CHAPTER 10

GENERAL CONSIDERATION

SECTION I

Morphological, histological, biochemical, physiological and experimental studies on skeletal muscles of vertebrates in general and aves in particular have provided enough basic informations about their metabolic peculiarities and adaptations (Stein & Padykula, 1962; Ballard & Oliver, 1963; Rossi et al., 1963; George & Iype, 1963; George & Bokdawala, 1963, 1964; Ogata & Mori, 1964; Adams & Finnegan, 1965, Nene & George, 1965, 1965a; George & Berger, 1966; Goldberg & Wuntch, 1967; Rinaudo & Giunta, 1967; Moyer et al., 1968; Buno & Germino, 1968; Dubale & Muraleedharan, 1970; Klicka & Kasper, 1970; Penney & Cascarano, 1970). These studies have conclusively proved the existence of two types of muscle fibres, a broad white type loaded with high concentration of glycogen as fuel and adapted for anaerobic metabolism, and a narrow red variety with fat as the main fuel and adapted for aerobic metabolism. In comparison to skeletal muscle, studies on smooth muscle in general and of alimentary canal of vertebrates in particular have been less attempted and explored eventhough scanty literatures are available. The major work on this line has been done by Prakash (1961) on fishes, by Moog (1946, 1950, 1951, 1955, 1956, 1957, 1960, 1961, 1962, 1965); Chang & Moog (1972) in the duodenum and intestine of aves and mammals, Hinsch (1965) in the oesophagus of chick~~en~~, and Hugon & Borgers (1966, 1967, 1968, 1969a, ~~1969b~~) in the duodenum and intestine of chicken. In recent times the smooth muscle complex of avian gizzard has attracted the attention of many investigators who have conducted morphological and experimental studies and as a result of which much is known about its development, innervation and electrophysiology (Bennett, 1969a, 1969b, 1969c; Bennett & Cobb, 1969; Cobb & Bennett, 1969; Grillo, 1969; ~~1970~~). Owing to the lack of adequate information regarding its metabolic aspects and the enzymes involved thereof, an investigation on these lines was deemed necessary and hence, in accordance, was carried out. The investigation deals with the histophysiological aspects of the gizzard of developing and adult pigeon, Columba livia. Since these aspects have been treated as individual topics for discussion in separate chapters in the thesis it was thought necessary to piece together all these findings and present them in a complete generalized picture.

The digestive tract in birds exhibits certain distinctive features. The stomach consists of two portions, a glandular stomach or proventriculus and a muscular stomach or gizzard which are distinct from each other both morphologically and physiologically. The proventriculus is characterised by its thick wall and numerous glands which secrete digestive enzymes and mucin. The gizzard, on the other hand, has an immensely thickened muscular wall with its inner mucosal lining covered with a layer of secreted keratinoid material apparently protecting the cellular surfaces of this muscular grinding mill against both wear and tear as well as the abrasive action of ingested pebbles and other hard objects. The gizzard secretes no digestive enzymes and its function is merely a mechanical one of grinding; its muscular activity thus replacing mastication.

The present quantitative as well as histochemical investigation on the amount and distribution pattern of metabolites such as glycogen and lipids and enzymes of aerobic and anaerobic metabolism revealed a metabolic flux with regard to the preferential choice and utilization of metabolites in the developing as well as adult pigeon gizzard. Carbohydrate seems to be the metabolite of choice in the gizzard tissue during the initial 10 days of development. This aspect is well exemplified by the observed high activities of the enzymes concerned with carbohydrate metabolism such as aldolase and LDH (chapter 2) and αGPDH (chapter 3). In contrast, the weak activities of lipase, esterase and BDH and a low level of lipids (chapter 3) together with a poor response of TCA cycle enzymes such as SDH and MDH (chapter 2) speak themselves the representation of an inadequate machinery for lipid catabolism. The low level of glycogen and lipids with corresponding increased levels of glycolytic enzymes and low levels of TCA cycle enzymes in the initial 10 days of gizzard development are indicative of an active anaerobic glycogenolysis. However, the utilization of glucose supplied through blood could also be a certain possibility and cannot be overruled. This fact gains validity when the observations of increasing activities of glycolytic enzymes such as aldolase and LDH (chapter 2) and negligible utilization of lipids (chapters 2 & 3) are reflected on to the meagre content of glycogen and its negligible decrease (chapter 2) during this period. An increased activities of aldolase and LDH when taken together with an elevated acid phosphatase activity (chapter 4) during these initial days of development is characterised by a tremendous pace of morphological and physiological development of the organ marked not only by the development of musculature and the connective tissue but also the progressive increase in the process of keratinization of the lining of the epithelium. These elevated activities signify the fact that the gizzard tubules are engaged in the synthesis of keratin and also aid in the functional differentiation of muscular tissue and help in the synthesis of contractile proteins as such proteins have been associated with smooth muscle too as in the case of striated muscle (Cobb & Bennett, 1969). The correlation made by Shah & Chakko (1966) and Radhakrishnan (1972) between acid phosphatase activity and keratinization in the epidermis of lizards,and Braun & Rupec (1967) in the human skin are noteworthy here. The alkaline phosphatase activity observed in the muscle fibres during the first 10 days of post-natal development could be attributed to the utility of the enzyme in laying down of connective tissue elements. Such a surmise is rather reasonable when looked into the probable association of this enzyme with the collagen formation (Fell & Danielli, 1943) as well as fibrous protein synthesis and collagen differentiation (Marchant, 1949; Junqueira, 1950). Its activity in the tubular epithelium, on the other hand, may be attributed to a function of imparting an initial influence to the process of keratiønization, as it is between the 5th and 10th days that the inner lining of the gizzard gets gradually keratinized.

However, during the period between 10 and 20 days of post-natal development of pigeon gizzard, there is an increased metabolic incidence as could be inferred from the higher activities of aldolase, LDH, SDH and MDH (chapter 2) and a concomitant depletion of lipids (chapter 3). At the same time, the lowest value of lipid obtained on the 15th through 20th days with corresponding high activities of lipolytic enzymes <u>viz</u>., lipase, and esterase (chapter 3) when taken together with an increased concentration of BDH, α GPDH and G6PDH (chapter 3) and maximum level of activities of SDH and MDH (chapter 2) positively indicate a high metabolic phase and interestingly enough this active period appears to be in good correlation with both the anatomical development of the organ as well as the attainment of full functional competence. The increasing activity of G6PDH observed during this period could be construed as a definite indication of the activation of the synthetic machinery for lipids. A concomitant depletion of glycogen and high activities of glycogenolytic enzymes noted during this period further emphasize the fact that the intermediary products of carbohydrate metabolism are being diverted towards the pathway of lipid synthesis. A high response of G6PDH together with aldolase (chapters 3 & 2) ensures the complete operation of HMP shunt, thus yielding a ready supply of reduced coenzyme II ($NADPH_2$) and pentose sugar, two important cofactors for both fatty acid synthesis as well as nucleotide metabolism. The presence of aldolase during this period of development seems to be of utmost importance, as according to the time and need of the organ, it may be of utility in both the continuity of glycolysis as well as the successful operation of HMP shunt. Another important aspect of aldolase catalysis at this juncture could be to channalize a part of its product towards glyceride synthesis mediated via the operation of α GPDH. By its participation, α GPDH not only entails a steady supply of triglycerides for esterification with the readily available fatty acids but also keeps open the avenue of phospholipid production. Evidences are available now to identify α-glycerophosphate derived from glycolysis as the most important starting point for phospholipid synthesis (Kornberg & Pricer, 1953; Rossiter et al., 1957; Kennedy, 1953, 1954, 1957a & b). As the process of development calls for much cellular turnover by division and differentiation an obvious increase in phospholipid production is rather pertinent as it is necessary for the laying down of structural frame work of the actively proliferating cellular elements. Apart from its significance in contributing for the structural entities of the cells, its other attribute as a metabolite for energy yielding reaction should not be overlooked. In all events, an increase in the activities of aldolase (chapter 2), αGPDH, G6PDH and phospholipid (chapter 3) have been noted in the present study. Thus it could be safely presumed that at this accelerated metabolic phase of the developing pigeon gizzard, the extra energy necessities are being successfully met with by the utilization of lipids (chapter 3); at the same time the organ is also involved in a slow replenishment of the depleted glycogen through glycogen synthesis. Comparatively high activities of aldolase and LDH (chapter 2) in the mucosal tubules of the developing pigeon gizzard during this period (between days 10 and 20) with the observed low level of glycogen in the gizzard as a whole signify the ability of the tubules to utilize glycogen or glucose as the energy source for both the synthesis of keratin as well as for cellular proliferation. A moderate incidence of G6PDH (chapter 3) in the mucosal tubules observed during the first 15 days of post-natal development could, well, be associated with a good rate of protein synthesis to be expected in these cells as the mucosal tubules are not only engaged actively in constant generation of new cells but also in the secretion of keratin over the mucosal epithelium. The work of Grillo (1969) in the developing chicken gizzard is noteworthy here and points out the participation of G6PDH in the development of the epithelial as well as mucosal cells of both stomach and intestine.

With the completion of initial spurt of muscular activity of the gizzard during the period between 20th and 30th days of development and also in the adult condition, the finer and final changes in development, differentiation and maturation aimed at the change over to adult pattern of gizzard are at work. At this period the metabolic necessities also seem to be very much reduced as could be visualized by gradual increase of glycogen and slightly reduced levels of aldolase, LDH, SDH and MDH (chapter 2). The concomitant increase in the activity of G6PDH and low level of lipase (chapter 3) could be indicative of a phase of stepped up lipid biosynthesis. Eventhough lipid biosynthesis is proceeding on one hand, the increased activity of esterase and BDH (chapter 3), on the other, are rather indicative of the possible utilization of lipids, possibly fatty acids, made available through the rich blood supply.

The spurt in the acid phosphatase activity between 20th and 25th days is in agreement with the attainment of the full mechanical functioning of the organ wherein muscles are initiated to contract vigorously when in use and to crush the solid grains with the agency of thickly keratinized layer over the mucosal tubules. With the aquisition of the full functional capacity by the gradual action and working on the following day (30th) ~~and also in the adult~~ and on the completion of the process of post-natal development and differentiation a decreased acid phosphatase activity was observed.

Histochemical investigation of cholinesterases on the developing and adult pigeon gizzard revealed the fact that there is a gradual and steady increase of acetylcholinesterase (AChE) from the day of hatching till the attainment of adult level on the 20th day. A complete absence of butyrylcholinesterase (BuChE) in the developing gizzard was also noticed in the present study. The gradual increase in AChE activity may be ascribed to a possible role of this enzymes in the progressive structural differentiation of the smooth muscle towards attainment of high AChE activity (20th day) and the retention of the same high level even in the adult is a period when young ones start their first feeding on solid grains. This coincidence of the peak value of AChE and the intake of solid grains at the same period are indicative of probable nervous involvement in the attainment of a capacity for full mechanical functioning of the gizzard.

In general the interesting aspects are brought out by the present study on the developing and adult pigeons are that:

1) excepting for a small period of high energy requirement the rest of the periods of post-natal development of pigeon gizzard are dependent on blood

FIGURE 1

Changes in the weights of body and gizzard and contents of glycogen and fat in pigeon gizzard during different days of of post hatching development.

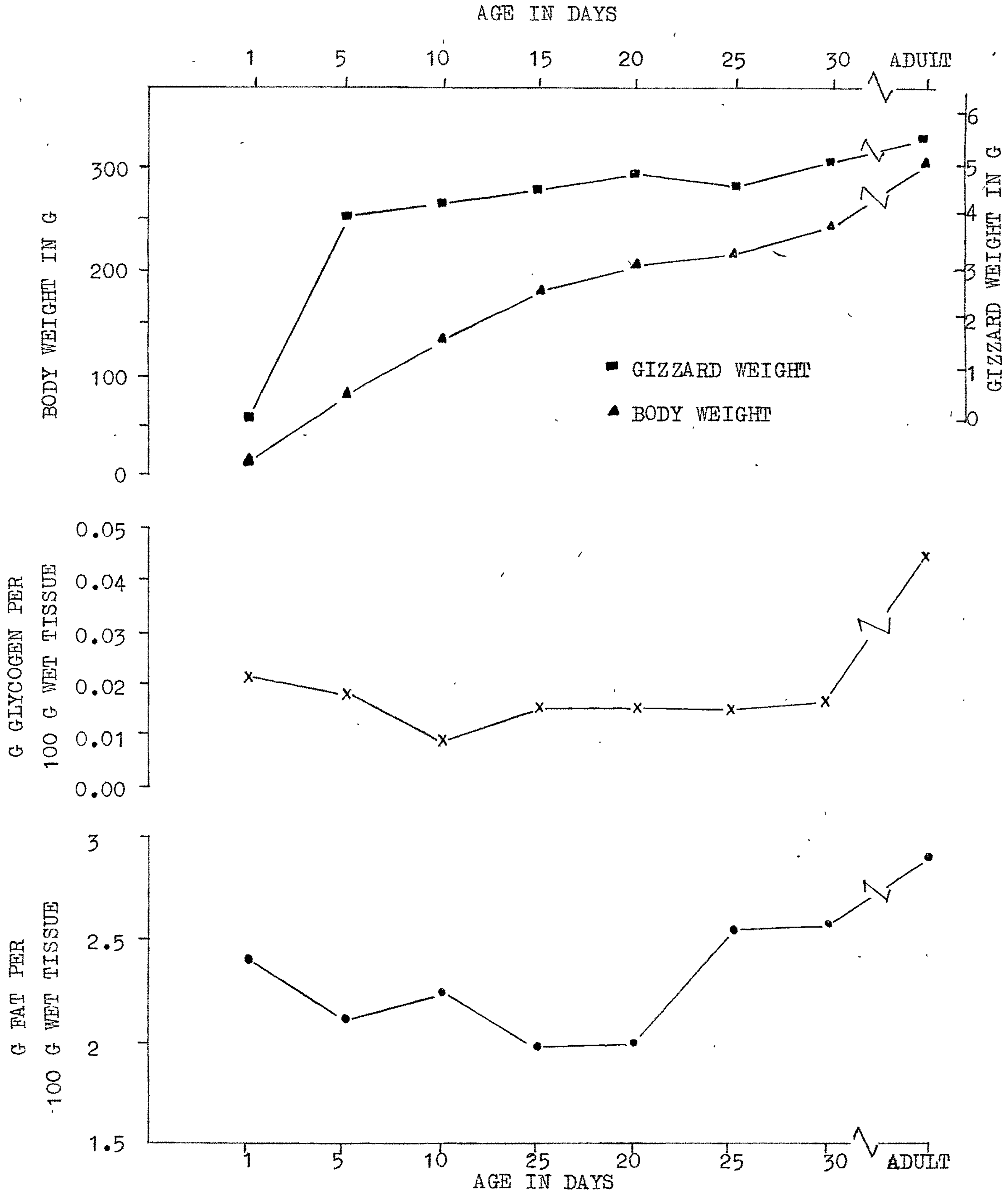
AGE IN DAYS
1
5
10
15
20
25
30
ADULT
BODY WEIGHT IN G
300
200
100
0
GIZZARD WEIGHT IN G
6
5
4
3
2
1
0
GIZZARD WEIGHT
BODY WEIGHT
G GLYCOGEN PER
100 G WET TISSUE
0.05
0.04
0.03
0.02
0.01
0.00
G FAT PER
100 G WET TISSUE
3
2.5
2
1.5
1
5
10
25
20
25
30
ADULT
AGE IN DAYS

TABLE I: COMPARISON OF THE CONCENTRATIONS OF METABOLITES AND CERTAIN ENZYMES REPRESENTED IN THE MUCOSAL TUBULES OF THE DEVELOPING AND ADULT GIZZARD OF PIGEON, *COLUMBA LIVIA*

Metabolites and Enzymes	Age in days							
	1	5	10	15	20	25	30	Adult
Aldolase	++	+±	++	+++	++++	++++	+++	+++
Lactate dehydrogenase	+±	++	++	+++	+++	+++	++±	+++
SDH	+	+±	++	+±	+++	+±	+±	+±
MDH	+	+±	++	+	++	+	+	++
Sudanophilic lipid	+±	+±	+±	+	+	++	++	++
Acidic lipid	+±	++	++	++	++±	+++	+++	+++
Lipase	++	++	++	+±	+±	++	++	+±
Esterase	+±	++	++±	++±	+++	+++	++±	+++
G6PDH	+++	++	+++	+++	++±	++±	+++	+++
α GPDH	+	+	+±	++	+++	+++	++±	++±
BDH	+	+±	++	++±	+++	+++	+++	+++
Acid phosphatase	+±	+±	++	+±	+++	+++	+	+
Alkaline phosphatase	++	++	+±	+±	+	+	±	±
AChE	+	++	+	++	+++	++++	+++	+++
BuChE	-	-	-	-	-	-	-	-

TABLE II: COMPARISON OF THE CONCENTRATIONS OF METABOLITES AND CERTAIN ENZYMES REPRESENTED IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING AND ADULT GIZZARD OF PIGEON, COLUMBA LIVIA

Metabolites and Enzymes	Age in days							
	1	5	10	15	20	25	30	Adult
Aldolase	++±	++	+++±	+++	+++±	+++±	++±	++±
Lactate dehydrogenase	++±	+±	++	++±	+++	++±	++±	+++
SDH	+±	+±	+++	++	+++	+++	++±	++±
MDH	+±	+±	++	+±	++	++	+±	+±
Sudanophilic lipid	+±	+±	+±	+	+	++	++	++
Acidic lipid	+±	+±	+±	++	++	++±	++±	+++
Lipase	++	++±	++±	+++	+±	++	++	++
Esterase	++	++±	++±	+++	+++±	+++±	++++	++++
G6PDH	++	++±	+++	+++±	++±	++±	+++	+++
α GPDH	++±	++±	++±	++±	+++	++±	++±	++±
BDH	+±	+±	++	++	+++	++++	++++	+++
Acid phosphatase	++	+++	++±	+±	++±	+++	++	+
Alkaline phosphatase	+±	+±	+±	+±	++	+	±	±
AChE	±	+	++	+++	++++	++	+++	+++
BuChE	-	-	-	-	-	-	-	-

glucose for the continuous and smooth operation of carbohydrate metabolism,

2) a continuous process of phospholipogenesis and an active phase of lipid utilization between 15th and 25th days of development,

3) a significant possibility of the supply of fatty acids through circulating blood for the energetics associated with the gizzard functioning,

4) an active participation of phosphatases - both acid as well as alkaline - in the functional differentiation of the muscular tissue and help in the synthesis and secretion of keratin by the mucosal tubule cells and also in the laying down of connective tissue elements around smooth muscle fasciculi, and

5) a gradual and steady increase of AChE from the day of hatching till 20th day when the adult pattern of enzyme concentration is reached and by the time when such is the concentration of enzyme the gizzard attains its functional maturity, thereby commencing its mechanical function in full swing.

SECTION II

The variations in the form and relative size of avian gizzard could be well correlated with the dietary conditions (Farner, 1960). It is generally known that the musculature and keratinoid lining of the epithelial tubules in the gizzard are greatly developed in birds that feed upon plants and seeds in contrast to the carnivorous species where the gizzard tend to be relatively thin walled and bag like with those of omnivores and insectivores displaying a large variety of intermediate conditions. Those birds which feed on fleshy fruits or nectar (frugivore and nectar feeders) have distinctly reduced gizzards so as to be seen as an insignificant swelling between the proventriculus and duodenum. Since the gizzards of birds vary structurally and functionally according to their food and feeding habits, there might be differences in their general morphological structure and biochemical and physiological adaptation. It was in this wake that the present comparative study on the metabolic and enzymatic peculiarities involved in the working of the smooth muscle was carried out in the gizzards of adult representative birds.

It is well established from the biochemical and histochemical observations on red and white skeletal muscle fibres of vertebrates that the red narrow fibres are well adapted for aerobic metabolism using fat as fuel while white broad fibres are better adapted for anaerobic metabolism using glycogen as the fuel (George & Berger, 1966). From the present study on glycogen and enzymes associated with carbohydrate metabolism, it becomes quite evident that the gizzards of granivores and carnivores are equipped with an efficient machinery for the utilization of carbohydrate. High concentration of aldolase, LDH and SDH and moderate activity of MDH (chapter 6) point to the above fact. In a comparative way, the gizzards of all the representative birds studied herein are found to have more concentration of lipid in comparison to glycogen. Lipid splitting enzymes lipase and esterase (chapter 7) also elicited a concomitant stronger response in the gizzards of granivores and insectivores. High concentrations of SDH and an appreciable concentration of MDH (chapter 6) with a pronounced response towards BDH and G6PDH (chapter 7) and a low level of glycogen in the gizzards of frugivores and nectar feeder, when taken together, might be indicative of a slightly higher level of lipid catabolism in comparison with that of carbohydrate in the gizzards of these groups of birds.

An almost equal and identical capacity to metabolize both carbohydrate and lipid by the gizzards of omnivores and insectivores when taken together with their degree of response towards lipase, esterase and BDH (chapter 7) tend to place them intermediate to that of granivores and carnivores on one hand and frugivores and nectar feeder on the other.

It is well known from the histochemical and biochemical studies on red and white skeletal muscle fibres of vertebrates that whereas the slow tonic contractile fibres (red) are chiefly dependent on lipid catabolism, the quick tetanic contractile fibres (white) are better adapted for carbohydrate catabolism (George and Berger, 1966). Based on this scheme of biochemical characterization of muscles, the expectance of a moderate and uniform incidence of lipid catabolism in the gizzard smooth muscles of all types of birds appear to be temptingly reasonable and tenable, as gizzard is an organ which could functionally be associated with the process of slow and sustained contractility. A comparatively higher level of fat than carbohydrate observed in the present study might, probably, be indicative of the former being metabolized more efficiently and economically for the energy yield than the latter by the gizzards of the adult birds. The utilization of carbohydrate source, though at a low minimal level, in the gizzards of all the herein mentioned groups of birds - in the light of activities of aldolase and LDH (chapter 6) - however, seems to gain justification not only from the fact that there is always a minimal level of carbohydrate metabolism in all the animal tissues but also from the possibility that the gizzard at times (immediately after the mechanical digestion of food in the gizzard) might have to engage itself in quick tetanic mode of contractions to evacuate their contents into the duodenum. However, the high degree of carbohydrate metabolism indicated in the case of granivores and carnivores and a comparatively low level of oxidation of the same by those of frugivores and nectar feeder and of an intermediary level of oxidation by the gizzards of omnivores and insectivores gain credulence and validity in this light when reflected on the type and nature of the food consumed by these various birds.

The glycolytic pathway and TCA cycle are the important routes whereby the organic food stuffs are completely metabolized. However, the HMP shunt pathway is also another useful one which serves not only as supplementary one but also aids in the biosynthesis of lipids. The operation of such pathway for the formation of reduced NADPH_2 and pentose sugars could be well visualized by the presence of G6PDH. A synergistic relationship between HMP shunt and lipid biosynthesis could be well perceived by which the shunt pathway provides NADPH_2 for the process of lipid synthesis which in turn regenerates NADP for the continuous operation of shunt pathway. High activities of G6PDH, α GPDH and BDH (chapter 7) were observed in the gizzards of all the groups of birds studied herein. High G6PDH activity has been reported in certain vertebrate skeletal muscles by Nene and George (1965), in pigeon breast muscles by Cherian (1967), in the fish skeletal muscles by Bokdawala & George (1967) and in the caudal muscles of reptiles by Shah & Ramachandran (1972). The work of Abraham & Chaikoff (1952), Abraham et al. (1954), Glock & McLean (1954) and Levy (1961) are noteworthy here. A prominent α GPDH activity noted in the present study is in perfect accordance with a significant activity of aldolase (chapter 6) and could be attributed to the continuous operation of glycolytic pathway. The operation of such pathway readily yields a continuous supply of dihydroxyacetone phosphate, an intermediary product, which is also the substrate for αGPDH which catalyzes the formation α-glycerophosphate, an important substrate much needed for synthesis of glycerides and phospholipids as suggested by Kornberg & Pricer, (1953), Kennedy (1953, 1954, 1957a, 1957b), Rossiter et al. (1957). Further, the presently observed moderate activities of lipase and esterase and BDH (chapter 7) when taken together with the appreciable activities of SDH and MDH (chapter 6) are indicative of an active lipid catabolism and its oxidation via TCA cycle.

It could be noted that though the gizzards of all the birds are capable of metabolizing carbohydrates and fat in a preferential way, the extent of ~~its~~ utilization appears to show a gradation as per the type of organic food stuffs ingested by them, being of a low level where the food is of fluid and soft consistency (frugivores and nectar feeder), of high order where the food is of hard and tough nature (granivores and carnivores) and of an intermediary grade where the food is of a mixed or intermediary nature.

The participation of phosphatases in the processes such as protein synthesis, transport of metabolites, collagen formation, laying down of connective tissue elements, autophagocytosis and keratinization has been well established from the study of many workers (Fell & Danielli, 1943; Marchant, 1949; Junquiera, 1950; Cobb & Bennet, 1969; de Duve, 1959; Weber & Niehus, 1961; Klockars & Wegelius, 1969; Pilo et al., 1972; Radhakrishnan, 1972; Shah & Chakko, 1966; Braun & Rupec, 1967). Such roles of phosphatases - acid as well as alkaline - have been well exemplified from the studies on developing and adult pigeon gizzard. It has been realized that the form and size of avian gizzard vary structurally and functionally according to the food and feeding habits (Farner, 1960). High acid phosphatase activity in the smooth muscle fibres and mucosal epithelium of the gizzards of representative birds could be correlated with the functional activity of that organ. The pronounced acid phosphatase activity in the mucosal tubules may be attributed to the elaboration and secretion of keratin around the epithelium. It has been observed that the keratin layer of the groups of birds other than granivores is invariably thin as could, possibly, be the adaptive feather in relation to soft food materials processed in

Tables III & IV. Comparative account of histochemically observed intensities of metabolites and enzymes in the mucosal tubules (Table III) and smooth muscle fasciculi (Table IV) of gizzards of various representative birds.

Metabolites and Enzymes	Pigeon	Shrike	Kite	Brahminy Myna	House Crow	Jungle Crow	Fowl	Indian Robin	Green Bee-Eater	Drongo	Crow Pheasent	Jungle Babbler	Red-Vented Bulbul	Koel	Common Myna	House Sparrow	Parakeet	Purple Sunbird
Aldolase	+++	++±	++±	+±	++±	++±	++	++	++±	++±	+++	++±	++±	++	++	++	++	++
Lactate dehydrogenase	+++	++±	+++	+±	++±	++±	++±	+±	++±	++±	+++	++±	+++	++	+±	++±	++	++±
SDH	++	++	++	+±	++	++	++	++	++	++	++	++±	+±	+±	+±	++	++	++
MDH	++	++	++	++	+±	+±	++	++	++	+±	+±	++	++	++	++	+±	+±	+±
Lipids	++	+±	+±	++	+±	+±	++	++	++	++	++	+±	++	+±	++	++	++	++
Lipase	++	++	++±	++	++	++	++	++	+±	++	++	++	++	++	++	++	++	+
Esterase	+++	++	+±	++	++	++	++	++	++	++	++±	+±	++	+±	++	+±	++	+±
G6PDH	+++	+++	+++	++±	++	++	++±	+++	+++	++	+++	++	++±	++±	++±	++	++	++±
α GPDH	++±	+++	++±	++	+±	+±	++±	++	++	++	+++	++	++	+	++	++	+±	+±
BDH	+++	++±	++±	++	++	++	++±	++	++	++	+++	++	+±	+±	++	++±	++	+++
Acid phosphatase	+	+±	+±	++	++	++	+++	++	++	++±	+++	++	+++	+++	++	++	++	+++
Alkaline phosphatase	±	+	+	±	−	−	+	±	+	−	−	+	−	+	±	−	+	±
AChE	+++	++	++	++	++	++	++	+	++	++	++	++	++	++	++	+	+	+
BuChE	−	+	+	++	++	++	+	+	+	+	+	++	++	++	++	+	+	+

Metabolites and Enzymes	Pigeon	Shrike	Kite	Brahminy Myna	House Crow	Jungle Crow	Fowl	Indian Robin	Green Bee-Eater	Drongo	Crow Pheasent	Jungle Babbler	Red-Vented Bulbul	Koel	Common Myna	House Sparrow	Parakeet	Purple Sunbird
Aldolase	++±	+++	++±	++	+++	+++	++±	++	+++	+++	+++	++	+++	++	++	++±	++	++
Lactate dehydrogenase	+++	+++	+++	++	+++	+++	+++	++	+++	+++	+++	++	+++	++	++	++±	++	+++
SDH	++±	+++	++±	++	+++	+++	++±	+++	++±	+++	+++	++	++	++	++	++±	+++	+++
MDH	+±	++	++	++	++	++	++	++	++	++	++	+±	++	+±	++	++	++	++
Lipids	++	++	++	+±	++	++	++	++	++	++	++±	++	++	++	+±	++	++	++
Lipase	++	+++	+++	++	+++	+++	+++	++	++	++	++	++	++	++	++	++	++	+
Esterase	++++	+++	++	++	+++	+++	+±	++±	++±	++	+++	++	++	++	++	++	++	++
G6PDH	+++	+++	+++	++	++	++	+±	+++	+++	++	+++	+++	++	++	++	++±	+++	++
αGPDH	++±	+++	+++	++	++	++	++±	++	++	++	+++	++	++	+	++	++	++	++
BDH	+++	+++	+++	++	++	++	+++	+++	+++	++	+++	++±	++	++	++	+++	+++	+++
Acid phosphate	+	+±	+±	++	++	++	+++	++	++	++±	+++	++	+++	+++	++	+++	++	+++
Alkaline phosphatase	±	±	+	±	−	−	++	±	+	−	−	+	−	+	±	−	±	±
AChE	+++	+++	+++	++	++	++	++	++	+++	+++	+++	++	+++	+++	++	++	++	++
BuChE	−	+±	+±	++	+±	+±	++±	+±	+±	++	++	++	++	++	++	++	++	++

such gizzards. The presence of high acid phosphatase and comparatively thicker keratin lining observed in the fowl gizzard, when viewed in this context, becomes interesting; the presence of this enzyme may be ascribed the function of both elaboration and secretion of keratin and perhaps helps indirectly in the secretion of acid required for the peptic digestion. Alkaline phosphatase activity was moderate to nil in the gizzards of all the groups of birds (all other than granivores) where the connective tissue surrounding the smooth muscle fasiculi is relatively thin; the enzyme activity was demonstrably higher in granivores and fowl (as an exception from omnivore) where the connective tissue tracts are thicker. The keratin layer in the gizzards of carnivores, frugivores, nectar feeder and omnivores - barring fowl - is quite thin and a correspondingly low concentration of alkaline phosphatase activity was also noted.