HISTOCHEMICAL CLASSIFICATION OF SKELETAL MUSCLE FIBERS IN THE CATTLE

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Muscle fibers in five different skeletal muscles of the steers were histochemically classified into five types. The type A reacted strongly for myosin ATPase and phosphorylase and moderately to strongly for succinate dehydrogenase (SDH) and NADH-diaphorase. The type B reacted strongly for myosin ATPase and phosphorylase and weakly for SDH and NADH-diaphorase. The type C reacted weakly for myosin ATPase, weakly to moderately for phosphorylase, and strongly for SDH and NADH-diaphorase. The type D reacted very weakly for myosin ATPase and phosphorylase, moderately to strongly for SDH, and strongly for NADH-diaphorase. The type E reacted moderately for myosin ATPase, weakly for phosphorylase, and very strongly for SDH and NADH-diaphorase. The β -hydroxybutyrate dehydrogenase (β -HBD) reaction was negative to very weak in the A fibers, negative in the B fibers, and very weak to moderate in the C fibers; it was moderate in the D fibers and very strong in the E fibers. The D fibers of the *M. serratus ventralis* were larger than four other types. The *M. longissimus thoracis* consisted of the A, B, and C fibers. The *M. semitendinosus*, *M. serratus ventralis*, and *M. supraspinatus* contained the A, B, C, and D fibers. The *M. masseter* consisted only of the E fibers.

Most vertebrate skeletal muscles are composed of the muscle fibers that differ in their histochemical characteristics (2, 4, 7, 8, 23, 28). The muscle fibers are classified mostly into three fiber types, and various terminology has been used to designate the fiber types (1, 4, 5, 8, 10, 14, 17, 23, 25, 26, 28, 37). The term "red, white and intermediate or medium" has been used as descriptive designations for three fiber types on the basis of differences in oxidative enzyme activities (14, 23, 36). "Type I and type II" have principally been used to designate the myosin ATPase-low red fibers and myosin ATPase-high white fibers, respectively (10, 11, 18). The alphabetical designations have been used to denote various fiber types classified by correlating enzyme activities including the distribution of SDH (22, 26, 28–32).

Romanul (26) showed that, in the rat gastrocnemius and plantaris, eight possible fiber types could be recognized by correlating the relative activities of eight different enzymes and that they were broadly divided into three groups. Nyström (22) recognized four fiber types (A, B, C, and S) in cat muscles by correlating the relative activities of SDH, NADH-diaphorase, myosin ATPase, and phosphorylase. Similarly, Suzuki (29–31) classified muscle fibers into five types (A, B, C, D, and E)

in the sheep.

In the cattle muscle fibers have been classified into three types, but the presence of the fiber equivalent to the types D and E found in sheep muscles was not revealed (2). The present study was designed in an attempt to determine whether the fibers similar to the types D and E of sheep muscles existed in bovine muscles. The alphabetical designations used in the sheep were applied to denote fiber types of bovine muscles.

MATERIALS AND METHODS

Muscle samples were obtained from three steers (Holstein) slaughtered at the age of 17 months. After slaughter, they were immediately excised from the middle of *M. semitendinosus*, *M. longissimus thoracis* (*M. longissimus dorsi*), *M. supraspinatus*, *M. serratus ventralis*, and *M. masseter*, and frozen in a mixture of acetone and dry ice. Sections (8 μ) were serially cut in a cryostat. The methods used for demonstration of SDH, NADH-diaphorase, and β -HBD followed those of Nachlas *et al.* (21), Burstone (6), and Ogata and Mori (23), respectively. The method of Padykula and Herman (24) was used for demonstration of myosin ATPase. The activity of phosphorylase was demonstrated by the method described by Takeuchi and Kuriaki (35).

RESULTS

The muscle fibers were classified into five types by correlating the relative activities of myosin ATPase, phosphorylase, SDH, NADH-diaphorase, and β -HBD. They were designated as A, B, C, D, and E according to the same manner employed in the sheep (29–31). The histochemical characteristics of five types are summarized in Table 1 (Figs. 1–15).

The type A fibers reacted strongly for myosin ATPase and phosphorylase, moderately to strongly for SDH and NADH-diaphorase, and negatively to very weakly for β -HBD. The type B fibers reacted strongly for myosin ATPase and phosphorylase, weakly for SDH and NADH-diaphorase, and negatively for β -HBD. The type C fibers reacted weakly for myosin ATPase, weakly to moderately for phosphorylase, and strongly for SDH and NADH-diaphorase. This type gave a very weak to moderate reaction for β -HBD. The type D fibers reacted very weakly for myosin ATPase and phosphorylase, moderately to strongly for SDH, and strongly for NADH-diaphorase. The D fibers showed a moderate reaction for β -HBD. The type E fibers reacted moderately for myosin ATPase, weakly for phosphorylase, and very strongly for SDH, NADH-diaphorase, and β -HBD.

In the A, B, and E fibers, diformazan deposit gave a granular appearance in reaction for SDH and NADH-diaphorase. The diformazan granules were generally much smaller in the B fibers than in the A and E fibers. The larger number of the C fibers showed a stellate pattern of diformazan, whereas the distribution pattern of diformazan granules in the remainders was similar to that of the A fibers. The D fibers showed generally a reticular pattern of diformazan. This pattern was more distinct in the D fibers of the *M. serratus ventralis* than in those of the *M. semitendinosus* and *M. supraspinatus* (Fig. 16).

FIGS. 11 to 15. The *M. masseter*. Five serial cross sections. $\times 203$.

FIG. 11. Myosin ATPase. E indicates the type E.

FIG. 12. Phosphorylase.

FIG. 13. Succinate dehydrogenase.

FIG. 14. NADH-diaphorase.

FIG. 15. β -Hydroxybutyrate dehydrogenase.

FIG. 16. The *M. serratus ventralis*. NADH-diaphorase. $\times 406$.

There were no significant differences in the histochemical characteristics of the A and B fibers among four different muscles. In the *M. longissimus thoracis* and *M. semitendinosus*, the C fibers were much weaker in β -HBD activity than the D fibers. In the *M. supraspinatus*, the β -HBD activity was much weaker in the larger number of the C fibers and slightly weaker in the remainders than in the D

FIGS. 1 to 5. The *M. semitendinosus*. Five serial cross sections. $\times 203$.

FIGS. 6 to 10. The *M. serratus ventralis*. Five serial cross sections. $\times 203$.

FIGS. 1 and 6. Myosin ATPase. A, B, C, and D indicate the types A, B, C, and D, respectively.

FIGS. 2 and 7. Phosphorylase.

FIGS. 3 and 8. Succinate dehydrogenase.

FIGS. 4 and 9. NADH-diaphorase.

FIGS. 5 and 10. β -Hydroxybutyrate dehydrogenase.

TABLE 1. *Histochemical characteristics of five fiber types in bovine muscles*

Enzyme	Type A	Type B	Type C	Type D	Type E
Myosin ATPase	++ ###*	###	+ ++	⊥→⊥	++ ++
Phosphorylase	++ ###	###	++ ++	⊥→⊥	+
Succinate dehydrogenase	++ ###	+ +	###	++ ###	###
NADH-diaphorase	++ ###	+ +	###	###	###
β -Hydroxybutyrate dehydrogenase	- +	-	+ ++	++	###

* —, negative reaction; ⊥→⊥, very weak reaction; ++, weak reaction; ++, moderate reaction; ###, strong reaction; ###, very strong reaction.

fibers. In the *M. serratus ventralis*, the larger number of the C fibers was as very weak in β -HBD as the ones of the *M. semitendinosus* and *M. longissimus thoracis*, whereas the remainders were as moderate as the D fibers. The C fibers having moderate β -HBD activity were higher in phosphorylase activity than the D fibers in all the muscles.

The mean diameter of each fiber type varied among the muscles except the *M. masseter* (Table 2). In the *M. serratus ventralis* the D fibers were much larger than three other types, and the C fibers were larger than the A and B fibers between which there was no significant difference in diameter. In the *M. supraspinatus*, *M. semitendinosus*, and *M. longissimus thoracis*, the B fibers were the largest, and the A fibers were generally smaller than the D fibers and larger than the C fibers. The E fiber were roughly as large as the A fibers or the C fibers.

Except for the *M. masseter*, the proportion of each fiber type varied markedly among the muscles (Table 2). The A fibers were greater in number than three other types in the *M. serratus ventralis* and *M. supraspinatus*. In these two muscles the total of the A, C, and D fibers similar to red fiber was more than 50 percent. The B fibers corresponding to white fiber were greater in number than three other types and more than 50 percent in the *M. semitendinosus* and *M. longissimus thoracis*. In the muscles used, the C fibers were less than about 30 percent, and the D fibers were fewer than three other types. The D fibers were more numerous in the *M. serratus ventralis* than in the *M. supraspinatus* and *M. semitendinosus*, but none in the *M. longissimus thoracis*. The *M. masseter* consisted only of the E fibers.

DISCUSSION

The muscle fibers were classified into three fiber types: red, white, and intermediate or medium, based on oxidative enzyme activities or mitochondrial content (14, 23, 36). Intermediate fibers exhibiting moderate SDH activity were not moderate in phosphorylase and myosin ATPase activity but either high or low in both the enzyme activities (2, 32). When many histochemical techniques including the reactions of SDH and myosin ATPase are used for the classification of the fibers, the term "intermediate" has no general significance (2, 3, 7, 17, 32). We used the alphabet as designations for five fiber types because it has an advantage in that many

TABLE 2. *Diameter and proportion of each fiber types in five different muscles*

Muscle	Fiber Type	Fiber Diameter (average) μ	Percentage of fiber types %
<i>M. semitendinosus</i>	A	47.2	16.6
	B	66.8	70.7
	C	46.8	10.6
	D	60.5	2.1
<i>M. longissimus thoracis</i>	A	45.8	17.3
	B	60.2	55.2
	C	39.1	27.5
	D	—	0
<i>M. serratus ventralis</i>	A	40.8	43.2
	B	40.9	11.9
	C	45.4	25.5
	D	76.4	19.4
<i>M. supraspinatus</i>	A	55.4	41.3
	B	58.8	32.7
	C	48.9	16.8
	D	55.5	9.2
<i>M. masseter</i>	E	45.3	100

types of fibers can be denoted and because the fiber types of bovine muscles need to be compared with the ones of sheep muscles.

On the basis of the biochemical, histochemical and physiological data, Peter *et al.* (25) proposed the new nomenclature for fiber types: fast-twitch-oxidative-glycolytic, fast-twitch-glycolytic, and slow-twitch-oxidative. These terms explicitly represent the nature of three fiber types. In this study, the type A corresponds to the fast-twitch-oxidative-glycolytic fibers, the type B to the fast-twitch-glycolytic fibers, and the type C to the slow-twitch-oxidative fibers. It is possible that the types D and E fall under the category of the slow-twitch-oxidative fibers.

Judging from the histochemical characteristics, the A fibers seem to obtain mainly energy of contraction by anaerobic glycogenolysis and by aerobic oxidation of carbohydrate. The B fibers depend mainly on anaerobic metabolism for energy production. The C, D, and E fibers reacting moderately to strongly for β -HBD appear to rely predominantly on lipid metabolism. The C fibers reacting very weakly for β -HBD and moderately for phosphorylase may have to some extent a capacity for glycogen metabolism in addition to aerobic metabolism.

The fibers having high oxidative enzyme activity are adapted for long sustained activity and resistant to fatigue, whereas the fibers low in oxidative enzyme activity show fast fatigue (5, 13, 19). It is therefore presumed that the A, C, D and E fibers

are resistant to fatigue, whereas the B fibers are fast fatigue.

It has been demonstrated that the histochemical characteristics and energy metabolism of the muscle fibers are determined by the nerve supply (9, 16, 18, 27). On the other hand, the myosin ATPase-high fibers have been suggested to have a capacity to transform from aerobic metabolism to anaerobic metabolism in postnatal development (1, 3) and *vice versa* in endurance-type exercise (12), whereas myosin ATPase-low fibers are stable in metabolic state. Therefore, there is a possibility that the A fibers may be transformed into the B fibers and *vice versa* in changes in physiological state such as exercise and nutrition. The C, D, and E fibers are assumed to be stable in adult, intact animals on the basis of the results of the histochemical study of muscles in starved sheep (33).

The enlargement of the D fibers has led to speculation that they may play an important role in maintaining a standing posture. Gutmann *et al.* (15) have reported that compensatory hypertrophy of skeletal muscle, induced by functional elimination of synergistic muscles, is mainly the result of stretch brought about by the action of the antagonistic muscles and results in an increase in diameter in muscle fibers. Since the *M. serratus ventralis* from each side form a sling which supports the trunk between the front legs, it is presumed that the enlargement of the D fibers may be caused by the action of the stretch resulting from slinging the trunk. The D fibers of the *M. supraspinatus* and *M. semitendinosus* seem to have a similar function.

The fibers of the *M. masseter* of non-ruminant animals do not always show the histochemical characteristics similar to the ones of ruminant animals (36). The E fibers having very high activities of oxidative enzymes are considered to be well adapted to long-sustained activity for rumination.

The histochemical characteristics of the A, B, and E fibers in the cattle correspond to those in the sheep (29, 31). In the cattle, the C fibers varied to some extent in the activity of phosphorylase and β -HBD among the muscles, whereas the C fibers in the sheep little varied in both enzyme activities among the muscles (34). In the *M. supraspinatus* and *M. semitendinosus* of the cattle the D fibers were smaller than the B fibers, whereas they were invariably much larger than four other types in the sheep (30, 31). The D fibers in the *M. serratus ventralis* of the cattle correspond closely to those in sheep muscles.

In the sheep, the D fibers were present in certain kinds of muscles such as the *M. supraspinatus* and *M. serratus ventralis*, but not found in the *M. semitendinosus* and *M. longissimus thoracis* and others (29, 30). The type D different from four other types was little changed in the enzyme activities and showed a resistance to atrophy in starvation (33). In the cattle the D fibers may possess similar properties.

The *M. longissimus thoracis* contained more than 50 percent B fibers. The data are in substantial agreement with those of Moody and Cassens (20), who regard this muscle as a typical white muscle. The *M. semitendinosus* with more than 50 percent B fibers is also regarded as white muscle. The *M. serratus ventralis*, *M. supraspinatus* and *M. masseter* that comprise more than 50 percent red fibers are considered red muscle.

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