

CHAPTER 9

CHOLINESTERASE ACTIVITY IN THE LIVERS OF ROSY PASTOR AND
SOME NON-MIGRATORY BIRDS

Almost all tissues of vertebrates have been shown to possess ACh (acetylcholine)- splitting activity (Gerebtzoff, 1959). The cholinesterases in muscles were extensively studied because their precise localization as well as the variation in their activities during different phases of muscular contractions, led to the conclusion that the ACh- AChE (acetylcholine- acetylcholinesterase) system plays a vital role in muscle contraction. In other organs besides the muscles, wherein, non-specific cholinesterase (ChE) has been studied, its role is still a matter of speculation since the precise substrate for this enzyme is unknown (Gerebtzoff, 1959).

Liver is one such organ which exhibits cholinesterase activity apart from muscle, but the presence of either specific (AChE) or the non-specific (ChE) cholinesterases varies from species to species (Gerebtzoff, 1959). A non-specific cholinesterase was demonstrated in the liver of guinea pigs and rats using benzoylcholine as the substrate (Sawyer, 1945; Blaschko *et al.*, 1947; Goutier-Pirotte and Goutier, 1956). The liver of cat also possessed non-specific cholinesterase (Korlle, 1951; Gerebtzoff, 1954) but rabbit had a predominance of AChE (Gerebtzoff, 1959). Although the precise localizations are clearly seen in the liver, the nature of their role remains still to be formulated.

Non-specific cholinesterase could participate in the metabolism of fat by splitting the choline moiety from the esters. The presence of choline however, is essential to prevent the formation of fatty liver.

The pseudo- or non-specific cholinesterase found in the plasma was thought to be of hepatic origin (Augustinsson, 1948; 1950; Gajdos, 1950), thus accounting for the presence of ChE in the liver. But, according to Gerebtzoff (1959), if such a 'secretion' of this enzyme could take place in the liver, then the centrilobular regions should show maximum activity, as the liver is formed of hexagonal lobules with rich vascularization at the periphery and a collecting vein in the centre. Bertrand, (1954) while studying the distribution of ChE in livers of mice, cats and guinea pigs, found that the localizations ranged from the peripheral to centrilobular areas and hence, he overruled the possibility of the suggested enzymic 'flow' from hepatic cells to the blood.

The role of acetylcholine (ACh) in the transmission of impulses along a nerve by influencing its membrane permeability is a well known fact. The inhibitors of AChE also ^{are} known to alter the permeability of the erythrocyte membranes (Grieg and Holland, 1949a; 1949b). Thus they suggested that the action of ACh-AChE system may be similar, namely the influence over the ionic changes, in other cells too besides R.B.C. The same function could be attributed to ACh-AChE system in the liver.

The possibility of the activation of bile secretion

by ACh incertain concentrations was put forward by Tanturi and Ivy,(1938), and Ramprasad and Sirsi (1960) because the vagus nerve possesses both excitory and inhibitory effects on the secretion.

Since, the ACh-AChE complex and ChE could affect fat metabolism, the permeability of cells and bile production, it was thought worth while to study the distribution of cholinesterases in the liver of a migratory bird (Rosy Pastor) as well as of some non-migratory ones (Common Myna and Pigeon).

MATERIALS AND METHODS

The livers of Rosy Pastor (Sturnus roseus), Common Myna (Acridotheres tristis) and Pigeon (Columba livia) were utilized for a comparative study. The activity of cholinesterases in the liver of Rosy Pastor was observed in both the post- and pre-migratory periods.

The tissues were fixed in cold formol saline and 10% formaline for 1-3 hours, washed repeatedly with redistilled water and sectioned on freezing microtome so as to obtain approximately 20 μ thick sections, The enzyme activity was demonstrated by employing the method of Koelle and Friedenwald (1949) as modified by Coupland and Holmes (1957) using Acetyl thiocholine-iodide and Butyryl thiocholine-iodide as the respective substrates, for acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE).

In this contest it is necessary to point out that the non-specific cholinesterase could hydrolyse a variety of choline-

esters besides, butyrylcholine, e.g. benzoylcholine, propionylcholine etc., but in the present study, only butyrylcholine was specifically employed as the substrate to demonstrate non-specific cholinesterase activity in the liver. Therefore the enzyme is designated as BuChE through^{ou}t, in order to avoid confusion, instead of ChE of Gerebtzoff (1959).

Control sections were treated with various concentrations of eserine sulphate and incubated at 37°C along with the sample sections. Eserine sulphate at a concentration of 3×10^{-5} M is known to inhibit both the cholinesterases (Chessick, 1954).

Formol saline fixed livers of Pigeon and Myna gave good results whereas 10% formaline (pH 5) was found to be suitable as fixative for the liver of Rosy Pastor.

RESULTS

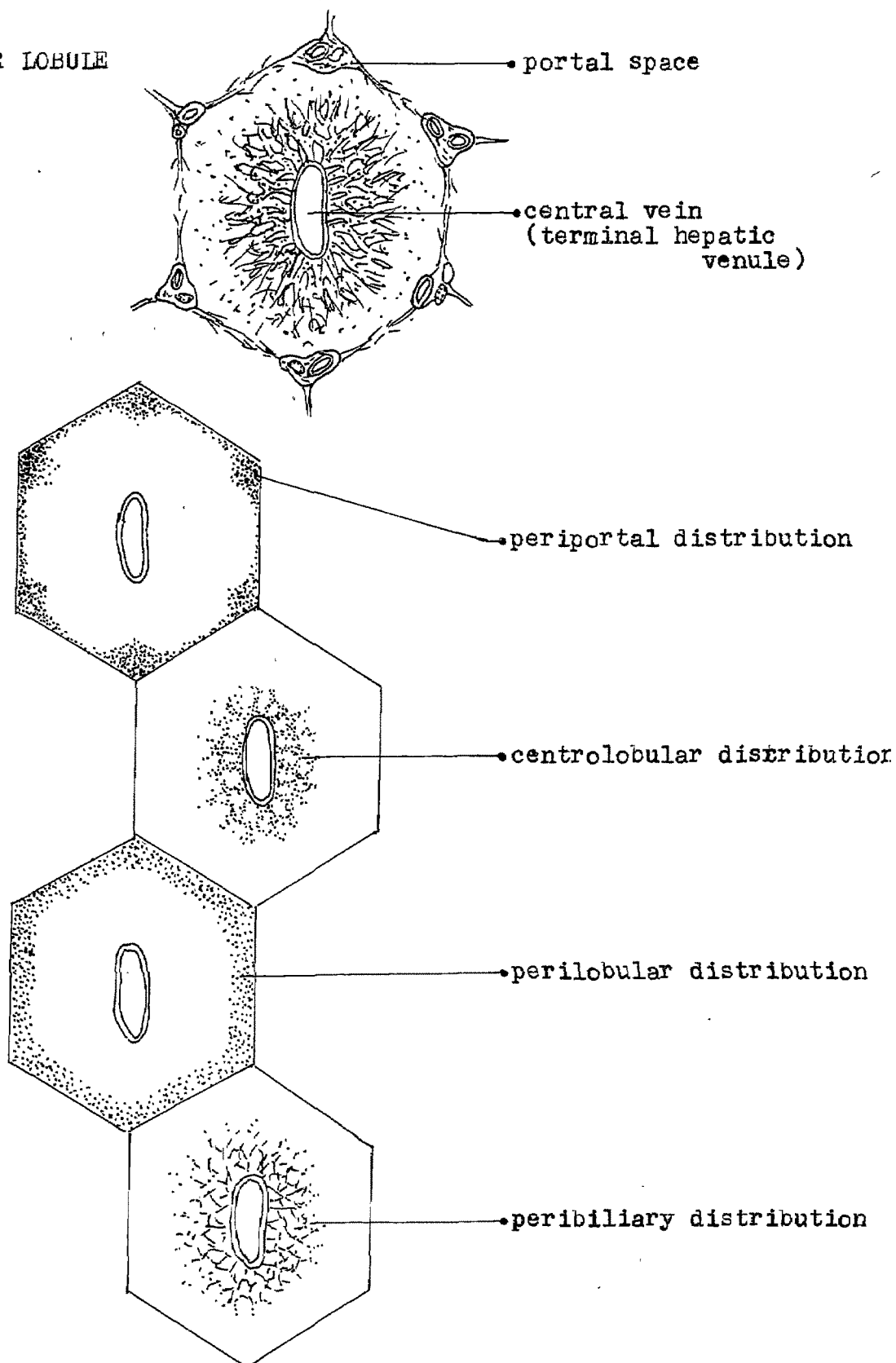
In the Rosy Pastor both AChE and BuChE activities were observed in the liver but the localization and intensity was, however, found to vary in different seasons.

In the postmigratory period, the AChE was obtained in the endothelial lining of the central vein of each lobule but only after 24 hour incubation (Fig.1). BuChE on the contrary, was more profound and was seen in the parenchymal cells surrounding the central vein as well as in the lining of the latter (Fig.2). The peripheral regions of the lobule and the portal spaces were however, devoid of these enzymes.

In the premigratory period, the activities of AChE and BuChE were considerably higher than in the postmigratory phase,

Diagrammatic representation of liver lobule and different types of enzyme distributions

LIVER LOBULE



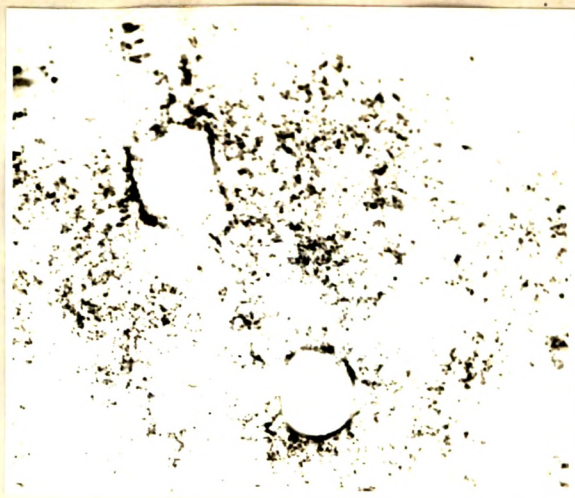


Fig.1. Photomicrograph of AChE activity in the liver of Rosy Pastor during postmigratory period (August). Note the activity more or less confined to the lining of the central vein. 50X.

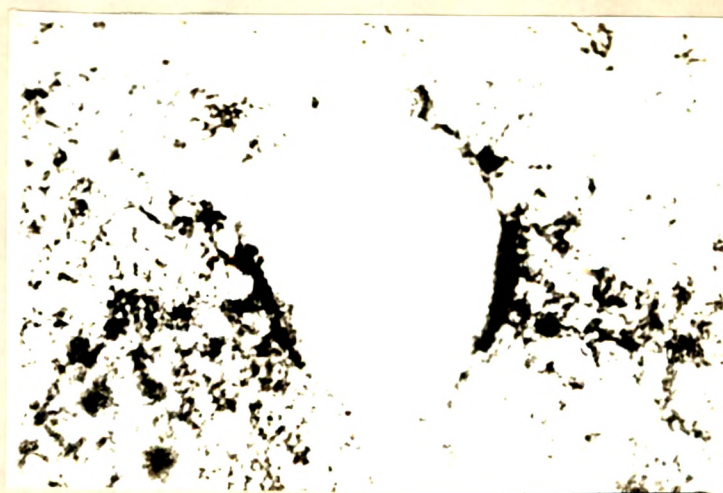


Fig.2. Photomicrograph of BuChE activity in the liver of Rosy Pastor during the postmigratory period (August). Note the activity in the lining of the central vein as well as in the parenchymal cells around the central vein. 128x.

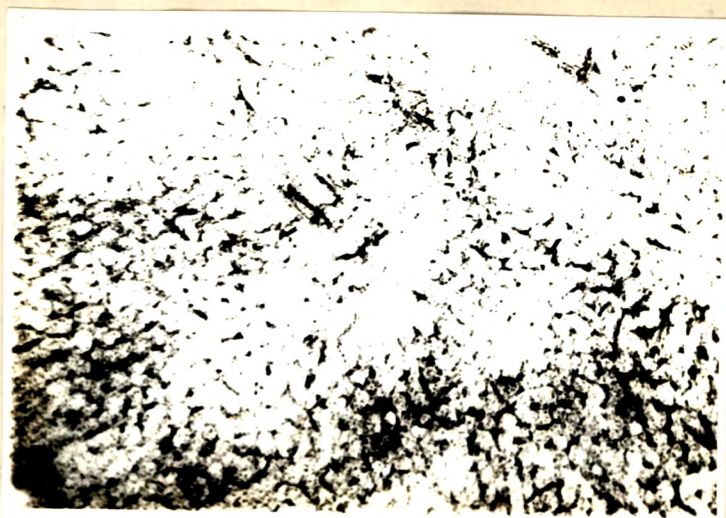


Fig.3. Microphotograph of AChE activity in the liver of Rosy Pastor during premigratory period (April). Note the AChE activity in the bile canaliculi (Peribiliary distribution). 128x.

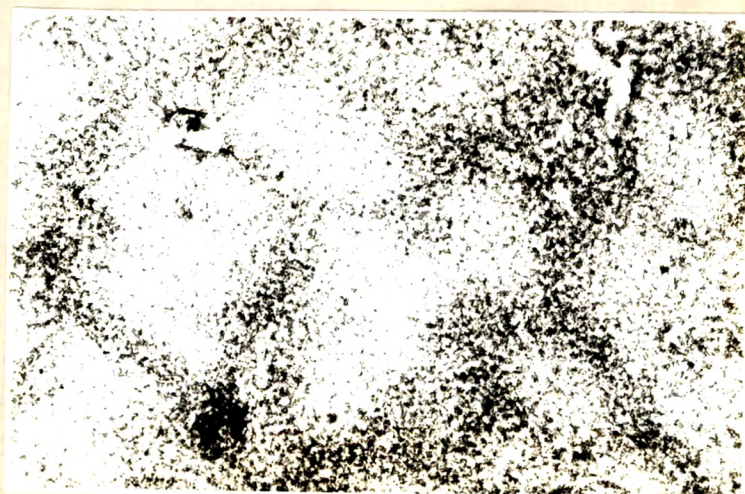


Fig.4. Microphotograph of BuChE activity in the liver of Rosy Pastor during premigratory period (April). Note the absence of activity in the lining of the central vein and the periportal distribution of the enzyme. 50x.

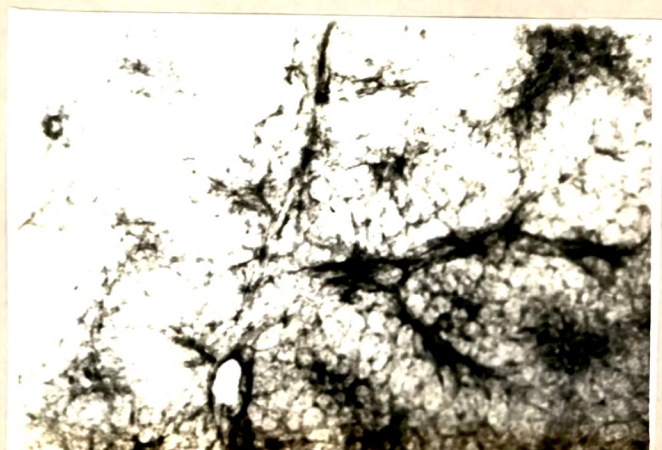


Fig.5. Photomicrograph of AChE activity in the liver of Common Myna. Note the peribiliary and perivascular distribution of the enzyme. 50x.

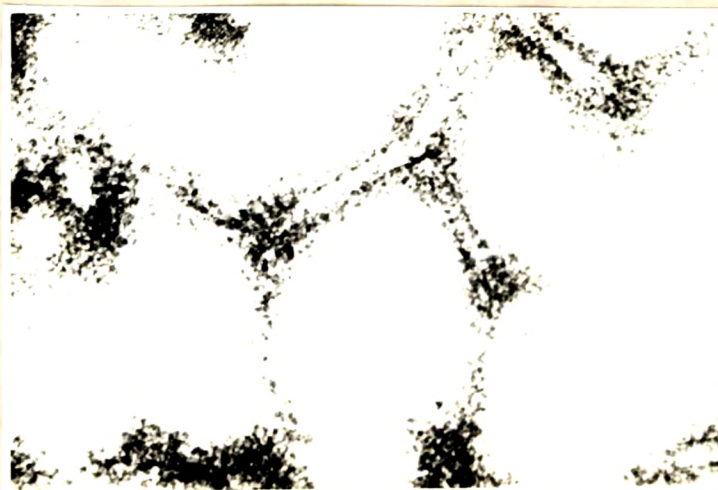


Fig.6. Photomicrograph of BuChE activity in the liver of Common Myna. The activity is seen confined to the periportal and peripheral (perilobular) regions. 50x.

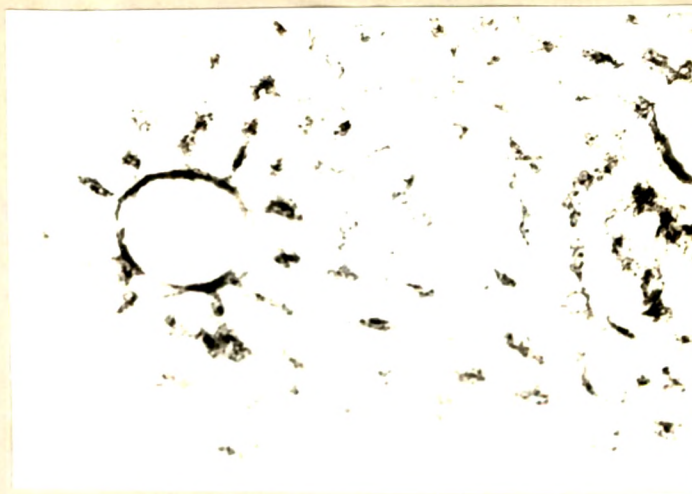


Fig.7. Photomicrograph of AChE activity in the liver of Pigeon. The activity is greatly seen in the lining of the central vein as well as in the parenchymal cells surrounding the central vein. 128x.

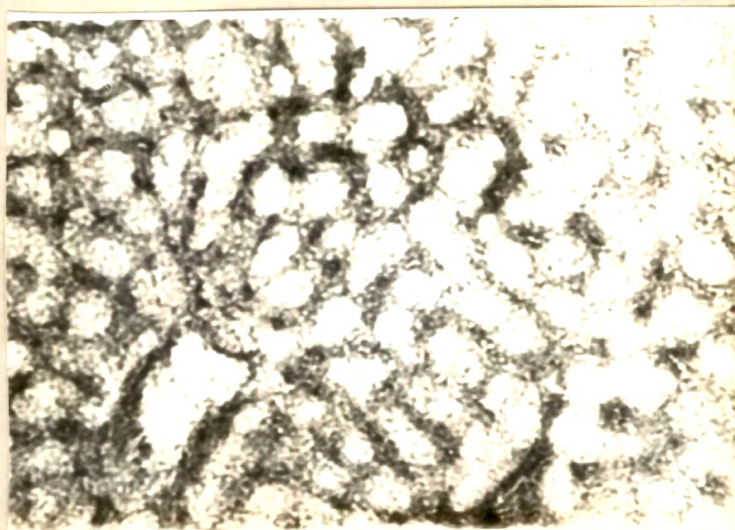


Fig.8. Microphotograph of BuChE activity in the liver of Pigeon. The activity is seen uniformly distributed in the lobules. 128x.

as evidenced by the shorter incubation periods (18-20 hours). AChE was observed in the lining of the blood vessels and central venules (terminal hepatic venules) and also in the bile canaliculi (Fig.3). Out of all these sites, the peribiliary localized AChE was found to be the most active. BuChE was also present in the cells surrounding the bile canaliculi but absent in the epithelial lining of the central vein (Fig.4).

Another important feature observed was the diurnal variation in the intensity of these cholinesterases in the Rosy Pastor liver during the premigratory period. In those birds which were collected in the early hours of the morning (5 a.m.), the liver showed increased activities of both AChE and BuChE (incubation 12 to 15 hours). These were mainly localized in the bile canaliculi. The liver at roosting time (7-7.30 p.m.) was found to contain a slightly lower enzymatic activity (incubation period 17-20 hours), but the site of activity was the same as before. This diurnal variation in the level of cholinesterases, however, is not well established with the histochemical techniques. But, a definite tendency was seen in the activities of these enzymes to be more active in the morning. This, at any rate is interesting with regard to bile production, as the gall bladder was found to be practically empty in the evening, while during the small hours of the day (5 a.m.) it was full.

The liver of Common Myna also possessed both AChE and BuChE, but the latter was more active than the former. BuChE was localized in the parenchymal cells surrounding the blood vessels

and the central vein and was more intense in the portal areas (Fig.6). AChE on the other hand, was seen in the lining of the central venules and in the bile canaliculi (Fig.5).

The Pigeon liver too, possessed both the esterases, wherein, AChE enjoyed a centrilobular distribution (Fig.7) and BuChE was uniformly distributed in the lobules (Fig.8). As these two enzymes showed more or less equal intensity in the histochemical preparations, it was difficult to assess the predominance of either in the liver of Pigeon.

DISCUSSION

The presence of AChE activity in the bile canaliculi in the livers of Rosy Pastor and Common Myna may be suggestive of the role played by acetylcholine in the bile production. Though Pigeon liver was found to contain AChE activity, the localization was definitely not peribiliary. It could be mentioned here that the passerines like Rosy Pastor and Myna have gall bladders whereas the Pigeon possesses none.

The localization of AChE along the bile canaliculi points to the possible site of ACh action. Acetylcholine could influence the production of bile by changing the physicochemical gradient on the inner and outer sides of the lumen of bile canaliculi. The secretion of bile with the same tonicity as that of blood is a complex process. According to the recent views (Sperber, 1959; Cobes, 1964), various organic anions get actively secreted first into the lumen of bile tracts. Electrochemical neutrality is maintained by the movement of cations such as Na^+ and K^+ , along with the passive movement of water. If the

relatively non-diffusable anions are to cross the membrane, a change in the membrane permeability should now occur. This could be mediated through the action of acetylcholine which is known to cause an increase in the membrane permeability by some action on proteins, or lipoproteins of the active membrane (Nachmansohn as cited by Augustinsson, 1950). Thus it could be stated that acetylcholine, by facilitating ionic exchanges through the membrane, increases the bile production. Then the function of intrinsic nerve fibres which are distributed all along the bile canaliculi (Sutherland, 1964) also becomes obvious. Shastin (1962) observed a decrease in the ChE activity in the rat liver, 8 to 10 days after denervation. Studies of Tanturi and Ivy (1938b) and Ramprasad and Sirsi (1960) have shown that the vagus nerve contains pathways that may enhance or inhibit the secretion of bile and splanchnic, adrenergic nerves on the other hand inhibit its formation (Tanturi and Ivy, 1938a).

The increase in the AChE activity could then be under the control of the nervous system which releases more acetylcholine to promote an active secretion of bile. But the increase in non-specific cholinesterase (BuChE) observed during the premigratory period cannot be explained on this basis as its specific substrates in the liver is unknown (Gerentzoff, 1959). Although a number of substrates such as benzoylcholine, butyrylcholine, propionylcholine etc., could be used for its

demonstration, these are not specific. The enzyme also hydrolysed a number of other choline-esters of higher fatty acids as well as non-choline esters at a higher or lower rates than the above substrates. Due to its non-specificity one could assume that the function of this cholinesterase might be to release choline or choline-like substances from their bound state. This choline 'supplying' function of ChE could markedly affect the fat metabolism, since it is well known that choline prevents the formation of a fatty liver. Similarly, Hawkins and Nishikawara, (1951) showed an increase of plasma pseudocholinesterase activity in rats fed on choline-deficient diets. This enhanced activity may be to provide choline by splitting the available esters of choline. An identical affect may also be observed in liver during choline deficiency, but experimental evidences are lacking to support this view. Assuming, that choline is made available by ChE, one could explain why a fatty liver does not occur in Rosy Pastor which synthesize and store considerable amounts of fat before migration.

Diurnal variation in the level of AChE could also be correlated with bile production. It was observed as early as 1930 by Forsgren that the secretion of bile by the liver alternates with the formation of glycogen. During glycogen formation in the liver (assimilatory phase), bile secretion was very slow, but during the 'secretory phase' when the liver glycogen was depleted, the bile secretion on the other hand was rapid. In Rosy Pastors the glycogen content was found to be highest in the

evening and very low during the early hours of the day (Chapter 1). This means that the assimilatory phase occurred during the day time and the secretory phase in the night. Therefore, a greater secretion of bile could also take place at night in these birds. The reported increase in the total cholesterol in the liver of Rosy Pastor during the premigratory period (John, 1967) could be for the increased production of bile acids from cholesterol.

Peribiliary distribution of AChE in Common Myna also could be related to bile production as in the case of Rosy Pastor. But BuChE was more active and seen localized in the portal areas where there is a rich supply of blood. Hence, in all probability, BuChE could have a food assimilatory function. Gerebtzoff (1959) while studying the activity of ChE in the liver of rat at different times after feeding, observed changes in its pattern of activity (from peripheral to centrolobular) as well as an increase in its level. There are other instances also where the diet is known to affect the ChE activity in the liver. Thus, a protein low diet with excess of vitamin A was found to increase liver cholinesterase activity (Esh and Bhattacharya, 1961). As observed in the liver of Rosy Pastor there were no changes in the intensity of BuChE in the liver of Common Myna during the different seasons. Throughout the year they consume a mixed diet consisting of insects and fruits. But, Rosy Pastors were found to shift exclusively to a carbohydrate diet in the premigratory period as a corollary to

hormonal influence. In the postmigratory phase (July-August) there is an abundance of insect life as this period comes after the rainy season and during this period Rosy Pastors were mainly insectivorous. But this does not mean that enough grains and fruits are not available during this period, similarly during the pre-migratory period the insects are also not scarce. Hence the change of diet was due to some hormonal influence. Such protein low carbohydrate rich diet could reflect in the BuChE activity in the liver during these months as observed by Esh and Bhattacharya, 1961).

The Pigeon which feeds on a carbohydrate rich food, possess high AChE and BuChE activities in its liver. It seems possible that the activity of cholinesterase has some relation with carbohydrate metabolism. Acetylcholine when added to thyroid slices resulted in an increase of glycogen breakdown via HMP shunt pathway (Pastan et al., 1961; Rose and Glow, 1966). Acetylcholine also produced hypoglycemia in adrenalectomized animals (Arvy, 1964). These observations point out that, ACh has a profound action on glycogen metabolism and by activating the HMP shunt pathway, it influences fat metabolism also. Further experimental proof is necessary to support this hypothesis.