Metabolic profiles of three fiber types of skeletal muscle in Guinea pigs and rabbits

ARTICLE *in* BIOCHEMISTRY · AUGUST 1972

Impact Factor: 3.02 · DOI: 10.1021/bi00764a013 · Source: PubMed

5 AUTHORS, INCLUDING:

Victor Edgerton
University of California, Los Angeles
607 PUBLICATIONS **25,813** CITATIONS

SEE PROFILE

Fuchs, F. (1971), *Biochim. Biophys. Acta 245*, 221.
Goodman, M., and Toniolo, C. (1968), *Biopolymers 6*, 1673.
Greaser, M. L., and Gergely, J. (1971), *J. Biol. Chem. 246*, 4226.
Han, M. H., and Benson, E. S. (1970), *Biochem. Biophys. Res. Commun. 38*, 378.
Hartshorne, D. J., and Mueller, H. (1968), *Biochem. Biophys. Res. Commun. 31*, 647.
Hartshorne, D. J., and Pyun, H. Y. (1971), *Biochim. Biophys. Acta 229*, 698.
Murray, A. C., and Kay, C. M. (1971), *Biochem. Biophys. Res. Commun. 44*, 237.
Richards, E. G., Teller, D. C., and Schachman, H. K. (1968), *Biochemistry 7*, 1054.
Schaub, M. C., Perry, S. V., and Hacker, W. (1972), *Biochem. J. 126*, 237.
Shapiro, A. L., Vinuela, E., and Maizel, J. V. (1967), *Biochem. Biophys. Res. Commun. 28*, 815.
Yphantis, D. A. (1964), *Biochemistry 3*, 297.

Metabolic Profiles of Three Fiber Types of Skeletal Muscle in Guinea Pigs and Rabbits†

James B. Peter,* R. James Barnard, V. Reggie Edgerton, Cynthia A. Gillespie, and Kerstin E. Stempel

ABSTRACT: Certain guinea pig and rabbit skeletal muscles, which are composed solely or predominantly of a single type of fiber as defined histochemically, were analyzed for various enzymatic and substrate characteristics. The results show that fiber types in the adult guinea pig and rabbit limb muscles can be conveniently classified into three categories on the basis of three criteria, (1) the contraction time of the fiber relative to others within the same animal, (2) glycolytic capacity, and (3) oxidative capacity. The rabbit semimembranosus accessorius and the white portion of the guinea pig vastus lateralis are fast twitch and have a very high anaerobic capacity, as indicated by glycogen concentration and phosphorylase, lactate dehydrogenase and mitochondrial α-glycerophosphate dehydrogenase activities. Cytochrome concentration and succinate dehydrogenase activity are low, indicating a low aerobic capacity. Consequently these fibers, formerly termed fast-twitch white, are more explicitly called fast-twitch-glycolytic fibers. The red portion of the guinea pig vastus lateralis has a high glycogen concentration, moderate lactate dehydrogenase, and high phosphorylase and α-glycerophosphate dehydrogenase activities. The red vastus lateralis has the highest cytochrome concentration and succinate dehydrogenase activity, indicating a high aerobic capacity in addition to moderate to high glycolytic capacity. Consequently these fibers, previously labeled fast-twitch red, can more explicitly be called fast-twitch-oxidative-glycolytic fibers. Although this fiber type is found in the rabbit, no muscle consisting predominantly of fibers that are fast-twitch-oxidative-glycolytic was found. The soleus muscle is slow twitch and chemically is characterized by low glycogen concentration and low phosphorylase, lactate dehydrogenase, and mitochondrial α-glycerophosphate dehydrogenase activities together with a moderate cytochrome concentration and succinate dehydrogenase activity. These features indicate a moderate to high aerobic and a relatively low glycolytic capacity. Formerly labeled the slow-twitch intermediate fiber they are more appropriately called slow-twitch-oxidative fibers. These data provide quantitative information on the metabolic characteristics of the three distinct muscle fiber types previously described by histochemical techniques. Furthermore, since muscles composed of fast-twitch-oxidative-glycolytic or slow-twitch-oxidative fibers are red in appearance the classification of skeletal muscle solely as "red" and "white" is imprecise and incomplete. Failure to recognize this can complicate the interpretation of studies with skeletal muscle.

In 1873 Ranvier reported that muscles which were slow contracting appeared red whereas fast contracting muscles appeared white. Exceptions to this general relationship between "redness" of a muscle and speed of contraction were later reported by Denny-Brown (1929). More recent investigations using a variety of histochemical techniques have demonstrated that most skeletal muscles are composed of different types of fibers and that muscles cannot be simply categorized as "red" or "white" (Barnard *et al.*, 1971; Edgerton and Simpson, 1969; Guth and Samaha, 1969; *cf.* Peter, 1972).

Histochemical, biochemical, and physiological studies of hind-limb skeletal muscle of guinea pigs have demonstrated the presence of three fiber types, each of which possesses a distinctive combination of histochemical and mechanical characteristics (Barnard *et al.*, 1971). These three fiber types were classified as fast-twitch red, fast-twitch white, and slow-twitch intermediate based on (1) their twitch characteristics which correlate with the specific activity of myosin ATPase and (2) histochemical assessment of their oxidative and glycolytic capacities (Edgerton and Simpson, 1969; Gillespie *et al.*, 1970; Barnard *et al.*, 1971; Peter, 1972).

The purpose of this paper is to provide quantitative data on the enzyme activities and glycogen concentration in muscles

† From the Neuromuscular Diseases Research Group and the Departments of Medicine and Physical Education, University of California, Los Angeles, Los Angeles, California 90024. *Received December 14, 1971.* Supported by National Institutes of Health Grants HD 02584, NS 07587, and NS 08590. Dr. R. James Barnard is a postdoctoral fellow of the Muscular Dystrophy Associations of America.

composed predominately or exclusively of one of these types of fibers. Based on the data obtained and on the histochemical and contractile properties described earlier (Edgerton and Simpson, 1969; Barnard *et al.*, 1971; *cf.* Peter, 1972, for review) a more explicit nomenclature is proposed in which the terms fast-twitch white, fast-twitch red, and slow-twitch intermediate are replaced by fast-twitch-glycolytic, fast-twitch-oxidative-glycolytic, and slow-twitch-oxidative, respectively.

Methods

Muscle Preparation. The muscles used in this study were obtained from adult guinea pigs and rabbits. The soleus (100% slow-twitch-oxidative fibers), the red portion of the vastus lateralis (78% fast-twitch-oxidative-glycolytic fibers), and the white portion of the vastus lateralis (71% fast-twitch-glycolytic fibers) were taken from the guinea pig. The soleus (96% slow-twitch-oxidative fibers) and the semimembranosus accessorius[1] (86% fast-twitch-glycolytic fibers) were taken from the rabbit. The guinea pigs were sacrificed by a sharp blow to the head and the rabbits by intravenous injection of air. The muscles were quickly removed, trimmed, and weighed.

Muscles used for cytochrome, succinate dehydrogenase, and mitochondrial α-glycerophosphate dehydrogenase assays were immediately frozen in liquid nitrogen and stored at $-70°$ for 3–6 days. Preliminary experiments showed no loss in enzyme activity with short periods of freezing. Myoglobin determinations were also done on frozen muscle.

For the determination of hexokinase and lactate dehydrogenase fresh muscle was homogenized in cold (1–4°) buffer medium (pH 7.4) containing 50 mM Tris, 1 mM EDTA, 15 mM K_2SO_4, and 6 mM $MgCl_2$. Fresh muscles used for phosphorylase determinations were homogenized in 100 mM Tris (pH 7.4) and muscles used for α-1,4-glucosidase determinations were homogenized in distilled water. For the determinations of cytochromes, succinate dehydrogenase, and mitochondrial α-glycerophosphate dehydrogenase the frozen muscles were thawed and homogenized in a buffer medium (pH 7.4) containing 50 mM Tes, 100 mM KCl, 5 mM $MgSO_4$, 1 mM EDTA, and 50 mM KP_i.

Immediately after sacrifice the trimmed muscles to be assayed for glycogen were placed in hot KOH (3%) for glycogen extraction and subsequent acid hydrolysis (Gillespie *et al.*, 1970). Glucose was then measured according to the method of Nelson (1944).

For myoglobin determinations, the muscles were thawed, homogenized with sand in a mortor and dialyzed overnight against 0.1 M Tris (pH 8.2). Supernatants were then chromatographed on a Cellex D (DEAE) column and myoglobin was determined according to Brown (1961) with the following exceptions. The precipitation of myoglobin with ammonium sulfate was eliminated and the supernatant from the first centrifugation was dialyzed with Tris buffer for 24 hr and then separated by column chromatography.

Enzyme Assays. All enzyme activities were determined on a Gilford 2000 recording spectrophotometer at 37° and are expressed as initial maximum rates.

Hexokinase (EC 2.7.1.1) activity was measured by a modification of the method of Sharma *et al.* (1963). This method is based on the rate of NADPH formation in a medium containing 50 mM Tris, 2.25 mM K_2SO_4, 8 mM $MgCl_2$, 3 mM ATP, 0.2 mM EDTA, 0.75 mM NADP, 2.5 mM glucose, and excess glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Measurements were done on both the gauze-filtered homogenate and 105,000*g* supernatant.

Phosphorylase (EC 2.4.1.1) activity was determined in a medium (pH 7.4) containing 75 mM Tris, 5 mM $MgCl_2$, 0.5 mM NADP, 10 mM KP_i, 0.6 mM AMP, 1.3% glycogen, and excess glucose-6-phosphate dehydrogenase and phosphoglucomutase. Measurements were made on the 270*g* supernatant which contains all of the phosphorylase activity. This assay medium measures total phosphorylase activity.

Lactate dehydrogenase (EC 1.1.1.27) activity was measured as the initial rate of oxidation of NADH at pH 7.4 in a medium containing 50 mM KP_i, 0.45 mM NADH, and 0.35 mM sodium pyruvate. Determinations were done on the 105,000*g* supernatant which contained all of the lactate dehydrogenase activity.

Succinate dehydrogenase (EC 1.3.99.1) activity was measured according to a modification of the ferricyanide method of Bonner (1955). The gauze-filtered whole homogenate was first preincubated at 37° for 5 min to deplete the endogenous substrate. The reaction mixture (pH 7.4) contained 0.1 M KP_i, 10 mM KCN, 1 mM $K_3Fe(CN)_6$, and 20 mM sodium succinate. Rotenone (20 μM) was added to block the oxidation of NADH.

α-Glycerophosphate dehydrogenase (EC 1.1.2.1) activity was measured by the ferricyanide method described by Bass *et al.* (1969). The gauze-filtered homogenate was preincubated for 5 min at 37°. The reaction mixture contained 0.1 M KP_i (pH 7.4), 5 mM EDTA, 10 mM KCN, 1 mM $K_3Fe(CN)_6$, 0.4 g of bovine albumin/100 ml, 25 mM α-glycerophosphate, and 20 μM rotenone.

α-1,4-Glucosidase (EC 3.2.1.20) activity was determined by the method described by Hudgson *et al.* (1968). The homogenate was incubated at 37° with shaking in a medium consisting of 5 mM maltose in 0.1 M acetate (pH 4.0). Aliquots were removed from the reaction mixture at 30 min intervals and immediately placed in 12% $HClO_4$. The solution was then neutralized with K_2CO_3 and centrifuged at 10,000*g* for 10 min. The amount of glucose formed was determined by the maximum change in optical density when an aliquot was added to a medium containing 100 mM Tris (pH 7.4), 5 mM $MgCl_2$, 0.5 mM NADP, 1 mM ATP, and excess hexokinase and glucose-6-phosphate dehydrogenase.

Cytochromes *a* and *c* concentrations were measured by the difference spectra method of Schollmeyer and Klingenberg (1962). Complete oxidation of the cytochromes was produced in a test tube containing 2 μM carbonyl cyanide *m*-chlorophenylhydrazone, and 1.5 ml of homogenate (1:7, w/v). To a second test tube containing 2 μM carbonyl cyanide *m*-chlorophenylhydrazone and 8 mM sodium succinate, 1.5 ml of homogenate was added and shaken for 1 min; freshly prepared potassium cyanide (2.5 mM) was then added and the solution incubated at 37° for 5 min. Both tubes were gassed for 30 sec with 100% O_2 and the difference spectra then recorded on a Cary 14 spectrophotometer. The difference in optical density between the completely oxidized and completely reduced forms was determined at the following wave lengths: cytochrome $c + c_1$, 550–541 mμ, and cytochrome *a*, 605–630 mμ. Extinction coefficients of 14 and 18 were used for cytochromes *a* and *c*, respectively.

[1] Anatomy and abbreviations used are: exact location of the semimembranosus accessorius is described by Kerr (1955). The small muscle located in the middle of the semimembranosus accessorius is the semimembranosus proprius and is composed predominately of slow-twitch-oxidative fibers; Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

TABLE I: Guinea Pig Skeletal Muscle Characteristics.

Muscle	Red Vastus	White Vastus	Soleus
Gross appearance	Red	White	Red
Histochemical fiber type (%)[a]			
Fast-twitch-oxidative-glycolytic	78 ± 2	29 ± 2	0
Fast-twitch-glycolytic	18 ± 2	71 ± 0.6	0
Slow-twitch-oxidative	4 ± 0.5	0.0	100
Time-to-peak tension (msec)[a]	19.0 ± 0.4	20.1 ± 0.4	82.3 ± 1.3
ATPase (μmoles/min per mg of natural actomyosin), pH 9.4			
Actomyosin	0.16 ± 0.02	0.27 ± 0.01	0.04 ± 0.01
Myosin	0.34 ± 0.02 (6)	0.32 ± 0.01 (6)	0.14 ± 0.01 (6)
Glycogen[a] (mg/g wet weight)	9.7 ± 0.8 (11)	7.4 ± 0.6 (11)	3.3 ± 0.4 (5)
Phosphorylase (μmoles/min per g)			
270g supernatant	6.67 ± 0.66 (8)	7.13 ± 0.72 (7)	1.54 ± 0.09 (6)
Lactate dehydrogenase (μmoles/min/g), 105,000g supernatant	218 ± 19 (9)	449 ± 41 (7)	105 ± 7 (6)
α-Glycerophosphate dehydrogenase (μmoles/min per g)			
Whole homogenate	1.67 ± 0.16 (6)	1.62 ± 0.10 (6)	0.61 ± 0.05 (6)
Hexokinase (nmoles/min per g)			
Whole homogenate	620 ± 54 (6)	297 ± 57 (5)	978 ± 59 (4)
105,000g supernatant	177 ± 17	71 ± 11	358 ± 30
α-1,4-Glucosidase (nmoles/min per g)			
Whole homogenate	22.1 ± 1.9 (4)	9.9 ± 0.7 (4)	25.5 ± 1.0 (5)
Succinate dehydrogenase (μmoles/min per g)			
Whole homogenate	2.49 ± 0.29 (6)	0.72 ± 0.05 (5)	1.95 ± 0.17 (4)
Cytochrome a (nmoles/g)			
Whole homogenate	12.8 ± 0.7 (5)	2.2 ± 0.3 (7)	4.8 ± 0.5 (4)
Cytochrome c (nmoles/g)			
Whole homogenate	18.0 ± 0.9 (5)	1.9 ± 0.3 (7)	6.5 ± 0.3 (4)
Myoglobin (mg/g)	1.44 ± 0.20 (14)	0.31 ± 0.07 (5)	1.39 ± 0.15 (8)

[a] Data cited from previous publications (Barnard *et al.*, 1971; Gillespie *et al.*, 1970). Values are means ± std dev. The number of muscles analyzed is given in parentheses.

Results

Table I summarizes the observations made on the three different guinea pig muscles. The red vastus, white vastus, and soleus are composed predominately of fast-twitch-oxidative-glycolytic, fast-twitch-glycolytic, and slow-twitch-oxidative fibers, respectively, as determined by the histochemical activity of myofibrillar ATPase, mitochondrial α-glycerophosphate dehydrogenase, NADH-diaphorase, and malate dehydrogenase (Figure 1e–h). The specific activity of myosin ATPase measured in homogenates of these muscles is consistent with physiological and histochemical findings (Table I; Figure 1; Barnard *et al.*, 1971) in that muscles with fast contraction times have a higher specific activity of myosin ATPase and stain darker at pH 9.4 for myofibrillar ATPase than do the slow-twitch muscles (Tables I and II).

The serial sections of rabbit (Figure 1a–d) and guinea pig (Figure 1e–h) gastrocnemius show that fibers staining darkest with myofibrillar ATPase also stain darkest with mitochondrial α-glycerophosphate dehydrogenase and phosphorylase (not shown) and lightest with NADH-diaphorase and malate dehydrogenase (Figure 1). These fibers are histochemically similar to the fast-twitch-glycolytic fibers found in the white vastus of the guinea pig and the semimembranosus accessorius of the rabbit.

By contrast slow-twitch-oxidative fibers stain negligibly for myofibrillar ATPase at pH 9.4 and for mitochondrial α-glycerophosphate dehydrogenase whereas the histochemical activity of NADH-diaphorase and malate dehydrogenase is intermediate between that of the fast-twitch-oxidative-glycolytic and slow-twitch-oxidative fibers (Figure 1). In addition to these differences the distribution of reaction product with NADH-diaphorase, malate dehydrogenase, and succinate dehydrogenase in slow-twitch-oxidative fibers is uniform throughout the fiber cross-section and its size is fine in contrast to the subsarcolemmal preponderance of coarse reaction product in fast-twitch-oxidative-glycolytic and fast-twitch-glycolytic fibers (Figure 1; Table III). In the sections shown, this difference in pattern is most obvious with malate dehydrogenase (Figure 1d,h).

As illustrated in Figure 1a–d the third type of fiber termed fast-twitch-oxidative-glycolytic stains for myofibrillar ATPase and mitochondrial α-glycerophosphate dehydrogenase with an intensity between that of fast-twitch glycolytic (dark) and slow-twitch-oxidative fibers (very light) but stains most intensely for NADH-diaphorase and malate dehydrogenase.

TABLE II: Rabbit Skeletal Muscle Characteristics.

Gross appearance	Muscle: Semimembranosus Accessorius White	Muscle: Soleus Red
Histochemical fiber type (%)[a]		
Fast-twitch-oxidative-glycolytic	12	4
Fast-twitch-glycolytic	86	0
Slow-twitch-oxidative	2	96
Time-to-peak tension (msec)	30	85
Phosphorylase (μmoles/min per g)		
270*g* supernatant	8.25 ± 1.72 (4)	2.06 ± 0.31 (4)
Lactate dehydrogenase (μmoles/min per g)		
105,000*g* supernatant	1485 ± 154 (5)	328 ± 88 (5)
α-Glycerophosphate dehydrogenase (μmoles/min per g wet wt)		
Whole homogenate	1.46 ± 0.31 (4)	0.28 ± 0.14 (4)
Hexokinase (nmoles/min per g)		
Whole homogenate	183 ± 53 (7)	993 ± 115 (7)
Succinate dehydrogenase (μmoles/min per g wet wt)		
Whole homogenate	0.64 ± 0.14 (4)	1.81 ± 0.16 (4)
Cytochrome *a* (nmoles/g wet wt)	<1.0 (4)	7.0 ± 0.6 (5)
Cytochrome *c* (nmoles/g wet wt)		
Whole homogenate	<1.0 (4)	7.3 ± 0.6 (4)

[a] These data are estimates taken from small samples of each of the three muscles. Values are means ± std dev. The number of muscles analyzed is given in parentheses.

TABLE III

	Type of Fiber: Fast-Twitch-Oxidative-Glycolytic	Fast-Twitch-Glycolytic	Slow-Twitch-Oxidative
Staining intensity			
NADH-Diaphorase	High	Low	Intermediate
Malate dehydrogenase	High	Low	Intermediate
Phosphorylase	High	High	Low
PAS	High	High	Low
Mitochondrial α-glycerophosphate dehydrogenase	High	High	Low
Myofibrillar ATPase, pH 9.4	Intermediate–High	High	Low
Staining with NADH-diaphorase			
Cytological localization	Many subsarcolemmal aggregates	Few subsarcolemmal aggregates	More uniform throughout fiber
Granular size of stained particles	Large	Large	Small
Previous terminologies			
Reference			
Dubowitz and Pearse (1960), Engel (1962)	II	II	I
Romanul (1964)	II	I	III
Yellin and Guth (1970)	$\alpha\beta$	α	β
Ashmore and Doerr (1971)	α-red	α-white	β-red
Brooke and Kaiser (1970)	IIA	IIB	I
Stein and Padykula (1962)	C	A	B
Padykula and Gauthier (1966)	Red	White	Intermediate
Burke *et al.* (1971)	FR	FF	S

Histochemical activities of phosphorylase, lactate dehydrogenase, and succinate dehydrogenase also show that this fiber has more glycolytic and oxidative activity than the slow-twitch-oxidative fiber. Hence, it is described as fast-twitch-oxidative-glycolytic. These fibers in the guinea pig gastrocnemius are presumed to be fast because of their great histochemical similarity to the predominant fiber of the red vastus, a muscle of the guinea pig known to be fast-twitch and com-

posed predominately of such fibers. Table III correlates the histochemical characteristics of these fibers with an outline of previous terminologies employed to describe various types of fibers.

The histochemical activity of myofibrillar ATPase at pH 9.4 in the three fibers correlates with the specific activity of natural actomyosin ATPase (Figure 1 and Table I); the fast-twitch-oxidative-glycolytic fibers show more activity than the slow-twitch-oxidative but less than fast-twitch-glycolytic fibers.

In the guinea pig the red vastus and white vastus both have high glycogen concentrations and high phosphorylase, lactate dehydrogenase, and mitochondrial α-glycerophosphate dehydrogenase activities relative to the soleus (Table I). These muscles are composed predominately of fast-twitch-oxidative-glycolytic, fast-twitch-glycolytic, and slow-twitch-oxidative fibers, respectively (Table III). Hexokinase activity which is highest in the soleus does not parallel either the glycolytic activities or cytochrome concentrations. Mitochondrial α-glycerophosphate dehydrogenase correlates with the glycolytic activities rather than with succinate dehydrogenase or cytochrome concentration (Table I). Like hexokinase the activity of α-1,4-glycosidase is high in both the soleus and red vastus relative to the white vastus. Succinate dehydrogenase activity and cytochrome concentrations are highest in the red vastus, intermediate in the soleus, and lowest in the white vastus. Myoglobin concentration is high in both the red vastus and soleus and is very low in the white vastus.

Table II summarizes the observations made on the two different rabbit muscles composed predominately of fast-twitch-glycolytic and slow-twitch-oxidative fibers. The population distribution of individual fibers in these muscles is an approximation made from histochemical examination of three each of the different muscles. Although the absolute level of enzyme activities between the two species show some quantitative differences, the enzyme profiles of fast-twitch-glycolytic and slow-twitch-oxidative fibers in the rabbit are similar to those observed in the guinea pig, as are the histoenzymatic activities of the three fiber types (Figure 1). No hind limb muscle composed predominately of fast-twitch-oxidative-glycolytic fibers was found in the rabbit.

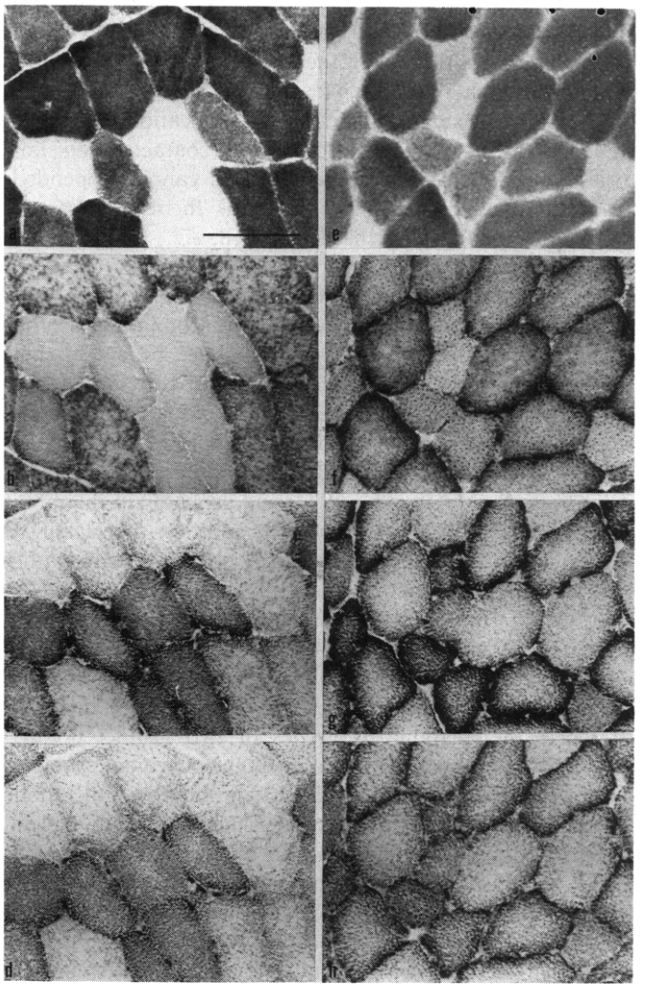

FIGURE 1: Rabbit 1a–d and guinea pig 1e–h gastrocnemius muscles consisting of the three basic fiber types are illustrated. Myofibrillar ATPase after an alkaline buffer preincubation and alkaline incubation at pH 9.4 (a,e), mitochondrial α-glycerophosphate dehydrogenase (b,f), reduced nicotinamide adenine dinucleotide diaphorase (c,g), and malate dehydrogenase (d,h) are shown. Fibers that are the darkest staining with ATPase (fast twitch) have high α-glycerophosphate dehydrogenase and low NADH-diaphorase and malate dehydrogenase activity and are called fast-twitch-glycolytic fibers. Other fibers that stain darkly with ATPase (but slightly less than in fast-twitch-glycolytic fibers) stain moderately with α-glycerophosphate dehydrogenase and heavy with NADH-diaphorase and malate dehydrogenase are called fast-twitch-oxidative-glycolytic. The fibers which show the lightest myofibrillar ATPase activity also have the lowest mitochondrial α-glycerophosphate dehydrogenase activity and moderate NADH-diaphorase and malate dehydrogenase activity and are called slow-twitch-oxidative. Morphologically fast-twitch-oxidative-glycolytic and slow-twitch-oxidative fibers differ in that the granularity of the staining is more coarse and localized more peripherally in the former. Although not illustrated here histochemical phosphorylase activity as well as glycogen concentration by PAS stain are higher in the fast-twitch-oxidative-glycolytic and fast-twitch-glycolytic than in slow-twitch-oxidative fibers of the guinea pig (Gillespie *et al.*, 1970). The bar in Figure 1a represents 50 μ.

Discussion

The biochemistry of red and white muscle has been of interest to scientists since the early 1900's. Early studies of the biochemical, structural and functional characteristics of red and white muscle were reviewed by Needham (1926). Pette and his associates (Pette and Bücher, 1963) and Beatty, Bocek, and their associates (Beatty and Bocek, 1970) have added extensive biochemical data on red and white muscle in different species.

Unfortunately the design of most of these biochemical investigations indicates a reliance on muscle color and a lack of application of information obtained from numerous histochemical studies describing the existence of at least three distinct types of skeletal muscle fibers (*cf.* Table III). Although Beatty and Bocek (1970) mention an intermediate fiber, they were unable to assess its biochemical properties because muscles composed predominately of one fiber type were not studied.

In the past we have classified the three fiber types found in mammalian hind limb muscles as fast-twitch red, fast-twitch white, and slow-twitch intermediate on the basis of their contraction time and according to their histochemical enzyme profile including oxidative and glycolytic enzyme activities. The relative speeds (times-to-peak tension) of hind limb muscles composed predominately or exclusively of one or another histochemical type of fiber determine their classification as fast-twitch or slow-twitch (Edgerton and Simpson, 1969; Barnard *et al.*, 1971; Peter, 1972).

Based on the quantitative data presented herein we now suggest that shorthand, nondescriptive nomenclatures such as

are listed in Table III as well as our own imprecise designation of fibers as fast-twitch white, fast-twitch red, and slow-twitch intermediate be replaced by the straightforward, descriptive terms fast-twitch-glycolytic, fast-twitch-oxidative-glycolytic, and slow-twitch-oxidative. The latter classification is purposefully open-ended so that additional terms characterizing similarities or differences between the fibers can be appended. In addition, the precision of the terms themselves can be improved by quantifying the terms used, *e.g.*, by appending to the speed term the measured time-to-peak tension of such a fiber or of a muscle composed of such fibers.

This system of nomenclature readily accommodates other permutations and combinations of muscle speed and metabolic pattern which are possible such as fast-twitch-oxidative, slow-twitch-glycolytic, and slow-twitch-oxidative-glycolytic, some of which are expected in various species. Indeed fast-twitch-oxidative fibers are known to exist in pigeon extraocular muscles (Maier *et al.*, 1972) and probably in the rabbit cricothyroid (Hall-Craggs, 1968). In addition the classification should prove useful for describing tonic, nonpropagating fibers as well as fibers of muscle spindles.

We would emphasize, however, that at this point there can be no definite answer to the fundamental question of whether the fast-twitch-glycolytic fibers and fast-twitch-oxidative-glycolytic fibers described herein reflect quantum differences in metabolic properties of two distinct populations of fibers or whether they are simply convenient examples for description of what is really a continuum of metabolic properties of fast-twitch fibers in certain hind-limb skeletal muscles of the species studied. An answer to this question should be possible with analysis of the biochemical, histochemical and quantitative electron microscopic features of many individual fast-twitch fibers. In any case investigations of skeletal muscle must take into account the differences in fast-twitch muscle fibers.

The present study defines certain quantitative biochemical differences in muscles known to be composed predominately or exclusively of a single fiber type. The enzyme activities given in Tables I and II represent maximum activities measured *in vitro* under the conditions described. Although the activity of these enzymes may be different *in vivo* due to compartmentalization, substrate–product inhibition, etc., the *in vitro* data establish the gross metabolic capabilities of slow-twitch-oxidative, fast-twitch-glycolytic, and fast-twitch-oxidative-glycolytic muscle fibers in the guinea pig as well as slow-twitch-oxidative and fast-twitch-glycolytic fibers in the rabbit. These capabilities are expected to parallel the *in vivo* capacity for glycogenolysis, glycolysis, and oxidative metabolism, but further studies are needed to establish the precision of this relationship.

Fast-twitch-glycolytic fibers (guinea pig, white vastus lateralis; rabbit, semimembranosus accessorius) are predominately anaerobic fibers. They have a high glycogen concentration and high phosphorylase, lactate dehydrogenase and α-glycerophosphate dehydrogenase activities. Their aerobic capacity is very limited as manifest by low succinate dehydrogenase activity as well as low cytochrome and myoglobin concentrations. The high activities of phosphorylase, lactate dehydrogenase, and mitochondrial α-glycerophosphate dehydrogenase relative to cytochrome *a* concentration (Tables I and II) also indicate that fast-twitch-glycolytic fibers rely mainly on anaerobic metabolism for the production of ATP.

Fast-twitch-oxidative-glycolytic fibers (guinea pig, red vastus lateralis) appear to have the highest capacity for aerobic metabolism because succinate dehydrogenase activity as well as cytochrome and myoglobin concentrations are greatest in these fibers. Fast-twitch-oxidative-glycolytic fibers are also characterized by a moderate to high glycolytic capacity. They have the highest glycogen concentration and moderate lactate dehydrogenase activity. In these fibers of the guinea pig the phosphorylase and mitochondrial α-glycerophosphate dehydrogenase activities are very high. The high α-glycerophosphate dehydrogenase activity suggests an important role for the α-glycerophosphate shuttle system in the regeneration of NAD for glycolysis in both fast-twitch-glycolytic and fast-twitch-oxidative-glycolytic fibers. The latter fibers in the guinea pig also have a high capillary to fiber ratio in comparison to fast-twitch-glycolytic fibers, suggesting a blood flow adequate for aerobic metabolism (Mai *et al.*, 1970). Other investigators have found the myoglobin concentration in cat muscle correlates directly with blood flow and inversely with speed of contraction (Reis and Wooten, 1970). Our work supports the direct relationship between capillary density and myoglobin concentration but study of fast-twitch-oxidative-glycolytic fibers clearly shows that the relationship between capillary or myoglobin concentration and speed of contraction is not necessarily inverse (Mai *et al.*, 1970; Table I).

Other studies from this laboratory show quantitative differences in the ATPase activity and other characteristics of natural actomyosin isolated from fast-twitch-oxidative-glycolytic fibers compared to that from fast-twitch-glycolytic fibers (Table I; Furukawa and Peter, 1971). In particular the specific activity of the Mg^{2+}-activated actomyosin ATPase at pH 9.4 is highest in fast-twitch-glycolytic fibers, intermediate in fast-twitch-oxidative-glycolytic fibers, and lowest in slow-twitch-oxidative fibers (Table I). These data (Table I) correspond to the intensity of staining of the same fibers for myofibrillar ATPase (Figure 1 and Table III) and suggest that a component of the thin filament contributes to the histochemical differentiation of the fast-twitch fibers. On the other hand, the characteristics of calcium transport by fragmented sarcoplasmic reticulum isolated from fast-twitch-oxidative-glycolytic and fast-twitch-glycolytic fibers (the red vastus and white vastus, respectively) are very similar (Fiehn and Peter, 1971).

The high capacities for glycogenolysis, glycolysis, and oxidative phosphorylation together with its speed of contraction give the fast-twitch-oxidative-glycolytic fiber a distinctive metabolic profile. The magnitude and scope of these metabolic activities appear to gear these fibers for high intensity, prolonged work, a proposed function which is supported by their selective recruitment during exercise (Edgerton *et al.*, 1970a,b).

The enzyme profile of fast-twitch-oxidative-glycolytic fibers could not be quantitated in homogenates of rabbit muscle, because muscle composed predominately of such fibers was not found. However, fast-twitch-oxidative-glycolytic fibers, identified histochemically in heterogeneous hind limb muscles of the rabbit, have an enzyme profile determined histochemically which is in complete agreement with results found in homogenates of fast-twitch-oxidative-glycolytic muscles in the guinea pig.

Slow-twitch-oxidative fibers (guinea pig and rabbit soleus) appear to rely predominately on aerobic metabolism. These fibers have a low glycogen concentration and low phosphorylase, lactate dehydrogenase, and mitochondrial α-glycerophosphate dehydrogenase activities. Their cytochrome concentration and succinate dehydrogenase enzyme activity are intermediate between those observed in the fast-twitch-oxidative-glycolytic and fast-twitch-glycolytic fibers.

In addition to these metabolic differences among the three fiber types other work showed different activities of acid hydrolases (Peter *et al.*, 1972) and different isoenzyme patterns of lactate dehydrogenase in the three fiber types with a full complement of all five isoenzymes in muscles composed predominately of fast-twitch-oxidative-glycolytic fibers (Peter *et al.*, 1971; Sawaki and Peter, 1972).

The biochemical and histochemical data reported in this paper, in addition to histochemical data already published, adequately demonstrate the existence of three muscle fiber types in hind-limb muscles of the guinea pig and rabbit. Histochemical data support the contention that these three metabolically distinct fiber types are present also in hind-limb muscles of the mouse, rat, cat, rabbit, and lesser bush baby. It is important to recognize, however, that the magnitudes of the aerobic systems are not static and can be changed in a given type of fiber with physiological stimuli such as exercise (Holloszy, 1967; Barnard *et al.*, 1970; Peter, 1972; Barnard and Peter, 1971). For this reason the quantitative aspects of the energy-generating systems of one fiber compared to another (*e.g.*, the cytochrome concentration in slow-twitch-oxidative fibers compared to that of the cytochrome-rich population of fast-twitch-oxidative-glycolytic fibers) should be expected to vary from species to species and perhaps even within the same species under different conditions or in different muscles. Such variations emphasize our contention that hind-limb skeletal muscles are best classified as fast twitch or slow twitch and subclassified according to as many metabolic features as possible. The latter features are more mutable and doubtless reflect the type, intensity, and duration of work required of a given fiber in a given muscle.

Since most muscles are composed of a mixture of the three fiber types, it is important to know the exact composition of muscles used. In biochemical studies of skeletal muscles selection of muscles composed predominately or exclusively of one fiber type is mandatory if unnecessary obfuscation is to be avoided.

References

Ashmore, C. R., and Doerr, L. (1971), *Exp. Neurol. 31*, 408.

Barnard, R. J., Edgerton, V. R., Furukawa, T., and Peter, J. B. (1971), *Amer. J. Physiol. 220*, 410.

Barnard, R. J., Edgerton, V. R., and Peter, J. B. (1970), *J. Appl. Physiol. 28*, 762.

Barnard, R. J., and Peter, J. B. (1971), *J. Appl. Physiol. 31*, 904.

Bass, A., Bradiczka, D., Eyer, P., Hofer, S., and Pette, D. (1969), *Eur. J. Biochem. 10*, 198.

Beatty, C. H., and Bocek, R. M. (1970), *in* The Physiology and Biochemistry of Muscle as a Food, Vol. 2, Briskey, E. J., *et al.*, Ed., Madison, Wis., University of Wisconsin Press, p 155.

Bonner, W. D. (1955), *Methods Enzymol.* 722.

Brooke, M. H., and Kaiser, K. K. (1970), *Arch. Neurol. 23*, 369.

Brown, W. D. (1961), *J. Biol. Chem. 236*, 2238.

Burke, R. E., Levine, D. N., Zajac, F. E., III, Tsairis, P., and Engel, W. K. (1971), *Science 174*, 709.

Denny-Brown, D. E. (1929), *Proc. Roy. Soc., Ser. A 104*, 371.

Dubowitz, V., and Pearse, A. G. E. (1960), *Histochemie 2*, 105.

Edgerton, V. R., Barnard, R. J., Peter, J. B., Simpson, D. R., and Gillespie, C. A. (1970a), *Exp. Neurol. 27*, 46.

Edgerton, V. R., and Simpson, D. R. (1969), *J. Histochem. Cytochem. 17*, 828.

Edgerton, V. R., Simpson, D. R., Barnard, R. J., and Peter, J. B. (1970b), *Nature (London) 225*, 866.

Engel, W. K. (1962), *Neurology 12*, 778.

Fiehn, W., and Peter, J. B. (1971), *J. Clin. Invest. 50*, 570.

Furukawa, T., and Peter, J. B. (1971), *Biochem. Biophys. Res. Commun. 43*, 1354.

Gillespie, C. A., Simpson, D. R., and Edgerton, V. R. (1970), *J. Histochem. Cytochem. 18*, 552.

Guth, L., and Samaha, F. J. (1969), *Exp. Neurol. 25*, 138.

Hall-Craggs, E. C. B. (1968), *J. Anat. 102*, 241.

Holloszy, J. O. (1967), *J. Biol. Chem. 242*, 2278.

Hudgson, P., Gardner-Medwin, D., Worsfold, M., Pennington, R. J. T., and Walton, J. N. (1968), *Brain 91*, 435.

Kerr, N. S. (1955), *Anat. Rec. 121*, 481.

Mai, J., Edgerton, V. R., and Barnard, R. J. (1970), *Experientia 26*, 1222.

Maier, A., Eldred, E., and Edgerton, V. R. (1972), *Exp. Eye Res. 13* (in press).

Needham, D. M. (1926), *Physiol. Rev. 6*, 1.

Nelson, N. (1944), *J. Biol. Chem. 153*, 375.

Padykula, H. A., and Gauthier, G. F. (1966), *Excerpta Med. Int. Cong. Ser. 147*, 117.

Peter, J. B. (1972), *in* Contractility of Muscle Cells and Related Processes, Podolsky, R., Ed., Englewood Cliffs, N. J., Prentice-Hall, p 151.

Peter, J. B., Kar, N. C., Barnard, R. J., Pearson, C. M., and Edgerton, V. R. (1972), *Biochem. Med.* (in press).

Peter, J. B., Sawaki, S., Barnard, R. J., Edgerton, V. R., and Gillespie, C. A. (1971), *Arch. Biochem. Biophys. 144*, 304.

Pette, D., and Bücher, T. (1963), *Hoppe-Seyler's Z. Physiol. Chem. 331*, 180.

Ranvier, L. (1873), *C. R. Acad. Sci. (Paris) 77*, 1030.

Reis, D. J., and Wooten, G. F. (1970), *J. Physiol. (London) 210*, 121.

Romanul, F. C. A. (1964), *Arch. Neurol. 11*, 355.

Sawaki, S., and Peter, J. B. (1972), *Exp. Neurol.* (in press).

Schollmeyer, P., and Klingenberg, M. (1962), *Biochem. Z. 335*, 426.

Sharma, C., Manjeshwar, R., and Weinhouse, S. (1963), *J. Biol. Chem. 238*, 3840.

Stein, J. M., and Padykula, H. A. (1962), *Amer. J. Anat. 110*, 103.

Yellin, H., and Guth, L. (1970), *Exp. Neurol. 26*, 424.