SUMMARY

Studies were conducted to identify different fiber types and to assess the fiber growth in relation to the somatic growth rate in seven muscles of growing male white leghorn chick (*Gallus gallus*) and male Swiss albino mouse (*Mus musculus*). Three broad objectives were set forth viz., (a) to identify the different fiber types in the skeletal muscle of the selected species on the basis of their histochemical nature and physiological properties, (b) to study the functional relationship of muscle fibers by comparing the diameter and distribution pattern of these fibers in various muscle mass in relation to the functional activities of that particular muscle and, (c) to study the post-natal muscle growth in growing chick and mice in relation to their somatic development.

The research objectives were hypotheses driven, designed and executed following four Hypotheses which were framed in classical ‘Null’ form. These are (a) muscle fibers typing on the basis of histochemical nature and physiological properties are not similar across the vertebrate classes in general and in chick and mouse in particular, (b) distribution pattern of different muscle fibers in various muscle mass are not relating to their functional activities in that particular species, (c) Fiber growth is not related to the somatic growth rate of that species, but related to the growth of that particular muscle mass, and (d) Post-natal muscle growth and development is not related to somatic growth rate across vertebrates in general and of that particular species in particular.

The selection of the animal models from two different vertebrate class for this study was done to compare the observed results with that of other vertebrate species (mostly fish) by different researchers of this laboratory to know if there is a generalized trend of muscle fiber growth dynamics exists in different vertebrate classes.

Seven muscles were selected viz., *Gastrocnemius, Soleus, Peroneus longus, Flexor digitorum longus, Sartorius, Biceps brachii* and *Triceps brachii*. Classical muscle nomenclature were used throughout the study for simplicity. Muscle samples were obtained from the sacrificed animals of eight age groups at 7 days of interval. As the newly born or hatched animals were very small for sufficient muscle sampling, animals ranged from 14 to 63 days old in mice and 7 to 56 days old in chick were selected for
the study. Fresh frozen sections were obtained by a cryostat microtome and stained for fiber identification and their morphometry. Fiber typing was done as per the histochemical and histological properties exhibited by the individual fiber types in the muscle mass. Detailed morphometrical studies on different fiber types in individual muscle mass were conducted for the growth studies (Kundu, 1990).

Results obtained from the histochemical localization of lipids, glycogen, succinate dehydrogenase, and lactate dehydrogenase gives the summary of the characteristics of the fiber types observed in the individual muscle. Nile blue sulfate staining showed colorations for various kinds of lipids. Phospholipids were stained blue whereas, neutral lipids stained as red droplets. As it appears, the fibers, which are smaller in size and almost round shaped were stained heavily for lipid. The larger fibers were lightly stained. Neutral lipids in the form of red droplets were found only in the interstitial spaces. Neutral fats are stained pink while acidic fats are stained blue-violet. Glycogen was stained brilliant red and nuclei stained blue. Lipids are present in red fibers while glycogens were found in white fibers.

The three muscle fiber types identified in the muscles studied are named by their color, viz., red, pink and white. In this study, the classical nomenclature of red, pink and white fibers are followed throughout for simple and better understanding of the objectives concerned.

Present investigation suggested aerobic properties of red fibers which are thus characterized by those usually associated with sustained activity. On the other hand, very low activity of the oxidative enzymes in white fibers suggests a mainly anaerobic metabolism through the glycolytic pathway in this part. Thus, in the present investigation, the properties of white fibers resemble the features more associated with anaerobic metabolism of vigorous action, a rapidly fatiguing activity that utilized glycogen. Pink fibers were found to be intermediate in its level of staining for oxidative enzymes and lipids. Since the theme of this study was revolving around the growth pattern of the selected muscle mass in relation to the somatic growth rate, histochemical studies were just used as a tool to identify the fiber types only. Obtained results showed strong difference in the histochemical characteristics between different fiber types. The high activity of the enzyme of the Kreb’s cycle and respiratory chain (SDH) in the red fibers indicated that the metabolism in this muscle part is mainly oxidative. This is
supported by high concentration of myoglobin and lipids. These results are in accordance with the data found in red muscles of the lateral line in fish for which several studies revealed a high activity of Kreb’s cycle enzyme.

The studied muscles in chick showed the basic pattern found in other species. The smaller fibers having almost round shape was Red fibers or Types I fibers. This was followed by larger Pink fibers or Intermediate fibers. However, the major bulk of the muscle was composed of very large irregular shaped White fibers or Type II fibers. However, white fibers occupied about 68-84 % of the total muscle mass in general. Soleus muscle was found to contain only red and pink fibers and no white fibers. Red fibers were less than pink fibers and smaller in size. Biceps contained larger red fibers. Almost similar results were obtained in mice except few differences. Here red fibers were smaller and rarer in most of the muscles. Soleus was consisting of red and pink fibers with very few white fibers. White fibers were predominant and larger. Pink and white fibers were found to become polygonal in successive age groups while red fibers were round.

Biceps and triceps muscle showed large concentration of red fibers near its insertion in chick and mice both which suggests its main role as postural muscle. Soleus on the other hand found to be occupied mainly of red and pink fiber with few white fibers in mice. It also works as postural muscle in both the animals. The fiber composition is responsible for activeness of these muscles. This muscle is required for sustained continuous activity like standing, walking etc. Flexor showed large concentration of white fibers. The results show that this muscle is not always needed in chick as chick exhibits less movement. Gastrocnemius also found to contain large concentration of white fibers in both the animals. This muscle is responsible for sudden movements in chick like in case of short flight in times of emergency, as chick is a near flightless bird. On the other hand mice contained large concentration of red fibers in its muscles which relates its fiber composition to its continuous and fast activity.

The obtained results revealed different orientation pattern of the fibers in the muscle masses studied. Except soleus all the muscle masses of the chick was found to be composed of all three basic fiber types with almost equal predominance of the red and white fibers. This suggests an active role of these muscles in the locomotion of the
animal as it is capable of all kinds of movements represented by all these fiber types. Muscle size is assumed to be proportional to the size of the whole organism. As such, growth of the muscle is used as an estimate of whole organism growth rate.

In the present investigation, variations were observed in the diameter of all three basic fiber types as the animal grew. In **chick** the diameter of red fibers ranged from 8.14µm (Sartorius) to 35.02 (Soleus) in the lowest age class and from 29.73µm (Triceps) to maximum of 109.86 µm (Soleus) in the highest age class in the developing chick. The mean diameter was 17.72-25.97µm (soleus) in the lowest age class which increased to 58.78-78.98µm (soleus) in the highest age class. Pink fibers ranged from 7.13µm (Sartorius) to 37.92µm (soleus) in the lowest age class and a minimum of 27.47µm (Sartorius) to maximum of 136.18µm (Gastrocnemius) in the highest age class in the developing chick. The mean diameter was 18.25-28.39µm in the lowest age class which increased to 68.60- 80.10 µm in the highest age class. White fibers ranged from 8.26µm (Triceps) to 37.38µm (Sartorius) in the lowest age class and a minimum of 28.00µm (Gastrocnemius) to maximum of 131.28µm (Gastrocnemius) in the highest age class in the developing mice. The mean diameter was 20.94- 24.72µm in the lowest age class which increased to 62.68-76.97µm in the highest age class. The results clearly show an increase in the fiber diameter from lower to higher age class as the animal grows as evident from Tables 19, 23 & 25.

In case of **mice**, the diameter of red fibers ranged from 13.14µm (Sartorius) to 37.87µm (Sartorius) in the lowest age class and from 30.00µm (Soleus) to maximum of 69.98µm (Triceps) in the highest age class in the developing mice. The mean diameter was 23.04-26.32µm in the lowest age class which increased to 49.75- 45.31µm in the highest age class. Pink fibers ranged from 15.81µm (soleus) to 58.46µm (Sartorius) in the lowest age class and a minimum of 31.36µm (Soleus) to maximum of 95.97µm (Triceps) in the highest age class in the developing mice. The mean diameter was 26.21- 30.61 µm in the lowest age class which increased to 49.53- 68.00 µm in the highest age class. White fibers ranged from 15.11µm (Sartorius) to 47.73µm (Biceps) in the lowest age class and a minimum of 38.51µm (Soleus) to maximum of 125.19µm (Sartorius) in the highest age class in the developing mice. The mean diameter was 27.14- 33.19 µm in the lowest age class which increased to 54.14- 90.18 µm in the highest age class. The results clearly
show an increase in the fiber diameter from lower to higher age class as the animal grows as evident from **Tables 21, 24 & 26**.

The growth and development of skeletal muscle is very much plastic (dynamic), influenced by many factors like water temperature, feeding habit, habitat, hormones etc. However, in the present study, an attempt has been made to understand the growth pattern of seven skeletal muscle masses of chick and mice in relation to their somatic growth rate. The overall pattern of the fiber growth in the animals studied showed marked variations from muscle to muscle.

The frequency distribution histograms in **chick** showed that in the lowest age class the frequencies of lower diameter modes (11 to 20 µm and 21 to 30 µm), where recruitment of small new fiber was evident, was not seen. However, the shifting of modal frequency values towards the higher diameter modes was evident from the early developmental phase itself. The maximum shifting of fiber diameter modes was evident at the highest age classes. The results suggest involvement of hypertrophy in the fiber growth of **red fibers** in all studied muscles. In the highest age classes, the frequency of certain intermediate fiber modes (31 to 40 µm) and showed tremendous increment. This may be due to splitting of existing large fibers into smaller fibers once the fiber attended the maximum fiber size. This phenomenon is well supported by regression analysis graphs.

**Pink fibers** showed more or less similar trend that of the red fiber. In this case however, the fiber with highest diameter (15 - 20 µm) was already presence in the lowest age class. The shifting of modal frequency distribution was evident which was indicating of budding and splitting of larger fibers. **White fibers** exhibited tremendous shifting of modal frequency values towards higher diameter modes as the age of the animal advanced. The maximum shifting was observed in higher age classes. However, splitting and budding of larger fibers was predominant as the animal approaches adulthood. Therefore, it can be assumed that the growth of white fiber in the selected muscles was predominantly by hypertrophy in the younger once and as the bird approaches as adulthood. The fiber growth dynamics was by both hypertrophy and hyperplasia. This hyperplasia however, is not recruitment of small new fibers but generated due to splitting of large white fiber into smaller one and this trend was well supported by the regression analysis given in Table 20.
More or less similar trend was observed in case of mice also. In the lowest age class the frequencies of lower diameter modes where recruitment of small new fiber was evident, was not seen. However, the shifting of modal frequency values towards the higher diameter modes was evident from the early developmental phase itself. The maximum shifting of fiber diameter modes was evident at the higher age classes. The results suggest involvement of hypertrophy in the fiber growth of red fibers in all studied muscles. In the highest age classes, the frequency of certain intermediate fiber modes showed tremendous increment. This may be due to splitting of existing large fibers into smaller fibers once the fiber attended the maximum fiber size. This phenomenon is well supported by regression analysis graphs. Pink fibers showed similar conditions like red fibers. However, the shifting of modal frequency values was like white fiber which was advance once the animal approaches in adulthood. Similar conditions were supported by the regression analysis graphs. White fibers exhibited tremendous shifting of modal frequency values towards higher diameter modes as the age of the animal advanced. The maximum shifting was observed in higher age classes. However, splitting and budding of larger fibers was predominant as the animal approaches adulthood. Therefore, it can be assumed that the growth of white fiber in the selected muscles was predominantly by hypertrophy in the younger once and as the bird approaches as adulthood. The obtained result is well supported by regression graphs. The fiber growth dynamics was by both hypertrophy and hyperplasia. This hyperplasia however, is not recruitment of small new fibers but generated due to spiting of large white fiber into smaller one and this trend was well supported by the regression analysis given in Table 22.

In this study, compared to chick, mice showed more hyperplasia in all the muscles studied. In lower age groups i.e. up to 35 days there was hypertrophy but after that recruitment of small new fibers were observed which might be due to budding and splitting of larger existing fibers. Hence the growth was observed by both hyperplasia as well as hypertrophy.

The overall result in the present investigation suggested that all three basic fiber types showed growth by hypertrophy almost exclusively irrespective of their location and functional activities. Red and pink fibers in these species were definitely different than that of fish muscle. Fiber growth by hyperplasia or recruitment of small new fibers was
not at all evident. Splitting of large fibers into smaller fibers was evident in some cases of pink and white fibers only. Muscle fiber growth in these muscle masses were definitely in relation the somatic growth rate of the chick.

Recruitment of small new white fibers ceased early and shifting of modal frequency values towards higher diameter mode was evident. However it is evident that splitting of extensive large fiber into smaller fiber occurred after attainment of certain size which is genetically fixed. The overall results indicate that selected muscles in chick are not used frequently but responsible for sudden and vigorous activity while in mice these muscles are responsible for continuous and rapid activities like running and walking. The growth dynamics of all 3 fiber types are predominantly by hypertrophy. Pink fibers occupy 6 to 10 percent of the total mass in chick and grew mainly by hypertrophy. White fibers occupy the major portion of the muscle masses and exhibited growth exclusively by hypertrophy.

All three fiber types were found unevenly distributed in all the muscle masses studied. The growth of red fiber indicated a major role of hypertrophy of existing fibers. Red fibers grew mainly by and increased fiber diameter in initial stages which was evident by the shifting of lower diameter mode to higher diameter mode. However, in higher age groups frequency of certain intermediate diameter modes showed increments. This indicates recruitment of small red fibers. The growth dynamics of pink fibers showed predominant trends of hypertrophy. The recruitment of small new pink fibers ceased early shifting of diameter modes was evident throughout the age groups. However, it appears that fiber number was increased in certain age groups which are a form of hyperplasia.

In the present study, all the hypotheses proposed were experimentally observed to be NOT TRUE. The study also revealed that the post-mitotic fiber growth mechanism in developing animals are possibly similar across the vertebrate classes. The fiber growth mechanism is highly plastic (thus dynamic), muscle specific and fully synchronized with somatic development. It was evident that the initial fiber growth after birth is always by hypertrophy of existing fibers and at a later stage when the fibers grew and reach to a genetically fixed diameter, it splits into two or more smaller fibers (hyperplasia). The continuation of both the processes is muscle as well as specis specific and genetically controlled.