Study of histochemical characterizations of skeletal muscle fiber types in the rabbits (*Oryctolagus Cuniculus*)

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Abstract

This project has been conducted on New Zealand White Rabbits (newborns and adults) . Biceps brachii, soleus and abdominal wall of muscles were used in this study. Fresh pieces of muscle tissues were frozen to be cut at (10 µm) thick sections by the cryostat.Skeletal musclefibre types were investigated histochemically to demonstrate myosin adenosin triphosphatase (ATPase) activity using acid and alkaline preincubation and succinate dehydrogenase (SDHase) activity . In this way it may be possible to recognize the fast and slow twitch muscle fibers, as well as to explore the difference in reaching maturity between different muscles of the body.

The results showed that, the biceps brachii and abdominal wall muscles consist predominantly of fast fibers, whilst the soleus muscle is made up of a near homogenous population of slow fibers.

The immature abdominal wall muscle showed some reversal of the mature limb muscles staining pattern of the same animal.

Key words: Muscle fiber, ATPase, SDHase.

Introduction

Skeletal muscle fibers can be classified according to their rate of contraction and to the type of energy metabolism. These workers described three basic myofibre types that are common to most mammalian muscles : (i) slow-twitch , oxidative metabolism(SO); (ii) fast-twitch mammalian, oxidative and Glycolytic metabolism (FOG); (iii) fast-twitch , glycolytic metabolism (FG) , therefore the two extremes of muscle fiber types are represented by SO and FG myofibres which have different functional properties . SO fibers are adapted for slow prolonged contraction, sustained for long periods of time , and are fatigue resistant whereas FG fibers are adapted for fast intermittent bursts of contraction and are fatiguable (1 , 2) The functional properties characteristic of a myofibre can be demonstrated histochemically by examining the activity of two enzymes , myosin adenosine triphosphatase (ATPase) and mitochondrial succinic dehydrogenase (SDHase) . A greater intrinsic speed of shortening relates to a higher ATPase activity (3) . As the activity of the ATPase releases phosphate by the hydrolysis of adenosine triphosphate (ATP) staining intensity for phosphate is indicative of ATPase activity at the pH employed (4 , 5) . The correlation between ATPase activity and the preincubation pH is due to the existence of different myosins of fast and slow muscle fibers. ATPase activity of slow myosin is relatively acid-stable and alkali-labile while that of fast myosin is alkaline-stable and acid-labile. Thus fast fibers have a high ATPase activity and show intense staining for phosphate at alkaline pHs, whereas slow fibers show intense staining for phosphate at low pHs.

Succinic dehydrogenase is an enzyme of the tricarboxylic acid cycle and is tightly bound to the inner mitochondrial membrane (5) . The role of this enzyme as an electron donor culminates in the generation of energy in the form of ATP, and its activity is indicative of aerobic metabolism. The histochemical test for SDHase is based on the deposition of diformazan caused by the activity of this enzyme (6) .

SO fibers are rich in mitochondria, are predominantly oxidative, and display a strong positive SDHase reaction while FG fibers have fewer mitochondria, depend on the generation of energy in anaerobic conditions, and display a weak SDHase reaction.

These properties are reflected in the different appearance of slow and fast fibers. Studies of mammalian muscle fibers shown a direct correlation between oxidative capacity and capillary density (7). Slow fibers are red, having a rich capillary supply to support their aerobic activity, and fast fibers are white (2) . Oxidative capacity is also inversely proportional to the cross-sectional dimensions of myofibrils. FG fibers being larger than SO fibers. Fibers with characteristics between the two are called intermediate fibers (8). The latter, like slow fibers are red .Most mature skeletal muscles are made up of a heterogeneous Population of fibers and are therefore 'mixed ' muscles, such as the limb muscles, the biceps brachii and the semitendinosus muscles. In the biceps there are fast, slow and intermediate fibers but the large proportion of fibers are of the fast type (9).

There are few muscles with a homogenous or near homogenous population of fibers, such as the postural muscle. The soleus, which, in some species, consists entirely of slow fibers (10) . Thus the biceps and soleus are considered to be good examples of fast-twitch and slow-twitch muscles respectively. This study is an attempt to characterize the types of muscle fibers in the abdominal wall by the histochemical demonstration of ATPase and SDHase activity. The profiles of muscle fiber types thus obtained are compared with those of limb muscles which are typically fast-twitch or slow-twitch in action. In this way it may be possible to explore the difference in reaching maturity between different muscles of the body, as well as, through looking to the arrangement of the fibers within the immature muscles (11).

Materials and methods

New Zealand White Rabbits (6 newborn and 3 adults) were used in this study . All animals were sacrificed by the administration of euthesate in a dose of 200mg / kg body weight intraperitonially . The tissues examined were limb muscles (biceps and soleus) , taken from animals at 4 days of age and abdominal wall muscles (near the inguinal region), taken from animals at 4 days of age and adults , Fresh pieces of tissue were mounted with Tissue-Tek embedding medium on a piece of cork attached to a cryostat metal chuck with a drop of of the same medium. Tissues were frozen in dichlorodifluoromethane, and then cooled to -190˚c in liquid nitrogen. 10µm thick sections were cut after allowing the tissue to warm up to -20 ˚c in the cabinet of the cryostat (Bright 5030 Microtom) . Sections were picked up on clean glass slides and left to thaw and dry at room temperature for 1-2 hours. The quality of sectioning was assessed at intervals by staining with methylen blue and examined under the light microscope.

Histochemical Procedures

Thawed and dried frozen sections were stained for the histochemical demonstration of myosin adenosine triphosphatase (ATPase) activity using acid and alkaline preincubation, and succinic dehydrogenase (SDHase) activity.

(i)Demonstration of alkaline stable adenosine triphosphatase
– modified technique after (4) .

(ii)Demonstration of acid stable adenosine triphosphatase
-- modified technique after (4) .

(iii) Demonstration of succinic dehydrogenase (6) .

Results

1. Biceps brachii muscle

(i) ATPase activity

Staining after acid preincubation demonstrated a mixed population of fibers consisting largely of fast fibers (both lightly staining FG, and unstained FOG) with just a few scattered darkly staining slow (SO) fibers (Fig. 1) .

The majority of the fibers were of the no oxidative FG type which had the largest diameter, and the minority was of the oxidative SO type with the smallest diameter. This proportion of fast to slow fibers was confirmed by the staining pattern after alkaline preincubation with unstained scattered slow fibers being surrounded by large numbers of darkly stained fast fibers (Fig. 2).

(ii) SDHase activity

About 40% of the fibers stained positively and were thus demonstrated to be oxidative (SO and FOG fibers) with the remaining non-oxidative (FG) fibers staining negatively. Thus the biceps brachii was shown to consist predominantly of fast fibers, most of these being of the FG type but many also being FOG fibers (Fig.3).

2. Soleus muscle

(i) ATPase activity

A lmost all the muscle fibres were acid stable (Fig. 4) with only the occasional, isolated alkaline stable fibre (Fig. 5) , indicating that this muscle consists entirely of slow fibers .

(ii) SDHase activity

The muscle stained weakly but uniformly with the purple coloured formazan granules indicating that all the fibers were oxidative, including the occasional fast fiber . The latter was also of small diameter.

Thus, unlike the other limb muscle and the body musculature, the soleus was shown to consist almost entirely of slow – twitch fibers (SO) with only the very occasional isolated FOG fibers (Fig. 6) .

3. Adult abdominal wall muscles

(i) ATPase activity

Staining after both acid preincubation (Fig. 7) and after Alkaline

Preincubation (Fig. 8) demonstrated a clear differentiation between the larger and more numerous fast fibers and the smaller scattered slow fibers.

(ii) SDHase activity

The distribution of SDHase positively staining fibers showed that apart from the slow fibers only a few of the fast fibers stained positively and were of the FOG type (Fig. 9). Thus, like the limb muscles so far described, the abdominal wall musculature was shown to have a majority of fast fibers but only a very few of these were oxidative.

4. Newborn abdominal wall muscles

The clearest differentiation between fiber types was seen in the sections stained after alkaline preincubation (Fig. 10). In these sections smaller darker staining fast fibers were clustered around larger pale-staining slow fibers representing the typical distribution of smaller secondary fibers around a single large primary fiber, as seen in maturing muscle.

Examination of the sections stained after acid preincubation showed some reversal of the staining pattern expected with the large primary fibers in the center of the fiber clusters staining less intensely than the surrounding , more numerous, secondary fibers (Fig. 11).Slow and fast fibers were stained positively for SDHase (Fig. 12) .

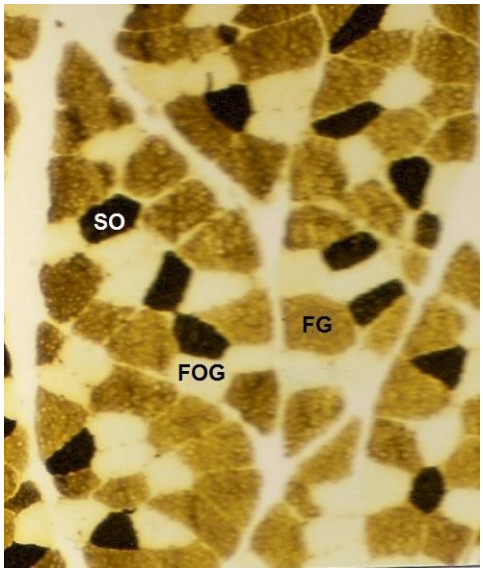
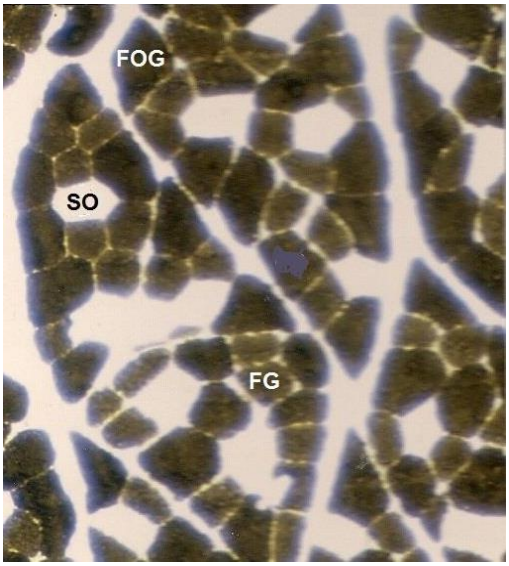


Figure (1):Atpase activity of biceps brachii muscle after acid preincubation.
(FOG : fast-twitch fiber). X40



Figure(2): ATPase activity of biceps brachii muscle after alkaline preincubation.
(SO : slow-twitch fiber) . X40

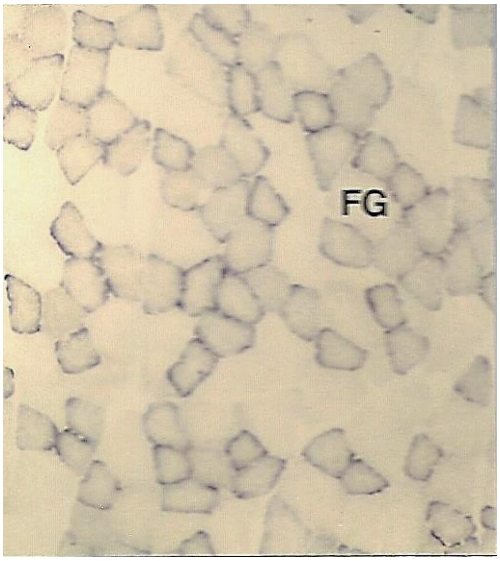


Figure (3): SDHase activity of biceps brachii muscle .(FG : fast glycolytic fiber) X40

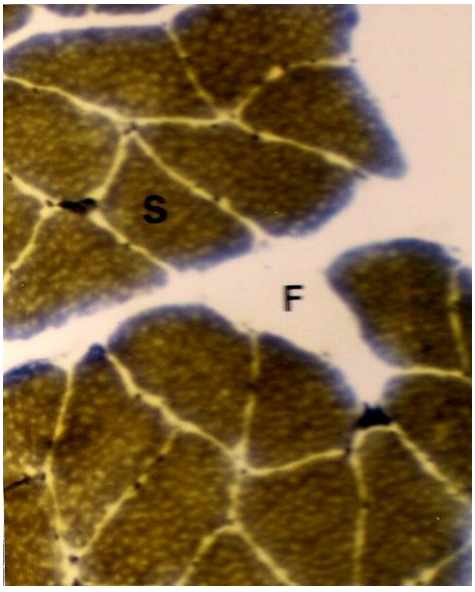
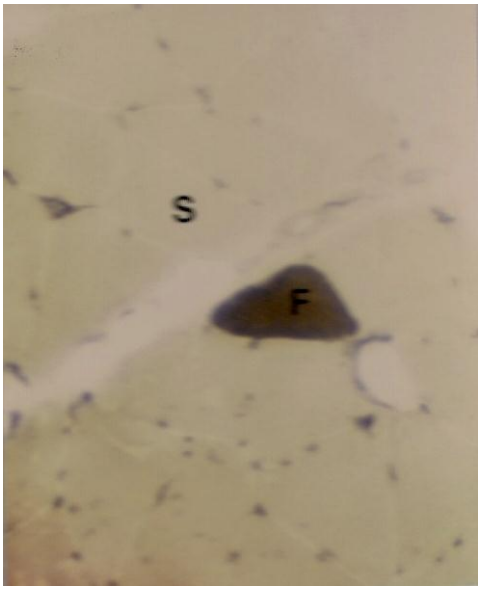


Figure (4): ATPase activity of soleus muscle after acid preincubation (S: slow fiber, F: fast fiber) X40



Figure(5): ATPase activity of soleus muscle after alkaline preincubatio (S: slow fiber, F: fast fiber) X40

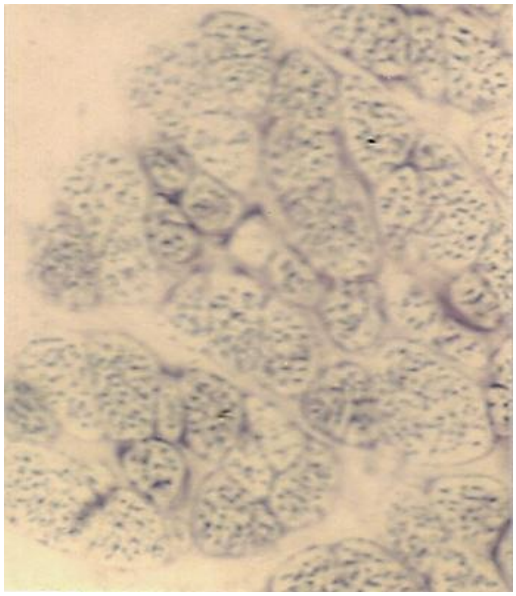


Figure (6): SDHase activity of soleus muscle X40

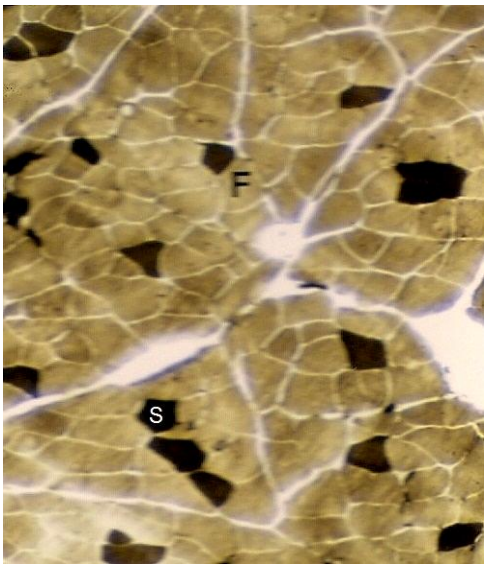


Figure (7): ATPase activity of adult abdominal wall muscles after acid preincubation (S : slow fiber, F : fast fiber) X40

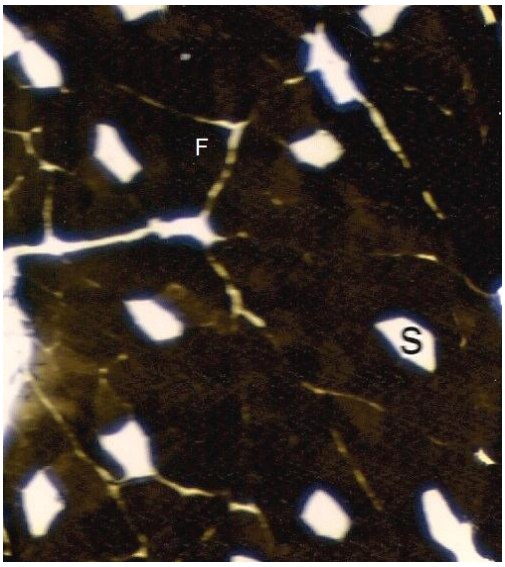


Figure (8):ATPase activity of adult abdominal wall muscles after alkaline preincubation (S : slow fiber, F : fast fiber) X40

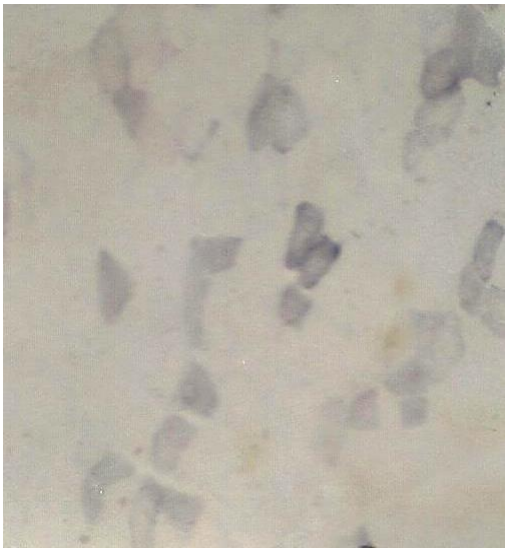


Figure (9): SDHase activity of adult abdominal wall muscles . X40

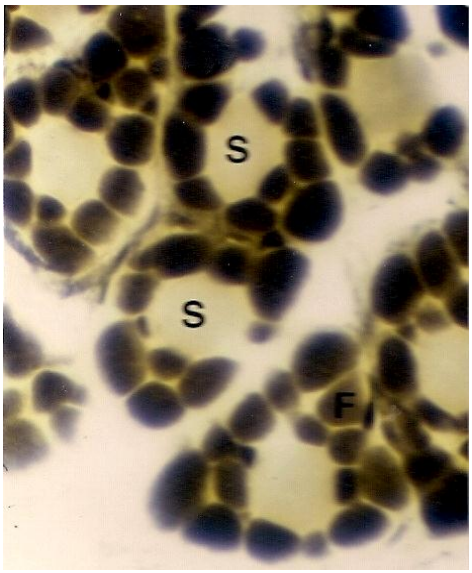
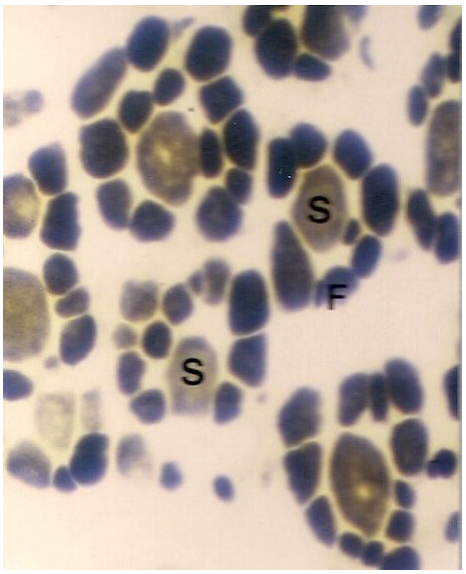


Figure (10): ATPase activity of newborn abdominal wall muscles after alkaline preincubation
(S : slow fiber, F : fast fiber) X40



Figure(11): ATPase activity of newborn abdominal wall muscles after acid preincubation

(S : slow fiber, F : fast fiber) X40

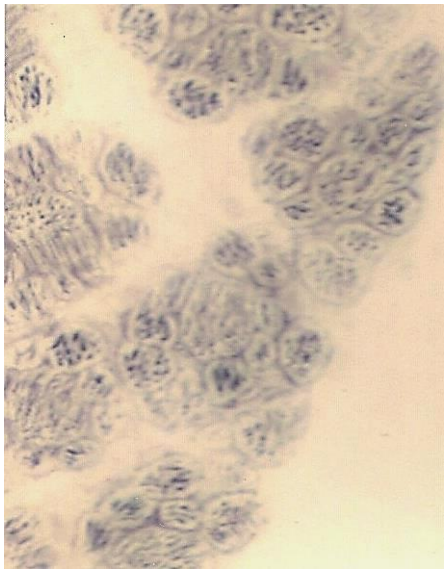


Figure (12): SDHase activity of newborn abdominal wall muscles . X40

Discussion

The conclusion that drawn from this study is that histochemical characterization of muscle fibers, so clearly illustrated in the muscles of the limb has obviously not reached full maturity in the early postnatal abdominal wall muscles.

It has been known for some time that adult muscles contractile properties are acquired during either late prenatal or early postnatal development, depending on the species and on the specific muscle (12) . The histochemical differentiation of fast and slow fibers in the rabbit seems to occur largely in the postnatal period rather than prenatally. Thus the rabbit is more like the rat than the guinea pig, two species which clearly illustrate the wide species variation in the timing of skeletal muscle differentiation (13) .Although a reversal of the expected staining pattern for phosphate, after acid preincubation, is seen in the muscle of abdominal wall indicating incomplete maturation , the limb musculature of four-day-old specimen is comparable to body wall muscle at the same age . This difference in the timing of the histochemical maturation of muscle fibers at these two sites reflects the slower progress of myogenesis seen in the abdominal wall muscles. So it is clear that the muscle fibers of the limb become differentiated earlier than those of the body wall.

Fast muscle fibers appear to be in the majority in the body wall of the neonatal rabbit, as they are in that of the mature rabbit. However, a high level of ATPase activity, normally associated with fast twitch fibers in adult muscle is also shown by slow type fibers in developing muscle (14). Thus the correlation between the speed of contraction and myosin properties (slow or fast types of myosin and ATPase activity) applicable to adult fibers is not necessarily appropriate for immature muscle. High ATPase activity and the presence of myosin chains of both fast and slow type are characteristic of embryonic and neonatal muscle (15). The speed of contraction of immature skeletal muscle tends to be slow even in the muscles which contract rapidly in the adult animal (12). Thus the developing muscle fibers of the rabbit body wall may behave like adult slow fibers during neonatal period. Contraction times of

developing muscles are even more prolonged than that of adult slow muscles, and gradually increase this speed with maturation (16).

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