PART - IV

BIOCHEMICAL PROPERTIES

OF

CERTAIN APPENDICULAR MUSCLES

OF

DABCHICK (PODICEPS RUFICOLLIS), COOT (FULICA ATRA) AND DOMESTIC DUCK (ANAS PLATYRHYNCHOS)

WITH

SPECIAL REFERENCE TO LIPID UTILIZATION

AND

SOME ENZYME CONCENTRATIONS
The evolutionary success of the birds has been facilitated by the birds' ability to store large quantities of fat and use this substrate as a source of energy for sustained muscle activity and as a source of metabolic water to supplement strategies of water conservation. It was long thought that the primary if not exclusive fuel of muscle metabolism was carbohydrate. However, in recent years more direct evidence has been presented which indicates that fat is also an important fuel for muscular activity. In fact it is now well recognized that during intensive muscle work the organism depends solely on their lipid reserves (Geyer et al., 1949; Volk et al., 1952; Tepperman et al., 1956; Neptune et al., 1959 and 1960; Havel et al., 1960; Hemmings and Black 1960; Ogata and Fortin 1961; Issaiz et al., 1964; George and Berger 1966; Beatty et al., 1965; Beatty and Kick 1970; Close 1972; Keesan et al., 1974; Downer and Mathew, 1976; Kieselring 1976).

In the performance of muscular work and maintenance of muscle tone energy is expended. This energy is generated from a chain of chemical reactions supported by a continuous supply of fuel. Carbohydrates and fats constitute the fuel reserves in all muscles, depending on the nature of their activity. The advantages of lipid over carbohydrate as a metabolic fuel include a higher caloric content/unit weight.
of substrate, and the fact that triacylglycerol may be stored in anhydrous form while glycogen is stored in the bulky hydrated form. Thus the use of lipid as a primary metabolic substrate permits accumulation of large reserves of energy which may be used during periods of prolonged energy demand.

During the last few years Prof. George and his associates have shown that the lipid constitutes a major fuel during the migratory flight of birds (George and Berger, 1966; Vallyathan and George, 1969; Vallyathan et al., 1970; John and George, 1973; and 1973b, Parker and George, 1974b). Birds have a remarkable ability to synthesize and deposit large amounts of lipid just prior to migratory flights (Farrer, 1965; 1966; Martin, 1962; Jordan and Kratzke, 1963; Goodridge, 1964; Goodridge and Ball, 1966 and 1967 and George and Berger, 1966). While working with pectoralis major of the pigeon, George and Vallyathan (1964 and 1969) found increased free fatty acid level in the muscle and corresponding decrease of it in the blood and liver. Total fatty acid content on the other hand increased significantly in blood, liver and muscle with corresponding decrease of these levels in the adipose tissue. These results indicated that the lipids are transported to the muscles through blood in the form of fatty acids from the adipose tissue. In their histochemical study of the pectoralis major muscle of the ruby-throated humming bird, Chaninbog and George (1964) have shown that lipids are transported...
through sarcoplasmic reticulum to the mitochondria for oxidation.

There is no evidence that neutral lipid as such could directly be oxidized. If neutral lipid to be utilized for energy, it has first of all to be hydrolyzed to fatty acids and glycerol; the fatty acids released can undergo oxidation in the mitochondria. The glycerol can enter into glycolytic cycle. The presence of enzyme lipase which catalyzes the hydrolysis of lipids to fatty acids and glycerol has been demonstrated in muscles (George and Berger, 1966), muscle lipase capable of hydrolyzing glycerides of long chain fatty acids has been shown to be localized in the mitochondria (Pokrovskii and George, 1964). The biochemical nature, precise histochemical localization and physiological role of lipase/s in certain muscle of birds have been discussed by George (1966). It has been suggested that the level of lipase activity in a tissue is an index of the extent of fat utilization and the capacity of the muscle for sustained activity. The final step in the utilization of fatty acids for energy is their oxidation to CO₂ and water. The mechanism of fatty acid oxidation in birds' muscle seems to be similar to that described for mammalian system (George and Berger, 1965). It is evident from the foregoing account that much attention has been focussed on the energetics of breast muscles of birds.
which are good flyers. Further, the survey of literature revealed that no comparative data is available on the muscle energetics of birds which exhibit dual type of locomotory adaptations.

The birds chosen in the present investigation exhibit remarkable contrasting characters. The dab duck (*Podiceps ruficollis*) and coot (*Fulica atra*) are active diving birds as well as fly frequently from one pool to another in search of food. The domestic duck (*Anas platyrhynchos*) on the other hand is more a wading bird and is only surface swimmer when it goes to water. It has been illustrated in our previous study (Part II and III) that the various appendicular muscles of these birds with highly variable functions and work performance have developed extremely variable metabolic pattern between the birds as well as in the individual muscles of the same bird. We thought that it would be rewarding to compare some aspects of lipid metabolism in the various appendicular muscles of these three birds. It is expected from these investigation to throw light on the biochemical adaptation of the various muscles to meet exigencies of energy demand by the flight and leg muscles used in the media with different physical properties.

The present discourse is dealt with in section-A and B. Section A describes nature of lipid utilization in breast
The dabchick and coot were collected from the local ponds with the help of local fishermen. The domestic duck was obtained from the local farm. The birds normally were maintained for a period of one week under the laboratory conditions before they were used for the experiments. The weights of birds were recorded soon after they were collected from their natural environment and also after the end of one
week period. The bird was killed by decapitation. The various breast and wing muscles used in the present investigation are listed below.

1. M. pectoralis major
2. M. supracoracoides
3. M. latissimus dorsi anterior
4. M. latissimus dorsi posterior
5. M. biceps brachii
6. M. biceps brachialis

Preparation of muscle tissue

Tenacious latissimus dorsi anterior and posterior, biceps brachi and triceps brachii were removed separately from their point of attachment to the point of insertion. The breast muscles, pectoralis major and supracoracoides were also removed and used for the various determinations.

Extraction of lipid and its analysis

The total lipid was extracted from each muscle following the method of Folch et al. (1957) using chloroform-methanol 2:1 (v/v) mixture. The detailed extraction procedure was similar to that described in Part II section of the thesis.
Separation and analysis of Neutral lipid

A known amount of lipid (usually 50 to 60 mg) from each muscle was subjected to silica acid 100-200 mesh (Merck, Darmstadt) column chromatography for their neutral and phospholipid separation. The neutral lipid along with free fatty acid (FFA) was eluted with 150 ml of chloroform. Further separation of neutral-lipid into individual glycerides and FFA was effected on thin layer chromatographic (TLC) plate.

Thin layer chromatography

A slurry of silica-gel (National Chemical Laboratories) and plaster of paris (3416 gm wt/vt) in 60 ml of distilled water was spread on 20 cm20 cm glass plates of 500 micron thick. The plates were activated at 100°C before use. A suitable aliquot (500 µl) of the mixture of neutral lipid and FFA from each muscle sample in chloroform was applied on plates and the lipids were resolved into triacylglycerol (TAG), free fatty acids (FFA), diacylglycerol (DAG) and monoacylglycerol (MAG) by developing the plate with n-hexanediethyl ether-acetic acid (90:10:1.5, v/v/v). A standard mixture of glycerides and fatty acids (Generously supplied by Dr. F. H. Ratson) was run each time along with the muscle sample, after development the spots were made visible by exposing the
plates to iodine vapour and were marked with fine needle. Individual spots were scraped out and were transferred separately into small chromatographic tubes from which individual glycerides and FFA were eluted with 50 ml of peroxide-free diethyl ether.

**Estimation of Glycerides**

Individual glycerides were estimated according to the method and described by Raghavan and Ganguly (1967). After evaporating the diethyl ether, the dried tlc samples were saponified with 1 ml of 2 per cent alcoholic potassium hydroxide at 60°C for 30 min, after which 1 ml of 8 per cent (v/v) hydrochloric acid was added. The liberated glycerol was treated with 0.1 ml sodium metaperiodate (0.05 M) for 15 min, in a seal-tight chamber and the colour was developed by the addition of 0.5 ml phenylhydrazine hydrochloride reagent (0.125 M). At the end of 10 min, 0.2 ml potassium ferricyanide solution (0.137 M) was added, followed by 2.5 ml of concentrated hydrochloric acid. The volume was made up to 10 ml with distilled water and the colour was read immediately on electronic 20 nm photoelectric colourimeter at 540 nm. The amount of individual glycerides were calculated from a standard graph prepared from glycerol.
STANDARD-CURVE FOR GLYCEROL

MICROGRAM GLYCEROL

OPTICAL DENSITY
STANDARD CURVE FOR FREE FATTY ACIDS 
USING PALMITIC ACID

\[ \mu \text{ MOLES OF PALMITIC ACID} \]

\[ \text{OPTICAL DENSITY} \]

\[ \begin{array}{c|c|c|c|c|c|c|c|c|c}
\hline
0 & 0.05 & 0.15 & 0.25 & 0.35 & 0.45 & 0.55 \\
\hline
\end{array} \]
**Determination of free fatty acids**

The free fatty acid content of the tissue samples were estimated colorimetrically following the method of Lauer (1969) with some modifications. The FFA spot from the TLC plate was collected as described above. The dried sample of FFA collected in a test tube were vigorously shaken with 3 ml petroleum ether-chloroform mixture (1:1) plus 2.5 ml of copper reagent consisting of 7 ml triethanolamine; 0.3 ml glacial acetic acid; 3.25 g copper nitrate; 6.25 g of potassium sulphate and water to give a final volume of 100 ml. The density of this solution was greater than chloroform-petroleum ether mixture. The tubes were centrifuged at 3000 rpm for 10 min. A known aliquot of the petroleum layer was taken and then added 0.5 ml of 0.1 per cent (w/v) sodium diethyl dithiocarbamate prepared in n-butanol. After mixing, the density of the colour was read at 440 nm against a reference solution of 3 ml petroleum-chloroform mixture (1:1 v/v) and 0.5 ml colour reagent. The results obtained were corrected with reference to a blank and were calculated from a standard curve obtained for palmitic acid.

**Protein content determination**

The protein content of the homogenate as well as the of the mitochondrial preparation was determined according to
the method of Lowry et al. (1951), using bovine serum albumin as reference standard.

Determination of lipolytic activity

The lipolytic activity in various muscle homogenates was determined using radiolabelled substrates. In this procedure uniformly labelled substrates were prepared as follows.

PREPARATION OF RADIOACTIVE SUBSTRATES

\(^{14}\)C triacylglycerol

Triacylglycerol uniformly labelled in the carboxyl position with \(^{14}\)C was obtained by germinating soybeans in a medium containing \(^{14}\)C-acetate. About 50 g of soybeans were allowed to soak in running tap water for about two hours. The beans were then transferred to a beaker containing 250 milliliters of \(^{14}\)C acetate dissolved in 10 ml of boiled distilled water. They were left at room temperature for about 8 hours, with occasional stirring. The labelled acetate was completely absorbed by the seeds during this time. They were then spread over moistened thick blotting paper and allowed to germinate in the dark at 30°C for 48 hours. The seedlings were then homogenized in a waring blender with 400 ml of methanol and lipids were extracted according to the
method of Bligh and Dyer (1957). The lipid extracts were
concentrated and loaded on to an alumina column prepared in
distilled chloroform. The neutral lipid fraction was eluted
with one litre of chloroform. After concentrating the eluates
the neutral lipid fraction which consists major portion of
triacylglycerol was further purified by subjecting it to an
alumina column and eluted with 2 per cent acetone in petrol.
The purity of the labelled triacylglycerol was checked on the
plate with a standard triglyceride (Sigma Co., U.S.A.). The tin
plate showed only one spot corresponding to the standard
triglyceride.

$^{14}$C diacylglycerol

Labelled diacylglycerol was prepared by hydrolysing
$^{14}$C-lecithin with phospholipase C. The incubation mixture
consisted of $^{14}$C-lecithin (equivalent to 30, millimicrons)
diluted with carrier lecithin; 2.0 ml tris salt buffer
(pH 7.4), 2.3 mg phospholipase C (Sigma U.S.A) prepared in
5 ml of 14 bovine serum albumin and 1 ml of 0.5 M CaCl$_2$. The
incubation was carried at 37°C for 60 min with a constant
shaking. The reaction was terminated by adding 25 ml methanol
and lipid was extracted according to the method of Bligh and
Dyer (1957). The extracted lipid was analysed on pre-active
silica gel tin plate along with standard triacylglycerol. After
exposing the plate in an iodine chamber, the diacylglycerol
beneath formed was scraped out and transferred to a small
chromatographic column and eluted with chloroform. The
chloroform was evaporated in a flask evaporator and labelled
diacylglycerol was stored in cool.

\[ ^{14}C \text{monoxylglycerol} \]

Labelled monoxylglycerol was obtained by hydrolysing
purified labelled triglyceride with an acetone dried powder
of goat pancreas. The reaction mixture consisted of 1.0 ml
1 M tris HCl buffer (pH 8); 0.3 ml (w/v) CaCl₂; 0.5 ml, 0.1;
sodium taurocholate; labelled triglyceride (20,000 c.p.m./mg); \nand 25 mg pancrease powder. After 3 hr of incubation at 4°C
with a constant shaking, the reaction was terminated by adding
10 ml methanol. The lipid fraction was extracted according
to the method of Sligh and Dyer (1957). Labelled monoxyl-
glycerol was separated on preparative TLC plate as described
above.

Substrate preparation

The labelled substrates obtained as a result of the
procedure described above were emulified in gum acacia sol
(1 gm/4 ml distilled water). Purified mono, di- and triolein
were used as carrier substrates. Radio-labelled and carrier
substrates were emulified for 3 min before use.
Enzyme assay

The standard reaction mixture contained in a final volume of 2 ml, 0.5 ml enzyme source and 0.5 ml emulsified substrate containing 25 to 30 micromoles of tri-, di- and monoycglycerol equivalent to two million e.m.u./min in each case, 0.7 ml tris malate buffer (pH 8) and 0.3 ml CaCl₂ (0.1M). The incubation was carried out at 37°C for 1 hr to 4 hr. Control preparations were run with each experiment by adding appropriate vol of methanol to the incubation mixture before the addition of homogenate and incubated along with the samples. The reaction in the experimental tubes was terminated by the addition of 2 ml of methanol, and the lipid portion was extracted following the method of Bligh and Dyer (1957). The reaction tubes and control tubes were centrifuged for 15 min at 3,500 rpm. A known vol. of chloroform layer containing lipid was removed, evaporated and redissolved in known volume of chloroform. The free fatty acid content of the lipid sample was estimated on silica gel plate using the solvent system of n-hexane:solvent ether:acetic acid (90:10:1.5, v/v/v). With this solvent system monoycglycerol and phospholipids remained at the origin, while diacylglycerol and triacylglycerol and free fatty acids were identified with their respective standards after exposing the plate to iodine vapour. The free fatty acid spot was scraped out from the plate and transferred to a counting vial containing liquid
scintillation solution (12 mg ppo/5 ml toluene). The radioactivity in free fatty acid was measured on Beckman's scintillation counter.

Enzyme units

Except in radioactivity there is no difference between the unlabelled and labelled substrates, since both were prepared in the same way. When this condition is met, and the amount of the substrates (molecules) as well as the radioactivity of the sample and free fatty acid fraction are known, the enzyme activity is calculated as usual following the formula given below.

\[
\text{Cpm in total sample} = \frac{\text{ specific activity of the fatty acid}}{\text{ substrate (mole)ester bond in sample}}
\]

\[
\text{Cpm obtained in FFA} = \text{ 1 mole of fatty acid released}
\]

preparation of mitochondrial fraction

Mitochondrial pellet was prepared from breast and wing muscles following conventional differential centrifugation technique. Each muscle was homogenized in 9 vol. of ice cold isolation medium containing 0.25 M sucrose and 1 mM, LIPAA using Potter and Elvehjem glass homogenizer at 0 to 4°C. The
homogenate was then filtered through a layer of cheesecloth moistened with isolation medium. The filtrate was centrifuged at 2000g for 10 min in an orbital refrigerated centrifuge at 4°C. The resulting supernatant was successively centrifuged twice for 10 min at 7000g. The resulting mitochondrial pellet was washed twice with isolation medium and resuspended in isolation medium.

The activity of the mitochondrial preparation was initially checked in the oxygraph (Milcon model) before use; succinic dehydrogenase activity was used as the marker enzyme for mitochondrial preparation.

Measurement of oxygen uptake

Oxygen uptake by the mitochondrial preparation was determined using conventional Warburg's apparatus at 37°C with a total liquid volume of 3.2 ml in the reaction flask. The metabolic CO₂ was trapped by 0.2 ml of 20% KOH in the centre well of the Warburg flask. The final incubation mixture contained 0.8 mole KCl; 4.9 mole MgCl₂; 50 mole triethanolamine HCl buffer (pH 7.4); and 1.5 mole ATP; 1 mole cytochrome b; sodium salts of butyrate (10 mM), octanoate (10 mM) palmitate (0.35 mM) were used as substrates. The effect of carnitine on palmitate oxidation was tested by adding 0.2 mM DL-carnitine. The amount of mitochondrial
suspension was adjusted so that each reaction vessel contained
1.6 mg protein. After thermal equilibration, incubations
were begun by the addition of mitochondria from the side arm
and were carried with constant shaking (160 oscillation/min).
O₂ uptake values were calculated from the period during which
O₂ uptake was linear with time. Results were corrected to
endogenous respiration.
SECTION 2 A.

NATURE OF LIPID UTILIZATION BY THE BREAST AND CURTAIN WING MUSCLES OF DABBLICK, COOT AND DOMESTIC DUCK.
RESULTS

Results obtained on neutral lipid analysis, lipolysis and fatty acid oxidation by the breast and certain wing muscles of dab-chick, coot and domestic duck are presented in Table 1, 2 and 3 as well as in Figs. 1, 2 and 3.

It is known that neutral lipid moiety of the tissue is involved in the energy production. It may be seen from Table 1 that the muscles, pectoralis major and supracoracoideus compared to the other four wing muscles, have higher concentration of total neutral lipid and free fatty acid (FPA). It is also interesting to note that these two breast muscles of dab-chick exhibited more amount of neutral lipid and FPA compared to the corresponding muscles of coot and domestic duck (Table 1). However, among the other four wing muscles, triceps brachii and biceps brachi have a higher amount of neutral lipid and FPA compared to muscles latissimus dorsi anterior and posterior.
<table>
<thead>
<tr>
<th>MUSCLE TYPE</th>
<th>SOURCE</th>
<th>Micrograms glycerol</th>
<th>Mean ± S.E.</th>
<th>Free fatty acid</th>
<th>Neutral lipid content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Monosylglycerol</td>
<td>Bisyalglycerol</td>
<td>Triacylglycerol</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PECTORALIS Major</td>
<td>(5)</td>
<td>900 ± 10.00</td>
<td>250 ± 20.00</td>
<td>2,450 ± 20.00</td>
<td>1,450 ± 15.00</td>
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<tr>
<td></td>
<td></td>
<td>720 ± 10.00</td>
<td>100 ± 20.00</td>
<td>2,340 ± 21.00</td>
<td>1,000 ± 15.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>190 ± 07.30</td>
<td>0 ± 10.00</td>
<td>1,200 ± 9.00</td>
<td>560 ± 9.00</td>
</tr>
<tr>
<td>SUPRA-CORACOIDUS</td>
<td>(5)</td>
<td>700 ± 07.13</td>
<td>150 ± 32.00</td>
<td>1,990 ± 20.00</td>
<td>1,760 ± 31.00</td>
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<tr>
<td></td>
<td></td>
<td>620 ± 15.00</td>
<td>130 ± 09.00</td>
<td>0,980 ± 31.30</td>
<td>590 ± 09.18</td>
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<td>LATISSIMUS DORSI, Ant.</td>
<td>(5)</td>
<td>120 ± 10.00</td>
<td>80 ± 17.00</td>
<td>1,200 ± 30.00</td>
<td>700 ± 20.00</td>
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<td></td>
<td></td>
<td>100 ± 02.33</td>
<td>100 ± 09.40</td>
<td>1,100 ± 11.98</td>
<td>760 ± 15.68</td>
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<td>LATISSIMUS DORSI, Post.</td>
<td>(5)</td>
<td>100 ± 05.30</td>
<td>210 ± 10.00</td>
<td>0,700 ± 07.00</td>
<td>390 ± 10.00</td>
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<tr>
<td></td>
<td></td>
<td>120 ± 05.00</td>
<td>100 ± 20.00</td>
<td>1,500 ± 20.10</td>
<td>690 ± 10.90</td>
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<td></td>
<td></td>
<td>100 ± 05.30</td>
<td>180 ± 16.90</td>
<td>1,680 ± 39.00</td>
<td>390 ± 9.80</td>
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<td></td>
<td>100 ± 02.80</td>
<td>210 ± 08.30</td>
<td>0,500 ± 32.00</td>
<td>320 ± 10.00</td>
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<tr>
<td>BICEPS BRACHII</td>
<td>(5)</td>
<td>320 ± 18.32</td>
<td>10 ± 16.78</td>
<td>1,600 ± 20.20</td>
<td>930 ± 20.00</td>
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<td></td>
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<td>290 ± 10.00</td>
<td>210 ± 20.00</td>
<td>1,000 ± 13.11</td>
<td>1,340 ± 30.00</td>
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<td></td>
<td></td>
<td>120 ± 11.00</td>
<td>210 ± 05.30</td>
<td>0,790 ± 20.00</td>
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<td>TRICEPS HUMERUS</td>
<td>(5)</td>
<td>150 ± 07.00</td>
<td>150 ± 19.00</td>
<td>2,000 ± 41.00</td>
<td>1,790 ± 50.00</td>
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<td></td>
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<td>120 ± 10.30</td>
<td>210 ± 21.00</td>
<td>2,330 ± 29.38</td>
<td>0,980 ± 18.00</td>
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<td></td>
<td></td>
<td>100 ± 5.10</td>
<td>100 ± 13.00</td>
<td>0,860 ± 15.68</td>
<td>420 ± 20.00</td>
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</tbody>
</table>

*Figure in paranthesis indicate number of experiments.*
Lipid is available to the muscle either stored within the muscle or as fuel present in the blood (long chain fatty acid, ketone bodies are triacylglycerol). Fatty acids in the blood are derived from triacylglycerol stored in adipose tissue and ketone bodies are formed from the partial oxidation of fatty acids in the liver.

Studies on the lipid content of skeletal muscles of birds show that reasonable amount of lipids is present in this tissue to provide energy (George and Sager 1966). The neutral lipid analysis of the breast and wing muscles is presented in Table 2. The results revealed that the triacylglycerol content of all the muscles was higher than the other glycerides and free fatty acids (except triceps femoris of dab-chick) n. pectoralis major and n. supracoracoideas exhibited higher triacylglycerol than the other four wing muscles. It may also be noted from the Table 1 that the amount of triacylglycerol of the breast and wing muscles of dab-chick and cost were higher than the muscles of the domestic duck.

The free fatty acid level showed considerable variation between the individual muscles of the each bird (Table 1). n. pectoralis major and supracoracoideas compared to other four wing muscles demonstrated higher amount of free fatty acids. n. latissimus doral ant and n. latissimus doral post, however, showed least amount of fatty acid content.
Table: Demonstration of lipolytic activity with mono-dio-and triacylglycerol as substrates in breast and cardiac muscles of Pekchick (DC), Coot (C) and Domestic duck (DD).

Microsomal fatty acid released/mg protein/30 min.

<table>
<thead>
<tr>
<th>MUSCLE TYPE</th>
<th>SOURCE</th>
<th>SUBSTRATES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Monoacylglycerol</td>
</tr>
<tr>
<td>PECTORALIS</td>
<td>DC</td>
<td>8.2 ± 0.01</td>
</tr>
<tr>
<td>MAJOR (5)</td>
<td>C</td>
<td>6.9 ± 0.023</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>3.1 ± 0.007</td>
</tr>
<tr>
<td>SUPRACORA-</td>
<td>DC</td>
<td>3.0 ± 0.02</td>
</tr>
<tr>
<td>COHISPUS (5)</td>
<td>C</td>
<td>2.1 ± 0.021</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>1.6 ± 0.003</td>
</tr>
<tr>
<td>LATISSIMUS</td>
<td>DC</td>
<td>1.8 ± 0.003</td>
</tr>
<tr>
<td>FORSI ANTR (5)</td>
<td>C</td>
<td>1.0 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>0.05 ± 0.001</td>
</tr>
<tr>
<td>LATISSIMUS</td>
<td>DC</td>
<td>1.6 ± 0.002</td>
</tr>
<tr>
<td>FORSI POST (5)</td>
<td>C</td>
<td>1.2 ± 0.002</td>
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<tr>
<td></td>
<td>BD</td>
<td>1.0 ± 0.002</td>
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<tr>
<td>BICEPS (5)</td>
<td>DC</td>
<td>2.3 ± 0.017</td>
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<tr>
<td></td>
<td>C</td>
<td>2.6 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>1.3 ± 0.012</td>
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<td>STYCUPS (5)</td>
<td>DC</td>
<td>1.9 ± 0.005</td>
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<td>C</td>
<td>2.13 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>1.12 ± 0.003</td>
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</tbody>
</table>

* Figures in parenthesis indicate number of experiments.
is known that mono and diacylglycerols are hydrolytic products of triacylglycerol, and during successive hydrolysis of triacylglycerol, free fatty acids are produced. It could then be anticipated that the amount of free fatty acids in a muscle may indicate its ability to use them during its intense activity.

It is well accepted now that triacylglycerol as such can not be utilized for energy in the muscles (George 1964). Triacylglycerol has to be broken down to fatty acids and glycerols, mediated by the enzyme lipase. The presence of the enzyme was investigated in wing and breast muscles of the birds mentioned hitherto. The results are presented in Table 2. It is apparent from these results that the homogenates of breast and wing muscles of the three birds have the ability to hydrolyze non-, di-, and triacylglycerol, although the rate of hydrolysis varies in different muscles with different substrates. It is of considerable interest, however, to note that both breast and wing muscles of all the three birds exhibited higher lipolytic activity with diacylglycerol as substrate compared to tri- and monoacylglycerol as substrates. Pectoralis major and supracoracoideus compared to the other four wing muscles studied demonstrated higher lipolysis with all the three substrates tested for. The results also revealed that all the wing and breast muscles of dab-chick
### Table: Fatty Acid Oxidation by the Mitochondrial Preparation of Breast and Certain Wing Muscles of Dabchick (DC), Goat (G), and Domestic Duck (DD)

<table>
<thead>
<tr>
<th>Muscle Type</th>
<th>Source</th>
<th>Endogenous</th>
<th>Butyrate</th>
<th>Octanoate</th>
<th>Palmitate</th>
<th>Palmitate + Carnitine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectoralis</td>
<td>DC</td>
<td>70.0±5.0</td>
<td>101.0±9.6</td>
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<td>50.0±1.8</td>
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Figures in parenthesis indicate no. of experiments.
and coot exhibited higher amount of lipolytic activity compared to the corresponding muscles of the domestic duck (Table 2).

It has been well demonstrated that the final step in the utilization of lipid for energy is the oxidation of fatty acids produced by the action of the lipase. It is believed that the mechanism of fatty acid oxidation in bird muscle is similar to that described for mammalian system (George and Berger 1966) in the present experiments an attempt has been made to demonstrate the oxidation of short and long-chain fatty acids by the mitochondrial preparation of the wing and breast muscles of three birds. The results are summarized in Table 3 and Figs. 1 to 3. The results show that both the breast as well as the wing muscles have the ability to oxidize short chain butyrate and octanoate but not the long chain fatty acid palmitate. Butyrate appeared to be oxidized more rapidly than octanoate. H. pectoralis major and H. supracoracoides showed more O2 uptake with butyrate and octanoate, than did the other four wing muscles.

Interestingly none of the mitochondrial preparation demonstrated increased O2 uptake with palmitate. However, palmitate oxidation by the mitochondrial preparation was significantly increased in all the muscle preparations after the addition of DL-carnitine to the incubation medium (Fig.1 to 3).
Discussion

There is no longer any doubt that the oxidation of fat plays a vital role in the provision of energy for sustained muscular activity. Fat is available to the muscle either as triglyceride stored within the muscles (endogenous) or from the adipose tissue. It has been well recognised that mitochondria are the sites of fat synthesis (Green 1959), and mitochondria for fatty acid oxidation (Kennedy and Lehninger, 1949, and Green 1951). George and coworkers (George and Vallyathan 1964; Vallyathan and George 1959; Vallyathan et al., 1970; John and George 1973; McLean et al., 1974) while studying the effect of exercise on lipid levels in the various tissues of pigeon obtained evidence of massive mobilisation and transport of lipid from liver and adipose tissue to the working muscle. The mode of transport of lipid appears to be in the form of free fatty acids. The results of the present investigation have revealed that triacylglycerol is the major neutral lipid fraction in all the muscles studied in dab-chick and coot (Table 1). The free fatty acid content of the blood was also higher in both birds compared to the other glyceride fractions (Table 4). It may be suggested from these studies that triacylglycerol is the major source of energy store in the breast and wing muscles of dab-chick and coot. The mode of transport of lipid from the storage organ to the muscular tissue seemed to be in the form of free
fatty acids. However, further evidences are required in these wild birds (dab-chick and coot) to support our findings; such as relative amount of various lipid fraction from the storage organs during vigorous exercise and the nutritional state of the bird; since the starvation causes elevation of free fatty acid level in the blood and finally esterification of excessive free fatty acids into triglyceride in the muscle cell.

It has been suggested that intracellular lipid constitute immediate source of energy during vigorous muscular activity (Navel et al., 1964; Isserits et al., 1964; Isserits et al., 1966; Valiyathan and George, 1969; Valiythan et al., 1970). Utilization of intracellular lipid during electrical stimulation of pectoralis major of pigeon (George and Berger, 1966) as well as in the diaphragm of rat (Heptane, 1959) has been very well demonstrated. It may be seen from the present results that the amount of triglyceride content of muscle vary among individual muscles of the same bird as well as among the three birds. It has been established that M. pectoralis major and R. supracoracoides are powerful depressor and elevator of wing respectively. Evidence has been presented in the earlier part of the thesis (Part II) that both these muscles compared to the other four wing muscles are compared to more red fibres, which are rich in myoglobin, succine dehydrogenase and packed with mitochondria.
It is known that higher amount of myoglobin and more succinic dehydrogenase activity in a muscle cell reflect its high oxidative capacity. It could be anticipated then that M. pectoralis major and M. supracoracoideus of dab-chick and coot (active flyers) are more involved in sustained actions and they utilize lipid as a chief source of energy. The corresponding muscles of the domestic duck on the other hand (a poor flyer) are composed of more white fibres, and contain considerably less myoglobin and show lesser succinic dehydroglucose activity. They seem to utilize carbohydrate reserves for their energy requirement instead of lipid.

Triacylglycerol stores of the muscle must first be hydrolyzed to nonesterified fatty acids (NEFA) before triacylglycerol is used as energy source. The production of NEFA from triacylglycerol is catalysed by lipase/s. Studies from other vertebrates have shown that the lipolysis is catalysed by three enzymes: triglyceride, diglyceride and monoglyceride lipases (Hinz, 1961; Cottin and Chaftrin, 1967 and 1968; Ohuda and Fujii 1969; Wallach 1966 and Newsholme and Sturt, 1976). It is evident from the results presented in Table 2 that the breast and wing muscles of dab-chick, coot and domestic duck were able to hydrolyse mono-, di- and triacylglycerols. It was interesting, however, to note that the rate of lipolysis with diacylglycerol as substrate was considerably higher with all the muscle homogenates compared to...
mono- and triacylglycerol as substrates. The fact that triacylglycerol lipase activity in the adipose tissue could be increased by a number of hormones like glucagon, growth hormone and vasotocin, lead to believe that the triacylglycerol lipase is a regulatory enzyme. The activities of mono- and diacylglycerol lipases were not affected (John and George 1973a and by John et al., 1973, 1974; Kennewon et al., 1973 and 1974 and Noshalme and Start, 1970). It has been shown that diacylglycerol is the intermediate during the synthesis of triacylglycerol from long chain fatty acids. If lipid was stored as triacylglycerol within the muscle cell and diglyceride lipase was more active, it followed that at least one fatty acid moiety must be cleaved from each triacylglycerol molecule before diacylglycerol lipase could act. This question of lipolytic activity in the muscles appear to be integral part of the general problem of energy demand and transport of lipid from storage organs. We assume that in the wing and breast muscles of these birds have lipases attacking tri-, di- and monocacyl glycerols. The activity of the each enzyme is determined by the energy need of the muscle. For the present, our results do not permit any generalization concerning different lipase's activity in the muscles, since the results are based on the captive birds and relatively small samples.
Carnitine (β-hydroxy-γ-trimethyl ammonium butyrate) has been shown to stimulate the oxidation of long chain fatty acids by muscle mitochondria as well recognized (Frits and Mazon 1959; Frits 1961 and 1964; Beenackers and Klingenberg 1964; Bose and Klingenberg 1955). It is generally accepted that fatty acids are activated to fatty acyl CoA by acyl CoA synthetase, since the mitochondrial membrane (probably the inner membrane) is impermeable to acyl CoA derivatives fatty acyl CoA has to be converted (by carnitine acetyl transferase) to fatty acyl carnitine which can freely cross the inner mitochondrial membrane. It is evident from the present observations that the mitochondrial preparation of the breast and wing muscles of the three birds (Table 3) investigated require carnitine to oxidize palmitate. Beenackers and Klingenberg (1964) observed high rate of carnitine transacetylase activity in the mitochondrial preparation of pigeon breast muscles compared to the flight muscles of Loxota and the skeletal muscles of rat. As was the case with other metabolic activity, the rate of palmitate + carnitine oxidation was significantly high in the pectoralis major and m. supracoracoideus of dab-chick and cock. This could be anticipated as these two birds are active flyers compared to the domestic duck which is very poor flyer. The mitochondrial preparation of breast and wing muscles of all the three birds although demonstrated accountable oxygen uptake with short chain fatty
acids, butyrate and octanoate, the physiological significance of these short chain fatty acid in muscles is not clearly understood.

In the final analysis, the aspects of lipid metabolism in the breast and certain wing muscles of three birds should be considered a reflection of the cellular organization of the flight muscles involving both structural and metabolic adaptations. In dab-chick and coot the II pectoralis major and supracoracoides have significantly higher number of red fibres, loaded with myoglobin, fat and also contain numerous large mitochondria and adapted for an oxidative metabolism. The corresponding muscles of the domestic duck, on the other hand are loaded with white fibres, rich in glycogen, very less myoglobin and a few small mitochondria (Part II) organized for anaerobic generation of ATP. The other four wing muscles of dab chick, and coot exhibited better adaptation for lipid utilization, compared to the corresponding muscles of the domestic duck. It is pertinent to mention here that all the four wing muscles of coot and dab-chick were white fibres, whereas white fibres took their place in the domestic duck (Part II). It appears imperative to correlate function with histochemical and biochemical results except in general terms; the weight of evidence, however, strongly support the original basic theory that red and predominately red muscles are adapted for sustained activity use lipid as chief energy
source and prolonged energy production and predominantly white muscle with glycolytic activity, is adapted for sudden burst of activity for which glycogen seems to be the chief fuel.

**Summary**

1. Comparative account of some aspects of lipid metabolism in the following breast and wing muscles was investigated in the three phylogenetically aquatic birds, the dab-chick (*Podiceps ruficollis*), the coot (*Fulica atra*) and the domestic duck (*Anas platyrhynchos*).

The dab-chick and coot are active flyers whereas the domestic duck is a very poor flyer.

i) **M. pectoralis major**
   - Breast muscle

ii) **M. supracoracoideus**

iii) **M. latissimus dorsi anterior**

iv) **M. latissimus dorsi posterior**

v) **M. biceps brachii**
   - Wing muscles

vi) **M. triceps brachii**

2. The lipid content of all the above mentioned muscles revealed that **M. pectoralis major** and **supracoracoideus** of all the three birds exhibited higher amount of neutral lipid and free fatty acid content than the
rest of the muscles. It was also interesting to note that the breast muscles of dab-chick and coot contained higher amount of neutral lipid and free fatty acid compared to corresponding muscles of domestic duck.

3 Analysis of neutral lipid moiety from the various breast and wing muscles suggested that triacylglycerol is the major neutral lipid fraction in all the muscles. The neutral lipid analysis of the plasma of all the three birds also revealed that free fatty acids and triacylglycerol were the major components. It was assumed that the mode of transport of lipid from storage organs to the working muscles was in the form of free fatty acids.

4 The most significant results on lipolysis were those indicating that wing and breast muscles preferentially hydrolyze diacylglycerol, whereas tri- and monoacylglycerol were less attacked. The question of lipolytic activity in the muscle cell appeared to be integral part of the general problem of energy demand by the muscle and transport of lipid to the muscle from other tissues. Our results suggested that different lipase/s attacking mono-, di- and triacylglycerol were present in the breast and wing
muscles of the three birds, studies from other vertebrates (Newsholme and Sturt, 1974) have shown that triglyceride lipase is a regulatory enzyme. Based on the observations made in this investigation it was suggested that diacylglycerol lipase was involved more during intense muscular activity, whereas triglyceride lipase appeared to be more active in the regulation of triacylglycerol concentration inside the muscle cell.

The present opportunity was also available to test the oxidation of different fatty acids by the mitochondrial preparation of the wing and breast muscles. The results revealed that all the breast and wing muscles were capable of oxidizing short chain fatty acids, butyrate and octanoate. Significant rate of oxidation of palmitate could be obtained only after the addition of carnitine to the medium. Therefore it was suggested that the carnitine is an obligatory cofactor, serving to facilitate the transfer of acyl groups into and out of mitochondria.

In the final analysis, it should be of considerable interest from the comparative point of view to understand how these phylogenetically aquatic birds with different mode of locomotory behaviour have set their
enzigencies of energy demand in the environment with
different physical properties. The dab chick and
coot migrate frequently from pond to pond the
domestic duck on the other hand is more of walking
bird and lost almost its flight ability. The problem
of energy for sustained flight during periods of
active flight is no more a matter of speculation. We
now do now that fat is the fuel of quantitative
importance during intense muscle activity. Our study
showed that breast and wing muscles of dab chick and
coot, exposed to the domestic duck are well organiz-
ed for lipid utilization.
LIPID AS SOURCE OF ENERGY IN CERTAIN LEG MUSCLES
OF PARCHICK, COOT AND DOMESTIC DUCK.
Materials and Methods

Materials consisted of leg muscles of three aquatic birds, the dab chick (Pediculus ruficollis) the coot (Fulica atra) and the domestic duck (Anas platyrhynchos). The methods used in this section are essentially similar to that described in the preceding section A. The following leg muscles were used.

1. Muscle arctorius
2. M. semitendinosus
3. M. plantaris
4. M. gastrocnemius internus
5. M. gastrocnemius externus
6. M. tibialis anterior

Results

The results obtained on neutral lipid composition, the lipolytic activity and the fatty acid oxidation in various leg muscles of the three birds are presented in Table 1, 2 and 3 as well as in figures 1, 2 and 3. Results obtained on the neutral lipid content indicated that M. semitendinosus and M. arctorius compared to the other four leg muscles showed characteristically higher level of neutral lipid and free fatty acid content. It may also be interesting to note that between M. gastrocnemius externus and M. gastrocnemius internus
of coot and dab chick the former muscle demonstrated higher level of neutral lipid and free fatty acid than does the latter muscle (Table I). Of all the leg muscles investigated, N. tibialis anterior exhibited the least amount of neutral lipid and free fatty acid content. It could also be seen from the results presented in Table I that the leg muscles of dab chick and coot contained higher amount of neutral lipid and free fatty acids than do the corresponding muscles of the domestic duck.

Similar to the breast and wing muscles (section A) the leg muscles also demonstrated triacylglycerol as the dominant neutral lipid fraction (Table I), in all the leg muscles investigated (Table I). N. semitendinosus and N. sartorius showed moderately higher triacylglycerol and free fatty acid compared to the other four leg muscles. It has been well documented during the last few years that during vigorous exercise, flight muscles of birds solely derive their energy requirement from lipid oxidation. There are also good reasons to believe that intramuscular lipid constitute important source of fuel reserve during exercise (George and Berger, 1966; Drumond, 1967). It could then be anticipated that muscles have all the necessary enzyme system to utilize lipids. First step in lipid utilization is its hydrolysis. The results obtained on the rate of hydrolysis of mono-, di-
Table 1: Hydrolysis of mono-di-and triacylglycerol by the leg muscle homogenate of dabchick (DC), Coet (C) and domestic duck (DD).

Microsomes fatty acid released/mg protein/30 min. mean ± S.E.

<table>
<thead>
<tr>
<th>MUSCLE TYPE</th>
<th>SOURCE</th>
<th>SUBSTRATE</th>
<th>Monoacylglycerol</th>
<th>Diacylglycerol</th>
<th>Triacylglycerol</th>
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<tr>
<td></td>
<td>C</td>
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<td>3.0 ± 0.03</td>
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<tr>
<td></td>
<td>DD</td>
<td>0.9 ± 0.003</td>
<td>2.1 ± 0.28</td>
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<tr>
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<td>C</td>
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<td>GASTROCNEMIUS EXT (5)</td>
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<td>1.0 ± 0.008</td>
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<td>C</td>
<td>0.89 ± 0.01</td>
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<td>DD</td>
<td>0.51 ± 0.009</td>
<td>1.9 ± 0.05</td>
<td>0.79 ± 0.098</td>
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<td>SARTORIUS ANTERIOR (5)</td>
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<td>DD</td>
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<td>2.73 ± 0.025</td>
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* Figures in parenthesis indicate number of experiments.
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<th>Octanate</th>
<th>Palmitate</th>
<th>Palmitate Carnitine</th>
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<td>50 ± 2.3</td>
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<td>G</td>
<td>50 ± 2.99</td>
<td>80 ± 2.9</td>
<td>59 ± 5.1</td>
<td>50 ± 2.1</td>
<td>101 ± 7.3</td>
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<td>G</td>
<td>53 ± 5.15</td>
<td>80 ± 4.3</td>
<td>63 ± 3.0</td>
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<td>88 ± 3.7</td>
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<td>45.0 ± 2.8</td>
<td>71 ± 2.5</td>
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<td>60.0 ± 2.8</td>
<td>52.0 ± 3.8</td>
<td>70.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>50 ± 3.6</td>
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<td>55.0 ± 2.9</td>
<td>50.0 ± 5.0</td>
<td>65.0 ± 4.2</td>
</tr>
<tr>
<td>GASTRONEUMUS</td>
<td>EC</td>
<td>54 ± 4.2</td>
<td>80.0 ± 3.22</td>
<td>86.0 ± 1.2</td>
<td>52.0 ± 3.0</td>
<td>88.0 ± 6.32</td>
</tr>
<tr>
<td>EXTERRINA (5)</td>
<td>G</td>
<td>54 ± 4.2</td>
<td>70.0 ± 3.4</td>
<td>63.0 ± 3.2</td>
<td>52.0 ± 3.0</td>
<td>80.0 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>49 ± 4.2</td>
<td>69.0 ± 2.20</td>
<td>56.0 ± 1.9</td>
<td>50.0 ± 5.9</td>
<td>64.0 ± 2.92</td>
</tr>
<tr>
<td>TIBIALIS</td>
<td>EC</td>
<td>46 ± 3.9</td>
<td>59.0 ± 5.0</td>
<td>55.0 ± 5.0</td>
<td>45.0 ± 3.5</td>
<td>69.0 ± 5.2</td>
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<tr>
<td>ANTERIOR (5)</td>
<td>G</td>
<td>49 ± 5.4</td>
<td>58.0 ± 2.8</td>
<td>54.0 ± 3.0</td>
<td>45.0 ± 3.5</td>
<td>67.0 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>49 ± 5.4</td>
<td>56.0 ± 2.52</td>
<td>52.0 ± 3.6</td>
<td>49.0 ± 4.0</td>
<td>60.0 ± 3.6</td>
</tr>
</tbody>
</table>

*Figure in parenthesis indicate number of experiments.
GASTROCNEMIUS EXTERNA

DOMESTIC DUCK
TIBIALIS ANTERIOR
COOT
GASTROCNEMIUS EXTERNA

O₂ UPTAKE (µL/mg protein/hr.)

DOMESTIC DUCK
DABCHICK
COOT

BUTYRATE OCTANOATE PALMITATE PALMITATE BUTYRATE OCTANOATE PALMITATE PALMITATE CARNITINE

BUTYRATE OCTANOATE PALMITATE PALMITATE CARNITINE
and triacylglycerol, by the homogenates of various leg muscles are presented in Table 2. It is interesting to note that all the leg muscle homogenates are capable of hydrolyzing mono-, di- and triacylglycerols. The rate of hydrolysis of diacyl-glycerol, however, by the leg muscle homogenate is moderately higher than those of mono- and triacylglycerols as substrates (Table 2). Presence of distinct lipase/s effecting hydrolysis of mono-, di- and triacylglycerols has been demonstrated in adipose and liver tissues (Nowsholmes and Start, 1976).

Ability of a muscle cell to utilize lipid for energy production could be assessed by determining the rate of oxidation of fatty acids. Summary of the results of rate of fatty acid oxidation are presented in the Table 3. The results indicated that mitochondrial preparation of the leg muscles of all the three birds could oxidize short chain fatty acid salts, butyrate and octanoate. The palmitate oxidation is established in all the leg muscles mitochondrial preparation only after the addition of carnitine to the incubation medium.

**Discussion**

It is well recognized now that sudden large scale bursts of muscular activity most commonly rely on glycolytic sources of ATP long term muscular activity on the other hand is generally sustained by the metabolism of fatty acids. The metabolic importance of lipid utilization during intense
sustained muscular activity has already been indicated (section A). It appears therefore that the rate and duration of muscular activity is a major determinant of the type of ATP generating scheme which will function to maintain muscle work. As the bird begins to exercise its muscle work both the out-put of free fatty acids from storage organs and the uptake of free fatty acids by working muscle increase (George and Berger, 1968; Forster et al., 1963; Parker and George 1975; George and Vallyathan 1964, Vallyathan et al., 1970; John and George 1972; Prentice et al., 1974). There are good reasons also to believe that during intense muscular activity intramuscular lipids is recruited first and the blood-borne lipids are selectively taken up for utilization by the working muscles (Donahue 1971). The importance of intramuscular lipid for energy production could then be anticipated. The results of the present observations revealed that \( M \), sartorius and \( M \), external iliacus of dab, chick and cock contain comparable amount of neutral lipid and free fatty acid compared to those other leg muscles of the same birds as well as all the leg muscles of the domestic duck (Table 1). In fact, the values obtained on \( M \), sartorius and \( M \), external iliacus come very close to those obtained on the breast muscles of the same birds (section A).

It is of considerable interest to note that between \( M \), gastrocnemius externus and \( M \), gastrocnemius internus of dab...
chick and coot, the gastrocnemius externa exhibited greater amount of neutral lipid and free fatty acid content than that of gastrocnemius interna. It may be recalled in this context our previous observations (Chapter 11) that gastrocnemius externa of these two birds is composed more of red as well as intermediate fibres, compared to the gastrocnemius interna. It has been shown that red fibres are involved in sustained continuous actions and are loaded more with fat and myoglobin. They are adapted for oxidative metabolism. The white fibres on the other hand contain more amount of glycogen, less myoglobin and generate ATP through anaerobically. The functional significance of metabolic differences in these two muscles may well reflect their role in the swimming and diving activity of these two birds. Morphological dispositions of M. gastrocnemius externa and M. gastrocnemius interna (Part 2) revealed that these muscles fuse distally with their tendons and join with the flexor tendon of toes. In dab chick and coot, the extensor lobes of feet are quite rigidly attached to the toes. According to Storer (1971) for recovery strokes, the foot is rotated 90° so that the inner side points forward and the toes with their lobes move through water like a knife. At the end of recovery stroke the foot is rotated back to the normal position so that the maximum surface area is available for the power stroke, we believe that the forward motion of the lobes through aquatic medium, which offers considerable resistance and involves sustained continuous
actions, is effected by the contraction of \( m. \) gastrocnemius externa, while the recovery stroke which is violent and quick is performed by \( m. \) gastrocnemius interna.

It is evident (Table 1) that in all the leg muscles studied, triacylglycerol is the major neutral lipid. This apparent difference between individual muscles or between the three birds appear to be quantitative. It cannot be easily assessed whether the observed difference reflects variations of an experimental nature or whether they can provide a basis for important differences in substrate utilization.

There is no evidence to show that the triacylglycerol could be used directly by the muscles. Hydrolysis of lipid has been considered as first step in the utilization of lipids. Presence of lipases in the muscles of various species of birds has been discussed by George and his associates (George and Berger, 1966). Hydrolysis of mono-, di- and triacylglycerols by the leg muscle homogenate suggested that diacylglycerol is more effectively hydrolysed than mono- and triacylglycerols. Presence of different lipase systems differing mono-, di- and triacylglycerols has been demonstrated in liver and adipose tissue of rat and other mammals (Gorin and Shefrin 1967; Glennis and Fligl, 1969; Newsholme and Start 1971). It is assumed that lipase systems of similar nature is operating in the muscles as well. Lipolytic activity of
A muscle can be taken as an index to the extent of fat utilization (George and Berger, 1966). In the light of this statement and the results obtained on lipid content of various leg muscles, it may be suggested that the \( M_1 \), sartorius, and \( M_2 \), semitendinosus of dab chick and coot depend on lipid metabolism for their energy demand during their swimming activity.

Long-term muscular activity which relies on fatty acid fuel is linked in an absolute manner to oxygen availability. As we would expect on the basis of their different roles, the leg muscles display varied abilities to oxidise fatty acids (Table 3). It has been indicated in our previous studies (Part III) that \( M_1 \), sartorius and \( M_2 \), semitendinosus of dab chick and coot are endowed with large proportions of red and intermediate fibres. It is well established fact that red fibres are highly oxidative in nature, contain large number of mitochondria and they are rich in myoglobin (hence the red colour) and fat. It is evident from the present results that all the mitochondrial preparations of the various leg muscles could oxidise only short-chain fatty acids, butyrate and octanoate. Significant amount of palmitate oxidation could be obtained only after the addition of carnitine. Carnitine as an obligatory co-factor in the oxidation of long chain fatty acids (palmitate) is very well recognized (Grenet, 1989).
our results on the fatty acid oxidation by the leg muscle mitochondria revealed that \( M_1 \), sartorius and \( M_2 \), semi-tendinosus of dab chick and coot show higher rate of fatty acid oxidation compared to the other four leg muscles. It should also be mentioned in this context that the precise physiological significance of short-chain fatty acid oxidation in the energy metabolism of muscle is not clearly understood. According to Gilbert (1967) the short chain fatty acids are more likely present as a result of \( \beta \)-oxidation of longer chain fatty acids.

**Summary**

Some aspects of lipid metabolism has been investigated in certain leg muscles of the following aquatic birds.

1) **Dab chick** *Gadocetes ruficollis*

2) **Coot** *Fulica atra*

3) **Domestic duck** occasional surface swimmer, *Anas platyrhynchos*

An attempt is made from this study to answer the question of which systems of energy supplying
metabolism are operating in the leg muscles involved. In various pattern of swimming activity of the birds under investigation. The extent of metabolic differences among the leg muscles is examined by comparing the amount of neutral lipid fractions ability to hydrolyse neutral lipid fractions and, finally the oxidation of short and long chains fatty acids by the mitochondrial preparations of the various leg muscles.

The various leg muscles used in this present investigation are listed below.

1) Muscle sartorius
2) M. semitendinosus
3) M. plantaris
4) M. gastrocnemius internus
5) M. gastrocnemius externus
6) M. tibialis anterior.

Neutral lipid and free fatty acid content of the leg muscles revealed that M. sartorius and M. semitendinosus of all the three birds compared to the other four leg muscles exhibited higher level of these substrates. Further analysis of neutral lipid revealed that triacylglycerol is the major lipid component in all the leg muscles.
Aspects of lipid utilization between M. gastrocnemius externa and M. gastrocnemius interna of ash chick and cot revealed characteristic differences. M. gastrocnemius externa compared to interna of both birds demonstrated higher level of neutral lipid and free fatty acids as well as in the rate of oxidation of fatty acids. It is interesting to note that M. gastrocnemius externa and interna fuse distally with their tendons and join with flexor tendon of toes. It appears that M. gastrocnemius externa effects forward motion of the lobes of the foot, which in aquatic media offers considerable resistances. The recovery stroke which is violent and quick action is performed by the action of the gastrocnemius interna.

The interesting observations of the present experiment is the finding that all the leg muscles exhibited higher rate of lipolysis with diacylglycerol as substrate compared to mono and triacylglycerol substrates. The results, although do not indicate the specificity of the lipase systems in the various leg muscles, however, do suggest that diacylglycerols are preferentially hydrolyzed.
Studies on the rate of fatty acid oxidation by the mitochondrial preparation of the different leg muscle preparation revealed that they could oxidize only the short chain fatty acids, butyrate and octanoate. Significant rate of palmitate oxidation is obtained in M. vastus lateralis and M. semitendinosus with the addition of DL-carnitine. We suggest from these observations that the first degradation of long chain fatty acids appear to take place in liver (may also in the adipose tissue) and the resulting short chain fatty acids are transported through blood to the muscles. However, further research is required to elucidate the nature of fatty acid composition of the blood liver and adipose tissues during exercise as well as carnitine content of the different leg muscles.
Introduction

It is seldom realized that the foundations of our present knowledge of aerobic metabolism were laid by studies in which the pigeon breast muscle was used as the experimental material. The observations of G. G. Gyorgyi (1953) that minced pigeon breast muscle respires very actively by the complete oxidation of pyruvic acid, in the presence of bicarbonate producing little or no lactic acid, lead to new innovations in the field of energy metabolism of a cell. Wide variation in structure and function occur in the appendicular muscles of birds adapted for different types of activity (George and Berger, 1966; Beatty et al., 1966; George, 1974, Keissling 1977). Muscles containing a majority of red fibres (red muscles) have high oxidative activity, whereas muscles low in red fibre content have especially high glycolytic enzyme activity (Beatty et al., 1963, 1966; George and Berger, 1966 and Kaiser and George, 1972). Physiological differences in the function of red and white muscle fibres are well recognized (Perry, 1960; Slater 1960; Fritz et al., 1969; Beecher et al., 1969, and George 1974). It has been suggested that the red muscle accumulates greater quantity of kreb cycle intermediates compared to the white muscle. The importance of this could be appreciated in view of suggested role of kreb cycle intermediates.
particularly citric acid in limiting glycolysis via phospho-
fructokinase inhibition (Garland, 1963; Parmeggiani and Bowan, 1963; Williams and Jones 1964, Williamson 1965 and Newholme and Stott 1970). It has been postulated also that kreb cycle
intermediates may activate particularly isozymes of LDH, (Frits, 1965) and thereby influence the type and rate of metabolism in the tissue (Vessel and Pool, 1956); succinic
dehydrogenase (SDH) and myofibrillar adenosine triphosphatase
(m-ATPase) are two important enzymes concerned with muscle study. The level of SDH in a muscle reflects the oxidative
capacity (Padykula, 1952; Nachmis and Padykula 1952; Dobowitz
and Pearse 1966) whereas ATPase appears to be the rate limit-
ing link in the shortening process (Close 1972).

The dynamic characteristics of a whole muscle are
dependent upon its structure and fibre composition (George
and Berger, 1966; Edgerton et al., 1969; Edgerton and Simpson, 1971; Close, 1972; Barke and Edgerton 1975 and Bernard et al., 1971). Since, skeletal muscles of birds are not homogeneous in
their fibre type, predominance of fibre type would reflect their metabolic differences. It is now well estab-
lished that red fibre type contains more oxidative enzymes than
that of white type fibres (George and Talesare 1961, George
Higher rate of respiration in red muscle homogenates and
mitochondria has also been demonstrated by many workers (Beall and Sparling, 1952; Ogata 1960; Blur and Blanchard 1963; George and Berger, 1966, Beatty et al. 1965 and 1966; Drummond, 1971).

In agreement with results of other workers (George and Berger, 1966, Gringer and George 1966, Beatty et al. 1966; Kaiser and George, 1973 and Kiesling, 1977) for avian appendicular muscles, the histochemical findings indicated (Section III) that the appendicular muscles of dabchick, coot and domestic duck are composed of mixed types of fibres. The histochemical localization of kreb cycle dehydrogenases indicated that red fibre types, compared to white ones are rich in the enzymes studied. However, it should be mentioned here that the histochemical evaluation is primarily qualitative (Dabowski and Pearson, 1961) and useful for the comparison of the relative number of different fibre population. It has also been suggested that in comparison with enzyme assay, histochemical evaluation of muscle metabolism is crude (Beatty et al. 1966).

In the light of the above mentioned observations it is thought desirable to carry-out quantitative analysis of some key enzymes of oxidative metabolism of the muscle homogenates of certain appendicular muscles of the three
birds, dabchick (*Podiceps ruficollis*), coot (*Fulica atra*) and domestic duck (*Anas platyrhynchos*).

Materials and methods

The experimental animals consisted of males of dabchick, coot and domestic duck. The dabchick and coot were collected from the local ponds. The domestic duck was obtained from the local farm. The birds were maintained for a period of one week under the laboratory conditions before they were used for the experiments. The following breast and certain wing muscles were used.

1. *M. pectoralis major*
2. *M. supracoracoideus*
3. *M. latissimus dorsi anterior*
4. *M. latissimus dorsi posterior*
5. *M. biceps brachii*
6. *M. triceps brachii*

Preparation of muscle homogenate

The birds were fasted, 20 hr., and then decapitated under ether anesthesia. The light anesthesia minimized muscle spasms and resultant changes in muscle components following decapitation. The various muscles listed above were freed from fat and connective tissue. Except for the bulky
pectoralis major and supracoracoides, the rest of the wing muscles were removed individually, from their point of attachment to the point of insertion. The central portion of the muscle (George and Berger, 1965) were used in the case of pectoralis major and supracoracoides. The tissue was minced in a pre-cooled micro meat grinder and was suspended in a 10 folded volume (w/v) of ice-cold extraction medium which consisted of 50 mM-Tris(hydroxymethyl)aminomethane, 1 mM EDTA, 2 mM MgCl₂ and 30 mM mercaptoethanol at pH 7.5. All homogenates were prepared and kept at 0°C until assayed for enzyme activity.

**Chemicals**

All substrates and bovine serum albumin were obtained from Sigma Co., U.S.A. 2 mercaptoethanol was obtained from Koch-Light laboratories Ltd., Colnbrook, Bucks, U.K. Inorganic reagent salts were purchased from B.D.H. Chemicals Poole, U.K.

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**Glycero-phosphate dehydrogenase ( -GPD)**

The -GPD activity in all the muscle preparations was measured spectrophotometrically as described by Ruesman et al. (1984) using Warburg apparatus at 37°C with a total liquid vol. of 3.2 ml in the reaction flask. The main compartment
of the flask contained 250 moles of potassium phosphate buffer (pH 7.4), 100 moles of KCl, 3 moles, KCN (freshly prepared and neutralised), 10 moles of MgCl₂, 1.8 mg bovine serum albumin and 1.8 mg phenazine methosulphate. The amount of the homogenate in each case was so adjusted as to contain approximately 12 mg protein in each vessel. The central well contained 0.2 ml of 20% KOH solution and the side arm 120 moles of disodium D.L. glycophosphate. After 10 to 15 min of temperature equilibration the reaction was started by tipping in D.L. glycophosphate from the side arm of the flasks. The uptake of oxygen was noted every 5 min and increase over the blank lacking the substrate was used for the calculation of the enzyme activity. The activity of the enzyme was expressed in terms of mole of product formed/min/100 g tissue fresh weight of muscle at 30°C.

Lactic dehydrogenase activity (LDH)

LDH activity was estimated spectrophotometrically as described in Worthington Bulletin (1967) in the same original homogenate used for the estimation of -GOD activity. The muscle homogenate was centrifuged in a refrigerated centrifuge at a low speed (2000g) before the enzyme assay was made. The supernatant was used to obtain the enzyme activity in Cary 14 recording spectrophotometer. The standard mixture consisted 0.1 ml of 0.3 M pyruvate, 0.1 ml
0.002 M (pH 8) NADH, 2.7 ml sodium phosphate buffer (pH 7.4) in a total volume of 3 ml. The reaction was initiated by the addition of the enzyme source (0.1 ml supernatant). The rate of decrease in absorbancy at 340 m\(\mu\) as NADH is oxidized was measured at 25°C. A unit of enzyme activity was that which causes an initial rate of oxidation of one mole of NADH per min under the condition specified at 25°C. The enzymatic oxidation in the absence of substrate was used for the calculation of the enzyme activity.

Succinic dehydrogenase (SDH) (EC 1.3.99.1)

The SDH activity in all the muscle preparations was determined according to the method developed by Green and Narahara (1960). For the assay, the following constituents were placed in standard test tubes at room temperature: 0.1 ml H\(\text{2}O\); 0.05 ml each of 0.19 sucrose, 0.1 M Tris-HCl (pH 7.5), 10 mM sodium azide, and 0.1 ml 2-((p-isophenyl)-3-((p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) solution (4 mg/ml); 0.1 ml of 0.5 M sodium succinate (prepared by adjusting a solution of succinic acid to pH 7.5 with a solution of NaOH before final dilution with H\(\text{2}O\)).

Myofibrillar Adenosine triphosphatase (M-ATPase) (EC 3.6.1.3)

Myofibrillar suspension of muscle was prepared according to Perry (1952) as described by Oser (1954). The entire
procedure of preparation of myofibrillar suspension was carried out at 0°C. The muscle was homogenized in a polytron homogenizer (Brinkmann Instruments Co., Calif), for 2 min with 8 vol of 0.05 M borate buffer (pH 7.1). The homogenate was centrifuged 15 min at 6000g and the sediment was resuspended in the original vol of borate buffer and homogenized once again for 2 min. The suspension was centrifuged for 20 min at 6000g. The supernatant was removed carefully with pasteur pipette. The white lighter upper layer of the supernatant was transferred to another centrifuge tube containing fresh borate buffer (pH 7.1). The suspension was then freed from coarser material by centrifugation for 5 min at 3000g. The suspension was then subjected to five cycles of centrifugation for 20 min each time at 6000g. The final pellet was suspended in known vol of 0.3 M KCl.

The ATPase activity of the above suspension was assessed by estimating the inorganic phosphate (Pi) of the incubation medium according to the method of Martin (1950). The incubation medium consisted of 40 mM Tris-HCl, 40 mM KCl 10 mM, CaCl₂, 0.2 ml of myofibrillar suspension and 3 mM ATP. The final vol of reactants was 1.5 ml. The reaction was started with the addition of ATP. The incubation was carried out at 37°C for 15 min. At the end of the incubation period the reaction was stopped by the addition of 1.5 ml of 1 N trichloroacetic acid. The protein content of the myofibrillar
suspension was determined according to Lowry et al. (1951).

**a**-ATPase activity was expressed as micromole of Pi liberated/
mg protein/30 min.

The tubes were warmed for 1 min in a water bath at 30°C then 0.1 ml of freshly prepared and diluted muscle
homogenate was added to initiate the reaction. The total
volume of the reaction mixture was 0.5. After incubation
for 10 min 4 ml of ethyl acetate. The tubes were kept in
ice then centrifuged for 15 min at 8000g at room temperature.
The absorbance of clear extract was read at 456 nm in spectro-
nic 3 quartz spectrophotometer. Basal reduction of 1 NAD was
determined in control tubes in which succinate was omitted
from the reaction mixture and the basal value was subtracted
from the absorbance that was measured for the experimental
tube.
SECTION - C.

ESTIMATES OF SUCCINIC DIHYDROGENATE, LACTIC DIHYDROGENATE, & OTHER DIHYDROGENATES
AND MYOBRILLAR ADENOSINE TRIPHOSPHATASES
IN BREAST AND CERTAIN WING MUSCLES OF
DARCHICK, COOT AND DOMESTIC DUCK.
The data obtained on the various enzyme activities are summarised in Table 1 and 2. The evidence that tricarboxylic acid (TCA) Cycle activity is higher in red muscle than in white muscle is overwhelming (Beatty et al., 1966 and George and Berger, 1966). It has also been suggested that white fibres are highly glycolytic (for review see Close, 1972). A direct correlation between the qualitative
histochemical classification and quantitative measurements of succ activity in red and white fibre population of avian skeletal muscles has also been suggested (Beatty et al., 1964).
The results of the present experiments indicate that among several muscle groups studied, M. pectoralis major compared to the other wing muscles, shows a sharp difference among the three birds. The two oxidative enzymes (Sdh and -GPDH) activities are moderately more in breast muscles of dab-chick compared to coot and considerably high when compared to domestic duck. The Ldh activity, an index of anaerobic metabolism, is considerably high in the two breast muscles (M. pectoralis major and supracoracoideus) of domestic duck compared to corresponding muscles of dab-chick and coot.
These results indicate therefore that the breast muscles of dab-chick and coot are organized more for aerobic metabolism whereas the corresponding muscles of domestic duck are adapted for anaerobic metabolism. It is worthwhile to mention in this context our histochemical findings (Section III) which revealed that these two powerful flight muscles of dab-chick and coot are composed more (only red type in dab-chick) of red fibre population, whereas white fibre population takes its place in these muscles of domestic duck.

The results also revealed some interesting enzymatic differences in the other four auxiliary wing muscles.
Table: Activities of Succinic Dehydrogenase (SDH), Glyceraldehyde Dehydrogenase (GPD), and Lactic Dehydrogenase (LDH) in Breast and certain wing-muscles of Babcock (DC), Coot (C), and Domestic duck (DB).

<table>
<thead>
<tr>
<th>MUSCLE TYPE</th>
<th>SOURCE</th>
<th>INT formation/ mg protein/min.</th>
<th>Micromole/min/gm tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SDH</td>
<td>GPD</td>
</tr>
<tr>
<td>Pectoralis Major</td>
<td>DC</td>
<td>10.1 ± 0.93</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>7.0 ± 1.20</td>
<td>5.32 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>0.51 ± 0.03</td>
<td>5.21 ± 0.29</td>
</tr>
<tr>
<td>Suprascapularis</td>
<td>DC</td>
<td>6.3 ± 1.2</td>
<td>5.73 ± 0.93</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6.0 ± 1.2</td>
<td>4.30 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>0.39 ± 0.01</td>
<td>4.20 ± 0.60</td>
</tr>
<tr>
<td>Latissimus Dorsal Ant</td>
<td>DC</td>
<td>5.31 ± 0.02</td>
<td>3.13 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>5.29 ± 0.013</td>
<td>2.19 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>2.78 ± 0.05</td>
<td>1.02 ± 0.01</td>
</tr>
<tr>
<td>Latissimus Dorsal Post</td>
<td>DC</td>
<td>5.89 ± 0.01</td>
<td>2.92 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6.00 ± 0.10</td>
<td>2.57 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>2.33 ± 0.02</td>
<td>2.59 ± 0.02</td>
</tr>
<tr>
<td>Biceps Brachii</td>
<td>DC</td>
<td>6.03 ± 0.50</td>
<td>3.92 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6.96 ± 0.20</td>
<td>3.35 ± 0.012</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>2.60 ± 0.10</td>
<td>2.33 ± 0.1</td>
</tr>
<tr>
<td>Pectoralis Femoralis</td>
<td>DC</td>
<td>5.31 ± 0.02</td>
<td>4.00 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>5.20 ± 0.70</td>
<td>4.12 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>2.80 ± 0.07</td>
<td>3.00 ± 0.19</td>
</tr>
</tbody>
</table>

* Values represent mean ± S.E. of five experiments.
Table-2: Adenosine triphosphatase activity (ATPase) in breast and certain wing muscles of dabchick, coot and domestic duck

<table>
<thead>
<tr>
<th>MUSCLE TYPE</th>
<th>ATPase Activity (Pi u moles/mg protein/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dabchick</td>
</tr>
<tr>
<td>PECTORALIS MAJOR</td>
<td>07.0 ± 1.09*</td>
</tr>
<tr>
<td>SUPRACORACOIDEUS</td>
<td>09.38 ± 0.98</td>
</tr>
<tr>
<td>LATISSIMUS DORSI. AN.</td>
<td>14.37 ± 1.98</td>
</tr>
<tr>
<td>LATISSIMUS DORSI. POST.</td>
<td>15.0 ± 1.93</td>
</tr>
<tr>
<td>BICEPS BRACHII</td>
<td>12.32 ± 1.37</td>
</tr>
<tr>
<td>TRICEPS HUMERUS</td>
<td>13.03 ± 1.19</td>
</tr>
</tbody>
</table>

* The results represent mean ± SD of five experiments.
activity in the two back muscles, latissimus dorsi ant and post as well as biceps brachii and triceps humeralis of dab-chick and coot compared to domestic duck is considerably high. It may also be seen that all the four wing muscles of three birds contain more SDH activity than the LDH. These results therefore suggest that the wing muscles are designed more for aerobic metabolism. It is of treat interest to note that the four auxiliary wing muscles compared to main two flight muscles (breast muscles) in domestic duck contains higher SDH activity (Table I).

The myofibrillar ATPase (m-ATPase) activity obtained in the two breast and four wing muscles is summarised in Table 2. The results reveal that the two breast muscles of domestic duck compared to the corresponding muscles of dab-chick and coot contain higher m-ATPase activity. It is interesting, however, to note that the four wing muscles of three birds show less variation in their m-ATPase content.

**Discussion**

The contrasting physiological and biochemical properties of predominantly red and white muscle have generally been considered appropriate adaptation to the different functions of these muscles. Recent evidence lays stress on the idea that glycolytic metabolism predominates in those fibres with
low oxidative enzyme activity and that a fibre with minimal glycolytic activity is characterised by predominance of citric acid cycle (Himmelhoch et al., 1961 and Romanul, 1964). Higher rate of respiration in red muscle homogenate with tricarboxylic acid cycle intermediates has also been very well demonstrated (Beatty et al., 1965). It is deduced from our histochemical studies (Section III of the thesis) that the breast muscles of coot and domestic duck seem to contain fibres with low oxidative activity (white type) and fibres with glycolytic activity but predominant with citric acid cycle enzymes (red type). The breast muscles of dabchick on the other hand appear to contain only the red type fibres. It should be of considerable interest therefore to understand how the two types of fibres with two different metabolic adaptation but situated side by side in the same muscle function.

The level of succinic dehydrogenase (SDH) activity in a muscle is an index of its capacity for oxidative metabolism and mitochondrial content (Padyala, 1952; Rabowitz, 1956 and George and Berger, 1966). Comparative data on SDH activity in the pectoralis and supracoracoideus muscles of some active flyers like pigeon, Romanpastor and house sparrow compared to crowpheasant, domestic fowl, the poor flyers, has indicated that the higher enzyme activity obtained in the pectoralis
muscles of active flyers. The results also seem to demonstrate that within the two breast muscles, the pectoralis contain higher SDH activity than that of supracoracoides (George and Talemesa, 1961 and George and Berger 1966). Obviously, the two breast muscles of active flyers are highly oxidative and their pectoral muscle compared to supracoracoides composed mainly of red fibre types, interestingly, in the humming birds (Zasiewski, et al, 1965) the supracoracoides compared to pectoralis muscle showed higher SDH content, which means that the former muscle has a higher oxidative capacity. It is well known that humming bird indulges in sustained hovering flight wherein the action of supracoracoides as powerful elevator is equally important. The dabchick and coot involve occasional short distance migratory flights. It could then be expected that the two major flight muscles, pectoralis and supracoracoides should be organised for long sustained action with high oxidative capacity compared to the corresponding muscles of domestic duck which rarely exercise the muscles. The results (Table 1) show that the breast muscles of dabchick and coot compared to domestic duck contain significantly high (15-20 folds) SDH activity. The results also demonstrated that between dabchick and coot, the pectoralis muscle of former bird shows higher SDH content than the latter one. It may be recalled in this context our earlier observations (Section III of the
thesis) that the pectoralis muscle of dabchick appears contain only red and intermediate type fibres.

The fact that the other four wing muscles compared to the breast muscles of dabchick and coot contain less SDH activity (Table 1) could possibly suggest their anaerobic metabolism. It is worthwhile to mention here the findings that wing muscles of these three birds have greater population of white fibres which are designed for short quick burst actions such as quick and sharp turnings during flight.

Skeletal muscles are capable of degrading glycolytic intermediates to pyruvates and \( \gamma \)-glycerophosphate ( \( \gamma \)GP). 

\( \gamma \)GP is produced by the reduction of dihydroxy-acetone phosphate catalysed by \( \gamma \)-glycerophosphate dehydrogenase ( \( \gamma \)GPD). \( \gamma \)GP cycle provides a mechanism whereby extra-mitochondrially formed reduced nicotinamide adenine dinucleotide (NADH) gets dehydrogenated to nicotinamide adenine dinucleotide (NAD). The significance of this reaction is due to the fact that the amount of NAD in a tissue is limited. Various pathways have been suggested for the oxidation of NADH: 1) direct mitochondrial oxidation of exogenous NADH; 2) oxidation of NADH by pyruvate, catalysed by cytoplasmic lactic dehydrogenase; 3) oxidation of NADH by dihydroxyacetone phosphate catalysed by cytoplasmic \( \gamma \)-glycerophosphate dehydrogenase (Zackter, 1965). In the light of
these observations if may be argued that both G6PD and LDH are equally important in the breast muscle of dabchick and coot for the oxidation of NADH to NAD. In the domestic duck on the other hand, LDH compared to G6PD is the one involved in the conversion of NADH to NAD in the breast muscles. The results obtained on the two back muscles of the wing latissimus dorsi ant and post indicated more LDH compared to G6PD, similarly muscles brehichi and triceps humerals show very little variation in the activities of G6PD and LDH which means that both enzymes are involved in the regeneration of NAD. It is of particular interest to mention here that the breast muscles of dabchick and coot are predominantly composed of red type fibres (Section III of the thesis). The higher LDH activity therefore seen to indicate that the red muscles are capable of high degree of anaerobic metabolism during periods of anoxia which these birds might experience during short underwater diving.

The type of myosin of a muscle influences the speed with which a muscle contracts and it is very probable that the myosin ATPase is the rate limiting link in the shortening process (Close, 1972). As might be expected from the fact that white muscles require more rapid energy production, m-ATPase activity is higher in white muscle than in red fibres (Cragg, 1969). Seidel Breier Thompson and Gergley
1964; Barney et al. 1965 and Maddox and Perry 1966 and
Tobiasara and Narang 1976). Studies on myosin from fast
(white type) and slow (red type) muscles of the same species
have revealed marked structural and physiological differences
(Close 1972). Homogenates of white muscle have been shown
to contain greater myosin ATPase activity than the red muscle
homogenate (Saidel et al. 1984 and Naray et al. 1985). The
ATPase level in different muscles of the three birds investig­
it revealed a correlation between the activity and the
predominance of the fibre type. The two breast muscles of
the domestic duck compared to the corresponding muscles of
dabchick and coot contain higher m-ATPase activity. The
lowest enzyme activity is obtained in the two breast muscles
of dabchick. It may be recalled in this context our observations (Section III of the thesis) that the relative distrib­
ution of white fibres in the pectoralis muscles of the
domestic duck contains 63%, the coot contains only 22% and
in the dabchick, the pectoralis seems to contain red type
fibres only, whereas supracoracoideus is of mixed type with
predominance of red type of fibres. Interestingly enough
the other four wing muscles in all the three birds show very
little, if any, differences in the m-ATPase activity. It
has been observed that the wing muscles of these birds show
predominance of white fibres in them. It is generally been
accepted that muscles containing more white fibres are
designed for short burst of activity and require more m-ATPase activity (Beatty et al. 1963). The higher m-ATPase activity in the wing muscles compared to the breast muscles of dabchick and coot is expected (Table 1).

In the final analysis, it seems reasonable to speculate that the comparative data obtained on certain enzymes, provided an useful information on the metabolic adaptation of each muscle at the level of quantitative organization.

Summary

1 Comparative assessment of succinic dehydrogenase (SDH), -glycerophosphate dehydrogenase (-GPD), lactate dehydrogenase (LDH) and myofibrillar adenosine triphosphatase (m-ATPase) are carried out in the breast and certain wing muscles of three birds namely, dabchick (Rodicops ruficollis), coot (Fulica atra) which are active flyers and the domestic duck (Anas platyrhynchos) the walking bird. It is expected from these studies to reveal metabolic differences, if any, in the muscles designed for different functions at the enzymatic organization.

2 Among the various muscles studied, pectoralis muscle of dabchick and coot compared to the corresponding
muscle of domestic duck shows 14-20 fold increased SDH activity. The result is in agreement with the other observations obtained on other birds. It is well accepted that the level of SDH reflects the oxidative capacity of a muscle and its mitochondria content. The pectoralis muscle of dabchick and coot is then adapted more for oxidative metabolism, whereas the same muscle of domestic duck is designed for anaerobic means of energy metabolism.

The supra coracoideus muscle compared to pectoralis of dabchick and coot shows less SDH activity which means that this muscle is less oxidative. This has been supported by the findings that supracoracoideus of these two birds is consisted of both red and white type fibres and the latter type fibres contain less amount of myoglobin and mitochondria.

It has also been observed that the other four wing muscles of dabchick and coot show almost the same SDH activity. This probably reflects on the similar activities of these muscles in the two birds. It is of considerable interest, however, to note that the four wing muscles compared to the two breast muscles of domestic duck show higher SDH activity. It may
be inferred from these results that the wing muscles are more oxidative compared to the breast muscles. It is common knowledge that the wing muscles in domestic duck are more oftenly used in spreading and folding of their wings while they are moving about.

The physiological significance of GPD and LDH activities in the muscle metabolism could be anticipated in the light of their participation in the regeneration of nicotinamide adenine dinucleotide (NAD) from reduced nicotinamide adenine dinucleotide (NADH). The results obtained on these two enzymes suggested that in the two breast muscles of dabchick and coot both GPD and LDH activities seem to be involved in the regeneration of NAD from NADH. The two back muscles of the wing latissimus dorsi and post appear to contain more LDH activity than that of GPD. Muscles biceps brachii and triceps brachii seem to contain almost the same activities of GPD and LDH. However the results obtained on breast muscles of domestic duck. Unlike the other two birds reveal that they contain more LDH than that of GPD. It is known that the LDH activity indicates anaerobic metabolism in the muscle cell. The
higher LDH activity in the two breast muscles of dabchick and coot may indicate their capacity of anaerobic metabolism during the period of anoxia which these two birds might experience during short underwater diving.

The enzyme ATPase which hydrolyses the terminal phosphate of ATP to release chemical energy is known to occur in muscle in three different forms, mitochondrial, myofibrillar and sarcotubular. In the present investigation an attempt is made to determine the amount of myofibrillar ATPase (m-ATPase) in the breast and wing muscles of three birds mentioned hitherto.

As might be expected from the fact that white muscles require more rapid energy production, m-ATPase activity is higher in white muscles than red fibres. The results reveal that the breast muscles of the domestic duck compared to the corresponding muscles of dabchick and coot show higher m-ATPase. It may be recalled here our earlier observations (section III of the thesis) that breast muscles of domestic duck contain predominantly white fibre population, whereas red fibres take their place in dabchick and coot. However, the other four wing muscles...
among the three birds indicated not such differences in the enzyme activity. Of considerable interest is the finding that in the dabchick and coot the wing muscles compared to the breast muscles, contain more amount of $\text{Na-Mg}$-ATPase. It may be worthwhile to mention here that the four wing muscles of these two birds have fairly more white fibres in them. Also these muscles are involved in quick and short burst of activity.
SECTION - B.

QUANTITATIVE DETERMINATION OF D U R C I N I C D E H Y D R O C E R U S I ,
GLYCOLYCOGEN D E H Y D R O C Y N U S , L A C T I C
D E H Y D R O C Y N U S A N D M Y O F I B E L L I R A D E N O S I N E
TR I P H O S P H A S U S I N C E R T A I N L E G M U C L E S O F
D A R C H I C K , C O O T A N D L O M E T I C D U C K.
Results

Results of the present investigation are presented in Table 1 and 2. Table 1 summarises the succinic dehydrogenase (SDH), -glycerophosphate dehydrogenase (-GPD) and lactic dehydrogenase (LDH) activities, whereas Table 2 indicates the results obtained on myofibrillin adenosine triphosphatase (a-ATase) activity in various leg muscles of dab-chick, coot and domestic duck.

The results revealed an interesting correlation between SDH content and colour of the muscle in all the three birds. The two thigh muscles, sartorius and semitendinosus compared to the other four calf muscles show higher enzyme activity. It may be recalled here that the two thigh muscles are rich in myoglobin and more redder in colour than the other four calf muscles. The results also demonstrated that the SDH activity of the thigh muscles of domestic duck compared to the coot and dab-chick is slightly higher, although the results seem to be insignificant, among the four calf muscles, the gastrocnemius externa and interna exhibit interesting enzymatic difference. Gastrocnemius externa compared to interna contains higher SDH activity.

The physiological role of -GPD and LDH in the reconversion of NADH to NAD has been now well recognised.
<table>
<thead>
<tr>
<th>MUSCLE TYPE</th>
<th>SOURCE</th>
<th>INT formation/ mg protein/min.</th>
<th>Micromoles/min/gm tissue</th>
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<tr>
<td></td>
<td></td>
<td>SDH</td>
<td>GPD</td>
</tr>
<tr>
<td>SARTORIUS</td>
<td>DC</td>
<td>4.55 ± 0.1452</td>
<td>4.00 ± 1.9</td>
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<tr>
<td></td>
<td>C</td>
<td>4.05 ± 0.117</td>
<td>4.231 ± 1.2</td>
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<tr>
<td></td>
<td>DD</td>
<td>5.12 ± 0.072</td>
<td>5.00 ± 0.95</td>
</tr>
<tr>
<td>SEMITENDINOSUS</td>
<td>DC</td>
<td>4.73 ± 0.112</td>
<td>4.93 ± 0.8</td>
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<td></td>
<td>C</td>
<td>4.00 ± 0.120</td>
<td>5.09 ± 0.38</td>
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<td></td>
<td>DD</td>
<td>5.13 ± 0.095</td>
<td>5.00 ± 0.12</td>
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<td>PLANTARIS</td>
<td>DC</td>
<td>3.35 ± 0.079</td>
<td>5.00 ± 0.6</td>
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<td>4.28 ± 0.5</td>
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<tr>
<td></td>
<td>DD</td>
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<td>4.12 ± 0.29</td>
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<td>C</td>
<td>2.67 ± 0.05</td>
<td>3.70 ± 0.6</td>
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<td>DD</td>
<td>1.28 ± 0.06</td>
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<td>GASTROCNEMIUS EXT.</td>
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<td>C</td>
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<td></td>
<td>DD</td>
<td>2.90 ± 0.07</td>
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<tr>
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<td>4.00 ± 0.23</td>
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<tr>
<td></td>
<td>C</td>
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<td>3.12 ± 0.40</td>
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<tr>
<td></td>
<td>DD</td>
<td>4.05 ± 0.1</td>
<td>5.15 ± 0.19</td>
</tr>
</tbody>
</table>

*Values represents mean ± S.E.of five experiments.*
<table>
<thead>
<tr>
<th>MUSCLE TYPE</th>
<th>ATPase Activity</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pi u mole/mg protein/30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BARNCHICK</td>
<td>Coot</td>
<td>Domestic Duck</td>
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<td>8.29 ± 2.0</td>
<td>10.00 ± 2.00</td>
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<tr>
<td>Plantaris</td>
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<td>14.0 ± 1.00</td>
<td>11.0 ± 2.39</td>
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<td>Gastrocnemius Int.</td>
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<td>15.00 ± 2.6</td>
<td>11.23 ± 3.0</td>
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<td>Gastrocnemius Ext.</td>
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<td>8.00 ± 1.00</td>
<td>6.91 ± 1.00</td>
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<td>Tibialis Ant.</td>
<td>11.00 ± 2.00</td>
<td>10.11 ± 2.00</td>
<td>9.00 ± 1.92</td>
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</table>

* Values represent mean ± SD of three experiments.
The result of the present investigation on the LDH and MD activity reveals interesting differences between the enzymes among various leg muscles of the three birds. It is of considerable interest to note that all the leg muscles of dab-chick, coot, and domestic duck seem to contain significantly higher LDH compared to MD activity. It may also be seen (Table 1) that the LDH compared to MD activity is higher in all the leg muscles of domestic duck than dab-chick and coot. It is also interesting to note that gastrocnemius interna compared to gastrocnemius externa of all the three birds contain higher LDH activity (Table 1), whereas MD activity in both the muscles of all the three birds is almost the same.

The various leg muscles of the three birds reveal some interesting differences in the m-ATPase activity (Table 2). The two thigh muscles, sartorius and semitendinosus, compared to the other calf muscles, plantaris, tibialis, and gastrocnemius interna contain less m-ATPase activity. The enzyme activity between gastrocnemius interna and externa revealed some interesting differences. The gastrocnemius interna of all the three birds compared to externa contain higher m-ATPase activity. One important finding of the present investigation is that the four calf muscles of dab-chick, and coot compared to domestic duck contain higher m-ATPase activity.
Discussion

Szent Györgyi (1953) has pointed out that red muscle such as pectoral muscles of pigeon contracts slowly and tonically, while such as pectoral muscle of domestic fowl contracts rapidly and strongly. Several investigators have reviewed the relation between the chemical and contractile function and structure of muscle, comparing, for example, heart tissue and normal skeletal muscle (Beatty et al., 1965; George and Berger, 1966; Saeed et al., 1969; Seecher et al., 1969; Close, 1972; George, 1974; Takeara and Narang, 1979 and Carol et al., 1980). The correlation of respiration of different types of muscles with mitochondrial density and color of the muscle has also been discussed (George and Berger, 1966; Burleigh and Schimel, 1969; James, 1972 and Wittenberg and Wittenberg, 1975). All these studies seem to indicate that wide variations in structure and function occur in muscles, adapted for different types of activity. It has generally been agreed that slow tonic red muscles of birds are rich in oxidative enzymes, rich in mitochondria and show higher respiratory activity with keto cycle intermediates (George and Berger, 1966). SOD and Na-KPase are two important enzymes concerned with muscle study. The SOD activity in muscle indicates its oxidative capacity and mitochondrial content. The Na-KPase appears to be the rate limiting link in the shortening process (Close, 1972). The results of the
present investigation seem to indicate inverse correlation between SDH and m-ATPase activity in the various leg muscles of three birds. The two thigh muscles compared to the calf muscles, contain higher SDH and lesser m-ATPase, whereas the four calf muscles compared to thigh muscles exhibit lesser SDH and higher m-ATPase activity.

The characteristic differences in enzyme content may be interpreted as a result of the functional load of different muscles which, in turn, is further complicated by the fact that individual fibres of a mixed muscles differ in their overall aerobic and anaerobic oxidative activity. The two fleshy muscles sartorius and semitendinosus seem to be used in maintaining posture and gait which requires a continuous effort. It has been shown that these two muscles contain more myoglobin (Section II of the thesis) and show more red fibre population (section III of the thesis). On the basis of these observations, it is suggested that the two thigh muscles are designed for sustained action. In contrast, the other four calf muscles appear to be involved in the swimming (dab-chick and coot) or walking (domestic duck) on land. Both swimming and walking performance involves short burst of actions. The histochemical observations on the calf muscles have revealed that they are predominantly composed of white fibres. It is well demonstrated that white fibres in birds are designed for short burst of activity and derive the
metabolic energy through anaerobic metabolism (George and Berger 1966). It is of considerable significance to mention in this context the observations of Lawrie (1953a and b). A muscle which indulges in sustained action requires a continuous generation of high energy compounds. Such a muscle has a high myoglobin content and cytochrome oxidase activity but a low ATP and creatine phosphate content and low myofibrillar ATPase activity. On the other hand a muscle capable of fast contraction for short periods of time utilizes, for energy, its stores of ATP and creatine phosphate. According to Lawrie (1953a), a muscle of this kind has low myoglobin content and cytochrome oxidase activity but high ATP and creatine phosphate content and high myofibrillar ATPase activity. The same characteristics are applicable to the thigh and calf muscles of dab-chick, coot and domestic duck.

The importance of GPD and LDH in muscle metabolism could be realized in the light of their possible role in the regeneration of nicotinamide adenine dinucleotide (NAD) from reduced nicotinamide adenine dinucleotide (NADH). The evidences, that -glycerophosphate ( -GP) and pyruvate are formed during glycolysis by several muscle preparations has been well demonstrated (Ogata and Mori 1963; Chafurka, 1965; Saclier 1965; Beecher et al, 1969; Besty and Bocek 1972). This means that the NAD-linked -glycerophosphate dehydrogenase
which is extraordinarily active in insect flight muscles
(Zabe and Mshen 1977; Saccio and Cochran 1957b and
Kallapur and George 1975) is largely responsible for the
oxidation of NADH, generated in glycolysis, the function
hitherto attributed to LDH activity, (Chefurka, 1965).
It may be deduced from these observations that both —GPD
and LDH are responsible in muscle to regenerate NAD from NADH.
The results of the present experiment on —GPD and LDH
content of various leg muscle have shown that the latter
enzyme is more active than the former one. Both histochemical
as well as quantitative assay of LDH in white and red fibres
have indicated higher activity in both the whole homogenate
and supernatant of white muscle (Blanchear et al. 1963 and
van Seehe 1963a). Not only are LDH activities higher in
white muscle but the distribution of the isozymes is speci­
fic in red and white fibres has also been suggested (van
Seehe et al. 1964). These isozyme patterns of red and white
fibres have been correlated with the kinetic properties of
LDH subunits and the different physiological roles of these
subunits (Bowsen et al. 1964). During active swimming or
running on ground the blood is unable to supply oxygen
rapidly enough for its requirements (more so to the white
fibres due to their greater surface area). Under these
conditions of temporary anoxia, the muscle uses pyruvate the
end product of glycolysis, as an electron acceptor, thus
enabling NADH to revert back to NAD by LDH activity. It is believed therefore that the higher LDH activity (Table 1) in all the leg muscles of three birds provides an elegant device which allows muscles to solve their redox problem when anaerobic conditions prevail.

The two leg muscles, gastrocnemius externa and interna call for special mentioning. The muscles fuse distally with their tendons and join with the flexor tendon of toes. It may be seen (Table 1 & 2) that between gastrocnemius externa and interna the former muscle contains higher SDH and lesser LDH and m-ATPase activity, whereas the latter muscle shows higher LDH and m-ATPase and lower SDH activity. It is believed that these two muscles have different contraction rate and represent two different metabolic adaptations. The gastrocnemius externa with its oxidative properties is adapted more for sustained efforts, whereas the gastrocnemius interna with its anaerobic characters is designed more for quick abrupt actions.

Summary

1 The leg muscles of three birds the dab-chick (Podiceps ruficollis), the coot (Fulica atra), and the domestic duck (Anas platyrhynchos) are used to understand biochemical strategies being in operation in certain leg muscles adapted for different modes...
of locomotion. The dab-chick and coot are active swimmers, while the domestic duck is more of walking bird on land. It should however, be mentioned here that the domestic duck is also phylogenetically aquatic in habitat. The following leg muscles are used to assess the relative concentration of succinic dehydrogenase (SDH), -glycerophosphate dehydrogenase (-GPD), lactic dehydrogenase (LDH) and myofibrillar adenosine triphosphatase (m-ATPase).

1. M. sartorius
2. M. semitendinosus
3. M. plantaris
4. M. gastrocnemius interna
5. M. gastrocnemius externa

The variation in the activities of all the enzymes studied is very much less in the corresponding muscles among the three birds. However, the differences in activity of each enzyme in different muscles is very conspicuous. Inverse correlation between the activities of SDH and m-ATPase is very well seen in the two thigh and four calf muscles of all the three birds. The inverse correlation between SDH and m-ATPase is attributed to the fibre composition.
of each muscle which again is correlated to the functional load of the individual muscle. The two thigh muscles, sartorius and semitendinosus appear to be involved in posture and gait, which requires continuous adjustment of the body. These two muscles contain greater number of red type fibres, more myoglobin, fat (Part I of the thesis) and high ATP activities but low CK activity. The muscle of this quality has been shown to be involved in sustained continuous actions. The four calf muscles, on the other hand are involved in the quick movements of the limbs in active swimming or used in walking or running on land. These muscles contain greater proportion of white fibres in them. This kind of fibre has been shown to contain less myoglobin and CK activity utilizing ATP and creatine phosphate as a source of energy hence these fibres show higher CK activity.

The results obtained in respect of activities of CPD and LDH in various leg muscles of the birds revealed some interesting observation. All the muscles seen to contain higher LDH compared to CPD. The significance of activities of these two enzymes in muscle could be anticipated to the fact that
these two enzymes seem to be involved in the regeneration of NAD from NADH produced during glycolysis. The high LDH activity in the leg muscles provides an elegant device which allows muscle to solve their redox problems when anaerobic conditions prevail in the muscle.

It is of considerable interest however, to note that the two calf muscles, gastrocnemius extern and interna with their common tendon but different in their biochemical properties. It is believed that these two muscles show different contraction rates organized for different functions. The gastrocnemius externa with its oxidation properties is adapted more for sustained efforts, whereas the gastrocnemius interna with its anaerobic characters is designed for quick abrupt actions.