CHAPTER 9

GENERAL CONSIDERATION

The fore going chapters account the various histochemical studies carried out on the liver of birds representing carnivorous, insectivorous, omnivorous, frugivorous and graminivorous groups. All of these ~~groups~~ except omnivores, ~~are having~~ have a fairly constant dietary preference, and are said to be stenophagous in their feeding habits. The omnivores, on the other hand feed on a variety of food materials and are said to be euryphagous in nature. All stenophagous birds exhibit specific morphological adaptations to suit their specific diets. From ~~the~~ countless studies, it has become apparent that food has been the major force behind the evolution of morphologically distinct varieties and species of birds. Such structural adaptations are not only seen in the food getting apparatuses like beak, tongue and feet but also in the internal anatomy, especially that of the alimentary tract (see Welty, 1964). If food can elicite structural adaptations, it can and should bring forth physiological adaptations too. One of the major physiological adaptations in the alimentary tract is that of the quantity of various enzymes secreted. According to the intake of proteins, refs fat or carbohydrates, the enzymes concerned are abundantly secreted into the digestive tract. Thus, all birds that consume largely carbohydrates are found to secrete more amylase (Battacharya and Ghose, 1971). Some authors have also shown that the liver of such birds is ~~also~~ found to secrete amylase through bile which means that the functional adaptations with respect to food are also found in glands associated with alimentary tract like the liver. This is not surprising as the liver function is always correlated to the type of diet. However, there are not many references in the literature as to the relative adaptations of liver with respect to the food. It is easier to speculate the adaptations of liver to deal with higher concentrations of one or the other types of metabolites (proteins, fat or carbohydrates) in terms of complementary enzyme machinery. ~~But~~ The finer details of such adaptations in the liver are not yet completely elucidated.

The present investigation is deemed to span this gap of knowledge to a certain extent. The studies presented in the foregoing chapters were planned to derive adequate insight into the physiological adaptations of liver to a particular diet. It is expected that from the histochemical demonstration of metabolites like fat, and various enzymes, the meatabolic as well as secretory adaptations of the liver could be envisaged. To maintain clarity, each chapter is presented as separate disclosures without recourse to information from other chapters. This necessitates an integrated discussion, taking all the information gathered, to present a comprehensive picture of hepatic adaptations.

<u>Morphological adaptations</u>

A general survey of the morphological feature of liver mass revealed that all avian members studied have a bilobed organ. The two lobes are either equal in size or dimensions (carnivores) or unequal with a larger or longer right lobe (insectivores, omnivores and graminivores). The size of the two lobes may have some adaptive correlations. The liver weight to body weight ratio can be linked with the size of the lobes. In carnivores, where the liver weight to body weight ratio is the smallest (1.8), the hepatic lobes are more or less equal. In other groups where the ratio is higher (insectivores - 3.0, omnivores - 2.7, and graminivores - 2.2) the right lobe is longer or larger. Perhaps relative crowding of visceral organs particularly the alimentary canal in the body cavity in these birds may be preventing the left lobe from growing to the fullest extent or the right one might be growing more to compensate the retarded growth of the left lobe to attain a specific liver mass in relation to the body size of the bird. One fact that is strik~~es~~ing in this connection is that smaller birds (like insectivores) ~~are~~ hav~~ing~~e a higher liver weight:body weight ratio and the heavily built birds (carnivores) ~~are having~~ have a lower ratio. Since the smaller birds ~~are having~~ have a higher metabolic rate than the heavier birds, it is possible to correlate a higher liver body weight ratio with a higher metabolic rate. Because of the higher metabolic rate, the small sized birds have to have a greater intake of food and therefore the liver is obliged to cope ~~up~~ with relatively higher influx of metabolites. The mass of a tissue or organ is usually correlated with the amount of work performed by it (Goss, 1972). The higher metabolic rate of smaller birds also ~~brings~~ places a heavy metabolic burden on the liver. The processes like removal of metabolites from the blood, interconversions, as well as maintenance of homeostatic level of blood sugar, heat production, production of bile and excretion of cholesterol and bile pigments, have to be rapid and efficient in such cases. Perhaps, this ~~must~~ is ~~be~~ the reason why the liver is composed of a single plate or simplex muralia in insectivores and omnivores, most of which are comparatively small~~er~~ in size. The carnivores

reference for this

and graminivores have only a duplex muralia. According to Hickey and Elias, (1954) the simplex muralia are much more advanced than the duplex type and the passerines are the most highly evolved group amongst all birds. Most of the passerines are omnivorous or euryphagous and this fact might have also aided in their adaptive radiation. The Simplex type of muralium provides an added advantage by exposing more surface areas of the hepatocytes to the circulating fluid. In this way the exchange of materials between the blood and cytoplasm becomes more efficient and rapid which is quite essential to birds having a high metabolic rate.

Metabolic adaptations

~~The~~ Metabolic activities being ~~the~~ of foremost concern ~~of~~ to the liver, most of ~~its~~ this organs adaptations are oriented ~~for~~ towards such functions. Undoubtedly the liver ~~gets~~ becomes adapted to homogeneous (consisting of only one metabolite out of three viz., carbohydrates, lipid or protein) or to heterogeneous (mixture of any two or all the three) types of diet, according to the feeding habits of birds. The stenophagous birds are very choosy about the food and hence the liver can exhibit a semipermanent metabolic adaptation. However, euryphagous birds may eat whatever is available in the surroundings and in this case liver may have heterogeneous adaptations to deal with changing food types.

The diet of carnivorous birds, being the flesh of other animals, pass on more nitrogenous metabolites and some lipids to the liver. This necessitates a metabolic orientation in the liver towards gluconeogenesis. Since the amount of carbohydrate is very poor in the diet, the hepatic glycogen deposition in these birds is very low (Chapter 2). The fairly good amount of lipids found to be present in the liver of this group of birds might have come directly from the food itself. The uptake of chylomicrons into hepatic cells is facilitated by the presence of non specific esterase on the sinusoidal linings (Chapter 3). Assimilated lipids are then converted into lipoproteins and phospholipids and then transported to the adipose tissue. The liver of carnivores has more ~~of~~ acidic lipids than the neutral ones (Chapter 2). Though lipase show an intense reactivity in the liver of these birds, β-hydroxybutyrate dehydrogenase did not show a high reactivity. These observations denote that the lipolysis is not very high in the organ (Chapter 3). The energy requirement of the liver must be met with by utilizing than what?

more of amino acids. Moreover, the glycolytic and Kreb's cycle dehydrogenases were found to be equally active in the liver of carnivores as in those of other groups (Chapter 4). Perhaps, these enzymes facilitate both gluconeogenesis as well as energy production. The observed high reactivity of alkaline phosphatase in the hepatocytes situated near the portal areas, also supports the suggestion of high gluconeogenesis in the liver (Chapter 5) and increased release of glucose into the blood in carnivores. By virtue of the localization of cholinesterases on the sinusoidal linings, these enzymes may be aiding the transport of phospholipids and glucose across the hepatocytic membranes of the carnivore liver. The important metabolic adaptation exhibited by the liver of carnivores to suit the higher protein diet is perhaps that which is concerned with gluconeogenesis and phospholipid synthesis.

The diet of insectivorous birds chiefly consists of a small quantity of carbohydrates in addition to ~~too besides~~ proteins and fats. From the quantitative data it is realised that the liver of insectivores has a very high lipid content (14%) and most of it is found as neutral fat (Chapter 2). Glycogen is slightly higher ~~more~~ than that is found in carnivore ones ~~'s~~ liver which indicates that more glucose is available to the liver of these birds (insectivores) through ~~food~~ diet. Lipid synthesis and/or storage are the major activities of the insectivore liver. The enzymes, like lipase, esterase and BDH are found uniformly distributed in the hepatic lobules of insectivores denoting that all the cells in a lobule have equal share in the metabolic activities concerning lipids. In these birds the neutral fat was found to be evenly distributed throughout all over the lobules. Since lipids form the major part of the food taken by the insectivore, gluconeogenesis must be taking place at the expense of fatty acids. The presence of mitochondrial α-GPDH in the livers of these birds adds support to this reasoning.

The liver of euryphagous birds show considerable flexibility in the metabolic adaptations. Since, the food of these omnivores may vary according to the availability of food of one type or the other , their liver can not exhibit any one set of metabolic adaptations as in the case of stenophagous birds. The mixed diet of omnivorous birds brings a moderate amount of all the three types of metabolites. The total fat content in the liver is lowest in omnivores compared to that in other groups. Although a fairly good amount of glycogen is present, its concentration is much lower than that in the liver of birds belonging to the other ~~three~~ four? groups (carnivores, insectivores, frugivores and graminivores). The distribution patterns of most of the enzymes studied vary considerably between the species included in the~~se~~ group of omnivorous birds. Due to this fact, it is believed that many of these birds ~~are~~ still possess~~ing~~ some of their original metabolic adaptations to the stenophagous diet of their ancestral group. In other words, their euryphagous habits have been evolved secondarily without enforcing ~~much~~ many deviations in the metabolic adaptations from the primary one. For example, α-GPDH reactivity was very strong in the periportal areas of the liver lobules in most of the omnivores except in that of Crow, Sparrow and Duck, where the reactivity of the enzyme was more or less uniform. In stenophagus birds (carnivores and graminivores) α-GPDH reactivity was seen uniformly distributed etc. From these facts it is surmised (Chapter 4) that the Crow, though omnivorous now, may ~~be~~ still hav~~ing~~e some of the original carnivorous adaptations, while the Sparrow and Duck ~~may be~~ still retain~~ing~~ the~~ir~~ original graminivorous adaptation of their livers as far as metabolic activities are concerned. The gizzard of the Crow is a bag like structure and is meant for receiving soft fleshy food while those the

of Sparrow and Duck are muscular like that of Graminivores. The pattern of esterase distribution in Koel liver is similar to that found in the frugivorous Parakeet (Chapter 3). The gizzard of Koel is thin and sacciform and is adapted for fruits, ~~like~~ as in the ~~case of~~ Parakeet (Panicker, 1974). However, one can not say that all omnivores have retained their original metabolic adaptation to suit their present diet. Again the example of the Crow can be cited, where the esterase distribution in its liver is very much different from that of carnivorous birds. In fact, the distribution pattern of these enzymes resembles that of graminivores. Based on these facts the conclusion that can be drawn is that most of the omnivores show a mixed metabolic adaptation to suit their varying diet. However, these birds may still retain some of their original metabolic adaptations and most of their original morphological adaptations (such as those seen in the beak, claws and gizzard).

The metabolic adaptations exhibited by both the frugivores and graminivores are more or less same, befitting their carbohydrate rich diet. An important characteristic feature is the high glycogen content in their livers. As the lipid content is also high, hyperlipogenesis is the striking metabolic adaptation exhibited by their livers unlike ~~in~~ the other two stenophagous groups (carnivores and insectivores) where gluconeogenesis is predominant. As far as the distribution patterns and localizations of enzymes in their liver are concerned, graminivores form a class by themselves itself, while in these respects carnivores and insectivores have similarities. For example, acid phosphatase exhibits a high reactivity in graminivore liver while alkaline phosphatase presents a higher reactivity in ~~those of~~ the carnivores and insectivores. Moreover, in graminivores most of the enzymes are uniformly distributed in the lobules except a few like SDH and MDH. Since the diet is invariably constant, containing mostly carbohydrates, all the parenchymal cells can show a more or less equal and similar ~~same~~ type of metabolic machinery. In the other two stenophagous groups (carnivores and insectivores) though the diet is consistent, it brings in both proteins and fats, which may necessitate some kind of division of labour amongst hepatocytes in the lobules to deal with these metabolites. This could be the reason why some of the enzymes, for example alkaline, phosphatase (Chapter 5), are distributed more in the hepatocytes situated around portal areas in carnivores and insectivores. Such a division of labour amongst the parenchymal cells of lobules, is much greater in the liver of omnivorous birds whose diet may contain a mixture of all the three metabolites. However, in frugivores and graminivores no such division of labour is necessary as their diet consists mostly of carbohydrates.

By virtue of a carbohydrate rich diet, the Sunbird's liver should have shown metabolic adaptations resembling those seen in the frugivore or graminivore. However, its liver exhibits altogether different metabolic adaptations. The total fat content of the liver of Sunbird is highest (21.0%) amongst all groups of birds studied. The carbohydrate rich food, however, does not influence the liver to deposit ~~high~~ a large quantity of glycogen. As the nectar contains more of monosaccharides other than glucose, the conversion of them into fat may be taking place at a higher rate than their conversion into glycogen. Hyperlipogenesis suits this small bird very well as it has a high metabolic rate. ref Moreover, the pectoralis muscle of the Sunbird is composed of tonic fibres which utilizes more of lipids. ref From the intensity of reactivities of enzymes like lipase, esterase, BDH and

α-GPDH, a predominant lipid metabolism in the liver of Sunbird could easily be ascertained ~~realized~~. However, the patterns of the histochemical distribution of many enzymes resemble, in general, that of the insectivore ~~liver~~. This fact may point to the insectivorous ancestry of the Sunbird. It is pertinent to mention here that the Sunbird is also known to ingest insects and spiders.

Secretory adaptations

Bile secretion is one of the major functions of the liver. Since bile is essential for the digestion of ingested food substances, especially lipids, the rate of synthesis and secretion of bile is always related to the digestive activities. A high fat diet usually demands increased bile production. Birds that feed on seeds and insects must eat fairly regularly throughout the day while the birds of prey and carrion feeders, may feed very irregularly (Welty, 1964). According~~ly~~ to these feeding habits, the graminivores, insectivores and omnivores should secrete bile continuously. The rate of bile production, usually, exceeds the rate of secretion and the bile produced in excess is stored in the gall bladder. Such storage is not possible in those birds (graminivores and frugivores) which do not have a gall bladder and hence the bile production must be continuous and at a very low rate in these birds. As such these birds do not require a high quantity of bile as the diet chiefly contains carbohydrates.

The high or low requirements of bile, should reflect on the secretory capacity of the liver. ~~The~~ Enzymes such as ~~like~~ ATPase and alkaline phosphatase, which are associated ~~connected~~ with ~~the~~ bile secretion and the intensity of their reactivities as well as their histochemical localization could be used as indicators ~~indicative~~ of the bile secretory capacity of the liver. ATPase is invariably seen localized around the bile canaliculi denoting the enzyme's role in the transport of bile components across the membrane into the canaliculi lumen. ~~With the~~ Electron microscopic studies of Gemmel and Heath (1973) have show~~ed~~n that ATPase is localized on the membrane of the microvilli of hepatocytes ~~that~~ is ~~thrown into microvilli~~ which project~~ing~~ into the lumen of the bile canaliculi.

The carnivore, frugivore and graminivore birds exhibited a~~n~~ uniform pattern of distribution of ATPase within the hepatic lobules. The enzyme reactivity was also moderate in intensity in the above mentioned groups compared to that in insectivores and omnivores(Chapter 7).

~~Though~~, Carnivores may require more bile than fruit- and grain-eaters, as their feeding is highly irregular with long intervals between two successive feedings, the rate of production of bile need not be very high; also the graminivores, though their feeding is continuous, may not require a high quantity of bile as their diet consists predominantly of ~~mainly~~ carbohydrates, and hence the lower rate of bile production in the frugivore and graminivore birds. As such the frugivore and graminivore (Parakeet and Pigeon respectively) do not have gall bladders, and therefore may require a low but continuous bile secretion ~~by their liver~~. In the liver of insectivores, the intensity of ATPase reactivity was fairly high especially in ~~those of~~ the Tailor bird, Bee-eater, and Drongo. This is understandable as these birds require more bile ~~due to the facts that~~ because: (i) their diet contains a good deal of fats and (ii) they feed almost continuously. However, the liver of the Cattle Egret (classified as insectivore) showed only a moderate enzyme reactivity. It is needless to say that this bird is the odd one out amongst the birds included in the group of insectivores. Not only does it eat~~s~~ insects but also many other small animals such as amphibians and reptiles. In this respect the Cattle Egret is more like a carnivore than an insectivore. Only in the omnivores, the ATPase showed regional differences in the pattern of the enzyme localization within each lobule. In these birds, the periportal areas showed a higher enzyme reactivity which may indicate that the hepatocytes around the portal spaces are entrusted with a greater responsibility for bile production. In other words, there may be a division of labour or a regional specialization as far as the synthesis and secretion of bile components are concerned in the hepatic lobules of omnivorous birds. In the liver of the Sunbird the ATPase showed only a moderate reactivity similar to that observed in ~~the case of the~~ the livers of graminivores and frugivores. Thus the intensity of enzyme reactivity could be directly correlated with the carbohydrate rich diet the Sunbird consumes. However, the periportal distribution of ATPase can only be explained in terms of this bird's lineage from an insectivorous ancestor.

The non specific alkaline phosphatase (~~Alk. Pase~~) is another enzyme that has been ascribed ~~with~~ a role in bile secretion (Essner _et al_., 1958). Alkaline phosphatase also enjoys a peribiliary localization, apart from being generally found in the parenchymal cells. The stronger reactivity of this enzyme was exhibited by the livers of insectivores and omnivores, while carnivores showed a moderate activity and ~~that~~ in graminivore and frugivore ~~could muster~~ only a poor response (Chapter 5, Table II and III). In frugivores and graminivores the Alk. Pase was not found in the peribiliary sites. This suggests ~~points out to the fact~~ that most probably Alk. Pase may not be participating in the process of bile production in these birds. A general pertinent question that crops up here is ~~about~~ the correlation between diet and the function of Alk. Pase in bile production. It is suggested that Alk. Pase participates in the transport of bile components (Essner et al., 1958). Bile salts and acids are the two main components that have a major role in digestion. However, the pH of bile in various birds has not been found to vary (Sturkie, 1965), which means that bile of carnivores, insectivores, omnivores, frugivores or graminivores contains more or less the same amount of bicarbonate salts. If this is the case, the variations in the Alk. Pase reactivity in the liver of birds belonging to various dietary group can not be correlated to the secretion of inorganic bile salts. However, it is reasonable to expect that in the bile the concentration of conjugated bile acids, which helps in the digestion and absorption of fat, may vary according to the diet. The Importance of bile in the digestion and absorption of fat is demonstrated by the fact that prevention of bile from reaching the alimentary tract leads to a rapid drop in plasma lipoprotein level in the chick (Clarke et al., 1964). The output of bile acids is also markedly affected by feeding different unsaturated and saturated lipids (Lindsay et al., 1969). Thus when the food contains a large quantity of fat, as in the case of insect diet, the secretion of bile acids has to be increased. On the other hand when the diet contains less fat, as in the case of graminivore ~~diet~~ less bile acids are required to be secreted through the bile. If Alk. Pase is connected with transport of bile acids and/or conjugation of cholesterol with taurine then the poor reactivity of Alk. Pase in graminivore and frugivore birds becomes self explanatory. On the basis of these understandings, the moderate reactivity of Alk. Pase in the liver of carnivores (Table II, Chapter 5) compared to that in insectivores, can also be explained. Thus it appears that high fat content of the diet influences the Alk.Pase reactivity in the liver more than a high protein diet.

Phagocytic adaptations

The Kupffer cells are capable of phagocytizing bacteria, virus, endotoxins, worn out erythrocytes, lipids and many colloidal particles (Benacerraf, 1964). The phagocytic capacity of these cells, commensurates with powerful hydrolytic enzyme components they have in the lysosomes are: proteolytic enzymes, lipase, acid phosphatase and ribonuclease. Thus the phagocytic activity could be judged from the concentration of these enzymes in the phagocytes. The level of Acid Pase reactivity could be taken as an useful index of the metabolic activity of Kupffer cells (Thorbeck et al., 1961) as well as their phagocytic actions (Wachstein, 1963).

The histochemical demonstrations of enzymes ~~Like~~ Such as Acid Pase, lipase and esterase in the liver of birds belonging to various dietary groups revealed that certain birds have highly active Kupffer cells. A high Acid Pase reactivity in the Kupffer cells was found in carnivorous birds (Vulture and Kite), and in some insectivores (Bee-eaters). The lipase reactivity was exhibited by Kupffer cells of graminivores (Parakeet, Dove and Pigeon), while that of easterase was fairly widespread, as in most of the omnivores and graminivores. The presence of elevated Acid Pase activity in the Kupffer cells of the Vulture and

Kite livers could be correlated with their diet which may be bringing in toxins and other foreign substances harmful to the birds.

The presence of lipase and esterase in the Kupffer cells, agrees with their, already known, fat clearing function. The uptake of dietary cholesterol by Kupffer cells (Neveu et al., 1956) and an enhancement of the phagocytic action of these cells by esters of long chain fatty acids (Stuart et al.,1960) have been clearly established. As such, the reticuloendothelial cells in the livers of many omnivores and some graminivores (Pigeon) were found to contain a large quantity of neutral fat (Chapter 2). The significance of the accumulation of neutral fat as well as the presence of lipolytic enzymes in the cells of the reticuloendothelial system of the liver cannot be ascertained at this stage.

In conclusion, based on the foregoing account it could be stated that the birds studied not only show morphological adaptations to suit the type of food they ingest but also exhibit corresponding physiological adaptations in their liver as well ~~too.~~ However, histochemical studies have certain limitations, and an extensive investigation using other techniques ~~as well~~ may reveal many more interesting details about the physiological adaptations of the liver in correlation with the type of food ingested by the bird concerned.