Histochemistry (1983) 77:543–555

Histochemistry

Differentiation of Fiber Types in Skeletal Muscle from the Sequential Inactivation of Myofibrillar Actomyosin ATPase During Acid Preincubation

P.D. Gollnick*, D. Parsons, and C.R. Oakley
Department of Physical Education for Men, Washington State University Pullman, Washington 99164-1410, USA

Summary. A method is described for identifying fiber types of skeletal muscle from several mammalian species on the basis of the sequential inactivation of myofibrillar actomyosin ATPase during acid preincubation. When this method is used in combination with the standard alkaline preincubation at least 5 types of fibers can be identified. Of these, 2 are type I fibers with those of the slow twitch soleus muscle being different from those that exist in mixed muscles. The 3 subtypes of type II fibers exist independent of their metabolic properties. The need for careful standardization of histochemical methods for the visualization of myofibrillar actomyosin ATPase and the implication of the existence of different fiber types in apparently homogeneous muscle for the preparation of antibodies used for immunocytochemical methods of fiber identification are discussed.

Introduction

Refinement of the methods for identifying, separating, and purifying isozymes of the myofibrillar complex of skeletal muscle has demonstrated that the possible combinations of these proteins could produce a considerable heterogeneity of fiber types (Billeter et al. 1981; Bronson and Schachat 1982). The application of immunocytochemical methods to identify specific proteins may resolve the question of how many fiber types exist in skeletal muscle (Gauthier and Lowey 1977; Pierobon-Bormioli et al. 1981). However, this method requires the isolation of pure proteins for inducing antibody production which limits its routine application for identifying fiber types of skeletal muscle.

A dichotomy of fiber types in skeletal muscle can be established via a histochemical staining for myofibrillar actomyosin adenosine triphospha-

* To whom offprint requests should be sent

tase (ATPase) after alkaline preincubation (Padykula and Herman 1955). These are frequently identified as type I and II fibers or as slow-twitch (ST) or fast-twitch (FT) fibers. Type II fibers can be subclassified into IIa (FTa), IIb (FTb), and IIc (FTc) on the basis of an activation or inactivation of the myofibrillar actomyosin ATPase by acid or alkaline preincubation (Brooke and Kaiser 1969, 1970). Guth and Samaha (1969, 1970) introduced a formalin fixation – alkaline preincubation method whereby fibers were identified as α, β, and $\alpha\beta$. This fiber identification scheme is not completely interchangeable with that of Brooke and Kaiser (Green et al. 1982). Formalin fixation (Hayashi and Freeman 1966), Ca–Mg stimulation (Mabuchi and Sréter 1980), and varying the pH of the incubation (Müntener 1979) or both the alkaline and acid preincubation media (Soukup et al. 1979) have been used to identify fiber types on the basis of their myofibrillar actomyosin ATPase. Most methods have confirmed the existence of type I fibers and 2 or 3 subclasses of type II fibers. The standard pH lability method for identifying fibers (i.e. preincubations at pH 4.35, 4.6, and 10.4) can produce inconsistent staining patterns following acid preincubation. Brooke and Kaiser (1974) did, however, stress the fact the method is critically dependent upon the time and pH of the acid preincubation and some of the variability that occurs with its use may be attributable to inadequate control over these conditions. Muscle fibers have also been categorized on the basis of contractile and metabolic characteristics as estimated histochemically (Peter et al. 1972; Ashmore et al. 1978; Spurway 1981). The variability and overlap of these characteristics, particularly of terminal oxidative potential, for fibers judged as being of the same or different from their actomyosin ATPase or other means (Pette and Spamer 1979; Spamer and Pette 1980; Reichmann and Pette 1982; Sickles et al. 1982; Lowry et al. 1978; Hintz et al. 1980), is such that no generalization can be drawn concerning it and the type of myosin contained in a fiber.

Differences in the staining patterns for myofibrillar actomyosin ATPase after alkaline and acid preincubation appear to be due to the presence of different isozymes of the proteins comprising the myofibrillar complex. These differences may be obscured when the staining is carried to the point where the fibers are either black or white. In this regard Kugelberg (1976) has suggested that histochemistry is most effective for differentiating the characteristics of muscle fibers when the staining intensity is intermediate and graded. It appeared to us that it was possible to increase the sensitivity, and usefulness, of the standard histochemical methods for visualizing myofibrillar actomyosin ATPase by following its time course under carefully controlled conditions. The hypothesis tested was that the differences which exist in the myofibrillar complex of skeletal muscle could be identified from the time course of the change in the myofibrillar actomyosin ATPase during acid preincubation. Experiments conducted with skeletal muscles from several animal species support this hypothesis and have revealed that subtypes of fast-twitch and slow-twitch fibers can be reliably identified from a modification of the standard acid preincubation method for the histochemical staining for myofibrillar actomyosin ATPase.

Material and Methods

Entire muscles or muscle samples from rats, horses, rabbits, and dogs were obtained at the time of death or by needle biopsy (some horse samples). Cross-sectional specimens of muscle were placed on a cork surface and quenched in 2-methyl butane cooled to near its freezing point with liquid nitrogen. They were subsequently mounted on specimen holders with a cryoprotectant (Ames Tissue-Tek). Serial cross-sections 10 μm thick were cut at about −20° C, mounted on glass cover slips, and air dried at room temperature.

Metabolic properties of the fibers were identified from stains for a-glycerophosphate dehydrogenase (a-GPDH) (Wattenberg and Leong 1960) and reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) (Novikoff et al. 1961). Myofibrillar ATPase was stained with modifications of the methods described by Padykula and Herman (1955) and Brooke and Kaiser (1970). Numerous combinations of the pH, temperature, and incubation time were tested in an attempt to establish optimal conditions for following the sequential change in the staining pattern. Type I fibers were identified by the inactivation of myofibrillar actomyosin ATPase after alkaline preincubation (pH 10.3) (Seidel 1967) in a 0.1 M 2-amino-2-methyl-1-propanol (AMpro) buffer containing 18 mM $CaCl_2$ (Guth and Samaha 1970) for 10 min at room temperature. A number of conditions for the acid preincubation were investigated. The method that produced the most definitive and reproducible results was a preincubation in acetate buffer (0.1 M – 18 mM $CaCl_2$) at pH 4.34 at 15° C for periods between 0.25 and 5.0 min. Incubations were timed with a stop watch with all cover slips from a given series placed in the same jar. The sections were removed from the incubation media at the designated times, rinsed in an AMpro buffer (0.1 M AMpro, 18 mM $CaCl_2$, 50 mM KCl) at pH 9.4, and placed into a staining jar containing the pH 9.4 AMpro buffer. When all samples had been removed from the acid bath and in AMpro buffer, they were rinsed again in the pH 9.4 AMpro buffer prior to incubation for myofibrillar actomyosin ATPase. The incubation for the ATPase reaction was carried out at 37° C in a medium at pH 9.4 containing 0.1 M AMpro buffer, 18 mM $CaCl_2$, 50 mM KCl, and 3.1 mM ATP. Following incubation, the sections were rinsed 3 times with distilled water, soaked for 1 min in distilled water, rinsed in water an additional 3 times, immersed for 3 min in 1% $CaCl_2$, the rinse cycle repeated, immersed in 2% $CoCl_2$ for 3 min, the rinse cycle repeated, placed in 1% $(NH_4)S$ for 1 min, and rinsed extensively in distilled water prior to mounting.

Comments Concerning Methods

The unique aspect of this method for identifying skeletal muscle fiber types is that of following the change in the intensity of the myofibrillar actomyosin ATPase staining in individual fibers as a function of time. Preincubation conditions must be rigorously controlled to reproduce the staining patterns. Although Brooke and Kaiser (1974) stressed the importance of controlling temperature and pH, the ramifications of altering these have not been fully explored. Moreover, the conditions for acid preincubation are frequently described as being pH 4.35 or 4.6 at room temperature for 5 to 10 min.

The early changes in the staining pattern after exposure to acid preincubation are extremely sensitive to pH and it must be carefully controlled. The pH (4.34) that produced the best results in these experiments is not very different from that of 4.35 frequently employed by others. We did examine the effects of pH from 4.3 to 4.6 and found pHs other than 4.34 can be used. Under these conditions the time course of the staining pattern shifts as a function of pH. At lower pHs the reaction occurred too rapidly to differentiate between the fibers whereas at higher pHs it was very slow. The pH of the buffer must be established at the temperature the solution is used with a temperature compensated pH meter. We have used an Orion

model 701A pH meter with digital readout capacity to 0.001 pH units. Although it is not likely that pH can be measured with this type system to 0.001 units, this type pH meter does make it possible to adjust the solutions to the hundredth unit with greater confidence and to reduce the variability of the pH (for example, pH of 4.34 could range from 4.340 to 4.349 when adjusted with a meter having a readout capacity only to the hunderdth place). The acid preincubation must be terminated precisely at the specified time. This was conveniently done by withdrawing the cover slips from the staining jar with a forceps, immersing and rinsing them in the pH 9.4 AMpro buffer, and placing them into a staining jar containing the pH 9.4 AMpro buffer. The forceps were rinsed between each specimen removal to ensure that the AMpro buffer was not carried back into the acetate buffer and change its pH. Initially we had placed the cover slips into distilled water and rinsed them with more distilled water. This procedure produced inconsistent staining patterns for sections from the same muscle sample. The acetate buffer apparently had adhered to the tissue section and continued to alter the myofibrillar actomyosin ATPase despite extensive rinsing of the samples with distilled water. The longer the sections were left in distilled water, regardless of the number of rinses, the greater the loss of staining intensity. It is unlikely that this was caused by the distilled water, which has a pH of about 6.0, since a 1 h incubation of tissue sections in distilled water and then incubation in the ATPase incubation medium result in a staining pattern similar to that after a standard alkaline preincubation. Moreover, a check of the pH of the water in which the sections were immersed after initial rinses revealed that it was not different from that of normal distilled water. This suggested that the acetate buffer may have been bound to the tissue sections. Rinsing the sections in the pH 9.4 AMpro buffer and leaving them in this buffer until use eliminated this difficulty.

Control of the preincubation temperature is crucial. Our attempt to use room temperature (about 23° C), as suggested by others, lead to extreme variability. Temperatures ranging from 10 to 25° C (maintained with a Lauda K-2/R water bath) were tested and 15° C found to be optimal. Deviaton of the temperature from 15° C changes the time course of the staining pattern in the direction of the temperature change, i.e. at higher temperatures the sequence of staining patterns occurred earlier. The acetate buffer and staining jars containing the cover slips with the tissue sections were equilibrated in the water bath at least 10 min prior to placing the acetate buffer in the staining jars. The ATPase reaction was conducted at 37° C in a Dubnoff shaking waterbath. The reaction medium and staining jars were brought to 37° C by being placed into the bath before the reaction medium was poured into the staining jars.

The time periods that we have used (0.25, 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 min) were selected to produce a sharp separation of fiber groups. Ideally it would be best to continuously follow the time course of the change in each fiber. Methodological constraints preclude this in the routine application of the method. Other time intervals for stopping the reaction could be used including more frequent sampling.

Results

Preincubation of sections of rat plantaris muscle at pH 10.3 and the subsequent demonstration of myofibrillar actomyosin ATPase produced the standard black and white dichotomy of fibers (Fig. 1a). The nomenclature of type I and II has been used to designate the light and dark staining fibers, respectively, after alkaline preincubation. This is synonymous with a nomenclature of ST and FT, which has a physiological connotation. Fibers could not be further subclassified by altering either the time or pH of the alkaline preincubation. The time course of the change in myofibrillar actomyosin ATPase staining intensity for the rat plantaris muscle after acid preincubation is illustrated in Fig. 1. Acid preincubation for 0.25 min (Fig. 1c) produced a staining pattern similar to that after alkaline preincubation. With longer periods of acid treatment there was a progressive diminution of myofibrillar actomyosin ATPase staining intensity in type II (FT) fibers and an enhancement of that for type I (ST) fibers. At least three types of type II fibers were identifiable on the basis of the time course for the loss of staining intensity. These are indicated on the Figs. as 1, 2, and 3 and are hereafter referred to as A1, A2, and A3. The A is used to signify acid lability of myofibrillar actomyosin ATPase and the numbers to indicate the rank order of this sensitivity. With longer periods of preincubation at pH 4.34 there was a progressive darkening of the staining intensity of the type I fibers that ultimately produced the standard reversal of myofibrillar actomyosin ATPase observed by others (Figs. 1a–h). After about 2 min of acid preincubation the staining intensity of the type I fibers for myofibrillar actomyosin ATPase was similar to that of the type IIA1 fiber (Fig. 1f).

Oxidative potential, as indicated by NADH-TR staining intensity (Fig. 1b), varied widely throughout most samples but was systematically high in type I fibers. Wide variation existed in the staining intensity of the type II fibers for NADH-TR. The type IIA1 fibers generally, but not always, stained intensely for NADH-TR. The combination of a dark stain for myofibrillar actomyosin ATPase after alkaline preincubation and an intense NADH-TR stain, therefore, could not be used a criterion for identifying IIA1 fibers. Figure 2 illustrates the lack of congruity between the staining for oxidative potential and myofibrillar actomyosin ATPase sensitivity to acid inactivation fibers of the rat plantaris muscle. Staining for a-GPDH (micrographs not included) resulted in a more intense staining of type II than type I fibers. However, there was no consistent staining pattern that corresponded to the subtypes of type II fibers.

The myofibrillar actomyosin ATPase staining pattern for the rat soleus muscle (Fig. 3) after acid preincubations differed from that of the plantaris muscle. After 15 s of acid treatment type I fibers were tan rather than white as was the case for the plantaris muscle. Type II fibers were initially black and similar to those in the plantaris muscles at this point. With longer periods of acid treatment some type II fibers became ATPase negative, however, others did not become ATPase negative after 5 min of acid preincubation. Type I fibers of the soleus muscle retained a tan staining intensity throughout the entire period of acid treatment and did not reverse to black

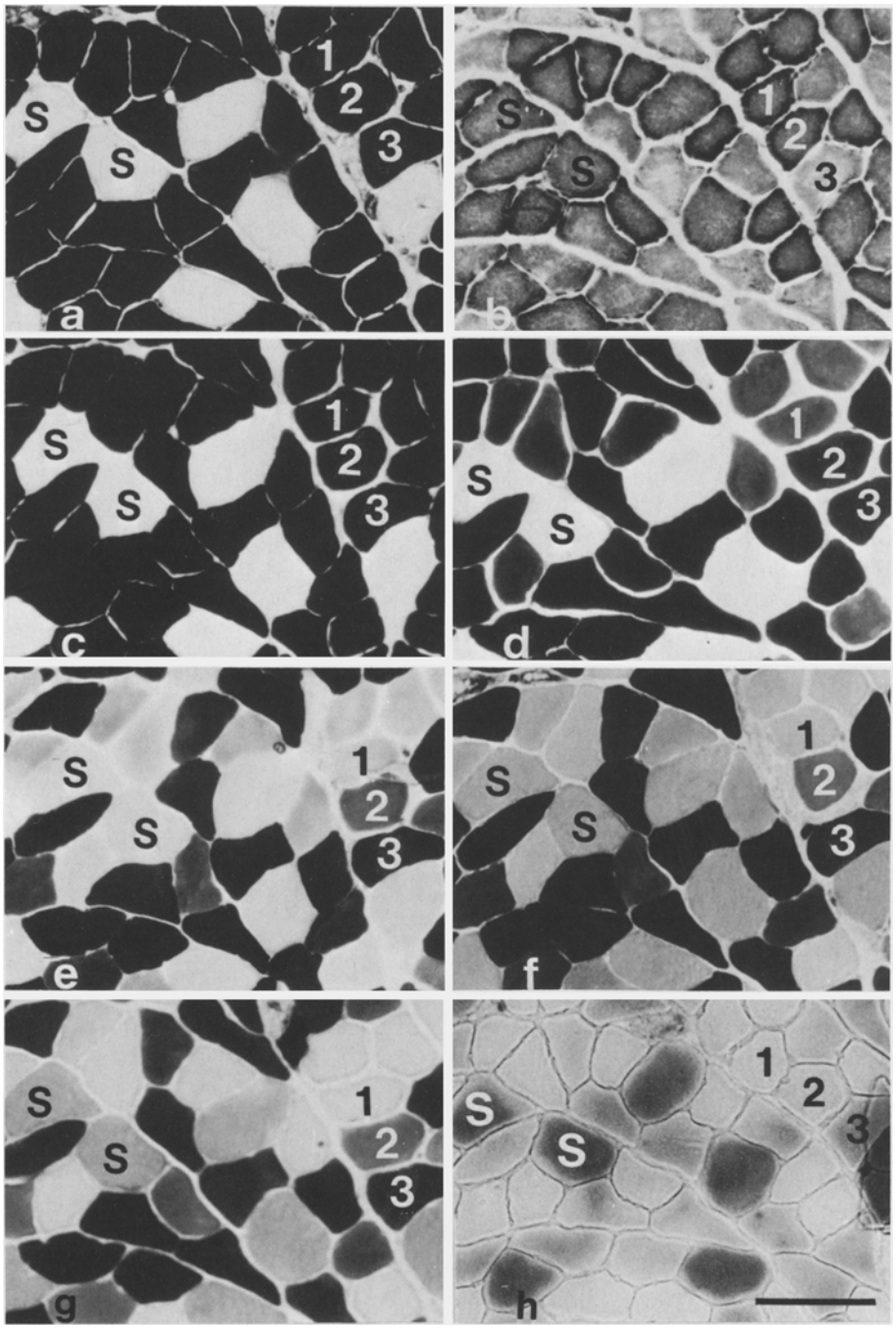


Fig. 1. The histochemical staining of serial cross sections of the rat plantaris muscle to visualize myofibrillar actomyosin ATPase (pannels a and c–h) and NADH-TR (pannel b). Pannel a is a myofibrillar actomyosin ATPase stain after preincubation at pH 10.3. ATPase negative fibers are identified as type I (s) fibers. The dark staining fibers are identified as type II fibers with the 3 subtypes indicated as 1, 2, and 3. Pannels c through h are sections preincubated at pH 4.34 at 15° C for periods of 0.25, 1.0, 1.5, 2.0, 3.0, and 5.0 min. ——=100 μm

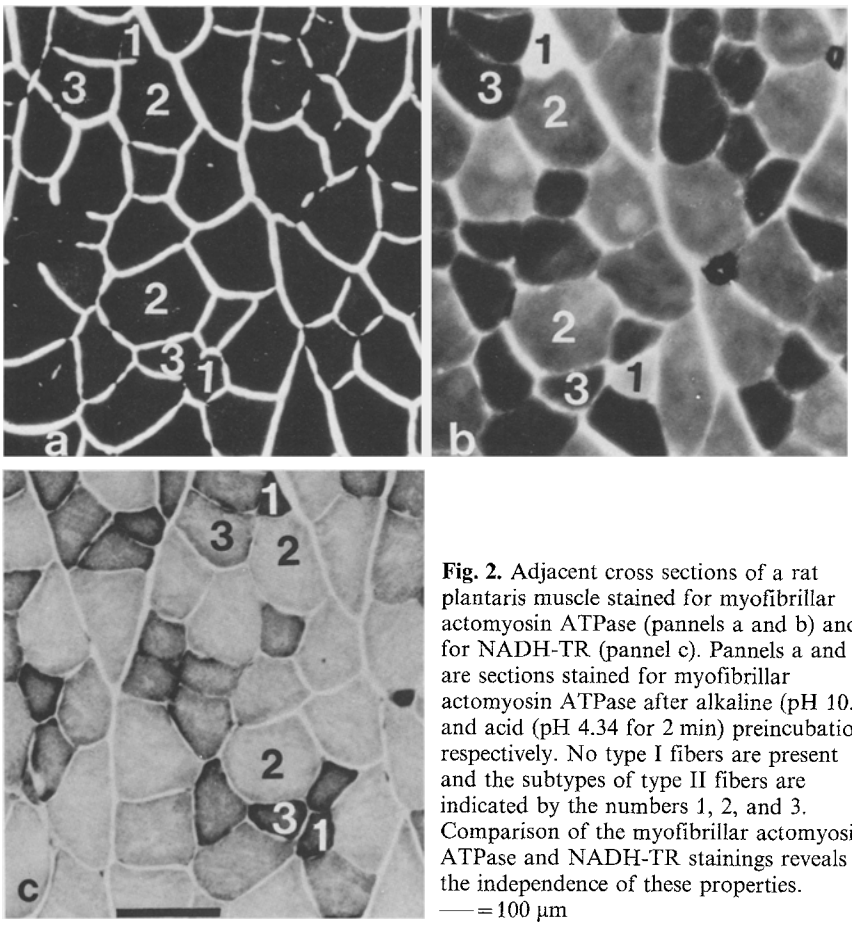
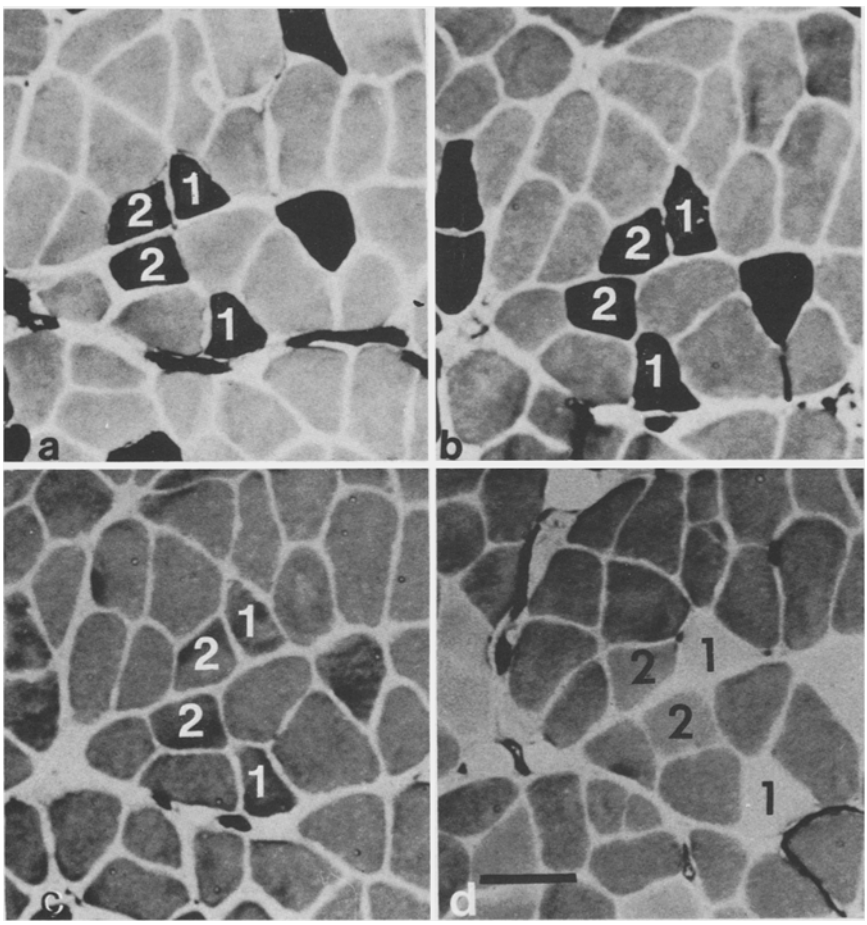


Fig. 2. Adjacent cross sections of a rat plantaris muscle stained for myofibrillar actomyosin ATPase (pannels a and b) and for NADH-TR (pannel c). Pannels a and b are sections stained for myofibrillar actomyosin ATPase after alkaline (pH 10.3) and acid (pH 4.34 for 2 min) preincubations, respectively. No type I fibers are present and the subtypes of type II fibers are indicated by the numbers 1, 2, and 3. Comparison of the myofibrillar actomyosin ATPase and NADH-TR stainings reveals the independence of these properties. —— = 100 μm

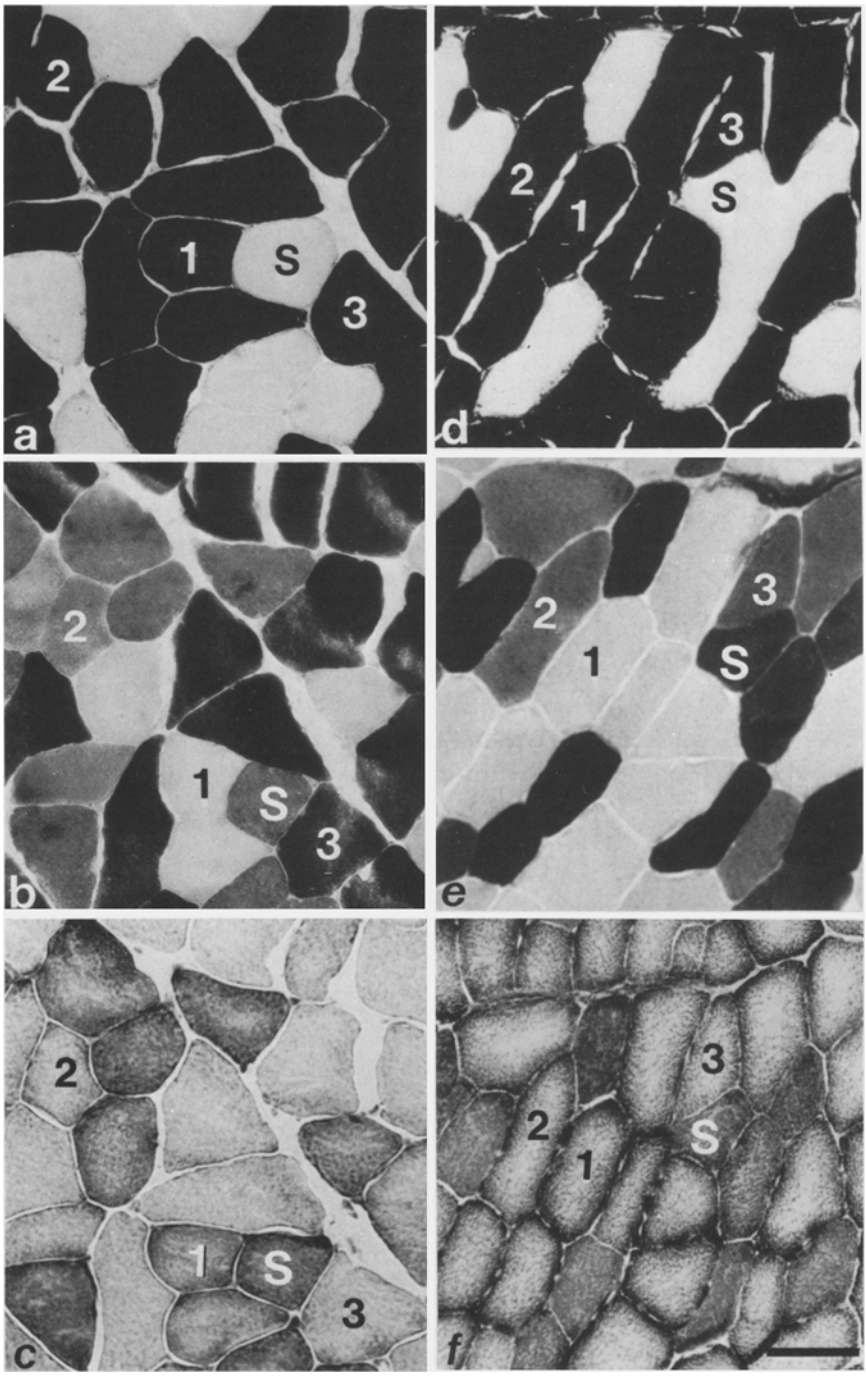
as was characteristic for the type I fiber of the plantaris or gastrocnemius muscles of the rat or other species. A similar staining pattern existed for the rabbit soleus muscle. This suggests that type I fibers of the soleus muscle are a phenotype different from type I fibers found in other muscles such as the plantaris muscle. These fibers have been identified as type I soleus type (ISLt) or ST soleus type (STSLt). The time course for loss of myofibrillar staining intensity of the type II fibers in the soleus muscle differentiated these into subtypes (see Fig. 3). All type II fibers in the soleus muscle stained intensely for NADH-TR.

Muscles from other animal species were essentially similar to those of the rat. Species differences do exist in the time course for the change in myofibrillar actomyosin ATPase after acid preincubation. However, the basic staining pattern was reproducible in the biceps and triceps from the dog; the biceps femoris, vastus lateralis, semitendinosus, deltoid, gluteal, diaphragm, and triceps of the horse; the plantaris, gastrocnemius, diaphragm, and soleus of the rabbit; and the plantaris and soleus of the cat.



Figs. 3a–d. Serial cross sections of rat soleus muscle stained for myofibrillar actomyosin ATPase after alkaline (pH 10.3) (**a**) and acid (pH 4.34) (**b–d**) preincubation. The pale and dark fibers in pannel a are types I and II fibers, respectively. Sections in pannels b, c, and d were stained after 0.5, 2.0, or 5.0 min or acid preincubation. Numbers 1 and 2 identify type II fibers with different sensitivities of the myofibrillar actomyosin ATPase inactivity with acid preincubation. —— = 100 μm

Figure 4 demonstrates this for the rabbit gastrocnemius (a–c) and horse deltoid (d–f) muscles. In these cases only the alkaline preincubation (a and d), one sample after 2 min of acid preincubation (b and e), and the NADH-TR (c and f) are presented. These figures illustrate a species difference for the type I fiber of the horse deltoid demonstrating a complete staining reversal after two min of acid preincubation whereas for the rabbit gastrocnemius muscle the type I fibers (indicated as S) were only tan. In each case it is possible to identify IIA1, IIA2, and IIA3 fibers after 2 min of acid preincubation.



Figs. 4a–f. Serial sections from the rabbit gastrocnemius (**a–c**) and horse deltoid (**d–f**) strained for myofibrillar actomyosin ATPase after alkaline (pH 10.3) (**a** and **b**) preincubation, acid preincubation (pH 4.34) for 2 min (**b** and **e**), and NADH-TR (**c** and **f**). Symbols are S for type I fibers and 1, 2, and 3 for the types II fibers with different sensitivities for inactivation of myofibrillar actomyosin ATPase during acid preincubation. —— = 100 μm

Discussion

This study demonstrates the existence of a time dependent differential for acid inactivation of the myofibrillar actomyosin ATPase of skeletal muscle fibers. The ability to subjectively identify three subtypes of type II fibers is not surprising since there are polymorphic forms of the proteins that comprise the myofibrillar complex (Gauthier and Lowey 1977; Pierobon-Bormioli et al. 1981; Hoh 1979; Bronson and Schachat 1982; Billeter et al. 1981). The present results are consistent with the existence of 3 isomyosins from fast muscle and 2 from slow muscle in several species (Hoh et al. 1979; Fitzsimons and Hoh 1981; d'Albis et al. 1982). This does not appear to be associated with the light chain composition of myosin (Pette and Schnez 1977). The histochemical demonstration of two types of type I fibers, one in mixed muscles such as the gastrocnemius and another in predominately slow twitch soleus muscle, is consistent with the observation that slow twitch motor units in the gastrocnemius muscle exhibit post tetanic potentiation (Burke et al. 1973) whereas there is a depression in twitch tension of the soleus muscle (Brown and von Euler 1938; Close and Hoh 1969).

The three subtypes of type II fibers identifiable from the time course of the acid inactivation of myofibrillar actomyosin ATPase are not synonymous with the IIa, IIb, and IIc fibers identified after preincubations at pHs of 10.4, 4.6, and 4.35. Type IIc fibers retain an intense stain after all preincubations. They usually constitute a small percentage of the total fiber population. This is not the case with the method described here. Armstrong et al. (1982) and Snow et al. (1982) have reported the absence of type IIb fibers in dog muscle. We have observed myofibrillar actomyosin ATPase staining pattern for dog muscle that is similar to that of other species. These fibers, however, are not concordant with the subtypes of type II fibers described by Snow et al. (1982) which includes a metabolic component in the classification scheme.

The classification scheme proposed by Brooke and Kaiser (1970) is based on the sensitivity of the myofibrillar actomyosin ATPase to acid and alkaline activation and inactivation. The method presented here is only a modification of these concepts with the addition of following the time course of the response in a system where the temperature and pH of the acid preincubation are carefully controlled. The ability to follow fibers through the progression from an initial black to a negative stain or the reverse gives this method a heretofore lacking dimension. The results demonstrate that all type II fibers that are ATPase negative after a period of exposure to preincubation at pH 4.34, for example 3 min, are not necessarily similar. Moreover, all fibers that are ATPase negative after alkaline preincubation are not the same. Thus, the type I fiber of the soleus is different from the type I fiber of the plantaris muscle for all samples that we have examined.

The subclassification of fibers as outlined in this paper relies only upon the sensitivity of the myofibrillar actomyosin ATPase to alteration following acid and alkaline treatment. ATPases other than those associated with the myofibrils, such as mitochondrial and sarcoplasmic, exist in skeletal muscle that could mask the myofibrillar actomyosin ATPase or produce artifacts

in the staining patterns. Attempts to identify such artifactual staining patterns included addition of NaN_3 to the reaction mixture to inhibit mitochondrial ATPase and extraction of lipids from the tissue by pretreatment with acetone or Triton X-100. In all cases the staining patterns were unaltered. The observation that some of the fibers that stained most intensely for NADH-TR, and therefore had a high mitochondrial density, were the first to become ATPase negative argues against the staining pattern being influenced by mitochondrial ATPases. Mabuchi and Sréter (1980) reached a similar conclusion when proposing an alternate method for identifying fiber types in skeletal muscle.

The results of the present study demonstrating a lack of concordance between myofibrillar actomyosin ATPase and oxidative potential are consistent with recent reports (Reichmann and Pette 1982; Sickles et al. 1982) and supports the conclusion that oxidative capacity is not a reliable determinant for the histochemical subclassification of fibers.

Kugelberg (1976) reported that the gradation in the staining intensity for motor units of the rat soleus as visualized by histochemistry is correlated to contraction speed. The method as presented here did not produce a similar gradation in staining intensity for the type I fibers in any of the muscles that were examined. Occasionally there are type I fibers that display unusual staining characteristics, such as off brown after alkaline preincubation. However, their number was so small as to make it questionable whether they could comprise a complete motor unit.

The modified stain procedure presented here for identifying fiber types does not add any new information about the protein composition of the fibers. Additional studies are needed to explore this point. Moreover, we have not conducted experiments to relate contractile time to the staining patterns for any muscle or differences in the use of the fibers as indicated by glycogen depletion of motor units during exercise. These studies do point to the difficulties in purifying proteins from the so-called homogeneous muscle and suggest that caution must be exercised in the production of antibodies for use in the immunocytochemical method for fiber type identification. The pure protein for antibody production may be extremely difficult to obtain.

The fact that the muscles we have studied generally conform to the same pattern of staining does not imply that all muscles of all animals possessed identical fiber types. Species differences do exist as indicated above which may make it necessary to alter the time, pH, or temperature of the acid preincubations to more effectively differentiate the fibers. The advantage of the method is that it is an objective procedure that can be used to subclassify fibers in skeletal muscle with the relative ease and economy of standard histochemistry.

References

Armstrong RB, Saubert CW, IV, Seeherman HJ, Taylor CR (1982) Distribution of fiber types in locomotory muscles of dogs. Am J Anat 163:87–98

Ashmore CR, Kikuchi T, Doerr L (1978) Some observations on the innervation patterns of different types of chick muscle. Exp Neurol 58:272–284

Billeter R, Heizmann CW, Howald H, Jenny E (1981) Analysis of myosin light and heavy chain types in single human skeletal muscle fibers. Eur J Biochem 116:389–395
Brooke MH, Kaiser KK (1969) Some comments on the histochemical characterization of muscle adenosine triphosphatase. J Histochem Cytochem 17:431–432
Brooke MH, Kaiser KK (1970) Three "myosin adenosine triphosphatase" systems: the nature of their pH lability and sulfhydryl dependence. J Histochem Cytochem 18:670–672
Brooke MH, Kaiser KK (1974) The use and abuse of muscle histochemistry. Ann NY Acad Sci 228:121–144
Bronson DD, Schachat FH (1982) Heterogeneity of contractile proteins. J Biol Chem 257:3937–3944
Brown GL, von Euler US (1938) The after effects of a tetanus on mammalian muscle. J Physiol 93:39–60
Burke RE, Levine DN, Salcman M, Tsaris P (1973) Motor units in cat soleus muscle: Physiological, histochemical, and morphological characteristics. J Physiol 238:723–748
Close RI, Hoh JFY (1969) Post-tetanic potentiation of twitch contractions of cross-innervated rat fast and slow muscles. Nature 221:179–181
d'Albis A, Pantaloni C, Bechet J-J (1982) Myosin isoenzymes in adult rat skeletal muscles. Biochimie 64:399–404
Fitzsimons RB, Hoh JFY (1981) Isomyosins in human type 1 and type 2 skeletal muscle fibres. Biochem J 193:229–233
Gauthier GF, Lowey S (1979) Distribution of myosin isoenzymes among skeletal muscle fiber types. J Cell Biol 81:10–25
Green HJ, Reichmann H, Pette D (1982) A comparison of two ATPase based schemes for histochemical muscle fibre typing in various mammals. Histochemistry 76:21–31
Guth L, Samaha FJ (1969) Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. Exp Neurol 25:138–152
Guth L, Samaha FJ (1970) Procedure for the histochemical demonstration of actomyosin ATPase. Exp Neurol 28:365–367
Hayashi M, Feiman D (1966) An improved method of fixation for formalin sensitive enzymes with special reference to myosin adenosine triphosphatase. J Histochem Cytochem 14:577–581
Hoh JFY (1979) Developmental changes in chicken skeletal myosin isoenzymes. FEBS Lett 98:269–270
Hintz CS, Lowry CV, Kaiser KK, McKee D, Lowry OH (1980) Enzyme levels in individual rat muscle fibers. Am J Physiol 239:C58–C65
Kugelberg E (1976) Adaptive transformation of rat soleus muscle motor units during growth. J Neurol Sci 27:269–289
Lowry CV, Kimmey JS, Felder S, Chi MM-Y, Kaiser KK, Passoneau PN, Kirk KA, Lowry OH (1978) Enzyme patterns in single human muscle fibers. J Biol Chem 253:8269–8277
Mabuchi K, Sréter FA (1979) Actomyosin ATPase II. Fiber typing by histochemical ATPase reaction. Muscle Nerve 3:233–239
Müntener M (1979) Variable pH dependence of the myosin-ATPase in different muscles of the rat. Histochemistry 62:299–304
Novikoff AF, Schin W, Drucker J (1961) Mitochondrial localization of oxidation enzymes: staining result with two tetrazolium salts. J Biophys Biochem Cytol 9:47–61
Padykula HA, Herman E (1955) The specificity of the histochemical method for adenosine triphosphatase. J Histochem Cytochem 3:170–195
Pette D, Schnez U (1977) Myosin ligh chain patterns of individual fast and slow-twitch fibres of rabbit muscles. Histochemistry 54:97–107
Pette D, Spamer C (1979) Metabolic subpopulations of muscle fibers. Diabetes 28: (Suppl 1) 25–29
Pierobon-Bormioli S, Sartore S, Dalla Libera L, Vitadello M (1981) "Fast" isomyosins and fiber types in mammalian skeletal muscle. J Histochem Cytochem 29:1179–1188
Reichmann H, Pette D (1982) A comparative microphotometric study of succinate dehydrogenase activity levels in type I, IIa and IIb fibres of mammalian and human muscles. Histochemistry 74:27–41
Seidel JC (1967) Studies on myosin from red and white skeletal muscles of the rabbit. II.

Inactivation of myosin from red muscles under mild alkaline conditions. J Biol Chem 242:5623–5629
Sickles DW, McLendon RE, Rosenquist TH (1982) Alternative method for quantiative enzyme histochemistry of muscle fibers. Histochemistry 75:577–588
Snow DH, Billeter R, Mascarello F, Carpene E, Rowlerson A, Jenny E (1982) No classical type IIB fibres in dog skeletal muscle. Histochemistry 75:53–65
Soukup T, Vydra J, Cerný M (1979) Changes in ATPase and SDH reactions of the rats extrafusal and intrafusal muscle fibres after preincubations at different pH. Histochemistry 60:71–84
Spamer C, Pette D (1980) Meteabolic subpopulations of rabbit skeletal muscle fibres. In: Pette D (ed) Plasticity of muscle. W. De Gruyter, Berlin, pp 19–30
Spurway NC (1981) Interrelationship between myosin-based and metabolism-based classifications of skeletal muscle fiber. J Histochem Cytochem 29:87–88
Wattenberg LW, Leong JL (1960) Effect of coenzyme Q_{10} and menadione on succinate dehydrogenase activity as measured by tetrazolium salt reduction. J Histochem Cytochem 8:269–303

Received November 19, 1982
Accepted January 19, 1983