

CHAPTER 2

RELATIONSHIP OF DIETARY PREFERENCE OF VARIOUS
 REPRESENTATIVE BIRDS WITH THE FAT AND GLYCOGEN CONTENTS
 AND DISTRIBUTION PATTERN OF HISTOCHEMICALLY
 DEMONSTRABLE LIPIDS IN THEIR LIVERS .

The vertebrate liver is intimately associated with the digestive and metabolic activities of these animals. The absorbed food materials and other nutrients first reach liver and the ^{the} ~~excess~~ ^{then excess} of them (carbohydrates and fats) are deposited in the liver itself. The liver has a limited capacity to store carbohydrates (as glycogen), and hence excess of carbohydrates is usually converted into fat which is either stored in the liver itself or is transported to the adipose tissue. ^{Ref. is the} If dietary intake of metabolites is not sufficient to meet the energy demand of the animals, the liver readily releases ^{what} ~~them~~ into the blood stream from its store. Apart from ^{what} ~~these~~, the liver can also easily convert one form of metabolite to another ^{Ref.} as per demand. In this way, ^{the} liver ^{can!} could regulate the concentration of various metabolites in the circulating blood. Thus, the metabolic activities in the liver are greatly influenced by the dietary intake of metabolites and the demand of the body for them. Accordingly the

reactions concerning glycolysis, glycogenolysis, glycogenesis, gluconeogenesis, lipogenesis, lipolysis, deamination or amination are always taking place in the liver; ¹⁴⁵ Thus, the metabolic adaptability of ~~the liver~~ in relation to dietary intake is remarkable. More striking is the ^{variability} viability of metabolic reactions, necessary in omnivorous animals which may, intermitantly or according to the availability, consume a carbohydrate rich, fat rich or protein rich food. However, in animals having ^a high preference to any one type of food, the liver may show semipermanent metabolic adaptations as their diet is almost constant. Thus, flesh and insect eaters, frugivores and graminivores may have a set metabolic adaptations in their liver with respect to their specific diet. Knowledge of such adaptations in the liver to specific diet, though advantageous, is meagre. *ref?*

In the present investigation, a number of birds having, carnivorous, insectivorous, omnivorous, frugivorous and graminivorous feeding habits, were collected from their natural environment and studied so as to understand the metabolic adaptations of their livers with respect to their normal diet. The present report deals with the histochemical distribution pattern

and localization of various types of lipids and the quantitative estimations of total fat in the livers of such representative birds.

MATERIAL AND METHODS

The birds selected for the present study, were grouped according to the food preference, into carnivores, insectivores, omnivores, frugivores and graminivores (Chapter 1, Table I). The carnivores (group I) are represented by kite and vulture; the insectivores (group II) by tailor bird, swift, martin, bee eater, cattle egret and drongo; the omnivores (group III) by brahminy myna, common myna, house crow, house sparrow, bulbul, babbler, barbet, robin, koel, fowl and duck; and frugivores and graminivores (group IV) are represented by parakeet, dove and pigeon (Table I).

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These birds were shot and collected from their natural habitats situated in and around the University Campus. Their livers were quickly excised and parts of them were fixed in Baker's Calcium formol solution. So fixed livers were frozen and sections of 15-20 μ thickness were cut using a microtome mounted in a cryostat. ^{type} The frozen sections were stained differently with Fettrot 7B,

Sudan black B, and Nile blue sulphate, employing the methods described by Pearse (1950).

Small pieces of livers were also utilized for quantitative estimations of glycogen. The KOH digested pieces were subjected to the methods described by Seifter et al. (1950) for the estimation of glycogen. The total fat was estimated gravimetrically after extracting the fat with chloroform:methanol (2:1) mixture.

OBSERVATIONS

GROUP I : (Carnivorous birds)

^{The} Both vulture and kite showed a moderate amount of neutral fat in their liver which was found to be distributed almost uniformly ^{throughout the} ~~all over the~~ liver lobules in the form of granules (Figs. 1A & 2A). However, ^{the} judging from the fact that fat content of about 10 to 12% (Table I) was found in the livers of both these birds, it could be reasoned that most of the lipids are in the form of lipoproteins and phospholipids. This was evident from the Sudan black B stained ~~liver~~ sections of both ~~these~~ birds (Figs. 1B & 2B). Moreover, the sudanophilic lipids, mainly composed of phospholipids, were found to be localized around the collecting veins (Figs. 1B & 2B).

GROUP II : (Insectivorous birds.)

Most of the insectivores were found to have large amounts of lipids (9 to 15%) deposited in their livers as compared to carnivorous birds (Table I). From the frozen sections suitably stained, it was clear that the livers of these birds have ^a large amount of neutral fat (Figs. 3A, 4, 5A, 7A & 8). Phospholipids were slightly more concentrated around the central collecting veins (Figs. 3B, 5B & 7B) as in the case of carnivorous birds. Contrastingly, the most abundant type of fat in the livers of these birds (group II) was found to be triglycerides, rather than phosphatides.

GROUP III : (Omnivorous birds.)

From Table I it can be clearly visualized that the livers of omnivorous birds also contained large quantity^{es} of lipids (8 to 11%). The neutral fat concentration, however, was much less than that found in ~~the livers of~~ insectivores. Interestingly, the neutral fat distribution was found to be uneven, being mainly localized around the portal areas (Figs. 9B, 10B, 11, 13A & 14A). Sudanophilic lipids were more or less uniformly distributed (Figs. 14B, 15B & 16B), though of course in bulbul the periportal areas showed ^e relatively slightly higher concentration of

these lipids (Fig. 13B). Another characteristic feature ~~of liver~~ of the omnivorous birds was the presence of ^ahigh concentration of neutral fat in a large number of specialized cells. Such cells, heavily loaded with neutral fat, were seen in the livers of fowl (Fig. 18), brahminy myna (Fig. 9B), common myna (Fig. 10B), house crow (Fig. 15A), bulbul (Fig. 13A) and babbler (Fig. 11).

GROUP IV : (Frugivorous and Graminivorous birds)

The fat content of the livers of birds of this group was also found to be high (9 to 12% - Table I).

The Most abundant type of lipids ~~in the livers of these~~ birds, was the neutral fat and that was found uniformly distributed in all the regions of the liver lobules (Figs. 19, 20A, 21 & 22A). In other words, all the cells of the hepatic lobules in these birds appeared to have more or less the same capacity to synthesize and/or store triglycerides.

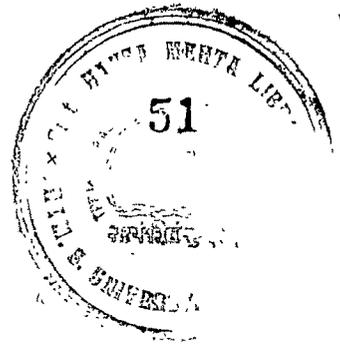
Data on the glycogen and total lipid contents of livers of various birds are presented in Table I.

DISCUSSION

In general, vertebrate liver contains various types of lipids. In rat liver, Clement (1953) found that the lipids are distributed as : phospholipids, 3.6%; glycerides, 1.7%; cholesterol esters, 0.28%; free cholesterol, 0.28%. With the high cholesterol diet (2%) the various fractions (as stated above) were found to be 3.4, 4.80, 5.60 and 0.39% respectively. The triglyceride composition and concentration are affected by diet while those of phospholipids do not ^{appear} ~~get~~ affected (Chalot-Haimovisi, 1957; Evans et al., 1958).

When the diet contains a large quantity of fat, most of it is absorbed into the circulatory fluids and transported in the form of chylomicrons (Bragdon and Karmen, 1960). The action of liver in this case is to take up the chylomicrons from the blood and rebuild them into lipoproteins (Borgstrom, 1960). 7

A carbohydrate~~s~~ rich diet elicit~~s~~ altogether different mechanisms in the liver. When the liver is confronted with a large quantity of glucose and other monosaccharides adaptive hyperlipogenesis is manifested (Tepperman and Tepperman, 1965).



A general survey of diet reveals that the carnivores consume a protein rich food while insectivorous birds' diet consists of high concentrations of both protein and fat and moderate amount of carbohydrates. The graminivores on the other hand ingest a carbohydrate rich food and the omnivores, according to the availability take protein and fat rich food (when they consume insects) or carbohydrate rich diet (when they eat grains, fruits or vegetables).

The dietary variations exert manifold influence on the metabolic adaptation of liver. The most conspicuous of them all is the deposition of fat and glycogen in the liver (Table II). The maximum concentration of fat in the liver was found in the insectivores followed by carnivores and graminivores and the least in omnivores. The glycogen content was found to be more in fruit^s and grain eaters and least in carnivores. It is seen that feeding on high lipid diet increases the triglycerides (neutral fat) content of the liver (Favarger, 1963). On the basis of this fact and present observation it could be surmised that the high concentration of fat in the livers of insectivorous birds is definitely due to consuming a more fatty diet. The major part of haemocoel of

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insects is filled with fat bodies and the most abundant type in the fat bodies of insects is triglycerides (c.f. Karnavar and Nair, 1973). Insect fat bodies also contain a good amount of carbohydrates (glycogen) unlike the adipocytes of vertebrates (c.f. Karnavar and Nair, 1973). These facts explain the presence of a relatively higher concentrations of glycogen in the livers of insectivores than in those of carnivores.

they don't explain it but suggest a correlation might exist

The glycogen deposition was found to be highest in the livers of fruit~~s~~ and grain eaters (Table II). The diet of these birds, ^{is predominantly} mostly contains carbohydrates ~~and~~ ^{with} negligible amounts of lipids. Fairly high fat content noticed in the livers of these birds, might be due to lipid synthesis from carbohydrates. The hyperlipogenesis must be one of the adaptations shown by the livers of frugivorous and graminivorous birds.

Similarly, the livers of group I (carnivores) and II (insectivores) birds must show adaptations to dispense ^{the} with large quantity of fat brought in through ^{the} diet. Since their diet contains ^{a small} less amount of carbohydrates, reactions resulting in gluconeogenesis must also take place at a higher rate. Thus a speedy removal of lipids

that have been taken up by the liver has to be the most characteristic adaptation of the livers of the birds belonging to groups I and II. Most of the lipids taken up by liver are oxidized or converted into lipoproteins and sent to the adipose tissues through blood. In light of this fact, the presence of more phospholipids around the central collecting veins in the livers of both carnivores and insectivores is indicative of the lipid mobilization from the liver to adipose tissues.

While the livers of birds belonging to groups I, II and IV show complete adaptations with respect to their diets (as their diets are more or less constant), the livers of omnivores (birds of group III) have to have more flexible metabolic adaptations. In other words the livers of these birds should show adaptations characteristic of both insectivores, and frugivores and graminivores depending on the type of diet. As expected, this adaptation is evident from the histochemical distribution of neutral fat in the hepatic lobules of the liver of omnivores. The neutral fat is mostly confined to the portal areas while central lobular areas are devoid of it. Conklin (1966) while studying the pattern of distributions of various enzymes in the developing chick liver, observed ^{or} definite shift in the

activity of enzymes from one region to another within the hepatic lobules. In his opinion, these shifts are due to ^{the} acquisition of functional maturity and specializations of various cells within the lobules. Specific variations in the intralobular localization of functions in the mammalian liver have also been reported by Novikoff and Essner (1960). In the light of these reports, it could be stated that enzymic machinery for the uptake of fat brought in by the blood is more pronounced in the portal areas of lobules of livers of omnivore (euryphagous) birds. While in the other stenophagous groups, all the parenchymal cells constituting hepatic lobules more or less have same adaptation, which is only to be expected as the diets of group I, II and IV birds are fairly constant.

The specialized cells loaded with neutral lipids present in the portal areas of the livers of omnivores are believed to be of reticuloendothelial in origin and are very much akin to Kupffer cells. The lymphocytopoietic nodules present in the liver of the migratory starling, Sturnus roseus, were found to take up large amount^s of neutral fat (George and Naik, 1963). However, ^{the} presence of such cells are not peculiar to omnivorous birds.

Similar nodules in the pigeon liver ^{also} too showed droplets of neutral fat (Pilo, 1970). The affinity of cells of ^{the} reticuloendothelial system for neutral fat is well known, the adipose tissue itself being a part of the system.

In conclusion it could be said that regional specialization is exhibited by lobular units of livers of euryphagous birds (omnivorous birds), while no such specialized regions are discernible in stenophagous birds like carnivores, insectivores, frugivores and graminivores.

TABLE I

Glycogen and fat contents and the neutral fat deposition in
the livers of birds of various dietary groups

Sr. No.	Groups	Birds	Glycogen %	Total Fat %	Neutral Fat
1.	I (Carnivores)	Vulture	0.07	10.56	++
2.		Kite	0.06	12.32	++
3.	II (Insectivores)	Cattle Egret	0.05	9.21	+++
4.		House Swift	0.05	12.58	+++
5.		Bee Eater	0.15	15.40	+++
6.		Tailor Bird	0.21	13.22	+++
7.		Drongo	0.21	11.28	+++
8.		Martin	0.39	13.75	+++
9.	III (Omnivores)	Brahmin's Myna	2.03	9.43	++
10.		Common Myna	2.25	10.18	++
11.		Jungle Babbler	1.76	9.06	++
12.		Indian Robin	4.03	11.73	++
13.		Bulbul	1.10	-	++
14.		Koel	2.03	8.13	++
15.		House Crow	1.39	8.50	++
16.		House Sparrow	0.06	9.76	++
17.		Barbet	2.44	6.85	++
18.		Fowl	0.07	11.20	++
19.		Duck	2.21	12.22	+++
20.	IV (Frugivore & graminivore)	Parakeet	3.48	9.43	+++
21.		Dove	3.58	9.23	+++
22.		Pigeon	4.86	12.11	+++

++ = moderate amount of neutral fat;

+++ = fairly good amount of neutral fat.

TABLE II

Average lipid and glycogen contents in the livers of birds of each group and distribution patterns of neutral and acidic lipids

Group	Total Fat %	Glycogen %	<u>Distribution pattern of</u> Neutral Acidic Lipids Fat	
GROUP I (Carnivores)	11.44	0.065	Uniform	Centrolobular
GROUP II (Insectivores)	14.24	0.180	Uniform	Centrolobular
GROUP III (Omnivores)	9.43	1.720	Uniform	Uniform
GROUP IV (Frugivores & Omnivores)	10.75	3.530	Uniform	Sparse and uniform

EXPLANATION TO FIGURES (CHAPTER 2)

Figs. 1 to 22. Photomicrographs of the livers of birds showing histochemical distribution of lipids.

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|---------|--|------------------------------|--------|
| Fig. 1 | Vulture | | |
| | 1A-Neutral fat (50X); | 1B-Sudanophilic lipids(125X) | |
| Fig. 2 | Kite | | |
| | 2A-Neutral fat (50X); | 2B-Sudanophilic lipids(50X) | |
| Fig. 3 | Cattle Egret | | |
| | 3A-Neutral fat(50X); | 3B-Sudanophilic lipids (50X) | |
| Fig. 4 | House Swift | Neutral fat | (125X) |
| Fig. 5 | Bee-Eater | | |
| | 5A-Neutral fat(50X); | 5B-Sudanophilic lipids (50X) | |
| Fig. 6 | Tailor Bird | Neutral fat | (50X) |
| Fig. 7 | Drongo | | |
| | 7A-Neutral fat (125X); | 7B-Sudanophilic lipids(50X) | |
| Fig. 8 | Martin | Neutral fat | (50X) |
| Fig. 9 | Brahminy Myna | | |
| | 9A-Neutral fat(125X); | 9B-Neutral fat (125X) | |
| Fig. 10 | Common Myna | | |
| | 10A-Neutral fat (50X); | 10B-Neutral fat (50X) | |
| Fig. 11 | Jungle Babbler | Neutral fat | (125X) |
| Fig. 12 | Indian Robin - Phospholipids(Nile blue sulphate) | | (125X) |

