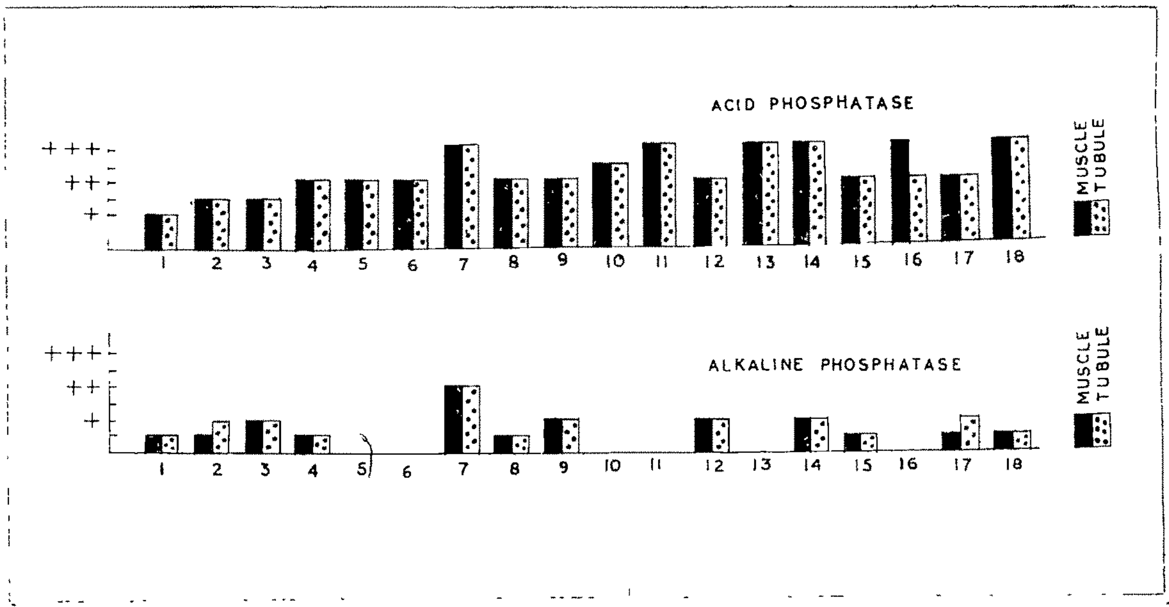

FIGURE 21

Graphical representation of the changes in acid and alkaline phosphatases distribution pattern in the gizzards of adult representative birds enzymes was observed. The birds with mixed diet (omnivores) also showed an appreciable acid phosphatase response though there were individual variations in the activities of the enzyme. Birds like brahminy myna, house crow and jungle crow showed a lower acid phosphatase activity when compared with that of fowl though the enzyme level was never below than that noted in the flesh eaters. The alkaline phosphatase activity was appreciable in the gizzard of fowl and moderate to nil in other birds of this group. The nectar feeder also registered a high phosphatase response identical to that of fowl while alkaline phosphatase response was moderate in the gizzard of this group.

Histochemically observed concentrations of these enzymes in the gizzard of these adult representative birds are represented diagrammatically in figure 21.

DISCUSSION

Acid phosphatase, being a hydrolytic enzyme, is associated with phagocytic process as well as cellular autolysis (de Duve, 1959; Weber & Niehus, 1961). A high concentration of this enzyme has been demonstrated in the macrophages and lymphocytes as well as histiocytes like

Kupffer cells (Dannenberg *et al*., 1960; Thorbecke *et al*.,1961; de Duve *et al*., 1962; Carranza & Cabrini, 1962; Novikoff, 1963; Elves, 1966). In general, whereas the granivorous groups of birds registered a low level of enzyme concentration, the frugivores, insectivores and omnivores recorded a relatively higher level of the enzyme activity. It has been concluded from the study of phosphatases during the post-natal development of the pigeon gizzard (chapter 4) that acid phosphatase is more active in contrast to alkaline phosphatase all throughout the periods of development. The role of acid phosphatase during the initial stages of development has been attributed to an initiation of morphological and functional differentiation of that organ and also to help in the removal of cellular debris resulted by constant process of proliferation and division of various cellular elements thus effecting functional maturation (Klockars & Wegelius, 1969).

The high acid phosphatase response of the smooth muscle fibres and the mucosal epithelial tubule cells of the gizzard could be correlated with the functional activity of that organ. Thus a variation of acid phosphatase activity in the intestinal epithelium in relation to fat metabolism was noted by Barka (1963). Further, Norberg (1964) demonstrated that liver regeneration in the rat is associated with an increase in acid phosphatase activity suggesting a relationship between this enzyme and protein synthesis. Similarly, Pilo et al. (1972) in their study on the process of wound healing and repair in the pigeon liver, reported a significant increase in the acid phosphatase activity in the injured region which they correlated with phagocytic reaction involved at the wound site during the early wound healing period. The elevated activity of alkaline phosphatase at the wound site during the early phase, according to these authors, is for the synthesis of collagen and other protein materials as well as transport of metabolites across the cell membrane. Hirsch (1965) suggests that this enzyme plays a central role in molecular transport of materials in cell metabolism rather than participating in secretion, although Sobel (1964) has reported that this enzyme has a secretory role in thyroid and anterior pituitary gland of rat. From the present study it could be safely assumed that the pronounced enzyme activity in the tubules may be in connection with the elaboration and secretion of keratin rather than for the elaboration of digestive juices. It is also interesting to note in this connection that the inner keratin layer of the groups of birds other than granivores is invariably thin as could possibly be the adaptive feature in relation to soft food materials processed in such gizzards. In such birds the assumption of association of acid phosphatase with keratin formation becomes untenable. The presence of high acid phosphatase activity and a comparatively thicker keratin lining observed in the fowl gizzard becomes quite interesting in this context. In this bird the phosphatase may be ascribed the function of both elaboration and secretion of keratin as well as secretion of acid required for peptic digestion. As reported by Farner (1942) the hydrogen ion concentration of the avian gizzard is extremely acidic, well lower than that of proventriculus on one hand and duodenum on the other. This sharp difference in the hydrogen ion concentration, according to him, provides necessary conditions for peptic and tryptic digestion.

As reported earlier (chapter 4) the presence of high acid phosphatase in the smooth muscle fibres of the developing pigeon gizzard is associated with autophagocytosis, a part of the mechanism by which the gizzard becomes structurally and functionally mature.

A low concentration of acid phosphatase has been noted in the gizzard of grain eating birds. Incidently grain eaters are provided with a thick inner layer of keratin. As observed in chapter 4 there is an accumulation of acid phosphatase during the development of pigeon gizzard and this pronounced enzyme response is for the complete morphological and physiological maturation of that organ. Once the adulthood is reached in the pigeon the acid phosphatase activity falls back slightly and this reduced level is retained. In grain eaters the presence of acid phosphatase is for the keratin secretion as has been observed during the development of pigeon gizzard to replace continuously wearing out of the existing keratin layer. Such a direct correlation between acid phosphatase activity and secretion of keratin has been reported by Braun and Rupec (1967).

Alkaline phosphatase activity was moderate to nil in the gizzards of all the birds studied. The activity of this enzyme has been related to laying down of connective tissue elements in a varieties of tissues by several workers (Fell & Danielli, 1943; Marchant, 1949; Junquiera, 1950; Pilo <u>et al</u>., 1972). This correlation between alkaline phosphatase and connective tissue appears to be well exemplified in the present study. Whereas the activity of this enzyme was low in those groups of birds (all other than granivores) where the connective tissue surrounding the smooth muscle fasciculii is relatively thin, the enzyme activity was demonstrably higher in the granivores and fowl (as an exception from omnivores) where the connective tissue tracts are thicker. The activity of alkaline phosphatase in the smooth muscle fibres of gizzard in general has been correlated with the synthesis of contractile proteins and that in the tubular epithelium with the process of keratinization as suggested in chapter 4. It is quite significant to note that the keratin layer in the gizzards of carnivores, frugivores and nectar feeder, and the omnivores, barring fowl, is quite thin and correspondingly a low concentration of alkaline phosphatase activity has also been noted.