STOMACH COMPLEX OF DEVELOPING AND ADULT BIRDS: BIOCHEMICAL AND HISTOCHEMICAL STUDIES ON NON-SPECIFIC ACID AND ALKALINE PHOSPHATASES.

CHAPTER VI

STOMACH COMPLEX OF DEVELOPING AND ADULT BIRDS: BIOCHEMICAL AND HISTOCHEMICAL STUDIES ON NON-SPECIFIC ACID AND ALKALINE PHOSPHATASES.

Non-specific phosphatases (acid and alkaline) are a set of enzymes involved in the transport of metabolites across the cell membrane. Their increased activities in the caudal alimentary tract indicate their involvement in the nutrient transport across the mucosa (Majumdar *et al.*, 1988). Acid phosphatase, according to the functional state of the tissue concerned, is reported to be involved in a number of activities such as phagocytosis (Klockars and Wegelius, 1969), dissolution of tissue components (Weber and Niehus, 1961), fat absorption in intestine (Barka, 1963), cellular differentiation (Ghiretti, 1950) and keratination (Novikoof, 1961; Mishima, 1964; Palade and Forquliar, 1965), while alkaline phosphatase has been associated with a number of activities such as formation of fibrous protein (Verzar and McDouqall, 1936; Moog, 1946; Bradfield, 1950) and calcification of bones (Moog, 1944; Pritchard, 1952). This enzyme is also reported to be involved in carbohydrate metabolism (Cori and Cori, 1952; Cusworth, 1958; Duncans, 1959; Rosenthal *et al.*, 1960). Further, this enzyme is related to growth and secondary ossification of the osseous tissue (Fleish and Newman, 1961; Anderson, 1976; Brown *et al.*, 1983 and Kan and Cress, 1987) though there is controversy about whether it acts in the proliferation of mesenchymal cells, expressing chondrogenic and osteogenic functions or only in the deposition of calcium phosphate salts (Newbrey and Banks, 1975; Warner *et al.*, 1983; Salo *et al.*, 1986).

There are authentic reports about variations in the plasmatic level of this enzyme throughout growth of birds (Polonis, 1982; Pjeschk and Kollarova, 1983; Niezgoda *et al.*, 1984; Hoffman *et al.*, 1985; Emanovic *et al.*, 1986 and Levy *et al.*, 1989). Metabolic adaptations and the concentration of enzymes in the avian stomach (i.e. proventriculus and ventriculus) are greatly influenced by the dietary preferences of the animals. Such dietary variations lead to the intake of any one or two of the metabolites like protein, fat or carbohydrates more than the others. In such circumstances non-specific phosphatases can be expected to show adaptive changes in their pattern of distribution and/or concentration.

Hence present biochemical and histochemical investigations were carried out on the stomach complex of domestic fowl and coturnix quail during their posthatched development and certain adult representative birds with various dietary preferences, with a view to understand the changes in the concentration and distribution pattern of these enzymes in relation to their functional development and to the extent of dietary influence on these two enzymes.

MATERIALS AND METHODS

Healthy young chicks of fowl and quail of different ages (in days) of posthatched development and adult birds from a well maintained aviary were used for the present study (Chapter 1).

Biochemical analysis

The organs were quickly removed and homogenated in various ice-cold buffers, for assaying the enzyme concentration according to the method of Linhardt and Walter (1963).

Histochemical procedure

For the histochemical observations small pieces of proventriculus and ventriculus were blotted well and fixed on a chuck of a cryostat microtome maintained at -20°C. Frozen section of 12 to 15 μ thickness were cut and fixed in 10% cold neutral formalin and processed further according to the method of Burstone (1962). Sections incubated in substrate blank medium served as controls.

RESULTS

Biochemical analyses

Acid phosphatase [Proventriculus] (Table 1; Figure 1a)

A moderate acid phosphatase level was observed in the proventriculus of fowl on day 0 which got reduced progressively on days 5, 10 and 20. After an increase on day 30, the level showed

a reduction and this reduced level was maintained in adult. In case of quail, compared to that of chicks, the enzyme concentration was high on day 0. The level got reduced during the subsequent days of development except on day 10 and this reduced level was the characteristic feature in the adult.

Amongst the representative birds, the altricial ones had higher enzyme concentrations with the swift depicting maximum amount followed by sparrow and pigeon. In the precocial birds viz., the quail and fowl, the concentration was less than observed for earlier. However, a peculiarity noted here was that the pigeon and quail registered almost the same amount of enzyme concentration (table 2; figure 2a).

Acid phosphatase [Ventriculus] (Table 1; Figure 1a).

In fowl, a low activity of acid phosphatase was observed in the ventriculus on day 0. Thereafter, the enzyme concentration showed fluctuations. In comparison to fowl, quail depicted a different pattern of enzyme concentration. Higher concentrations were the features on days 0, 20 and adult. Whereas on days 5, 10 and 30 the concentrations were still lower than observed for the above mentioned days.

From the data (table 2; figure 2a) it is clear that the level of acid phosphatase was almost uniform with altricial or precocial mode of development in all the adult birds except fowl where the enzyme concentration was lowest.

Alkaline phosphatase [Proventriculus] (Table 1; Figure 1b)

In the proventriculus of growing fowl and quail, the enzyme concentration showed fluctuations akin to ventricular acid phosphatase. On a comparative basis, the quail had lower enzyme concentration than the fowl.

Among adult birds, highest activity of alkaline phosphatase was observed in fowl followed by sparrow, swift and pigeon in that descending order. Quail showed the lowest alkaline phosphatase activity (table 2; figure 2b).

Alkaline phosphatase [Ventriculus] (Table 1; Figure 1b).

The alkaline phosphatase in the ventriculus of developing fowl was negligible to minimum during different days of development, whereas in case of quail moderate level of enzyme concentration was observed except on day 20 where the lowest concentration was noted.

Among adult birds, highest level of alkaline phosphatase activity was observed in pigeon followed by fowl, sparrow, swift and quail (table 2; figure 2b).

Histochemical observations

Acid phosphatase [Proventriculus] (Plate I, II; Table 3).

Of the different components, the multiple gland of the proventriculus of fowl showed higher activity of acid phosphatase when compared to that of quail during the different days of development. The outer layer depicted at the same time, very low to negligible activity. As far as the adult birds are concerned, the maximum enzyme activity was noticed in the swift followed by sparrow, pigeon, fowl and quail in both the components of proventriculus (plate III; table 4).

Ventriculus (Plate I, II; Table 3).

Compared to proventriculus, reverse pattern of activity of the enzyme was observed in the ventriculus of developing birds. The mucosal tubules of quail had a higher concentration when compared to fowl. The submucosal connective tissue layer and muscle layers registered very low to poor enzyme concentration. In the mucosal tubules and muscle fibres of adult birds, the sparrow, swift, pigeon and quail had a high to moderate enzyme concentration whereas the submucosal connective tissue layer showed a poor enzyme response (plate III; table 4).

Alkaline phosphatase [Proventriculus] (Plate IV, V; Table 5).

The multiple glands of proventriculus of both fowl and quail depicted an identical enzyme activity during the different days of development. The outer layer had showed less enzyme

Table 1. Comparative data on the concentration of the phosphatases* in the proventriculus and ventriculus of developing and adult fowl and quail.

AGE (DAYS)	ACID PHOSPHATASE				ALKALINE PHOSPHATASE			
	PROVENTRICULUS		VENTRICULUS		PROVENTRICULUS		VENTRICULUS	
	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL
0	2.42 ± 0.04@	3.18 ± 0.12	0.93 ± 0.07	1.21 ± 0.05	0.26 ± 0.02	0.15 ± 0.008	0.04 ± 0.003	0.22 ± 0.01
5	1.63 ± 0.03	2.78 ± 0.13	0.34 ± 0.02	0.97 ± 0.04	0.13 ± 0.008	0.10 ± 0.009	0.34 ± 0.008	0.17 ± 0.01
10	1.57 ± 0.06	2.93 ± 0.22	0.58 ± 0.03	0.91 ± 0.05	0.15 ± 0.008	0.13 ± 0.008	0.15 ± 0.004	0.21 ± 0.01
20	1.52 ± 0.07	2.60 ± 0.13	0.40 ± 0.02	1.18 ± 0.08	0.14 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.06 ± 0.005
30	2.05 ± 0.08	2.45 ± 0.21	0.64 ± 0.03	0.84 ± 0.06	0.89 ± 0.06	0.06 ± 0.007	0.20 ± 0.003	0.14 ± 0.008
ADULT	1.53 ± 0.05	2.45 ± 0.18	0.56 ± 0.03	1.02 ± 0.09	0.91 ± 0.06	0.08 ± 0.004	0.14 ± 0.01	0.10 ± 0.01

* μmoles PNP released/mg protein/30 minutes.
@ Values expressed as mean ± SEM of six animals.

$n = 6$

Table 2. Comparative data on the concentration of phosphatases* in the proventriculus and ventriculus of adult birds.

ANIMAL MODEL	ACID PHOSPHATASE		ALKALINE PHOSPHATASE	
	PROVENTRICULUS	VENTRICULUS	PROVENTRICULUS	VENTRICULUS
SWIFT	4.45 ± 0.20@	1.05 ± 0.04	0.15 ± 0.01	0.13 ± 0.01
SPARROW	3.89 ± 0.21	1.72 ± 0.09	0.23 ± 0.02	0.14 ± 0.01
QUAIL	2.45 ± 0.18	1.02 ± 0.09	0.08 ± 0.004	0.10 ± 0.01
PIGEON	2.36 ± 0.20	1.05 ± 0.05	0.12 ± 0.01	0.26 ± 0.02
FOWL	1.53 ± 0.05	0.56 ± 0.03	0.91 ± 0.06	0.14 ± 0.01

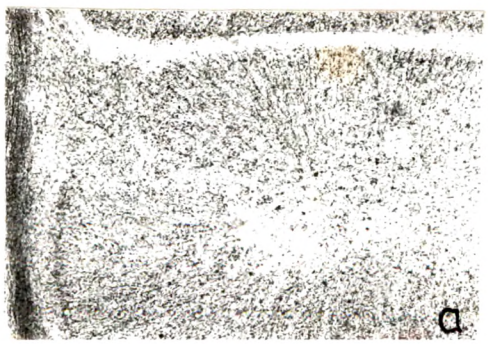
* μmoles PNP released/mg protein/30 minutes.
@ Values expressed as mean ± SEM of six animals.

$n = 7$

EXPLANATION TO PLATE

Plate I. Photomicrographs of sections of the proventriculus and ventriculus of the developing and adult domestic fowl showing acid phosphatase activity.

a. 20 day old proventriculus (64x).
b. Adult proventriculus [only multiple gland] (128x).
c. Adult proventriculus (64x).
d. 0 day old ventriculus (64x).
e. 10 day old ventriculus (64x).
f. Adult ventriculus [only mucosal tubules] (104x).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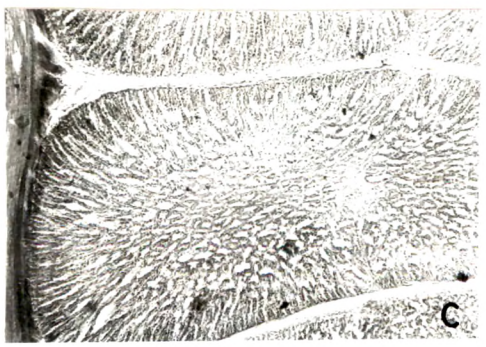
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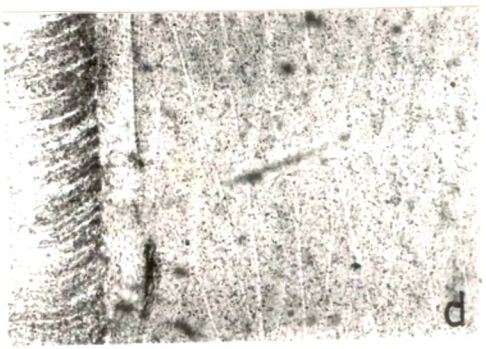
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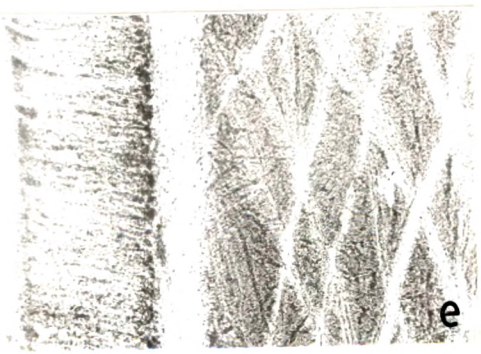
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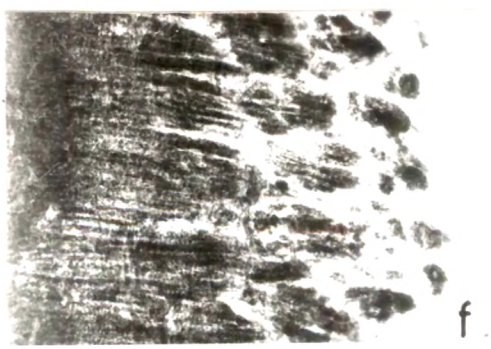
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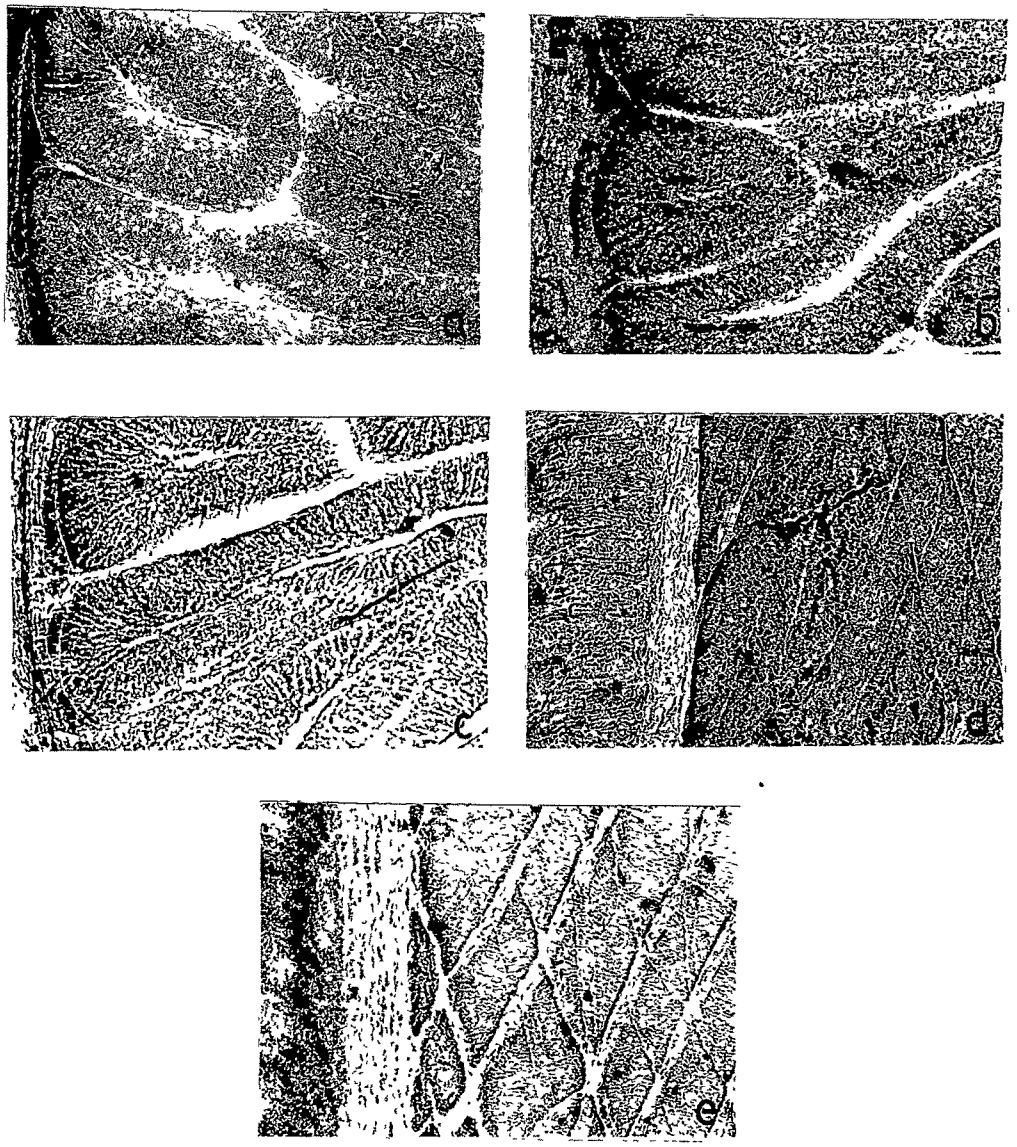
EXPLANATION TO PLATE

Plate II. Photomicrographs of sections of the proventriculus and ventriculus of the developing and adult Japanese quail showing acid phosphatase activity.

a. 0 day old proventriculus (64x).
b. 10 day old proventriculus (64x).
c. Adult proventriculus (64x).
d. 20 day old ventriculus (64x).
e. Adult ventriculus (64x).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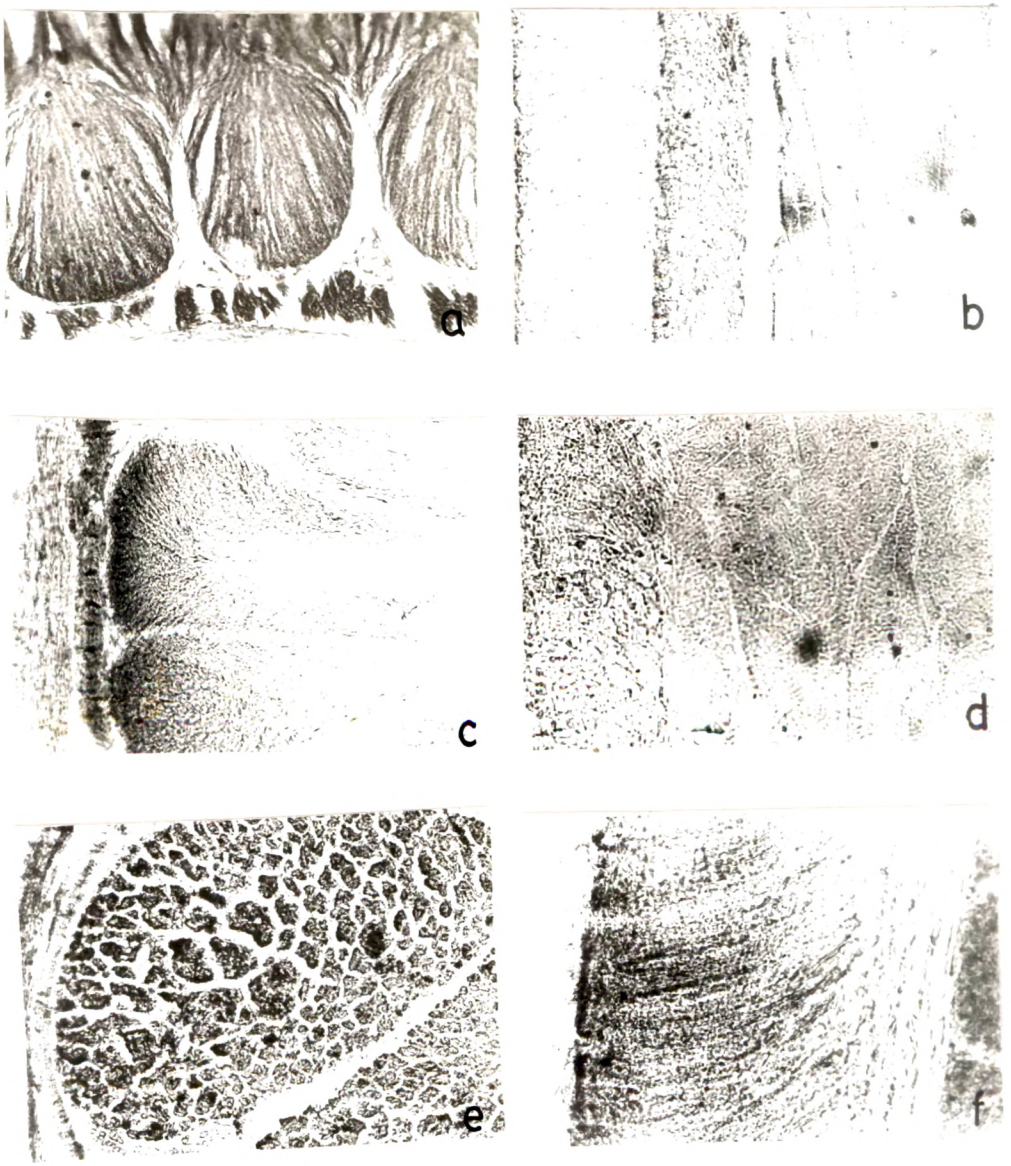
EXPLANATION TO PLATE

Plate III. Photomicrographs of sections of the proventriculus and ventriculus of certain adult avian species showing acid phosphatase activity.

a. Swift proventriculus (128x).
b. Swift ventriculus (128x).
c. Sparrow proventriculus (128x).
d. Sparrow ventriculus (128x).
e. Pigeon proventriculus (128x).
f. Pigeon ventriculus (128x).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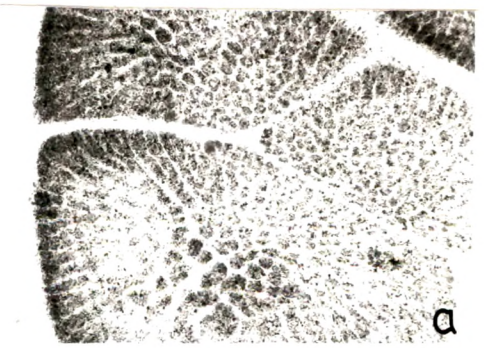
EXPLANATION TO PLATE

Plate IV. Photomicrographs of sections of the proventriculus and ventriculus of developing and adult domestic fowl showing alkaline phosphatase activity.

a. 10 day old proventriculus (64x).
b. Adult proventriculus (64x).
c. 0 day old ventriculus (64x).
d. 10 day old ventriculus (64x).
e. Adult ventriculus [mucosal tubules] (64x).
f. Adult ventriculus [muscle] (64x).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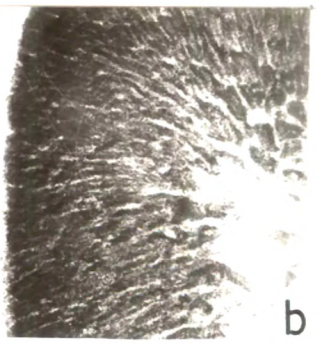
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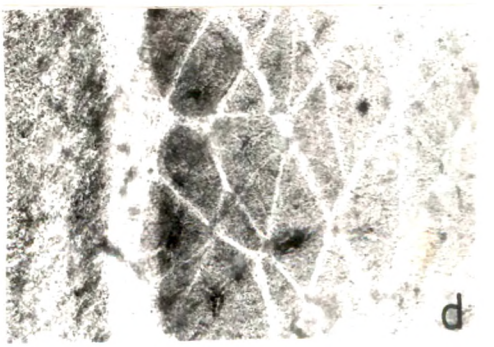
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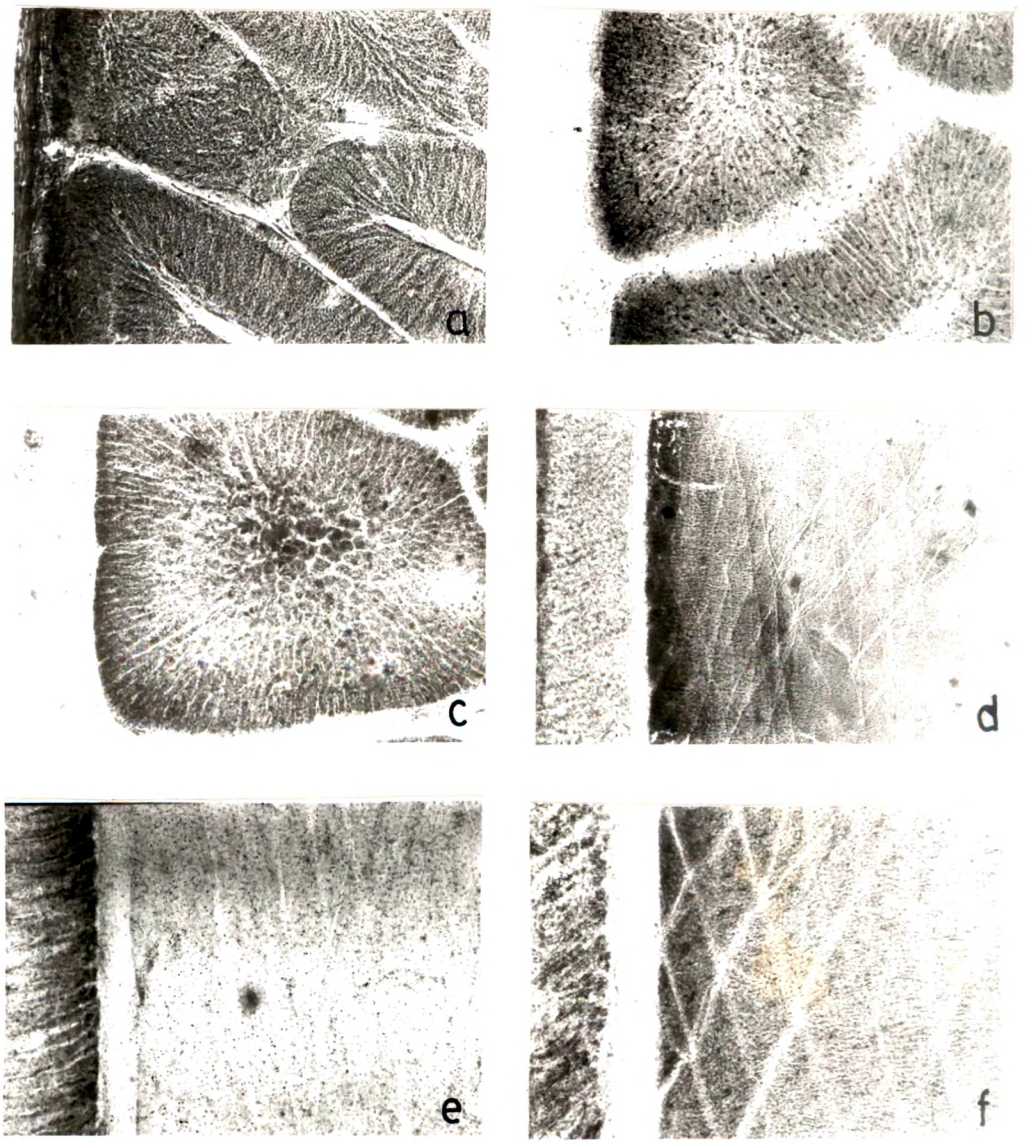
EXPLANATION TO PLATE

Plate V. Photomicrographs of sections of the proventriculus and ventriculus of the developing and adult Japanese quail showing alkaline phosphatase activity.

a. 0 day old proventriculus (64x).
b. 10 day old proventriculus (64x).
c. Adult proventriculus (64x).
d. 0 day old ventriculus (64x).
e. 10 day old ventriculus (64x).
f. Adult ventriculus (64x).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EXPLANATION TO PLATE

Plate VI. Photomicrographs of sections of the proventriculus and ventriculus of certain adult avian species showing alkaline phosphatase activity.

a. Swift proventriculus (128x).
b. Swift ventriculus (128x).
c. Pigeon proventriculus (64x).
d. Pigeon ventriculus (64x).

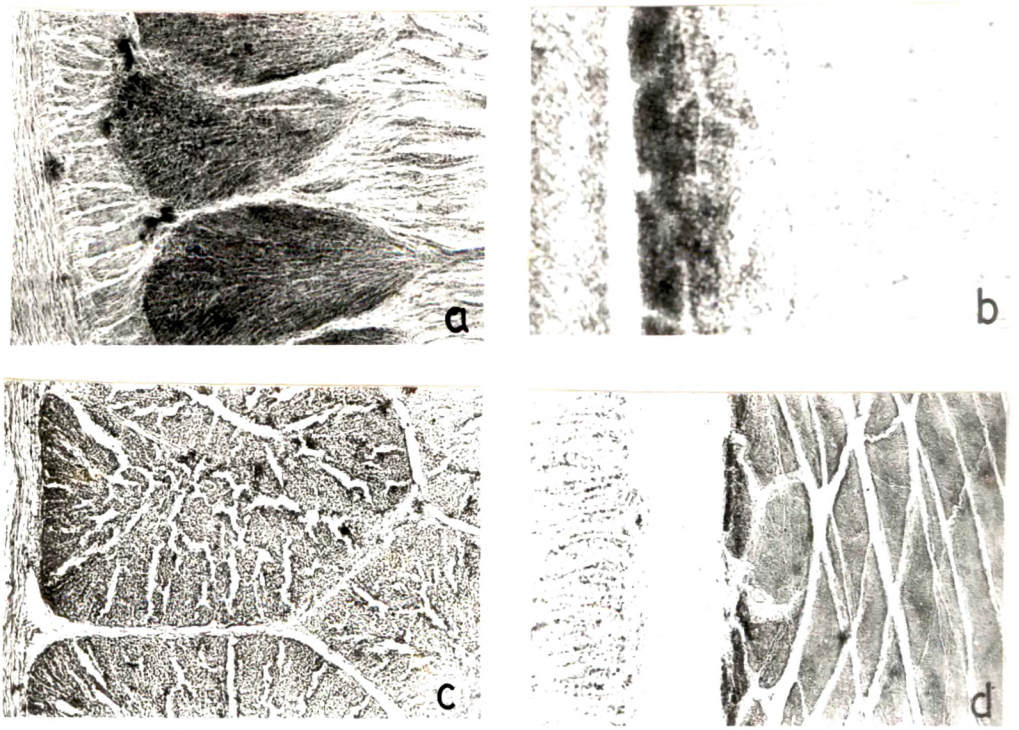
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Table 3. Intensity of histochemical reactivity of acid phosphatase activity in proventriculus and ventriculus of developing and adult fowl and quail.

AGE (DAYS)	ACID PHOSPHATASE ACTIVITY									
	PROVENTRICULUS				VENTRICULUS					
	MULTIPLE GLAND		OUTER LAYER		MUCOSAL TUBULE		SUBMUCOSAL CONNECTIVE TISSUE LAYER		MUSCLE LAYER	
	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL
0	+++	++±	+±	+±	+++	+++±	+	+	+	+
5	+++	+±	±	+	+±	++±	±	±	±	±
10	+++	++	+	+	++	+++	+	±	+	±
20	++±	+±	-	±	+±	++±	±	±	±	±
30	++±	+±	-	±	+±	++	±	±	±	±
ADULT	++±	+±	++	+	+±	++	±	-	±	-

n = ?

Table 4. Intensity of histochemical reactivity of acid phosphatase activity in proventriculus and ventriculus of certain adult birds.

ANIMAL MODEL	ACID PHOSPHATASE ACTIVITY				
	PROVENTRICULUS		VENTRICULUS		
	MULTIPLE GLAND	OUTER LAYER	MUCOSAL TUBULE	SUBMUCOSAL CONNECTIVE TISSUE LAYER	MUSCLE LAYER
SWIFT	++++	+++±	++	±	+±
SPARROW	+++	++	+++	+	++±
PIGEON	++±	++	++	±	+±
QUAIL	+±	+	++	-	-
FOWL	++±	+±	+±	±	±

n= ?

Table 5. Intensity of histochemical reactivity of alkaline phosphatase activity in proventriculus and ventriculus of developing and adult fowl and quail.

AGE (DAYS)	ALKALINE PHOSPHATASE ACTIVITY									
	PROVENTRICULUS				VENTRICULUS					
	MULTIPLE GLAND		OUTER LAYER		MUCOSAL TUBULE		SUBMUCOSAL CONNECTIVE TISSUE LAYER		MUSCLE LAYER	
	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL
0	+++	+++	++	++	+±	++	+	+	+±	+
5	+±	+±	+	+	+++	+±	-	±	++±	+±
10	++	++±	±	+	+±	++	±	±	±	±
20	+±	+±	±	±	++	+±	±	±	+±	+±
30	++±	+±	+±	+	++±	+±	±	+	+±	±
ADULT	++±	+±	+±	±	++	+±	+	±	±	±

n = ?

Table 6. Intensity of histochemical reactivity of alkaline phosphatase activity in proventriculus and ventriculus of certain adult birds.

ANIMAL MODEL	ALKALINE PHOSPHATASE ACTIVITY				
	PROVENTRICULUS		VENTRICULUS		
	MULTIPLE GLAND	OUTER LAYER	MUCOSAL TUBULE	SUBMUCOSAL CONNECTIVE TISSUE LAYER	MUSCLE LAYER
SWIFT	++	+±	+±	±	±
SPARROW	+	±	+±	+	±
PIGEON	+	+	++	±	+
QUAIL	++	+	++	+	+
FOWL	++±	+±	++	+	±

n = ?

Fig 1a. Levels of acid phosphatase in growing birds.

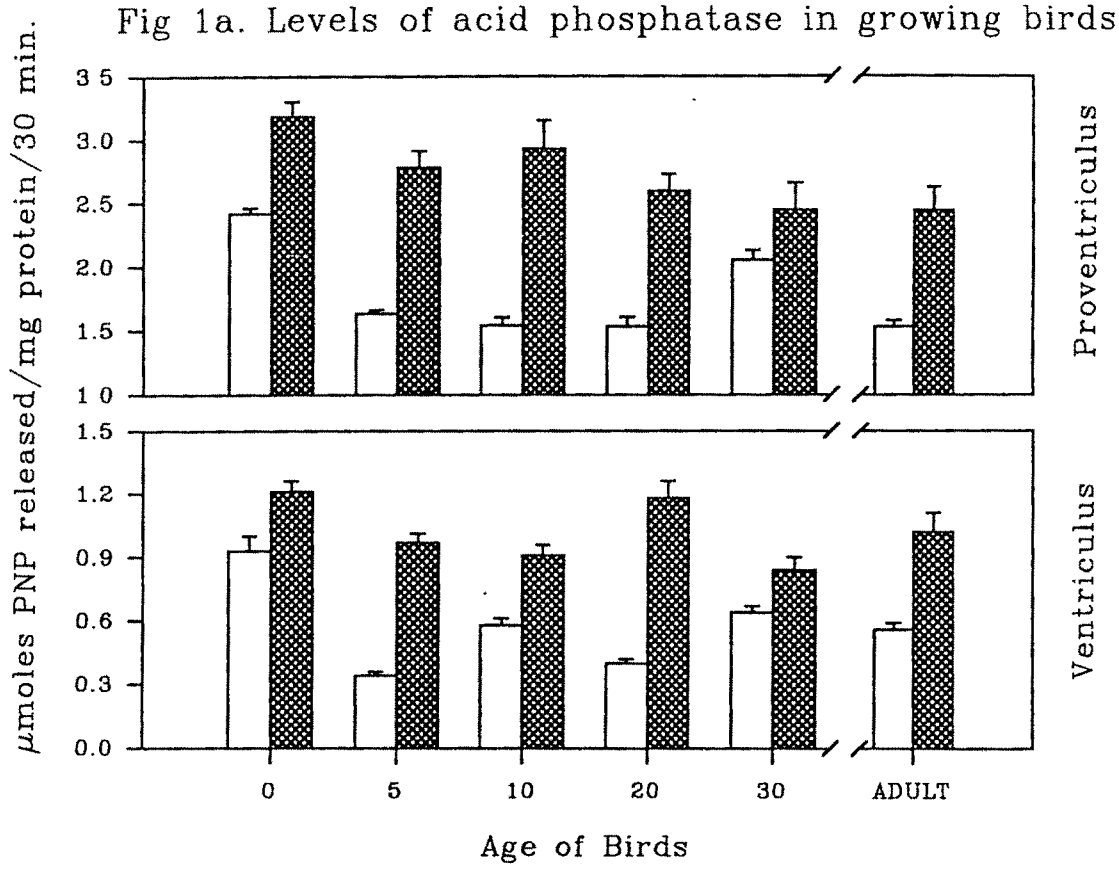


Fig 1b. Levels of alkaline phosphatase in growing birds.

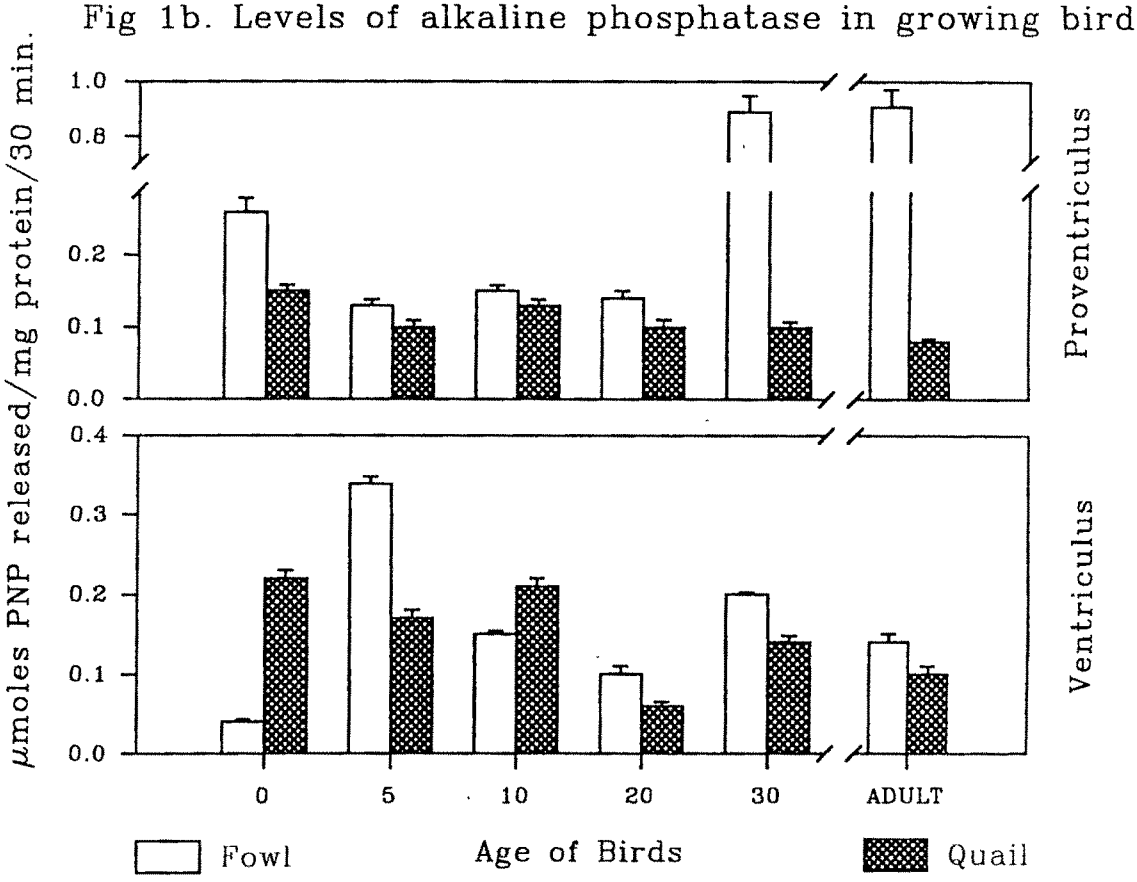


Fig 2a. Levels of acid phosphatase in adult birds.

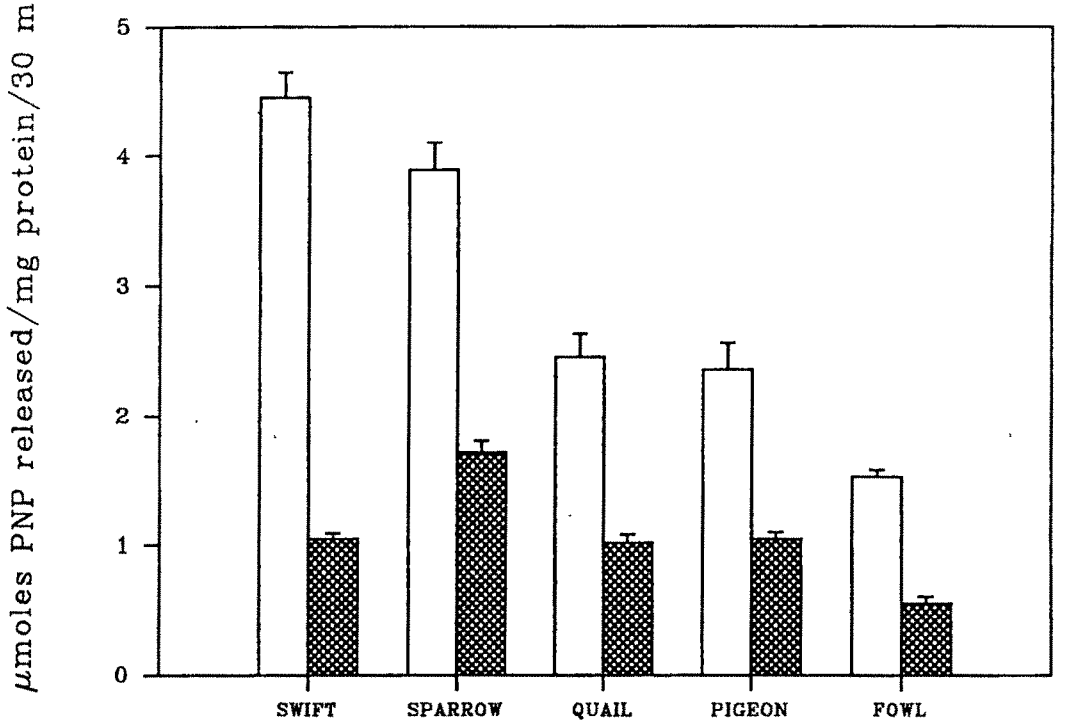
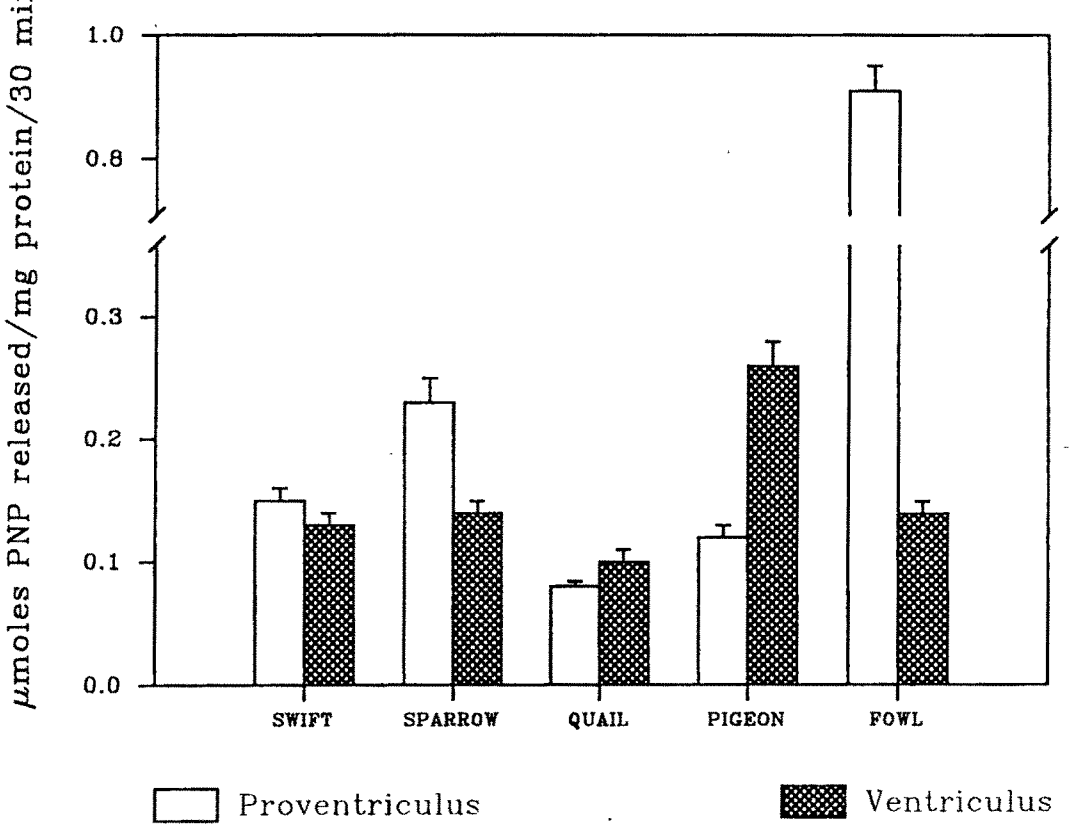


Fig 2b. Levels of alkaline phosphatase in adult birds.



activity. Amongst the adult birds, fowl had the maximum enzyme activity followed by swift and quail. The other two birds, the sparrow and pigeon had negligible activity (plate VI; table 6)

Ventriculus (Plate IV, V; Table 5).

The mucosal tubules as well as muscle layer of both fowl and quail were enzyme reactive while submucosal connective tissue layer reacted very poorly to alkaline phosphatase activity. Amongst the different components of adult birds, the mucosal tubules depicted high enzyme reactivity whereas the other two components showed low but identical enzyme concentration (plate VI; table 6).

DISCUSSION

It is quite significant from the present observation that the posthatched developmental period of proventriculus and ventriculus of the two precocial birds, both acid and alkaline phosphatases showed divergent trends in their concentrations. From a moderate level of acid phosphatase observed in the proventriculus of fowl on the day of hatching the enzyme concentration progressively reduced till day 20. Thereafter the same level was maintained in the adult after a short increase on day 30. As far as ventriculus of quail was concerned, from a high level of enzyme concentration on day of hatching, a progressive reduction was observed and the reduced level was maintained in the adult. On a comparative basis, alkaline phosphatase concentration appears to be little in the proventriculus of quail than fowl whereas in the ventriculus of fowl it was negligible and in quail it was moderate.

Interestingly enough, the initial period of growth is characterized by a quick development of the connective tissue around the proventricular glands and ventricular smooth muscle fasciculi. It is surmised that alkaline phosphatase, even in small concentration, could be functioning in the laying down of connective tissue elements in the developing proventriculus and ventriculus as could be observed from the thickness of connective tissue (chapter II). Such an assumption is rather tenable as this enzyme has been associated with the collagen synthesis (Fell and Danielli, 1943) and fibrous protein formation and collagen differentiation (Marchant, 1949; Junquiera, 1950). Whereas the activity of alkaline phosphatases in the smooth muscle may be correlated

with the synthesis of contractile protein during the initial days, its presence during the same period could also be related with high metabolic incidence as number of non-specific functions have been ascribed to this enzyme in cell metabolism (Cusworth, 1958; Duncun, 1959; Rosenthal *et al.*, 1960; Saev, 1963: Morton, 1965; Vallyathan and George, 1965; Pilo *et al.*, 1972).

In fowl excepting only for this short period of low alkaline phosphatase activity in proventriculus of 30 day old and adult the activity was moderate all throughout the rest of the period of its development. An elevated level of acid phosphatase activity in both proventriculus and ventriculus was the feature on the day of hatching. Correspondingly this is the period characterized by a tremendous pace of morphological and physiological development of these organs (chapter II) marked off not only by the development of the musculature and the connective tissue, and multiple glands but also the progressive increase in the process of keratinization. The role of this enzyme at this stage could, well, be for functional differentiation of muscular tissue and help in the synthesis of contractile proteins. A progressive fall in the enzyme activity was discernable coinciding with the completion of the process of development and differentiation of the structural and functional entities of the proventriculus and ventriculus. An increase in the acid and alkaline phosphatase activity was observed on day 30 in proventriculus and ventriculus of fowl and on day 30 in the ventriculus of quail could be in good correlation with the attainment of the full mechanical functioning of the ventriculus and also the secretory activity of the proventriculus.

The acid phosphatase activity in both the components of stomach of adult birds could be correlated with both the structural and functional peculiarities of the organs. Perhaps the most important structural elements of the avian proventriculus and ventriculus are the glands and the koilin lining respectively. The pronounced activity of acid phosphatase in the mucosal tubules and the proventricular glands could be attributed to the secretory activity of these organs. The secretory role of this enzyme has been reported (Sobel, 1964; Majumdar *et al.*, 1988). It is interesting to note in this connection that the inner koilin layer in the group of birds other than graminivore is invariably thin as could possibly be the adaptive feature in relation to the soft food consumed and processed by such ventriculus. The elaboration and secretion of koilin layer

becomes necessary to replace the constantly wearing out koilin layer whereas in the case of proventriculus the multiple glands are smaller in size in the groups other than the graminivores.

The alkaline phosphatase activity in the proventriculus and ventriculus studied herein was moderate to nil. The activity of this enzyme has been correlated with laying down of connective tissue element in a variety of tissue by various investigators (Fell and Danielli, 1943; Marchant, 1949; Junquiera, 1950). This correlation appears to be well exemplified in the present study. The activity of this enzyme was low in those groups of birds where the connective tissue of proventriculus and ventriculus is relatively thin (all birds other than graminivores); the enzyme activity was demonstrably higher in graminivores and fowl where the connective tissue strands surrounding the muscle fasciculi are thicker.