CHAPTER 9

A COMPARATIVE STUDY ON CHOLINESTERASES IN THE GIZZARDS OF CERTAIN ADULT REPRESENTATIVE BIRDS

It has been well established that coordinated activity of the visceral smooth muscle depends on the regulatory control exerted by neural elements consisting of the gastric branch of vagus and an extension of the autonomic nervous system into the gut. The involuntary activity leading to coordinated movements of the greater part of the gut is, thus, not due to contractility of visceral smooth muscle alone but is also due to the reflex activity involving neural elements. The presence of nerve cells in the pharynx and stomach has been known since the time of Remak (1840, 1852); and the studies of Meissner (1857), Auerbach (1862, 1864), Billroth (1858) and Drasch (1881) indicated that the layer of the gut contained neural elements similar in many respects to those in other parts of the nervous system. An increased or decreased nervous activity at any site could be inferred through studies on the level of activity of cholinesterases, the intensity of which fluctuates according to the amount of esters of choline secreted by the nerve endings. Histochemical studies of visceral organs showing regional

distribution and types of cholinesterases are, by now, available (Gerebtzoff, 1950; Koelle, 1950; Hey, 1960; Mumenthalar & Engel, 1961; Yamamoto, 1961; Ballantyne & Burwell, 1965; Maynard, 1965; Leeson & Leeson, 1965a; Desai, 1967; Julia & Bryan, 1967; Pilo, 1969; Yamauchi & Burnstock, 1969a & b; Asnani, 1971). The present histochemical investigations of acetyl and butyryl-cholinesterases (AChE & BuChE) were, therefore, undertaken with a view to obtain more information about these in the gizzards of a number of adult representative birds showing variations in their food and feeding habits.

MATERIALS AND METHODS

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

The gizzards from all the adult birds were 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 as given in chapter 5 for the histochemical demonstration of AChE and BuChE.

OBSERVATIONS

(Figs. 1–12; 1a – 12a).

In general, the activity of AChE was found to be more prominent than BuChE in both the components of the gizzards of all the birds studied herein. In granivore (pigeon), both the components of the gizzard viz., mucosal tubules as well as smooth muscle fibres failed to show any response towards BuChE while the concentration of AChE in them was found to be relatively higher as well as equal. In carnivorous birds like shrike and kite, the smooth muscle layer depicted the AChE concentration identical and equal to that observed in granivores while BuChE was half the AChE concentration. The mucosal tubules of these birds registered the levels of these enzymes which was slightly lower than those observed in the smooth muscle fibres. Amongst insectivores (Bee-eater, Drongo, Crow Pheasent, Redvented Bulbul and Koel), both these enzymes registered a concentration in both the components of the gizzard in the same style and pattern as that noted for carnivores. The rest of the members of this group (Indian Robin, House Sparrow, Jungle Babbler and Common Myna) registered a lower level of AChE activity in the smooth muscle fibres while the BuChE activity in the same component was identical and equal to the one observed for

EXPLANATIONS FOR FIGURES

(Figures 1 - 6 Acetylcholinesterase activity in the mucosal tubules of gizzards of various birds),

(Figures 1a - 6a Acetylcholinesterase activity in the smooth muscle fasciculi of gizzards of various birds),

(Figures 7 - 12 Butyrylcholinesterase activity in the mucosla tubules of gizzards of various birds) and

(Figures 7a - 12a Butyrylcholinesterase activity in the smooth muscle fasciculi of gizzards of various birds)

Figs. 1, 1a, 7, 7a - Shrike

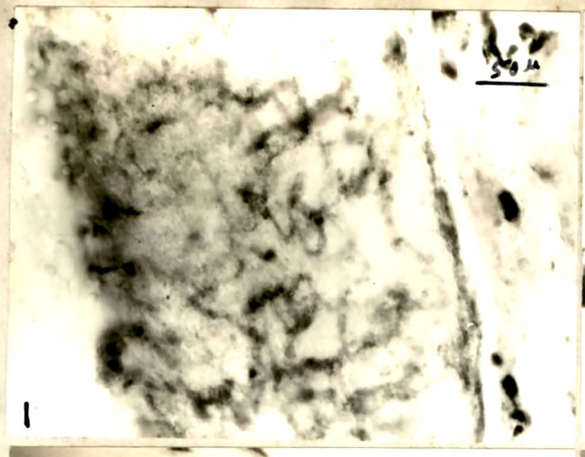
Figs. 2, 2a, 8, 8a - Kite

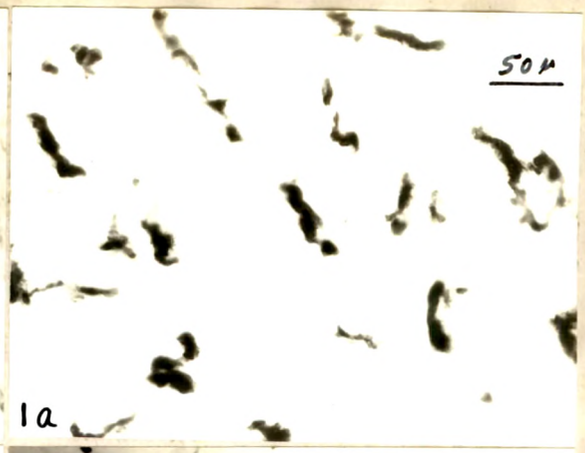
Figs. 3, 3a, 9, 9a - Crow

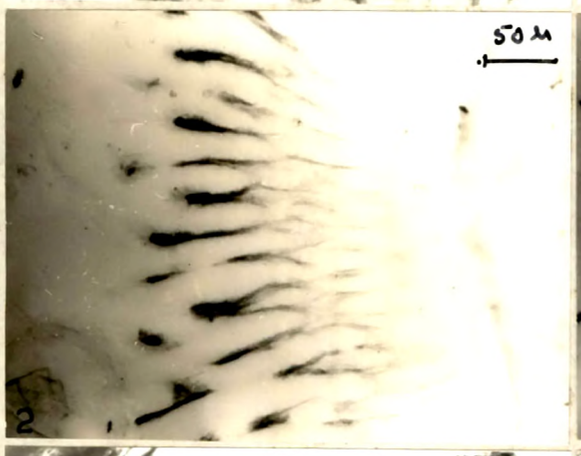
Figs. 4, 4a, 10, 10a - Drongo

Figs. 5, 5a, 11, 11a - Crow Pheasant

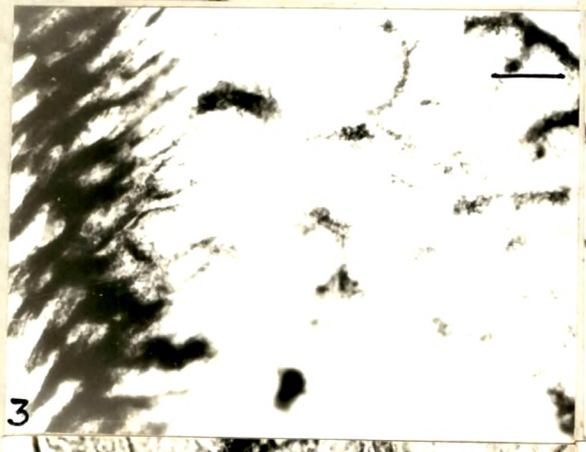
Figs. 6, 6a, 12, 12a - Sunbird

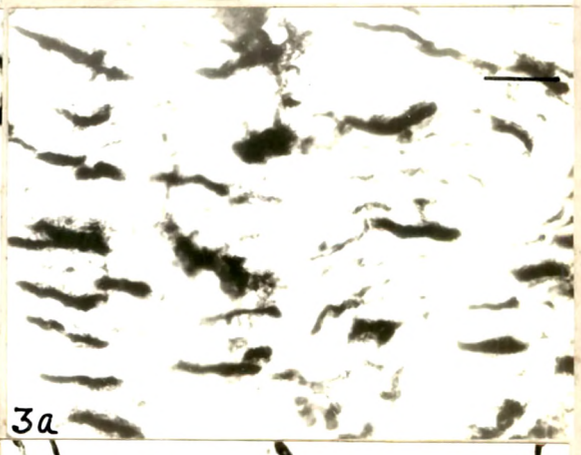

50 μ
1

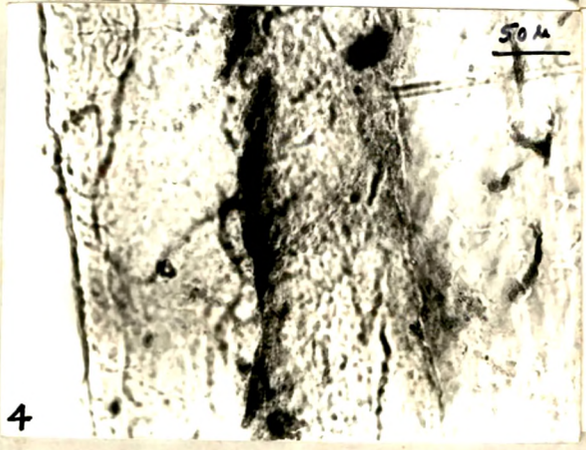

50 μ
1a

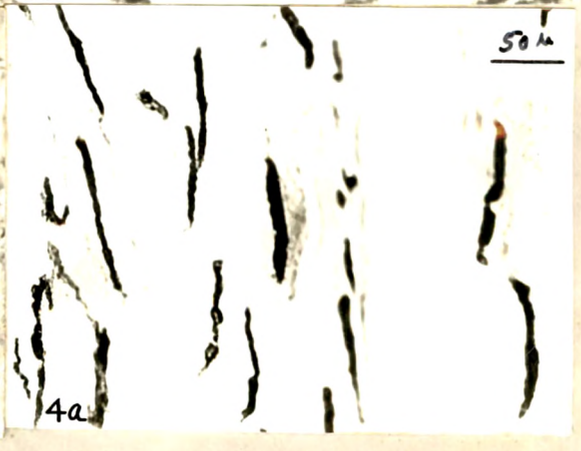

50 μ
2

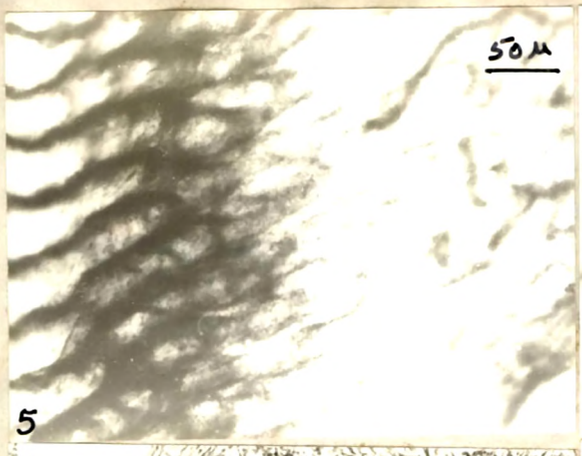

50 μ
2a

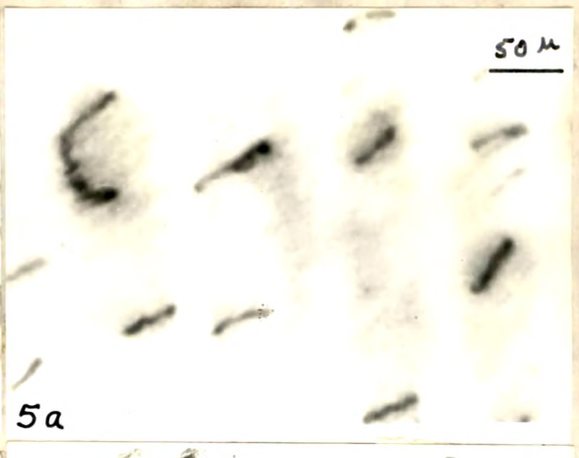

3

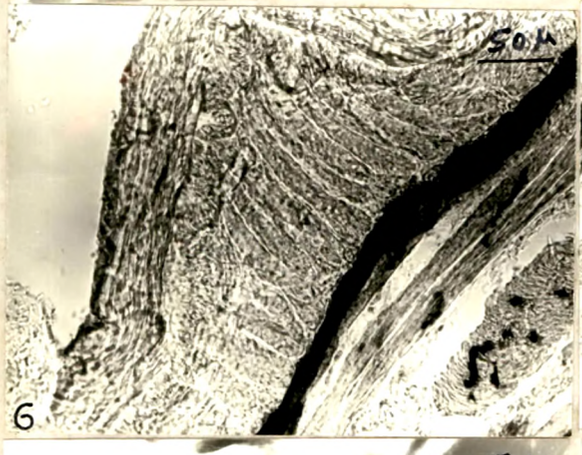

3a

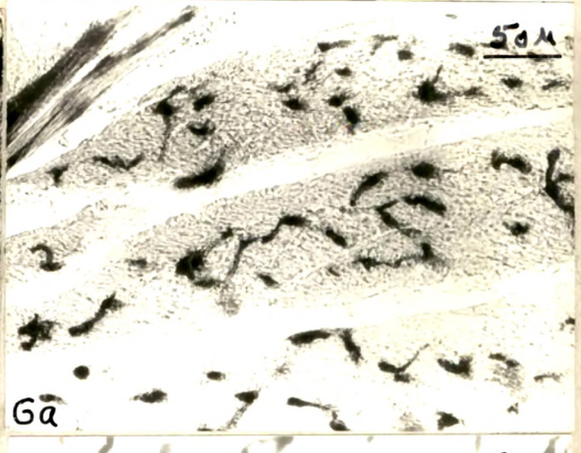

50 μ
4

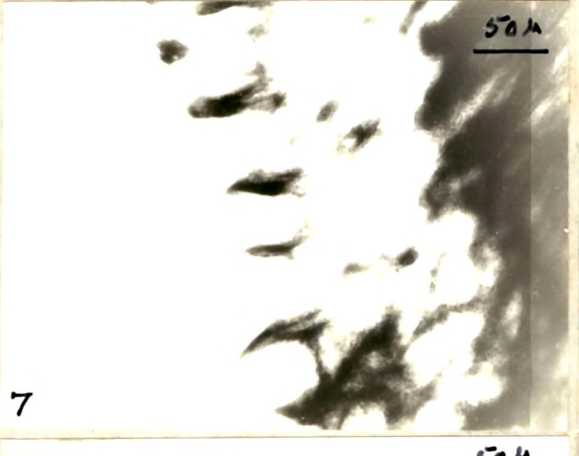

50 μ
4a


50 μ
5

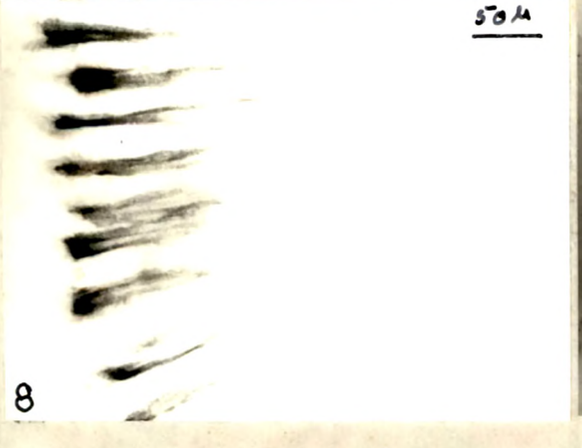

50 μ
5a

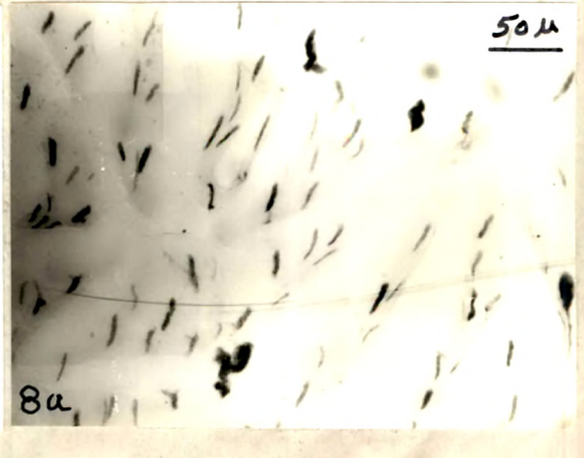

50 μ
6

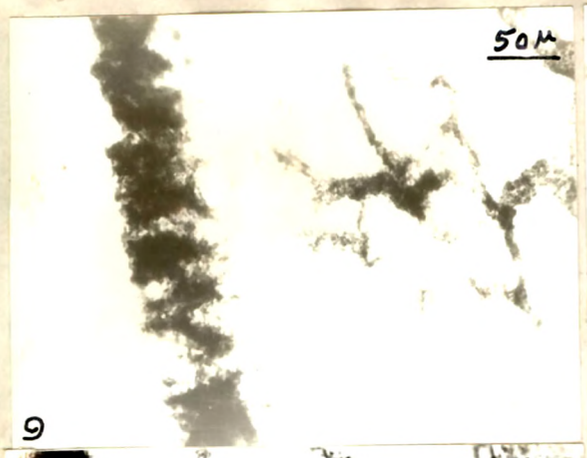

50 μ
6a


50 μ
7

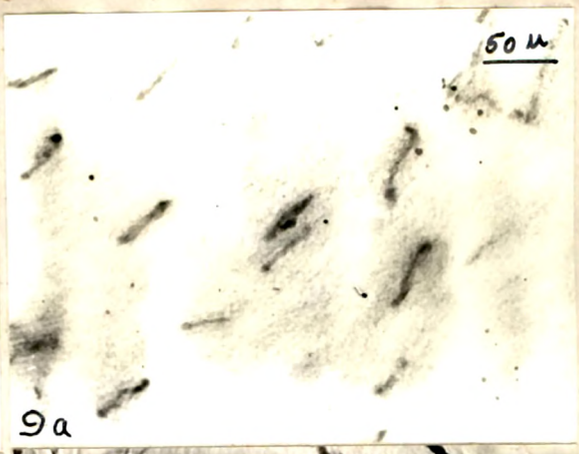

50 μ
7a


50 μ
8

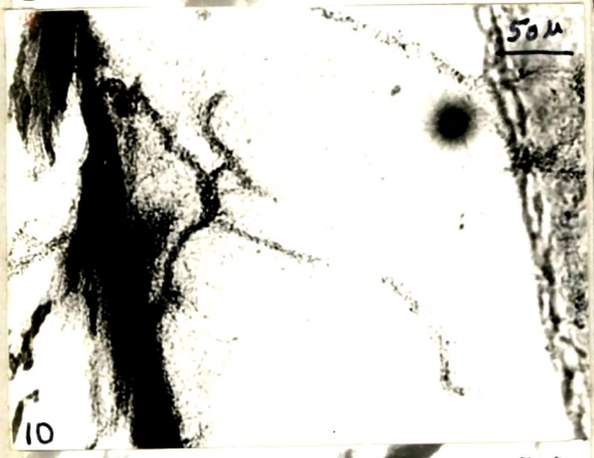

50 μ
8a



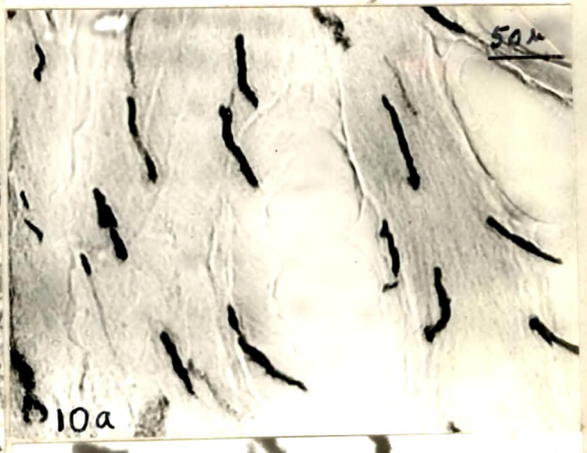
9



9a

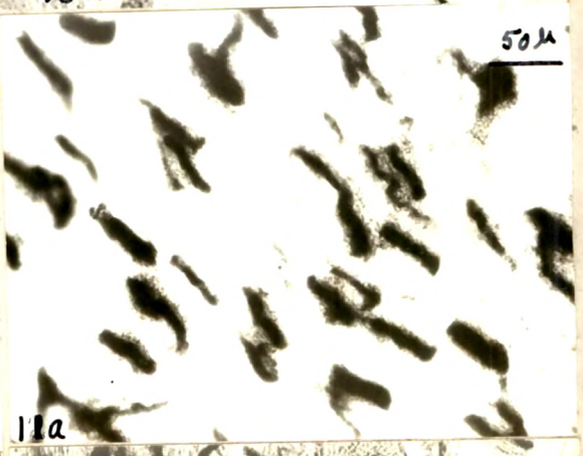


10



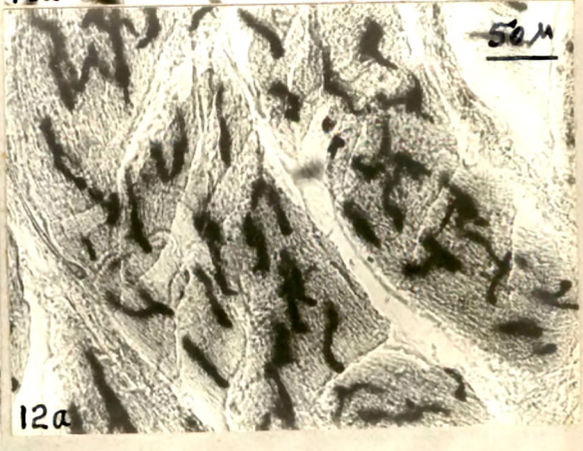
10a

11



11a

12



12a

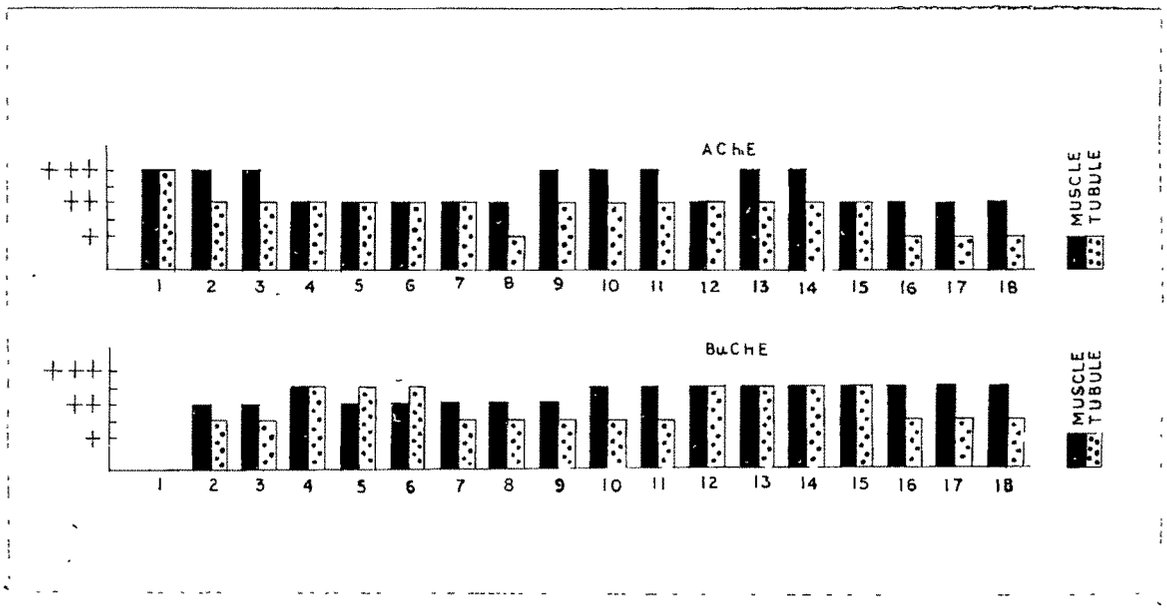


FIGURE 13

Graphical representation of the changes in acetl and butyryl cholinesterases distribution pattern in the gizzards of adult representative birds

carnivores. The mucosal tubules of these birds showed two different concentrations of these enzymes with one (Indian Robin and House Sparrow) recording the lowest level and the other (Jungle Babbler and Common Myna) registering a concentration identical and equal to the one observed in the smooth muscle fibres of the gizzards of the same birds. In omnivores, the concentration of AChE in both the components and that of BuChE in the mucosal tubules were identical and equal to those observed for Common Myna and House Sparrow (except for fowl where it was half this concentration) while the concentration of BuChE in the smooth muscle fibres was identical to the one observed for carnivores and insectivores. In the frugivore and nectar feeder, both the components of the gizzard responded with same intensity towards both these enzymes as observed in Jungle Babbler and House Sparrow (more in the smooth muscle fibres and less in the mucosal tubules).

DISCUSSION

AChE and BuChE have been shown to be present in every nerve ending irrespective of the nature of the fibres in the red and white muscles of vertebrates

(Klinar & Zupanac, 1962; George & Berger, 1966). The cholinesterases in the striated muscle were extensively studied due to their precise localization as well as variations in their activities during different phases of muscular contractions. In other tissues besides muscle wherein non-specific cholinesterases have been studied, its role is still a matter of speculation since the precise substrate for this enzyme is unknown (Gerebtzoff, 1959). Skeletal muscles of a number of vertebrates are known to be made up of two distinct types of fibres, the white quick contracting fibres possessing motor end plates of the "en plaque" type and the red, slow contracting fibres having a characteristic "en grappe" type of nerve ending (Krüger, 1958; 1960; Ginsborg & Meckay, 1960).

The findings from the present study revealed that the nerve fibres (vagus branch) innervating the gizzard smooth muscle of all birds were cholinesterase positive, there being only slight differences in the concentration of these two enzymes. No cytoplasmic cholinesterase activity could be discernible in the smooth muscles whereas nerve fibres which course through muscular layers registered both AChE and BuChE activity. From the result

obtained from various workers on cholinesterases in the alimentary canal, it is evident that great variability exists in their activity and localization (Gerebtzoff, 1959). Studies conducted by Bennett (1969) have conclusively proved that when gizzard tissue from chicken and pigeon was incubated with acetyl- or propionylcholine an intense stain developed after 10-20 minutes and was localized in the main body of Auerbach's plexus and in the nerve trunks and ganglion cells of its extension, in axon bundles running in the muscle and in the nerve trunks accompanying the arteries. When the tissue was incubated in butyrylthiocholine, the staining reaction was developed after periods of one hour or more. The stain was localized in those structures stained with acetyl- or propionylthiocholine and also in the smooth muscle cells. These results, according to Bennett (1969), indicate that true cholinesterase is localized in nervous structures associated with gizzard and its vasculature. Staining of smooth muscle cells is a result of pseudocholinesterase, these results agree with those of other workers (Koelle, 1951; Gerebtzoff, 1959; Cuthbert, 1963; Bell, 1967) who have demonstrated the presence of pseudocholinesterase within the spontaneously active smooth muscle tissue.

Though the increased activity of AChE could be correlated with the increased nervous function, the increase in the activity of non-specific cholinesterase (BuChE) in the gizzard is difficult to explain. However, an increased BuChE activity in the gizzards of all the birds studied could be related to an enhanced lipolytic activity. Szendzikowski et al. (1961/62) observed that about 50% of the lipolytic activity in the rat aorta depends on non-specific cholinesterase. Ballantyne & Burwell (1965) studied histochemically the distribution of BuChE in the lymph node and suggested that the enzymes may be associated with macrophages. According to them this enzyme probably functions to remove some lipid component from the tissue fragments so as to make them presentable to "receptor cells". Since choline assists in lipid clearance, BuChE could also provide more choline thereby clearing most of the fat. The presence of an identical level of BuChE though lower than AChE noticed in the adult gizzards might, thus, be correlated with the lipid utilization as could be surmised from the activities of the enzymes associated with lipid catabolism (chapter 7).

Finally, it is pertinent to note that Bulbring (1953), Ellis (1957) and Lundholm (1960) have brought out an interesting aspect that a stimulating substance like acetylcholine increases the expenditure of energy and therefore also oxygen consumption, glycogenolysis and lactic acid production in the smooth muscle of intestine. These revelations when reflected on to the inference drawn from the present study that there is a differential gradations of metabolic activity in the various types of gizzards as per their ingested food material (chapters 6 & 7) are rather suggestive and, thus viewed in this context, should be expected to show a similar gradation of AChE activity in the different types of gizzards. However, such a gradation could not be evidenced as per the observations on AChE in the present study. Instead, two levels of this enzyme activity were observed; one where the activity was maximum as in the case of granivores and carnivores and a few insectivores and the other where the activity was slightly lower as in the case of omnivores, frugivores and nectar feeder. Coincidently the expectance of a high incidence of AChE in metabolically active gizzards of granivores and carnivores is rather self explanatory, in the light of the discussion presented in chapters 6 & 7,

and is in good correlation with the reported views of Bulbring (1953), Ellis (1957) and Lundholm (1960). On the other hand the slightly lower level of AChE activity noted in the other groups of gizzards may be looked upon as a minimal normal complement, very much necessary - irrespective of the size and levels of metabolism of the gizzard - for the normal complementary metabolic functions.