CHAPTER - I A

STUDIES ON ADAPTIVE MODIFICATIONS IN VERTEBRATE SKELETAL MUSCLE

1. EVIDENCES FOR THE OCCURRENCE OF STRUCTURAL AND METABOLIC EVOLUTIONARY TRENDS IN VERTEBRATE SKELETAL MUSCLES

INTRODUCTION

The heterogeneity of mammalian and avian skeletal muscles with respect of their fiber types is well known (Stein and Padykula, 1962; Romanul, 1964; Yellin, 1967; Schiaffino et al., 1970; George and Berger, 1966; Padykula and Gauthier, 1967; Brooke and Kaiser, 1970; Gauthier, 1970; Brooke, 1973). Thus extrafusal muscle fibers of two or more kinds have been described in many vertebrates (Peachey, 1961; George and Berger, 1966; Hess, 1970; George et al., 1971; Gordon, 1972 a,b). More commonly, the muscle is of the mixed variety comprised of type I (red), type II (white) and the intermediate type of fibers (Dubowitz, 1973; Ogata and Murata, 1973; Eisenberg, 1975). Immunological typing of fibers has also been done by Dawkins et al., (1973). The muscles containing more of type I fibers are adapted for an aerobic metabolism and they metabolize fat as the chief energy fuel, whereas the type II fibers utilize mainly glycogen as they are
adapted for an anaerobic metabolism to generate energy (George and Berger, 1966; Grinyer and George, 1969 a,b; Pandie and Blanchaer, 1971). The avian Mm. pectoralis being a heterogeneous muscle, also comprises of the above mentioned three types of muscle fibers on the basis of their contents of myoglobin, lipids, glycogen, oxidative and glycolytic enzymes (George and Berger, 1966). In all birds except category 6 of George and Berger (1966), the pectoralis muscle is a mixed muscle with these three muscle fiber types in varying proportions.

The metabolism of a muscle is dependent upon its cellular organization and biochemical components. Although a number of comparative studies have been reported which reveal metabolic patterns in skeletal muscles of various vertebrate species, very limited ones are available correlating structure and functional adaptations of a muscle.

Therefore, the present study was an attempt towards elucidating patterns of muscle metabolism in pectoralis major muscle of vertebrates (from amphibia to mammals) with a view to indicate any evolutionary trends in muscle metabolism and correlate its structural and physiological makeup with the functional demands in view of the respective modes of life of the animals. Several
oxidative/hydrolytic enzymes and metabolites including vitamin C turnover were studied. It may be mentioned that the choice of the bird species was made keeping in mind the fact that although some information is available on myna and starling, of which the latter is a migratory bird, very scanty reports are available on the Grey Partridge, an important Indian game bird which is a poor flier and the Redwattled Lapwing, a rather common bird with a slow flight.

MATERIAL AND METHODS

The Mm. pectoralis of healthy, adult animals of either sex was utilized for the present study. The following animals were used for the investigation.

A. Amphibia : Frog (Rana tigrina)
B. Reptile : Calotes (Calotes versicolor)
C. Birds : 1. Blue Rock pigeon (Columba livia)

Columbiformes, Columbidae.

2. Common Grey Partridge (Francolinus pondicerianus) (Gmelin) - Galliformes, Phasianidae.

3. Red-Wattled Lapwing (Vanellus indicus)
   (Boddart) or (Hoplopterus indicus indicus)
   Charadriidae.

5. Rosy pastor (*Sturnus roseus*) (Linnaeus) - Passeriformes, Sturnidae.


2. Mice (*Mus musculus* L.) - Rodentia, Muridae.


The birds were either trapped alive in the field or supplied by local game dealers. The mammals, the reptile and frogs were also supplied by the local suppliers.

The animals were beheaded and pieces of the muscle were blotted free from blood, frozen immediately and sectioned (10-15 µm) for all histochemical study.

**HISTOCHEMICAL:**

1. Succinate dehydrogenase (*SDH*): (EC 1.3.99.1)

For the histochemical demonstration of SDH, the method of George and Talesara (1961) was adopted. Fresh frozen sections (5 to 10 µm) were dropped in ice cold phosphate buffer of pH 7.6. They were then incubated at 37°C in a medium whose composition was as follows:
A total volume of 6 ml contained 2.5 ml of 0.1 M phosphate buffer (pH 7.6); 0.6 ml of 0.5 M sodium succinate; 0.5 ml of 0.004 M CaCl₂; 0.5 ml of 0.004 M AlCl₃; 0.3 ml of 0.6 M NaHCO₃; 1.0 ml, 2 mg/ml Neotetrazolium salt; 0.5 ml of 0.005 M MgSO₄ and 0.05 ml of 2 mg/ml methylene blue. The incubation medium was boiled, cooled and then used.

After incubation, the sections were transferred to phosphate buffer and thereafter washed with distilled water. They were then fixed in 10% neutral formalin for 20 minutes at room temperature. The sections were washed with distilled water and mounted in glycerine jelly. The control sections were incubated in substrate blank medium.

2. Alkaline and acid phosphatases: (EC. 3.1.3.1. and 3.1.3.2.)

The alkaline and acid phosphatase activities were demonstrated histochemically by the Naphthol-AS phosphate method of Burstone (1961).

The fresh frozen sections were washed in buffer at the required pH and were transferred to the incubation medium at 37°C. 5.0 mg of Naphthol AS-Bl substrate in 0.25 ml of dimethyl formamide or dimethyl sulfoxide was used for alkaline and acid phosphatases. 25 ml of buffer (Tris buffer, 0.2 M at pH 9.2 for alkaline and Acetate
buffer, 0.2 M at pH 5.2 for acid phosphatase) was added together with 25 ml of distilled water and 30 mg of Fast Red LTR salt, diazonium salt. The incubation medium was filtered before use. No activator is necessary for alkaline phosphatase but for acid phosphatase, two drops of 10% MnCl₂ was added to the incubation medium. The sections were incubated at 37°C for 5 min. to several hours and thereafter washed in water and mounted in glycerine jelly. The control sections were incubated in an incubation medium devoid of the substrate.

3. Lactate dehydrogenase (LDH) : (EC. 1.1.1.27)

The LDH activity was localized histochemically by the method of Pearse (1960) as adopted by Susheela and George (1966). 10-15 μM thick sections were washed in cold phosphate buffer and incubated at 37 ± 2°C in an incubation medium prepared as follows:

0.5 ml of 0.1 M phosphate buffer at pH 7.0; 0.2 ml of 1 M sodium DL lactate; 0.2 ml of 100 mg/ml DPN (Sigma); 0.2 ml of 0.1 M sodium azide; 0.2 ml of 0.05 M MgCl₂; 0.2 ml of 2 mg/ml Neotetrazolium; 75 mg Polyvinyl pyrrolidone and 1.0 ml distilled water.

After the required incubation period, the sections were transferred to cold phosphate buffer and thereafter, washed in distilled water and fixed in 10% neutral
formalin at 4°C for 30 minutes. The sections were washed again in water and mounted in glycerine jelly.

4. **Glucose-6-phosphate dehydrogenase (G-6-PDH) (EC:3.1.3.9):**

The mitochondrial G-6-PDH was demonstrated by the method of Pearse (1960) and Nene and George (1965). The muscle sections were directly transferred to the incubation medium consisting of: Glucose - 6 - PO₄ (Barium salt, as barium salt is known to activate G-6-PDH (Nachlas et al., 1958); (0.1 M substrate) 0.1 ml; NADP (0.1 M) 0.05 ml; Sodium azide (0.1 M) 0.1 ml, MgCl₂ (0.05 M) 0.1 ml; Tris buffer (pH 7.0) 0.25 ml; Nitroblue tetrazolium (1 mg/ml) 0.25 ml; Sodium fluoride (0.01 M) 0.05 ml., Polyvinyl pyrrolidone 75 mg and Distilled water to make the volume up to 1 ml. The sections were incubated at 37 ± 2°C for required periods at a pH of 7.0. The control sections were treated in a substrate blank medium. After incubation, both control and sample sections were washed thoroughly in Tris buffer, followed by distilled water and fixed in 10% buffered neutral formalin at 4°C for 2 hours. Then they were washed with distilled water and mounted in glycerine jelly.

**Fiber types:**

The fiber types were determined on the basis of SDH staining.
**Fiber diameter:**

The diameter of the muscle fibers was calculated by the methods of George and Naik (1959) and Dubale and Muralidharan (1970) by using sections treated for SDH activity. This was found to be necessary since in unstained fresh frozen sections, different fiber types were not clearly visible. The mean of 50 fibers of each type of muscle fiber was calculated from six randomly selected animals.

**Percent Distribution:**

The total number of different fiber types in percentage per unit area of the pectoral muscle of the animals was determined according to the method of Karpati and King Engel (1968). The mean counts from 10 different areas were selected at random from muscle sections stained for SDH.

**QUANTITATIVE:**

1. **SUCCINATE DEHYDROGENASE (SDH) (EC: 1.3.99.1):**

   The colorimetric estimation of SDH was carried out by the tetrazolium reduction method of Kun and Abood (1949). In centrifuge tubes, the following solutions were pipetted, 0.5 ml of 0.1M phosphate buffer (pH 7.6) and 0.5 ml of 0.2 M sodium succinate followed by 1 ml of freshly prepared 0.1% triphenyl tetrazolium chloride
solution (TTC, BDH) and finally 1 ml of tissue homogenate prepared in ice cold distilled water. The blank contained all the above solutions except the TTC, which was replaced with 1 ml of distilled water. After mixing the solutions, the centrifuge tubes were placed in an incubator at 37°C ± 2°C for 3 hours and thereafter the enzyme activity was stopped by addition of 3 ml of acetone, which also extracts the coloured formazan. The tubes were shaken well and centrifuged for 5 minutes at 5000 rpm. The clear supernatant was poured off and its optical density was read at 420 nm. The calculations were done using the regression formula:

\[ X = 230.0Y - 6.44 \]

Where, \( Y \) = optical density of the unknown sample and \( X \) = concentration.

The enzyme activity was expressed as \( \mu \text{g formazan formed} / \text{mg protein} / 3 \text{ hours} \).

2. **ALKALINE PHOSPHATASE (EC: 3.1.3.1):**

The alkaline phosphatase activity was estimated by the method of Belfield and Goldberg (1971). 0.05 ml of tissue homogenate was incubated at 37°C with 2 ml of buffer-substrate mixture (the buffer comprised of 30 M mol/liter (1), anhydrous sodium carbonate, 20 m mol/l sodium bicarbonate and disodium phenyl phosphate as
substrate at a pH of 10.00) for 15 minutes. The blank contained all the solutions as in sample except that the enzyme source (0.05 ml of homogenate) was replaced with an equal amount of water. In control tubes, 0.05 ml of homogenate was added after incubation. The standard was run containing phenol instead of the homogenate (1 gm/100 ml). Then 0.5 ml of aminoantipyrine-arsenate reagent (60 m mol/l 4-amino antipyrine and 240 m mol/l sodium arsenate) was added to all tubes. The colour produced by the addition of 0.5 ml of potassium ferricyanide reagent was measured at 510 μm. The activity obtained as King Armstrong units/100 ml homogenate (mg phenol liberated/15 minutes) was converted to μmoles/liter (U/l) by multiplying with the factor 7.09 (Belfield and Goldberg, 1971).

3. ACID PHOSPHATASE (EC: 3.1.3.2):

The acid phosphatase activity was assayed by the method of Belfield and Goldberg (1971). The buffer-substrate mixture at pH 4.9 consisting of citric acid - NaOH and the substrate disodium phenyl phosphate was used. The rest of the procedure for the sample, blank, control and standard tubes was the same as for alkaline phosphatase. After 1 hour incubation at 37° ± 2°C, 1 ml of carbonate-bicarbonate buffer, sodium arsenate and 4-amino antipyrine
reagent were added followed by 1 ml of potassium ferricyanide reagent for colour development, which was measured at 510 nm. The activity of acid phosphatase which was obtained as King Armstrong units/100 ml homogenate was expressed as U/l as for alkaline phosphatase.

4. PHOSPHORYLASE (EC: 2.4.1.1.):

Phosphorylase activity was determined in the tissue homogenate by the method of Fiske and Subbarow (1925), Cori et al., (1943), as adopted by Cahill et al., (1957). The incubation mixture taken in test tubes contained 0.2 ml of 0.2 M sodium citrate buffer of pH 5.9; 0.3 ml of 0.154 M potassium fluoride and 0.05 ml of 0.2 M glucose-1-phosphate (E. Merck). Then 0.1 ml of the freshly prepared homogenate was added to the cold incubation mixture. The incubation was carried out for 15 minutes at 30°C. The reaction was terminated by the addition of 1 ml of 10% trichloracetic acid (TCA). Simultaneously, controls were run with each experiment by adding 1 ml of 10% TCA to the incubation mixture before the addition of the homogenate and these tubes were incubated along with the samples. The blanks were prepared by addition of 1 ml of 10% TCA to the incubation medium but contained no homogenate. For standard, 5 ml of working standard solution was taken (40 gammas/5 ml) in a 10 ml calibrated test tube and treated
along with the sample for colour development. A TCA blank was used for the standard. After incubation and addition of the TCA, the samples as well as control solutions were filtered into 10 ml graduated test tubes by several washings of the filter paper into tubes kept in a cold water bath. Then 1 ml of ammonium molybdate solution and 0.4 ml of aminonaphthol sulphonic acid reagent were added to all the tubes and the volume was made up to 10 ml in each tube. The solutions were mixed well and after keeping for 6 minutes at room temperature, the optical density of the resultant blue colour was read at 660 mp. The enzyme activity was expressed as mg phosphorus released/mg protein/15 minutes.

5. FRUCTOSE:

The method of Foreman et al., (1973) which is a modified method of Roe procedure (1934) was used. A weighed amount of the tissue was homogenized in 5% (0.9 N) perchloric acid, 0.5 ml of 0.1% (0.09 M) resorcinol (BDH) in 95% ethyl alcohol and 1.5 ml of 30% HCl. The tubes were heated in a water bath at 80°C for 1 hour cooled to room temperature and the colour intensity was read at 420 mp. The concentration of fructose was calculated by using the regression formula:

\[ x = 747.5 \cdot y + 4.685 \]
Where, \( Y = \text{O.D. of the unknown sample and} \)
\( X = \text{Concentration.} \)

The concentration was expressed as mg/100 mg fresh tissue weight.

6. **GLYCOGEN:**

The colorimetric determination of glycogen was carried out by using the method of Seifter et al., (1950). A known amount of fresh tissue (about 200 mg) was dropped in 2 ml of 30% KOH taken in test tubes which were placed in a boiling water bath. The tissue was digested in hot KOH for 20 minutes. The solutions were then cooled and 3 ml of 95% ethyl alcohol was added, mixed with a glass rod, and gently brought to a boil in a water bath and again cooled. The tubes were centrifuged for 15 minutes at 3000 rpm. The supernatant was drained off and again 2-3 ml of 95% ethyl alcohol was added and the procedure was repeated as before. After discarding the supernatant, the residue containing glycogen was dissolved in 5 ml of distilled water. To test tubes placed in an ice bath and containing 1 ml aliquots of this solution, 4 ml of anthrone reagent (BDH) was added. The solutions were mixed well and the tubes were then kept in a boiling water bath for 10 minutes, after which they were cooled in an ice bath immediately. The intensity of the resultant
green colour formed was read on a colorimeter at 620 nm. For blank, the glycogen solution was replaced by 1 ml of distilled water. A standard containing 40 gammas/ml was also treated along with the sample. The concentration of glycogen was expressed as μg/100 mg fresh tissue weight.

7. PROTEIN:

The determination of total protein was carried out by the method of Gornall et al., (1949). The tissue was weighed and homogenized with a known volume of distilled water. To 1 ml of the homogenate, 4 ml of Biuret reagent was added, mixed and allowed to stand for 30 minutes at room temperature (30 ± 2°C). The final colour produced was read at 520 nm. The blank was also treated along with the sample but which contained 1 ml of distilled water instead of the homogenate. The calculations were done using the regression formula:

\[ X = 38.51Y - 1.0626 \]

Where, \( Y \) = Optical density of the unknown sample and \( X \) = Concentration.

The concentration of protein was expressed as mg/100 mg wet weight of tissue.
8. ASCORBIC ACID:

Free ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complex (AA-MM) were estimated by the method of Chinoy et al. (1974). A known amount of the tissue was homogenized in a known volume (16 ml) of ice cold saturated deionised CO₂ water and then the following determinations were carried out. The homogenate was divided into 4 equal parts.

(a) FREE ASCORBIC ACID (AA):

One part of the homogenate and 3 % metaphosphoric acid (H₃PO₄) buffered with citric acid - NaOH at pH 3.6 were mixed in the ratio of 1:1 and the free ascorbic acid concentration was estimated colorimetrically with the help of the dye 2,6-dichlorophenol indophenol.

(b) ASCORBIGEN (ASG):

The second part of the homogenate was mixed with 15 % W/V metaphosphoric acid in the ratio of 2:1 and hydrolysed in a water bath at 75°C for 15 minutes to release the bound ascorbic acid (ASG). ASG was estimated after addition of citric acid - NaOH buffer at 3.6, with the above mentioned dye. The total ascorbic acid after hydrolysis minus free ascorbic acid gave the values of ascorbigen in the tissue.
(c) **ASCORBIC ACID UTILIZATION (AAU):**

To the remaining two parts of the homogenate, a known amount of freshly prepared ascorbic acid solution (0.1 mg/ml) made in CO₂ saturated glass distilled water was added in the ratio of 1:1 and the mixture was incubated at 30°C for one hour. After incubation the mixture was divided into two subparts and in one the unutilised ascorbic acid was estimated by the method as described above under 'a'.

(d) **ASCORBIC ACID MACROMOLECULE (AA-MM) COMPLEX:**

After incubation of tissue homogenate with externally added AA for determination of AAU, the remaining part of the tissue homogenate from 'C' was hydrolysed using the same procedure as described for ASG and ascorbic acid in the hydrolysate was estimated as mentioned earlier, under 'b'. Deducting from this quantity, the total amount of AA unutilised, gave the value of AA released from the bound state.

The levels of each of the above mentioned parameters were calculated by using the regression formula:

\[ X = 0.1075 - 0.1099 Y \]

Where, \( Y \) = the O.D. of the unknown sample and
\[ X = \text{the concentration.} \]
The levels of AA, ASC, AAU and AA-MM complex were expressed as mg/gm fresh tissue weight.

9. ASCORBIC ACID FREE RADICAL (AA-FR) SPECIAL PEROXIDASE (EC: 1.11.1.7):

AA-free radical forming special peroxidase which is an isozyme of peroxidase was estimated by the method of Chinoy (1973), which is based on the induced reduction reaction of Gurevich (1963). The fresh tissue was weighed and homogenised in 10 ml of ice cold glass distilled water. To each 2 ml aliquots one ml of freshly prepared ascorbic acid solution (5 mg/ml), 1 ml of a saturated aqueous solution of o-dinitrobenzene (O-DNB), and 0.1 ml of 20 volume hydrogen peroxide (H₂O₂) were added in sequence. The blank contained all the above solutions except that H₂O₂ was replaced with an equal volume of distilled water. The solutions were incubated for 20 minutes at 37°C after which the resultant yellow colour was measured on a spectronic 20 colorimeter at a wavelength of 420 nm. The enzyme activity was expressed as special peroxidase activity / 20 minutes / gm fresh tissue weight.

For all estimations, a minimum of six replicates were done and all the data was statistically analyzed using the student's 't' test.
RESULTS

The results are presented in Tables I to V and figures 1 to 12.

FIBER TYPES: The animals were divisible into four groups on the basis of their fiber types (Table I). The pectoralis muscle of all the animals investigated were of the mixed variety except that of frog which possessed only type II fibers (group I). The pectoral muscle of the pigeon and lapwing with type I and type II fibers formed group II, while type I and intermediate types were present in myna and Rosy pastor pectoralis major muscle (group III). The four mammals, Partridge and Calotes which contained type I, type II and intermediate fibers were classified in group IV (Figs. 1 to 4).

FIBER DIAMETER: The type II fibers of frog pectoral muscle were divisible into small (25 μ), medium (40 μ) and large (75.25 μ) fibers on the basis of their diameter (Table I).

Group II: In lapwing the type I and type II fibers had greater diameter than the corresponding fibers of the pigeon. However, type I fibers in both birds were smaller in diameter than the type II fibers (Table I).

Group III: The type I and intermediate fibers of myna were larger in diameter than those of the starling but in
both birds the intermediate fibers possessed greater diameter than the type I fibers (Table I).

Group IV: In group IV animals, the type II fibers were largest than type I or intermediate except in Calotes wherein the latter (intermediate) were the largest in diameter (Table I). The type I fibers of Calotes were observed to be the largest and those of black rat the smallest, amongst the animals of this group. On the other hand, type II fibers of Partridge had the largest diameter and least was found in Calotes. Similarly, the intermediate fibers of Calotes were the largest and those of the squirrel, the smallest. The diameter of the type II fibers increases in birds and mammals (groups III and IV) as compared to Calotes, whereas, type I and intermediate fibers become smaller (Table I).

PERCENT DISTRIBUTION OF FIBERS:

In frog, the large sized Type II (W fibers) were maximum in distribution followed by the medium sized ones and least were the smallest sized fibers. The number of type I fibers in percent/unit area were higher in pigeon than in lapwing (group II) but the reverse was observed for type II fibers. In group III, the Rosy pastor muscle had larger number of type I fibers but less of intermediate
than in myna. Amongst the animals of group IV, type I fibers were highest in number in mice and least in partridge. On the contrary, type II were maximum in partridge and least in white rat. The distribution of intermediate fibers however was more in white rat and least in the black rat and mice. The animals of groups II and III possessed greater percentage of type II or intermediate fibers. In group IV on the whole, the fiber distribution was type II > intermediate > type I (Table II).

HISTOCHEMICAL:

1. Succinate dehydrogenase:
   Please see Figs. 1 to 4.

2. Alkaline and acid phosphatases:
   Any detectable phosphatase activity could be localized histochemically only on prolonged incubation. The activity appeared more in the type I fibers.

3. G-6-PDH and LDH:
   The localization pattern of mitochondrial LDH and G-6-PDH were exactly the same as that obtained for SDH and were highest in type I fibers as compared to either the intermediate or type II fibers (Figs. 5 to 8).
1. Succinate dehydrogenase (SDH):

SDH activity was comparatively higher in pectoral muscle of animals of groups II, III and in mammals (groups IV) (Table III). Moreover, the enzyme activity of group III animals was significantly \( P < 0.001 \) higher than in the others. Partridge, frog and Calotes muscles were the poorest in SDH activity (Table III). As is seen from figure 9, the SDH activity increased as compared to Calotes in all the animals except Partridge.

2. Alkaline and Acid Phosphatases:

Highest \( P < 0.01 \) alkaline phosphatase was found in lapwing and least in mice and white rat. In the other animals however, almost equal amounts were observed.

The pectoral muscle of Rosy pastor was the richest in acid phosphatase followed by black rat and myna. Nearly equal activities of the enzyme were found in frog, lapwing, Partridge and squirrel followed by pigeon, white rat and mice, while the least was noted in Calotes (Table III).

Alkaline phosphatase increased in birds and in black rat as compared to Calotes but decreased in rest of the animals, whereas acid phosphatase increased in all the animals as compared to Calotes (Fig. 9).
3. **Protein:**

The concentration of protein was highest in Lapwing and Calotes whereas pigeon, mice, black and white rat, squirrel and partridge had almost equal concentrations followed by frog and myna. The Rosy Pastor muscle was the poorest in protein (Table III). As compared to Calotes, protein levels decreased in all animals except in Lapwing (Fig. 9).

4. **Phosphorylase:**

Phosphorylase activity was highest in myna, pigeon and mice followed by Calotes, Lapwing, frog and white rat while lowest enzyme activity was observed in Partridge (Table IV). Animals of group III possessed the highest activity as compared to Calotes (group IV). However animals of groups II and IV had almost similar activity (Fig. 10).

5. **Fructose:**

The concentration was highest (P < 0.001) in Lapwing followed by partridge, Calotes, mice, myna, white rat and pigeon and least in frog (Table IV). The fructose levels were decreased in all animals except Lapwing and partridge as compared to Calotes (Fig. 10).

6. **Glycogen:**

The Lapwing and pigeon muscles were significantly (P < 0.001) richer in glycogen followed by those of Partridge,
whereas, mice, Calotes, myna and white rat had almost similar levels. The least glycogen was found in frog (Table IV). As compared with Calotes, glycogen levels remained the same in mammals and group III birds but increased in rest of the animals (Fig. 10).

7. Free ascorbic acid:

The levels of free ascorbic acid were highest in pigeon and Calotes followed by lapwing, frog and partridge and least was noted in myna and mice. Ascorbigen concentration was highest in lapwing and mice than in Calotes, whereas pigeon, frog and myna had almost equal amounts and partridge had the least. The rate of utilization of ascorbic acid was maximum in pigeon followed by Calotes, myna and Partridge, while lapwing, mice, white rat and frog had nearly the same rates. The rate of AA-MM complex was highest in mice and white rat lesser in comparison in Calotes and lapwing; still less, but almost equal amounts were observed in pigeon, Partridge and frog and was not detectable in myna (Table V). On the whole ascorbic acid metabolism was decreased as compared to Calotes except AAU, which was not affected (Fig. 11).

8. AA-FR special peroxidase:

AA-FR special peroxidase activity was highest in mice and pigeon followed by myna, lapwing, frog, Partridge,
As compared to Calotes the activity of special peroxidase increased in birds and mammals except white rat (Fig. 11).

**DISCUSSION**

It is known that the structural components of a muscle complement its function, so that the metabolism of a muscle depends on its fiber architecture, and its biochemical makeup. In the generalized pattern of vertebrate appendicular muscles, three types of fibers have been recognized on the basis of their colour, mitochondrial density and enzyme, metabolic load, viz, type I or red fibers with greater number of mitochondria, more myoglobin, lipids and oxidative enzymes which are needed for metabolizing the lipids as fuel for muscular contraction; the type II (white) fibers with comparatively lesser number of mitochondria, less myoglobin but more glycogen and several glycolytic enzymes necessary for utilization of glycogen as the fuel for contraction and the third type, intermediate fibers (Int) which have intermediate characteristics as compared to type I and type II fibers. The type II fibers are specialized for fast and sudden contractions as needed for a quick take-off in birds, while type I fibers are specialized for slower, tonic contraction as required in sustained muscle activity. The three basic types are represented in reptiles viz. Calotes (John, 1966),
all the mammals investigated which belong to families Sciuridae and Muridae of Rodentia and also in a poor flier like Partridge. In birds of group II (pigeon and lapwing), the type I and type II are represented which are considerably specialized (George and Berger, 1966). However, in group III (Myna and Rosy pastor) between the type I and intermediate fibers, the former type predominate corroborating the observations of George and Berger (1966). In frog muscle the type I and intermediate were lacking.

Considering the diameters of the fiber types in the animals studied, the main evolutionary trends were (i) type II fibers increased in diameter in birds and mammals as compared to those of Calotes and (ii) the diameter of type I and intermediate fibers decreased in birds and mammals. On the basis of percent distribution of the different fibers; (i) type II fibers were found to decrease in pigeon and lapwing but significantly increase in partridge and black rat only, while (ii) % distribution of type I fibers increased in birds of group II and III as well as in mice and black rat but decreased in Partridge, and (iii) the intermediate fibers of myna, white rat and squirrel increased in % distribution while those of Rosy pastor decreased. The level of SDH activity in a muscle is considered as an index of its capacity for oxidative metabolism (George and Berger, 1966). This point is further
substantiated by the results of the present study where a correlation exists between the concentration of this enzyme and the greater number of type I fibers (birds of groups II and III). The data also shows that SDH activity was increased as compared to Calotes in all animals except partridge which is a member of Galliformes, family phasianidae, known for its flightless birds. Partridge is mostly a ground bird whose flight is short involving rapid wing beats. Thus the least SDH in its pectoralis is justified. The pigeon and lapwing are active fliers with sustained activity. The lapwing can swerve and mount into the sky rapidly when pursued by larger bids (Dharmakumarsinhji, 1955). The slow flight calls for an oxidative metabolism with increased SDH and fat utilization to obtain energy for muscular action (George and Berger, 1966). The rapid mount would involve carbohydrate utilization since it has been shown that in vigourously exercising muscle carbohydrate utilization predominates over that of fat (Pande and Blanchaer, 1971). It is evident from figs. 9 and 10 that the pectoral muscle of pigeon is not only more specialized to carry out oxidative metabolism but also has a greater turnover of glycogen than those of other birds investigated. Although myna and Rosy pastor belong to the same family (Sturnidae) and have similar fiber types (I and intermediate) in their
pectoral muscles, there is a marked discrepancy in their content of SDH. This is probably due to the greater predominance of type I fibers in the starling which is a flapping, flier indulging in prolonged flights (George and Berger, 1966) and its migratory habit. The birds were utilized in their premigratory phase when their muscles have increased oxidative metabolism. Similarly, the increase in SDH in muscles of all mammals as compared to Calotes could also be explained on the basis of the greater percentage of type I and intermediate fibers and their active modes of life. If the oxidative activity, the vascularity and the glycogen content appear to be related to fatiguability (Guth and Yellin, 1971), then it is evident that the pectoral muscle of pigeon, lapwing and Rosy pastor are comparatively more metabolically active than those of the other birds, while those of Partridge and frog are the least active. Similarly, Govindappa and Swami (1966) have reported low activities of SDH and MDH in gastrocnemius of Rana hexadactyla.

The alkaline and acid phosphatases though not clearly demonstrated histochemically in our study, have been localized in the sarcoplasmic reticulum of type I (red) and type II (white) fibers of the pigeon pectoralis muscle and in those of the dog diaphragm (Naik, 1965; Vallyathan and George, 1965). By virtue of their
localization in sarcoplasmic reticulum of the fibers, they would probably partake in inter-fiberal transportation reactions involving the various metabolites. The transport of glycogen from type II to type I fibers was suggested by George et al., (1958), while Radha and Krishna Moorthy (1972) assigned an antibacterial role to lysozyme in the pathophysiology of muscles. The presence of lysosomal type enzymes has been shown biochemically in vertebrate muscles (Greenstein, 1945; Ogata and Mori, 1963; Ono, 1970; Krishna Moorthy, 1971; Chinoy and Kshatriya, 1973 a,b).

The present data reveals that amongst the birds, alkaline phosphatase was maximum in lapwing while acid phosphatase was higher in Rosy pastor and myna. The black rat pectoralis contained higher activity of both these phosphatases than the other mammals.

The pectoralis of birds were observed to have a greater capacity to utilize ascorbic acid than those of mammals. Pigeon muscle possessed the maximum turnover of vitamin C. On the contrary, the bound form of vitamin or ascorbigen and the rate of ascorbic acid macromolecule (AA-MM) complex were less in birds (Table V). Ascorbic acid is known to form charge transfer complexes with macromolecules like proteins, nucleic acids (Chinoy et al., 1969). The comparatively less amount of bound ascorbic acid in birds probably correlates with the lesser contents of total protein in their muscle except that of lapwing. The participation of vitamin C in metabolic functions of the muscle has been reported by several workers (Mason, 1973; Khan and Swami, 1967; Floyd and Shand, 1952).
Chapter I B). A deficiency of this vitamin results in edematous and hyperplastic state of connective tissue and impaired vascularization of muscle. The metabolic turnover of ascorbic acid (AA) was found to be responsible for muscular fatigue (Khan and Swami, 1967), which has long been recognized as a characteristic symptom of Scurvy in man (Lloyd and Sinclair, 1953). It is also known that ascorbic acid alters the activity of enzymes and the contractile phenomenon. Basu and Biswas (1940) claimed that AA improved muscular function and the inactivation of action was inhibited by AA and other reducing agents (Straub and Feuer, 1950). Similarly, Boyle and Irving (1951) reported that ascorbic acid is probably essential for the maintenance of the attachment of myofibrils to the sarcolemma. Some enzyme systems concerned in the provision of energy have been related to ascorbic acid. The succinate dehydrogenase activity in cardiac and skeletal muscle of scurbutic guinea pigs was decreased than in those animals fed a vitamin C fortified diet (Harrer and King, 1941).

All these data suggest an important metabolic role of ascorbic acid via the formation of its free radical, monodehydroascorbic acid (MDHA) (Fig. 12) as a source of electron energy for oxido-reduction reactions of muscles.
In conclusion, it may be pointed out that (i) there exist definite evolutionary trends at the structural and biochemical levels in pectoral muscle which indicate the greater specialization of muscle in the Passeriformes and Columbiformes than in the Galliform birds and those of mammals than the reptiles; (ii) Within the apparent unity in the morphological pattern of fibers amongst the different vertebrate, there exists a biochemical diversity.

SUMMARY

A histophysiological study was carried out on pectoralis major muscle of an amphibian, reptile and several birds and mammals. The results suggest that the structural components of a muscle complement its function and within the apparent unity in the morphological pattern of fibers amongst the different vertebrates, there exists a biochemical diversity and definite evolutionary trends at both the structural and functional levels. Amongst the birds studied here, the pectoralis muscles of passeriformes and columbiformes are more specialised than those of Galliformes birds. The pigeon, Lapwing and Rosy pastor pectoral muscles had comparatively greater metabolic activity than those of mammals, Calotes or other birds, while Partridge and frog muscles were the least active.

The results suggest an involvement of ascorbic acid in muscle metabolism.
REFERENCES


8. Chapter I B. Studies on adaptive modifications in vertebrate skeletal muscle. 2. Regional differences in guinea pig pectoral muscle.


Table I

Showing the diameter of the different fiber types in the pectoralis muscle of the Frog, Pigeon, Lapwing, Rosy pastor, Myna, Calotes, Partridge, Mice, Squirrel, Black rat and White rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fiber Types</th>
<th>Animals</th>
<th>Type I</th>
<th>Type II</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>II</td>
<td>Frog</td>
<td>a. 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(20-32.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>I/II</td>
<td>Pigeon</td>
<td>36</td>
<td>77.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(30-45)</td>
<td>(65-92.50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Red wattled Lapwing</td>
<td>50.35</td>
<td>81.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(37.50-75)</td>
<td>(62.50-100)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>I/Int.</td>
<td>Rosy pastor</td>
<td>28.55</td>
<td></td>
<td>45.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(22.50-30)</td>
<td></td>
<td>(32.50-52.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Common Myna</td>
<td>44.10</td>
<td></td>
<td>77.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(35-62.50)</td>
<td></td>
<td>(62.50-87.50)</td>
</tr>
<tr>
<td>IV</td>
<td>I/II/Int.</td>
<td>Calotes</td>
<td>100.25</td>
<td>75.37</td>
<td>125.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(92.50-117.50)</td>
<td>(62.50-87.50)</td>
<td>(112.50-155)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Partridge</td>
<td>61.25</td>
<td>108.75</td>
<td>87.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(50-70)</td>
<td>(100-125)</td>
<td>(77.50-97.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mice</td>
<td>57.40</td>
<td>92.70</td>
<td>69.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(37.50-75)</td>
<td>(62.50-100)</td>
<td>(50-87.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Squirrel</td>
<td>62.00</td>
<td>85.90</td>
<td>66.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(50-75)</td>
<td>(75-100)</td>
<td>(57.50-87.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black rat</td>
<td>48.75</td>
<td>94.70</td>
<td>71.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(37.50-62.50)</td>
<td>(25-112.50)</td>
<td>(62.50-87.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White rat</td>
<td>69.85</td>
<td>86.55</td>
<td>73.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(50-87.50)</td>
<td>(75-100)</td>
<td>(62.50-92.50)</td>
</tr>
</tbody>
</table>

The figures in parenthesis represents the range
Showing the percentage distribution of the different fiber types in the pectoralis muscle of Frog, Pigeon, Lapwing, Rosy pastor, Myna, Calotes, Partridge, Mice, Squirrel, Black rat and White rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fiber Types</th>
<th>Animals</th>
<th>Type I</th>
<th>Type II</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>II</td>
<td>Frog</td>
<td>-</td>
<td>(a) 19.89</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b) 33.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(c) 46.46</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>I/II</td>
<td>Pigeon</td>
<td>93.75</td>
<td>6.25</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Red wattled Lapwing</td>
<td>79.31</td>
<td>20.67</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>I/Int.</td>
<td>Rosy pastor</td>
<td>84.44</td>
<td>-</td>
<td>15.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Common Myna</td>
<td>58.89</td>
<td>-</td>
<td>41.09</td>
</tr>
<tr>
<td>IV</td>
<td>I/II/Int.</td>
<td>Calotes</td>
<td>18.73</td>
<td>43.31</td>
<td>37.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Partridge</td>
<td>10.59</td>
<td>59.40</td>
<td>29.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mice</td>
<td>21.08</td>
<td>51.24</td>
<td>27.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Squirrel</td>
<td>16.25</td>
<td>44.30</td>
<td>39.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black rat</td>
<td>19.25</td>
<td>53.28</td>
<td>27.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White rat</td>
<td>15.20</td>
<td>40.99</td>
<td>43.78</td>
</tr>
</tbody>
</table>
Table III

Showing the concentration of SDH (μg formazan formed / mg protein / 3 hours), Alkaline and Acid phosphatases (u/l), and Protein (mg / 100 mg fresh tissue weight) in the pectoralis major muscle of the frog, pigeon, lapwing, myna, Rosy pastor, Galotes, Partridge, mice, black rat, white rat, and squirrel.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals</th>
<th>SDH</th>
<th>Alkaline phosphatase</th>
<th>Acid Phosphatase</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Frog</td>
<td>2.92 ± 0.25</td>
<td>10.13 ± 1.06</td>
<td>25.87 ± 6.23</td>
<td>12.50 ± 1.05</td>
</tr>
<tr>
<td>II</td>
<td>Pigeon</td>
<td>18.15 ± 1.70</td>
<td>12.00 ± 0.81</td>
<td>12.05 ± 0.95</td>
<td>27.00 ± 3.60</td>
</tr>
<tr>
<td></td>
<td>Lapwing</td>
<td>18.34 ± 2.10</td>
<td>26.51 ± 2.48</td>
<td>28.99 ± 1.91</td>
<td>68.00 ± 8.25</td>
</tr>
<tr>
<td>III</td>
<td>Myna</td>
<td>25.63 ± 3.64</td>
<td>9.64 ± 0.56</td>
<td>46.08 ± 3.19</td>
<td>13.51 ± 1.75</td>
</tr>
<tr>
<td></td>
<td>Rosy pastor</td>
<td>76.94 ± 7.55</td>
<td>7.37 ± 0.07</td>
<td>87.27 ± 4.39</td>
<td>9.30 ± 0.85</td>
</tr>
<tr>
<td>IV</td>
<td>Calotes</td>
<td>4.32 ± 0.31</td>
<td>7.54 ± 0.46</td>
<td>1.34 ± 0.14</td>
<td>52.00 ± 6.35</td>
</tr>
<tr>
<td></td>
<td>Partridge</td>
<td>3.04 ± 0.5</td>
<td>9.66 ± 0.28</td>
<td>28.14 ± 4.46</td>
<td>31.50 ± 3.50</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>10.38 ± 1.00</td>
<td>1.91 ± 0.07</td>
<td>8.64 ± 0.99</td>
<td>32.50 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>Black rat</td>
<td>12.64 ± 1.21</td>
<td>13.82 ± 0.12</td>
<td>50.76 ± 0.68</td>
<td>31.70 ± 5.10</td>
</tr>
<tr>
<td></td>
<td>White rat</td>
<td>16.82 ± 2.3</td>
<td>1.65 ± 0.70</td>
<td>7.22 ± 0.86</td>
<td>29.90 ± 2.90</td>
</tr>
<tr>
<td></td>
<td>Squirrel</td>
<td>11.28 ± 0.9</td>
<td>7.23 ± 0.49</td>
<td>29.35 ± 7.72</td>
<td>23.90 ± 8.00</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
Table IV

Showing the levels of Fructose (μg/100 mg fresh tissue weight), Glycogen (μg/100 mg fresh tissues weight), and the concentration of Phosphorylase mg/mg fresh tissue weight) in the pectoral muscle of frog, pigeon, Lapwing, Myna, Calotes, Partridge, Mice and White rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals</th>
<th>Fructose</th>
<th>Glycogen</th>
<th>Phosphorylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Frog</td>
<td>50.15 ± 2.35</td>
<td>30.00 ± 1.70</td>
<td>0.011 ± 0.001</td>
</tr>
<tr>
<td>II</td>
<td>Pigeon</td>
<td>73.80 ± 5.80</td>
<td>302.60 ± 2.19</td>
<td>0.034 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>Lapwing</td>
<td>225.80 ± 10.30</td>
<td>437.00 ± 11.25</td>
<td>0.014 ± 0.001</td>
</tr>
<tr>
<td>III</td>
<td>Myna</td>
<td>133.27 ± 7.35</td>
<td>59.44 ± 3.85</td>
<td>0.040 ± 0.002</td>
</tr>
<tr>
<td>IV</td>
<td>Calotes</td>
<td>152.60 ± 13.90</td>
<td>59.57 ± 10.23</td>
<td>0.020 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Partridge</td>
<td>195.10 ± 8.34</td>
<td>86.30 ± 2.30</td>
<td>0.007 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>127.29 ± 18.70</td>
<td>58.50 ± 2.80</td>
<td>0.026 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>White rat</td>
<td>114.83 ± 6.70</td>
<td>54.79 ± 2.35</td>
<td>0.012 ± 0.001</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
Tabe V

Showing the levels of free ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU), ascorbic acid macromolecule complex (AA-MM complex) and AA-FR special peroxidase activity in the pectoralis major muscle of Frog, Pigeon, Lapwing, Myna, Calotes, Partridge, Mice and White rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animals</th>
<th>Ascorbic acid (AA)</th>
<th>Ascorbigen (ASG)</th>
<th>Ascorbic acid utilization (AAU)</th>
<th>AA-MM complex</th>
<th>AA-FR special peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Frog</td>
<td>1.85 ± 0.12</td>
<td>0.33 ± 0.02</td>
<td>10.96 ± 0.76</td>
<td>0.63 ± 0.15</td>
<td>8.80 ± 0.10</td>
</tr>
<tr>
<td>II</td>
<td>Pigeon</td>
<td>2.71 ± 0.49</td>
<td>0.38 ± 0.07</td>
<td>19.81 ± 3.10</td>
<td>0.69 ± 0.04</td>
<td>15.93 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>Lapwing</td>
<td>1.89 ± 0.15</td>
<td>0.94 ± 0.07</td>
<td>11.78 ± 0.93</td>
<td>0.93 ± 0.07</td>
<td>9.41 ± 1.01</td>
</tr>
<tr>
<td>III</td>
<td>Myna</td>
<td>0.43 ± 0.05</td>
<td>0.28 ± 0.03</td>
<td>15.40 ± 1.21</td>
<td>ND</td>
<td>11.33 ± 0.95</td>
</tr>
<tr>
<td>IV</td>
<td>Calotes</td>
<td>2.31 ± 0.34</td>
<td>0.52 ± 0.06</td>
<td>15.74 ± 1.00</td>
<td>1.16 ± 0.25</td>
<td>5.47 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Partridge</td>
<td>1.71 ± 0.31</td>
<td>0.13 ± 0.01</td>
<td>13.12 ± 1.89</td>
<td>0.60 ± 0.07</td>
<td>7.76 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>0.33 ± 0.01</td>
<td>0.79 ± 0.14</td>
<td>10.73 ± 0.49</td>
<td>1.95 ± 0.09</td>
<td>19.00 ± 2.35</td>
</tr>
<tr>
<td></td>
<td>White rat</td>
<td>1.09 ± 0.11</td>
<td>0.14 ± 0.02</td>
<td>10.82 ± 1.05</td>
<td>1.90 ± 0.24</td>
<td>3.01 ± 0.14</td>
</tr>
</tbody>
</table>

ND = Not detectable

The values are mean ± S.E.
Figs. 1 to 4.

Showing the localization of SDH in Myna, Lapwing, Calotes and black rat pectoral muscles. Figs. 1, 2 & 4 × 450; Fig. 3 × 300.

I = Type I (Red fiber)
II. = Type II (White fiber)
Int. = Intermediate fiber
Fig. 5 and 6.

Histochemical localization of pectoral muscle of Myna and Lapwing showing the localization of G-6-PDH activity. x450.

Figs. 7 and 8.

Mm. pectoralis of Myna and Lapwing showing the staining for mitochondrial LDH. x450.

I = Type I (red fiber)
II = Type II (White fiber)
Int. = Intermediate fiber
Fig. 9.

Diagram showing the evolutionary trends in vertebrate pectoral muscles on the basis of concentrations of SDH, alkaline and acid phosphatases and protein.

I = Increased
D = Decreased
S = Same
Fig. 10.

Diagram showing the evolutionary trends in vertebrate pectoral muscle on the basis of levels of fructose, glycogen and phosphorylase

I = Increased
D = Decreased
S = Same
Diagram showing the evolutionary trends in vertebrate pectoral muscle on the basis of free ascorbic acid (AA), Ascorbigen (ASG), the rate of ascorbic acid utilization (AAU), ascorbic acid macromolecule complex (AA-MM complex) and the AA-ER special peroxidase.

I = Increased
D = Decreased
S = Same
AAU CALOTES CALOTES
f FROG AA-MM
FROG COMPLEX
MYNA (D)
PIGEON (D)
LAPWING (S)
PARTRIDGE (D)
WHITE RAT (D)
MOUSE (D)
CALOTES

AA
FROG

ASG
FROG

AAU
FROG

AA-MM COMPLEX

MYNA (D)
PIGEON (D)
LAPWING (D)
PARTRIDGE (D)
WHITE RAT (D)
MOUSE (D)
CALOTES

AA-FR SPECIAL PEROXIDASE
Fig. 12.

Showing the pathway of ascorbic acid metabolism in animal tissues.
CHAPTER I B

STUDIES ON ADAPTIVE MODIFICATIONS IN VERTEBRATE SKELETAL MUSCLE.
2. REGIONAL DIFFERENCES IN GUINEA PIG PECTORAL MUSCLE

INTRODUCTION

It is known that adult mammalian skeletal muscles are not homogenous (Yellin and Guth, 1970; Yellin, 1972), and that the so-called red and white muscles are also comprised of two fiber types, viz., the red fibers (type I) or those which have been shown by histochemical enzyme reactions to be rich in oxidative enzymes and the white fibers (type II), which have enzymes of the glycolytic pathway and function under anaerobic conditions (Brooke et al., 1971; George and Berger, 1966; Dubale and Muralidharan, 1970). A third type of fiber, the intermediate, has been described by several workers (Nachmas and Padykula, 1958; Ogata, 1958; Stein and Padykula, 1962; Cosmos et al., 1965; Bigerton and Simpson, 1969; Cooper et al., 1970; Yellin and Guth, 1970; George et al., 1971; Fiehn and Peter, 1971; Ashmore et al., 1972; Khan et al., 1973; Peter, 1973). The histochemical and ultrastructural heterogeneity of mammalian skeletal muscle fibers is now well established (Stein and Padykula, 1962; Romanul, 1964; Yellin, 1967; Schiffino et al., 1970; Eisenberg,
1975), and immunotyping of rabbit and guinea pig muscle has been done by Dawkins et al., (1973). The different biochemical makeup of the fiber types have been correlated with their functional differences (Nachmias and Padykula, 1958; Ogata, 1958; Dubowitz and Pearse, 1960 a, b; Drews and Engel, 1961; George and Susheela, 1961; Chinoy and George, 1965; Cooper et al., 1970; Barnard et al., 1971; Peter et al., 1973).

Moreover, regional differences in fiber types in muscle have been reported in avian and mammalian species (George and Susheela, 1961; George and Berger, 1966; Peter et al., 1972). But since there have been scanty reports on guinea pig MM pectoralis major, this study was attempted to investigate regional differences if any, along the depth of the pectoralis which appears predominantly to be a white muscle in gross appearance. Most of the work so far on guinea pig muscles has centered around the red and white muscles in toto (Blanchaer, 1964; Hogenhuis and Engel, 1965). But as mentioned earlier, these muscles with few exceptions are comprised of two or more fiber types having varied distributions in different regions of the same muscle. Therefore, it was thought worthwhile to study the regionwise distribution of fiber types present in the guinea pig pectoralis major muscle.
MATERIAL AND METHODS

The pectoralis major muscle of healthy, adult guinea pigs (Cavia porcellus L) of either sex weighing between 400-450 gm were utilized for the present study. They were maintained in an animal house at a temperature of 26° ± 2°C and 14 day light hours, on standard diets (Hindustan Lever Ltd., Bombay) and water supplied ad libitum.

The animals were beheaded and pieces of the muscle were blotted free of blood and frozen immediately. Fresh frozen sections 10-15 μm were utilized for all histochemical study. The muscle pieces from regions W and R (Figs. 1 to 3) were separately analyzed, throughout. However, estimations were also carried out on muscle pieces mixed together from both regions.

HISTOCHEMICAL:

1. Succinate dehydrogenase (SDH):

   The histochemical demonstration of SDH in both the regions was carried out by the method of George and Talesara (1961) as described in Chapter I A.

2. Alkaline and acid phosphatases:

   The alkaline and acid phosphatase activities were demonstrated histochemically by Naphthol- AS phosphate method of Burstone (1961). These phosphatases were assayed in muscle
pieces from both the regions as well as in muscle pieces mixed together from these regions.

3. Ascorbic acid:

A modified method of Chinoy (1969 a,b) and Dave et al., (1969) was used for the localization of ascorbic acid. Freshly excised pieces of tissue were dropped into Chilled acidic, alcoholic silver nitrate reagent at 4°C for 24 hours. The tissue pieces were then rinsed in three changes of alcoholic ammonia reagent for 15-20 minutes and then transferred to 70% alcohol. After dehydration the tissue was embedded in paraffin and sectioned. The sections were deparaffinized in xylol and mounted in Canada balsam. The control sections were devitaminized in 10% formaldehyde for 3-4 hours prior to staining.

4. Baker's method for lipids:

Baker's method (1946) using Sudan Black B was employed for histochemical demonstration of lipids.

5. Muscle weight, fiber types, diameter and percent fiber distribution:

(a) Muscle weight:

The total weight of the pectoralis muscle as well as the individual weights of regions W and R were taken at the commencement of each experiment.
(b) Fiber types and Fiber diameter:

Muscle sections stained for the localization of SDH activity were utilized for observing the fiber types and their diameters from regions W and R respectively. The diameter was calculated with the help of an ocular eye piece and micrometer scale as described in Chapter I A. Thin sections mounted in glycerol solution were also used for this purpose but since the different types of fibers could not be clearly discerned, therefore SDH stained sections were used instead.

(6) Percent fiber distribution:

The total number of different fiber types in percentage per unit area in the two regions were determined according to the method of Karpati and King Engel (1968) as described in Chapter I A.

Quantitative estimations were carried out as per details given in Chapter 1 A.

1. Succinate dehydrogenase (Kan and Abood, 1949).
2. Alkaline and acid phosphatases (Belfield and Goldberg, 1971).
3. Phosphorylase (Fiske and Subbarow, 1925 and Cori et al., 1943).
4. Fructose (Foreman et al., 1973).
5. Glycogen (Seifter et al., 1950).
6. Protein (Gornall et al., 1949).
7. Citric acid (Ettinger et al., 1952).
8. Free ascorbic acid (AA), ascorbigen (ASG), ascorbic acid utilization (AAU), ascorbic acid macromolecule (AA-MM) complex (Chinoy et al., 1974).

A minimum of six replicates were done for both regions and each parameter. The results were statistically analysed using the students 't' test.

RESULTS

The pectoralis major muscle of guinea pig was observed to be comprised of two distinct regions. The superficial region (Region W) forms the bulk of the muscle and covers the region R dorsally and peripherally, whereas region R is a comparatively smaller region (Figs. 1 to 3).

The weight of the region W was about 70% of the total weight of the pectoralis muscle while region R comprised of only about 30% of the total weight (Table 1).
The regions W and R are mixed muscles consisting of three fiber types, namely the type I, type II and the intermediate variety (red, white and intermediate) (Table I).

**Fiber Diameter:**

In region W, the type II fiber possess the largest diameter (59.62 \(\mu\)m) and type I fibers the least (44.92 \(\mu\)m), while the intermediate type had a diameter in between these two varieties (52.67 \(\mu\)m). In region R, the type II fibers are the largest in diameter (62.37 \(\mu\)m) followed by type I (58.20 \(\mu\)m) and the intermediate (66.75 \(\mu\)m) (Table I).

**Percent distribution of fibers:**

In region W, the type II fibers form a majority followed by the intermediate, while the type I are least, thus this region is the white, mixed region (Table I). Due to the presence of predominantly type II fibers, this region appears white in gross appearance. In region R, the intermediate fibers are maximum in number and the type I fibers follow next. Since type II fibers are the least in number (Table 1), therefore, this region could be designated as the red, mixed region which appears red in gross appearance (Table 1).

4. **Succinate dehydrogenase (SDH):**

The presence of diformazan granules was highest in type I fibers and least in type II fibers in both regions. On
the other hand the intermediate type fibers revealed an intermediate degree of staining (Figs. 4 to 6). The concentration of SDH was significantly ($P \leq 0.02$) higher in the region R than in the W region. The enzyme level in the muscle pieces of the two regions mixed together revealed lesser activity as compared to region R but more in comparison to W region (Table II).

5. **Alkaline and acid phosphatases:**

The localization of acid and alkaline phosphatase activities was observed only after prolonged incubation. Acid phosphatase activity was greater on the whole than the alkaline phosphatase and was localized in type I fibers.

The concentration of both phosphatases was higher in region R than W and the levels of acid phosphatase were significantly higher ($P \leq 0.01$) in the red region and also nearly three times higher than that of alkaline in region R and W respectively (Table II).

6. **Fat:**

The histochemical localization pattern for sudanophilic lipids was almost the same as for SDH.

7. **Phosphorylase and Glycogen:**

The phosphorylase activity and the levels of glycogen were comparatively higher in the white region than the red region (Table II).
8. Fructose and Protein:

The red region was richer in fructose than the white region (Table II) whereas protein levels were comparatively higher in the white region than the red region (Table II).

9. Ascorbic acid:

The ascorbigen and the rate of ascorbic acid utilization were comparatively higher in the white region than the red while free ascorbic acid and the rate of AA-MM complex were almost the same in both muscle regions (Table III).

The red region was more darkly stained for ascorbic acid than the white region (Figs. 7 and 8).

10. Ascorbic acid-free radical forming (AA-FR) special peroxidase:

The enzymic activity of AA-FR special peroxidase was slightly higher in the white region than the red region (Table III).

**DISCUSSION**

The results of the present study revealed that the guinea pig Mm pectoralis major has region wise division into two distinct pattern of fiber types. These have been designated as (1) region W, extending to major part of the muscle and comprised of a majority of type II fibers (white)
and (2) the region R which is deeply situated occupying a rather narrow strip and made up of more type I (red) and intermediate fibers than the type II fibers (Table I). Since there occur three fiber types in each of the above mentioned regions, they could be further designated as the white, mixed region (region W) and the red, mixed region (region R) respectively, as suggested by Denny-Brown (1929) and Ogata (1958). The biochemical data obtained separately on each of these regions as well as in muscle pieces mixed together from both regions, elucidated clearly how erroneous results would be the outcome, if this muscle is considered to be of uniform fiber types throughout (Table II). Similar heterogeneity of fiber types in several guinea pig muscles has been reported by Blanchaer (1964).

The biochemical data as well as those on fiber count and histochemical localizations revealed that the region R equipped with larger number of type I fibers having greater diameter as compared to the corresponding fibers of region W, and also by virtue of possessing higher fat and SDH activities is adapted for an oxidative metabolism with greater sustained contraction than the white region. It is known from several studies (Beatty et al., 1963; Blanchaer, 1964; Dubowitz and Pearse, 1960 a,b; George and Berger, 1966) that type I fibers with their load of fat and oxidative enzymes yield more energy for the muscle to contract slowly.
and tonically, whereas the type II fibers loaded with glycogen and enzymes of the glycolytic pathway allow rapid, strong muscular action. Blanchear (1964) had shown that the guinea pig type I and II muscle fibers favored different modes of coupling the respiratory chain to the glycolytic reactions generating NADH in cytoplasm, which together with succinate and lactate is directly oxidized more actively in mitochondria of type I (red) fibers than in the type II (white). The latter have NAD linked cytoplasmic and mitochondrial L-glycerophosphate dehydrogenase systems. These enzymes form a shuttle in white muscle to couple the oxidation of L-lactate and glyceraldehyde-3-phosphate in cytoplasm to mitochondrial respiratory chain. The red muscle mitochondria on the other hand, are poor in oxidase needed for L-glycerophosphate shuttle to operate. In all probability, a similar shuttle mechanism might prevail in the white fibers (type II) of the pectoral muscle of guinea pigs, since they are poor in SDH activity (Table II). From the results of the present study it is also evident that both regions W and R have almost same pattern of carbohydrate metabolism, since both have by and large, the same amounts of phosphorylase and fructose whereas glycogen was more in red region similar to the observations of Gillespie et al. (1970). This would imply further that the numerous intermediate fibers of the R region
are active participants in glycolytic pathway along with the type II fibers. Similar results have been obtained by Gillespie et al. (1970) and Peter et al. (1972) who have shown higher glycogen contents in the red portion of guinear pig vastus lateralis muscle which has significant numbers of intermediate fibers.

The significantly greater acid phosphatase activity in both regions as compared to alkaline (Table II) corroborates with observations of Chinoy and Hess (1974) in chicken latissimus dorsi anterior and posterior. Ogata and Mori (1963) could not detect acid phosphatase histochemically in skeletal muscles of many mammals and Kay (1928) proved that this enzyme was not present in muscle by his biochemical approach. In our studies however, the histochemical localization was only possible after prolonged incubation but biochemically phosphatases were detectable. Badha and KrishnaMoorthy (1972) reported the occurrence of lysosomal enzymes in fast, slow and cardiac muscles of chick. The adult posterior latissimus dorsi (LDP), a predominantly white muscle possessed higher lysozyme activity than the anterior latissimus dorsi (LDA), a red muscle. It was concluded by the authors that the well organized lysosomal activity in adult muscles has pathophysiological significance in acting as an antibacterial agent (Jolles, 1968) for the
protection of the muscle. However, Chinoy and Hess (1974) have demonstrated higher acid and alkaline phosphatases in the normal LDA of adult chicken. In the dystrophic birds (1½ years old), the LDP was richer in both phosphatases than the LDA. But the 3 years old dystrophic birds although had significantly high enzymic activity than the normal, yet both enzymes were more in LDA (Chinoy, 1974).

The occurrence of free ascorbic acid, bound ascorbigen, ascorbic acid utilization, the rate of ascorbic acid macromolecule complex formation and also activity of AA-FR special peroxidase in both W and R regions is suggestive of the fact that ascorbic acid metabolism takes place by the mobilization of the bound vitamin to its free form and its subsequent utilization and conversion to the free radical of ascorbic acid viz, monodehydroascorbic acid (MDHA). MDHA was by virtue of possessing unpaired electrons is known to participate in several biosynthetic reactions in muscle and other tissues (Chinoy, 1969a; 1970; 1972a, b; 1973), and is mainly responsible for maintenance of cellular metabolic activity via a transfer of electron energy. These results are significant since they reveal that electron energy is obtained for metabolism of red and white muscle not only by the conventional breakdown of ATP but also via the formation of ascorbic acid free radical MDHA (Chapter I A). Similar results have been reported for a number of
other actively metabolic tissues including the reproductive organs (Sheth, 1976). MDHA is finally oxidized to dehydro-ascorbic acid (DHA) which in animal tissues is reconverted to the reduced form of ascorbic acid by the action of glutathione reductase (Loh and Wilson, 1970). Thus the tissue stores of ascorbic acid are maintained and are comparatively higher in the red region than in the white (Figs. 7, 8). As ascorbic acid is known to form charge transfer complexes with macromolecules such as proteins (Chinoy, J.J. et al., 1969) and since both red and white regions of the guinea pig muscle have almost similar protein concentration, the involvement of ascorbic acid in muscular contraction in view of the above data, cannot be ruled out (Chapter I A).

The regional differentiation of the pectoralis major muscle of guinea pig into distinct larger, white-mixed and smaller red-mixed regions is probably a specialized adaptation to facilitate rapid and strong contractions of the muscle enabling the animal to execute its peculiar jumping strides. The red region might allow repeated action for some time.

**SUMMARY**

Histophysiological studies on Mm. pectoralis major of guinea pig revealed that it is a heterogeneous muscle, regionally divisible into a red, mixed region (R) with
predominantly, intermediate and type I fibers than type II, and a white mixed region (W) having more of type II fibers. It is suggested that the disposition of the muscle into separate regions is a specialized adaptation to enable the animal to perform its sudden, strong and rapid jumping strides. The red region probably allows repeated action for a longer period. The involvement of ascorbic acid via the formation of its free radical, monodehydroascorbic acid as a source of electron energy for muscular contraction has been highlighted.
REFERENCES


8. Chapter I A. Studies on adaptive modifications in vertebrate skeletal muscle. 1. Evidences for the occurrence of structural and metabolic evolutionary trends in vertebrate skeletal muscles.


Table I

Showing the fiber diameter, percentage distribution of the fiber types and weight of the white and red regions of the Mm pectoralis major of guinea pig

<table>
<thead>
<tr>
<th>Diameter of fiber (µm)</th>
<th>% distribution of fibers/ unit area</th>
<th>Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
<td>Type II</td>
</tr>
<tr>
<td>White region</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.92</td>
<td>59.62</td>
</tr>
<tr>
<td></td>
<td>(32.50-60)</td>
<td>(45-75)</td>
</tr>
<tr>
<td>Red region</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>58.20</td>
<td>62.37</td>
</tr>
<tr>
<td></td>
<td>(45-75)</td>
<td>(50-75)</td>
</tr>
</tbody>
</table>

The figures in parenthesis represents the range
Table II
Showing the concentration of SDH, alkaline and acid phosphatases, phosphorylase, fructose, glycogen, protein, ascorbic acid (AA), ascorbigen (ASG), the rates of AAU & AA-MM complex and AA-FR special peroxidase in the white and red regions of the pectoralis major muscle of guinea pig.

<table>
<thead>
<tr>
<th>Enzyme/Metabolite</th>
<th>White region</th>
<th>Red region</th>
<th>Mixed region</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDH</td>
<td>7.60 ± 0.95</td>
<td>11.30 ± 1.35</td>
<td>9.00 ± 1.05</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>20.98 ± 2.90</td>
<td>28.64 ± 4.67</td>
<td>19.06 ± 0.90</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>58.56 ± 1.06</td>
<td>87.91 ± 2.48</td>
<td>29.06 ± 4.25</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>0.017 ± 0.001</td>
<td>0.014 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>556.98 ± 21.93</td>
<td>625.58 ± 56.58</td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>64.23 ± 4.42</td>
<td>62.67 ± 4.87</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>55.00 ± 0.05</td>
<td>43.00 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (AA)</td>
<td>3.08 ± 0.18</td>
<td>2.52 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Ascorbigen (ASG)</td>
<td>1.40 ± 0.25</td>
<td>0.60 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid utilization (AAU)</td>
<td>18.82 ± 1.93</td>
<td>15.70 ± 0.90</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid macromolecule complex (AA-MM complex)</td>
<td>1.01 ± 0.28</td>
<td>0.71 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>AA-FR special peroxidase</td>
<td>8.57 ± 0.78</td>
<td>8.04 ± 0.77</td>
<td></td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
Fig. 1.
Dorsal view of the pectoralis major muscle of the guinea pig showing the superficial white region (W).

Fig. 2.
Ventral view of the pectoral major muscle of the guinea pig showing the red region (R) which is clearly seen after flapping back the entire muscle.

Fig. 3.
T.S. of the pectoralis muscle of the guinea pig showing the transverse disposition of the white (W) and red (R) regions.
Fig. 4.

Showing the SDH activity of white region of guinea pig pectoralis major muscle. x450.

Fig. 5.

Showing the SDH activity of red region of guinea pig pectoralis major muscle. x450.

Fig. 6.

T.S. of the pectoralis major muscle of the guinea pig stained for SDH, passing through the W and R regions respectively. x450.

I = Type I (Red fiber); II = Type II (white fiber)
Int = Intermediate fiber; W = White muscle region
R = Red muscle region.
Fig. 7.

Showing the localization of ascorbic acid in the red region of guinea pig pectoralis major muscle. x 160.

Fig. 8.

Showing the localization of ascorbic acid in white region of guinea pig pectoralis major muscle. x 640.