

CHAPTER 8

DISTRIBUTION PATTERNS OF LIPIDS AND SOME ENZYMES IN THE
LIVER OF NECTAR FEEDER, SUN BIRD (NECTERINIA ASIATICA)

Vertebrate liver is known to perform large number of functions most of which are concerned with metabolic activities. Since the absorbed nutrients reach directly to liver, its functions are also intimately associated with the diet. Though the liver is composed of histologically distinguishable units (lobules), the parenchymal cells forming the major part of the lobules are all alike (Elias, 1963). Since there are no histologically different types of parenchymal cells which perform different functions, it appears that some sort of division of labour in them is manifested by concentrating the different physiological activities by localizing different enzymes in different specific areas of lobules or same enzyme with different intensities in different regions of the same lobule.

The histochemical distributions of various enzymes in the liver have been studied in many mammalian species (see Wachstein, 1963), but avian liver is found to be spared in this respect. Recently Ratzlaff and Tyler (1973)

studied histochemistry of livers of some birds. However, information about spatial (zonal) distributions of lipids and certain enzymes and their possible relationship with dietary preferences is not available. ^{As a result of the above} So a series of studies on the livers of birds with various dietary preferences were planned. The Sun Bird (Nectarinia asiatica) is primarily a nectar feeder and therefore histochemical studies on the distribution pattern of lipids and some enzymes in the liver of bird ^{should} ~~would~~ throw ^{some} light on ^{the} adaptive features of liver with the special type of food consumed by this bird. With this view in mind the present study was undertaken.

MATERIAL AND METHODS

Sun Birds (Nectarinia asiatica) were shot by an air rifle from the natural environments around the University Campus and brought to the laboratory immediately. The liver was quickly removed, blotted and weighed. Part of the liver was immediately frozen in a cryostat for fresh frozen section, while another part was fixed in Baker's Calcium formol and frozen sections were cut for histochemical demonstration of lipids using Fettrot 7B, Sudan Black B and Nile Blue Sulphate (Pearse, 1960).

Fresh frozen sections of 10 to 15 μ thickness were cut to demonstrate histochemically the localization and distribution pattern of the enzymes viz., lipase; esterase; CC-glycerophosphate, lactate, succinate, malate and β -hydroxybutyrate dehydrogenases; alkaline and acid phosphatases; ATPases; and cholinesterases. Lipase was demonstrated by the Gomori's Method (Pearse, 197²) using 'Tween 85' as improved by George and Ambadkar (1963). Non specific esterase, was demonstrated using Burstone's azo dye technique (Burstone, 1962). Respective methods of Ogata and Mori (1964) were followed for the demonstration of CC-GPDH, LDH, SDH, MDH and BDH, using CC-glycerophosphate, sodium lactate, sodium succinate, sodium malate and β -hydroxybutyrate as substrates respectively.

Alkaline and Acid phosphatases were demonstrated employing the technique described by Burstone (1962). Cholinesterases were demonstrated by the method of Koelle and Friedenwald (1949) as modified by Coupland and Holmes (1957).

The total lipid was calculated gravimetrically after extracting it with chloroform:methanol (2:1) mixture. The glycogen was estimated by the method of Seifter et al. (1950) using anthrone reagent.

quantitative data?

OBSERVATIONS

FAT

The liver of Sun Bird contained large amount of fat (21.0%) most of which ^{was} ~~were~~ seen as neutral fat in histochemical preparations. Droplets of fat were found to be uniformly distributed in the cells of hepatic lobules (Figs. 1F and 1S). Phospholipids were not present in high concentration.

LIPASE

Lipase was fairly active in the liver cells and had ^a more or less uniform distribution in the hepatic lobules (Fig. 2).

ESTERASE

The esterase was uniformly active in the parenchymal cells all over the lobules (Fig. 3).

B-HYDROXYBUTYRATE DEHYDROGENASE (BDH)

This enzyme was moderately active in the parenchymal cells. ^{and showed} A uniform distribution ~~of the enzyme was found~~ in the lobules (Fig. 4).

LDH

LDH was highly active in all the parts of the liver and was found to be uniformly distributed (Fig. 5).

SDH

Like LDH, SDH was also found to be highly active but showed ^a higher reactivity in the periportal regions (Fig. 6).

MDH

MDH was relatively less active and was uniformly distributed in the hepatic lobules (Fig. 7).

CC -GPDH

This enzyme, like BDH, showed moderate reactivity, but its distribution was slightly ^a ~~more~~ ^{greater} around the portal areas (Fig. 8).

ALKALINE PHOSPHATASE

The enzyme was highly active ^{with the} but portal areas showed ^{my a} relatively higher concentration of ~~this enzyme~~ (Fig. 9). The enzyme was ^{also to be} seen localized around the bile canaliculi.

ACID PHOSPHATASE

The acid phosphatase was not as active as alkaline phosphatase. ^a Relatively higher concentration of acid phosphatase was found around the portal areas (Fig. 10). Its localization ^{ed within the} was in parenchymal cells.

ATPase

ATPase was moderately active but periportal areas showed ^a relatively slightly higher reactivity ^(Fig 11). It was localized more around the bile canaliculi (Fig. 12).

CHOLINESTERASES

Both acetyl (specific) and butyryl (non specific) cholinesterases were localized on the sinusoidal linings (Figs. 13 & 14). The connective tissue ^{of the} of central vein, ^a portal areas and blood vessels showed higher reactivity of these enzymes.

GLYCOGEN

The glycogen content of the liver of Sun Bird ^{she} was found to be 0.074 g/100 g wet tissue.

DISCUSSION

From the histochemical investigations on various enzymes studied in the liver of Sun Bird, it is clear that they show variations in their distribution patterns and localizations. As far as localization is concerned, all dehydrogenases, lipase, esterase and acid ^Pase were, in general, ^{with} in the parenchymal cells while cholinesterases were in the linings of sinusoids, blood vessels and central collecting veins, and ^A alkaline and ATPase were

mostly concentrated around bile canaliculi, Not only the localization, but the distribution of the enzyme within the areas of the lobule, was also found to vary. The reactivities of the enzymes ^{such as} like alkaline phosphatase and ATPase were ^{greater} ~~more~~ in the hepatocytes situated nearer to the portal areas, while dehydrogenases and lipase were found uniformly distributed ^{throughout} ~~all over~~ the hepatic lobule, but the esterase was found predominantly around the collecting veins. Such variations in the localizations and distributions of enzymes can be envisaged to have a direct correlation with anatomical and functional specializations of different areas of hepatic lobules. For example, the diameter of bile canaliculi is ^{the} ~~more~~ ^{greater} near the portal areas and hence enzymes like ATPase and alkaline phosphatase are found to be more concentrated in this region. As far as the oxidative metabolism is concerned, all the parenchymal cells share the responsibility equally and hence, dehydrogenases are found to be more or less uniformly distributed in the hepatic lobules except SDH which is ^{greater} ~~more~~ near the portal areas. Ratzlaff and Tyler (1973) who also observed increased periportal distribution of SDH in some avian liver ^Sopined that this is due to the presence of more mitochondria in the cells that are nearer to the portal areas.

The presence of enzymes at specific microanatomical regions of hepatic lobules points to the possible functions of enzymes in those areas. The presence of alkaline phosphatase near the bile canaliculi, can be taken as definite pointer to the function of this enzyme in the transport of materials across the membrane. Conklin (1966) is also of the same opinion as to the function of alkaline phosphatase in the bile canaliculi areas. The bile secretion is an active process requiring energy, and hence the presence of ATPase around bile canaliculi could be easily explained. Novikoff and Essner (1960) in livers of mammals and Ratzlaff and Tyler (1973) in those of birds also found that ATPase is localized around the bile canaliculi.

The Sun Bird's liver contains ^a large quantity of fat (21.00%) most of which was present as neutral fat. But ^a as only a moderately active BDH was discernible it can be deduced that lipolysis is not a prominent activity in the liver of this bird. Since the food mainly consists of carbohydrates, lipogenesis rather than lipolysis is to be naturally expected.

An increased rate of lipogenesis in the liver, can also be realized from the data on ^{ix2} glycogen content.

In spite of the fact that this bird consumes ^a large quantity of carbohydrates, the liver contained only a very low amount of glycogen (0.074 g/100 g), which could mean that most of the assimilated glucose ^{is} gets converted into fat. The reason for this hyperlipogenesis may be that tissues in Sun Bird, specially muscles are probably adapted for utilizing lipids. The pectoral muscles of Sun Bird consist of tonic fibres which utilizes more of fat than glucose (George and Berger, 1966). The liver then has to synthesize more fat and release it into the blood stream. This is clearly seen from the fact that lipase and esterase which are concerned with the release of free fatty acids into ^{the} blood stream, are highly active in the liver while ^{the} enzyme associated with fatty acid catabolism (BDH) is poorly active.

In general, the Sun Bird's liver, as far as the pattern of distribution and localization of enzymes are concerned, resembles more or less that of an insectivore bird (see Chapter 9X for further discussion). Perhaps the ancestral Sun Birds might have been insectivores and during the course of evolution they might have changed their main food to nectar. This

necessitated a complete change in the feeding apparatus.
However, since this bird occasionally consumes insects
and spiders (Das, 1967) in ^{the} adult condition as well as
during ^{the} fledgling period, their physiological adaptations
would not have been changed to suit only the nectar diet.

EXPLANATION TO FIGURES (CHAPTER 8)

Figs. 1 to 14. Photomicrographs of liver of Sun Bird showing the histochemical localizations of lipids and various enzymes.

Fig. 1F	Neutral fat	50X
Fig. 1S	Sudanophilic lipids	125X
Fig. 2	Lipase	50X
Fig. 3	Esterase	125X
Fig. 4	BDH	125X
Fig. 5	LDH	50X
Fig. 6	SDH	50X
Fig. 7	MDH	125X
Fig. 8	OC -GPDH	125X
Fig. 9	Alkaline Phosphatase	50X
Fig. 10	Acid Phosphatase	50X
Fig. 11	ATPase	50X
Fig. 12	ATPase	125X
Fig. 13	AChE	50X
Fig. 14	BuChE	50X



