# Histochemical and Electron Microscopic Properties of the Masseter Muscle in the Japanese Field Vole Microtus montebelli

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# Histochemical and Electron Microscopic Properties of the Masseter Muscle in the Japanese Field Vole *Microtus montebelli*

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**ABSTRACT**—The masseter muscles of the Japanese field vole *Microtus montebelli* were studied histochemically and electron microscopically. The masseter muscles were composed of only fast-twitch oxidative (FO) fibers. As to ultrastructural features of the fibers, mitochondria, sarcoplasmic reticula and transverse tubules were well developed. It is concluded that the masseter muscles in *M. montebelli* have the structural characteristics that they can contract fast and enduringly. Thus, they appear to be highly adapted to herbivorous food habit.

## INTRODUCTION

The masticatory activity is largely carried out by masseter, temporalis, medial and lateral pterygoid muscles. The jaw muscles of herbivorous mammals differ from those of carnivorous mammals, i.e. the masseter muscles in herbivores are relatively large in size, and in carnivores the temporal muscles are relatively large in size (Hildebrand, 1988). The former needs functionally slow and long-term exercise, but the latter fast and short-term exercise. In omnivorous mammals, the intermediate type between herbivores and carnivores is recognized in the masseteric muscles (Suzuki, 1977).

In rodents, the masseter muscle is the largest masticatory muscle and it seems to be the most important muscle for biting hard food. The masseter muscles of the Japanese field vole *Microtus montebelli* are larger than those of the other members of Myomorpha (Miyao *et al.*, 1962). Moreover, dissection of the masticatory musculature of microtines (arvicolines) shows an increase in the potential anterior vector component and in the potential vertical vector component of these muscles relative to the molar tooth row, thus voles and lemmings are the most successful group of rodents for herbivorous habit (Kesner, 1980).

Skeletal muscles of vertebrates have two or more fiber types that differ in histochemical properties, having various proportion of fiber types to functional demands (Edström and Kugelberg, 1968; Moody and Cassens, 1968; Barnard *et al.*, 1971; Burke *et al.*, 1971). Although a number of functional studies on mammalian masticatory muscles have been made, there is no study on histochemical and ultrastructural characteristics of the masticatory muscles in the Japanese field vole.

The purpose of the present study is to clarify histological characteristics of the masseter muscle fibers in *M. montebelli*, comparing to those of Tokara goat which is one of typical herbivores and laboratory mice belonging to Rodentia.

## MATERIALS AND METHODS

Adult eleven voles, five mice and one Tokara goat were used in this study, and their *Musculus masseter* were immediately removed under ether anaesthesia.

For the light microscopic examination, muscle tissues were rapidly frozen in isopentane solution cooled with dry ice. Serial cross-sections of the muscles, 8 μm thick, were obtained and stained for myosin adenosine triphosphatase (ATPase) (Padykula and Herman, 1955) after acid (pH 4.3) or alkaline (pH 10.5) preincubation (Brooke and Kaiser, 1970a, b; Suzuki, 1977), for reduced nicotinamide adenine dinucleotide dehydrogenase (NADH-DH) (Burstone, 1962) and for phosphorylase (Takeuchi and Kuriaki, 1955) activities. In the vole, other sections were also stained with Periodic Acid-Schiff (PAS) and Sudan black B, respectively.

Fibers were classified as slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), fast-twitch oxidative (FO) by Peter *et al.* (1972) and Armstrong *et al.* (1977).

For the electron microscopic examination, the masseter muscles of the vole were fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.2 for 2 hr, then washed briefly in the same buffer and further fixed for 2 hr in 1% osmium tetroxide buffered with sodium cacodylate at pH 7.2. The tissues were dehydrated in alcohol series and embedded in Epon 812. Thin sections (600Å) for electron microscopy were cut with a glass knife on Porter-Blum MT-1 microtome, and doubly stained with lead and uranyl acetate. The sections were examined in an Hitachi-H600A electron microscope.

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## RESULTS

*Histochemical observations*

The masseter muscles in *M. montebelli* were composed of only FO fiber which was strongly reactive for myosin ATPase after preincubation at pH 10.5 (Fig. 1A), unreactive at pH 4.3 (Fig. 1B), strongly reactive for NADH-DH (Fig. 1C) and weakly reactive for phosphorylase (Fig. 1D). In NADH-DH activity, large granular diformazan deposits and strong reaction at subsarcolemmal region were recognized in the muscle fibers. The glycogen (Fig. 1E) and sudanophilic granules (Fig. 1F) were less rich. The diameter of the fiber was 18.19 ± 1.81 (S.D.) μm.

The masseter muscles of the mouse were composed of FOG (51%) and FG (49%) fibers (Fig. 2A). NADH-DH activity of FOG fiber of the muscle was weak and the diformazan deposits were smaller than that of the vole. The diameters of FOG and FG fibers were 22.00 ± 3.76 and 33.17 ± 6.86 μm, respectively.

The masseter muscle of the Tokara goat was composed of only SO fiber. In NADH-DH activity, large granular diformazan deposits and strong reaction at subsarcolemmal region were recognized in the muscle fibers as observed in the muscle fibers of the vole (Fig. 2B). The diameter of fiber was 40.38 ± 4.80 μm.

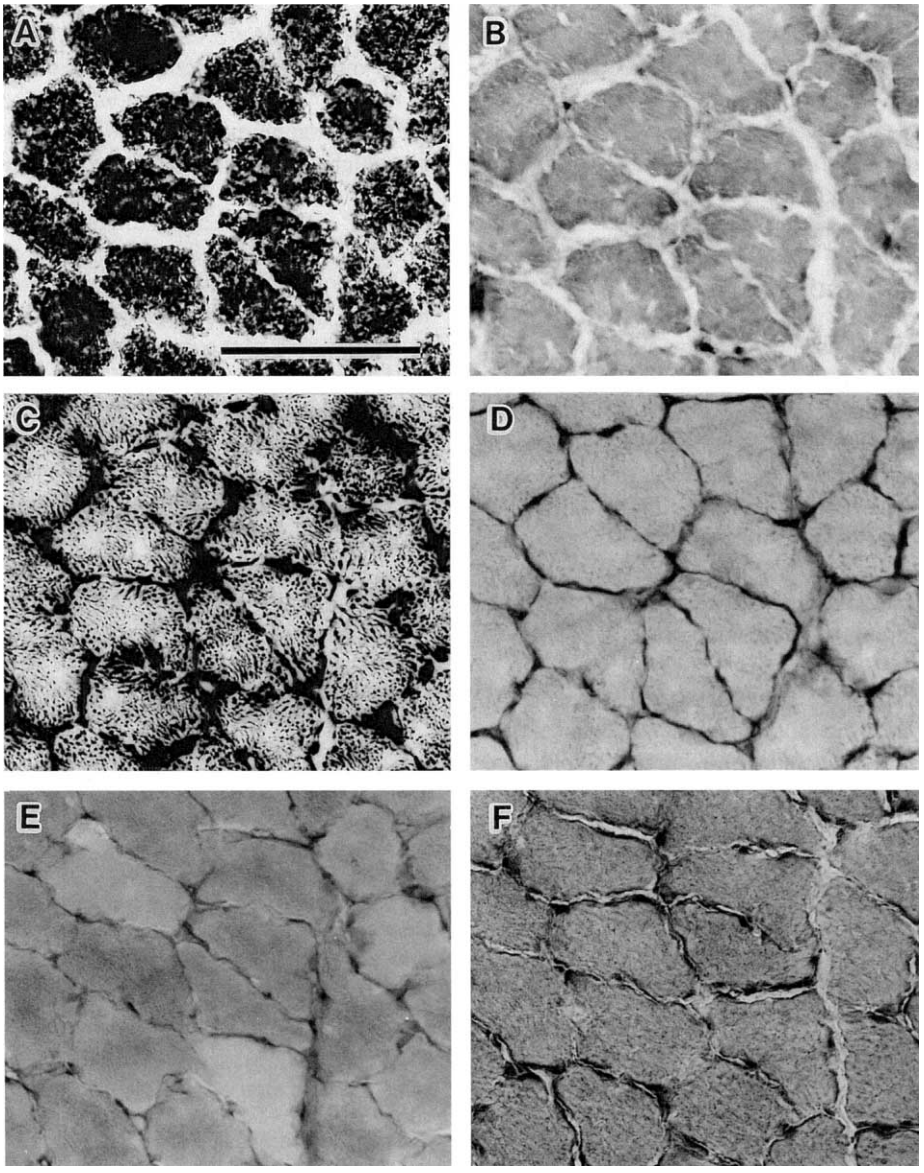


Fig. 1. Histochemical profiles of the masseter muscle in *M. montebelli*. These myofibers are all strongly reactive for myosin ATPase after preincubation at pH 10.5 (**A**), unreactive for myosin ATPase after preincubation at pH 4.3 (**B**), strongly reactive for NADH-DH (**C**), weakly reactive for phosphorylase (**D**), less rich in glycogen (PAS reaction) (**E**), and less rich in fat content (Sudan black B staining) (**F**). Bar = 50 μm.

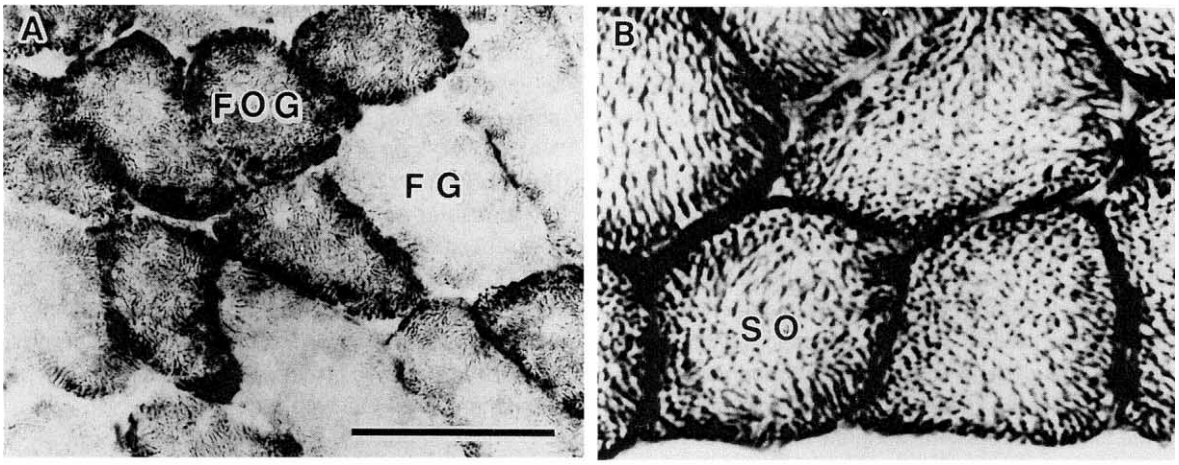


Fig. 2. NADH-DH activities of the masseter muscle. (**A**) Mouse. (**B**) Tokara goat. FOG, fast-twitch oxidative glycolytic fiber; FG, fast-twitch glycolytic fiber; SO, slow-twitch oxidative fiber. Bar = 50 μm.

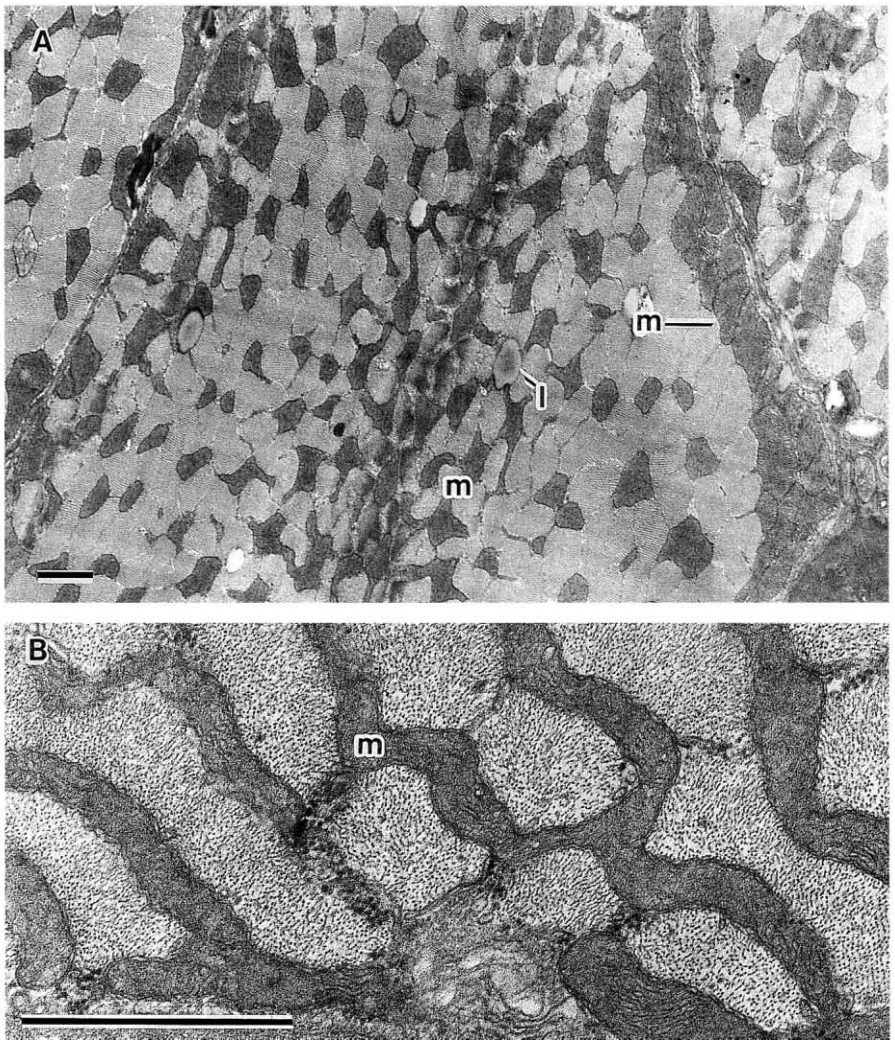


Fig. 3. Ultrastructure of the masseter muscle in the vole. (**A**) Cross-section showing large mitochondria with dense mitochondrial matrix arranged in transversal rows between myofibrils. The mitochondria with abundant cristae primarily consisting of parallel sheets are arranged just beneath the sarcolemma. Note the lipid droplets associated with them. (**B**) There are bizarrely shaped mitochondria in the transverse section of fibers. l, lipid droplet; m, mitochondrion. Bar = 1 μm.

*Electron microscopic observations*

The masseter muscles of the vole were composed of a single type of fiber characterized by densely packed mitochondria (Fig. 3A, B). The large mitochondria with dense mitochondrial matrix were arranged in transversal rows between myofibrils. Especially, there were many bizarrely shaped mitochondria in the transverse sections of fibers. The mitochondria of which abundant cristae primarily consisted of parallel sheets aggregated beneath the sarcolemma. Many lipid droplets were frequently associated with mitochondria.

The transverse tubules and sarcoplasmic reticula were well developed, and glycogen granules were moderate in the interfibrillar sarcoplasm. Triads occurred near A-I junctions (Fig. 4). In the terminal axoplasm, moderate numbers of vesicle and mitochondria occurred (Fig. 5A, B). Junctional folds are regular, long and numerous.

## DISCUSSION

Skeletal muscle fibers in mammals are highly differentiated in adulthood, and can be designated as fast or slow on the basis of the pH lability of histochemical staining for myosin ATPase. Fast-twitch fibers are subdivided into three types in accordance with the nature of metabolic enzyme for their energy source, fibers utilizing oxidative (FO) or glycolytic (FG) or both (FOG) enzyme, while slow-twitch fibers are primarily oxidative (SO) (Pette and Vrbová, 1985; Powers *et al.*, 1991).

All the fibers of the vole masseter muscles react strongly for myosin ATPase after alkaline preincubation (fast isoform), stain strongly for oxidative mitochondrial enzymes such as NADH-DH, and weakly for glycolytic markers such as phosphorylase. These histochemical data clearly show that the masseter muscles of the vole, as well as of the mouse (present study), rat (Rokx *et al.*, 1984) and guinea pig (Suzuki, 1977), are composed of fast-twitch fibers, but the masseter muscle of the vole are of FO type, being distinctly different from those of the other three rodents that comprise a high amount of glycolytic enzyme (both FOG and FG types for the mouse and rat; FOG type for the guinea pig).

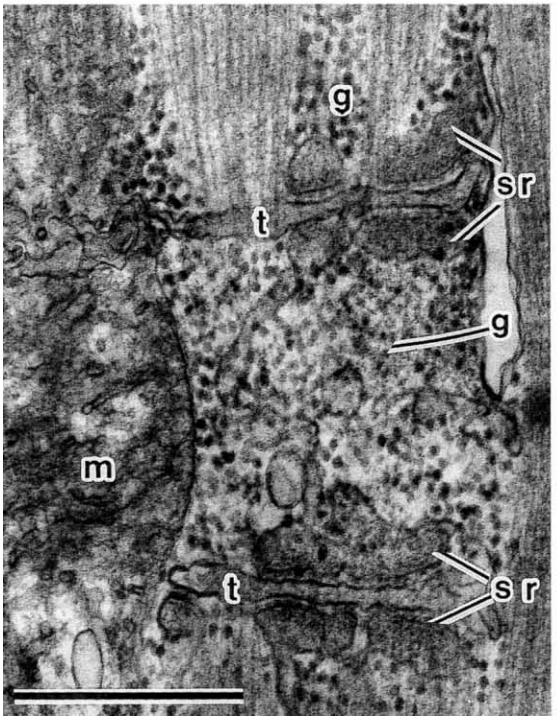


Fig. 4. Longitudinal section illustrating organization of sarcotubular system of the masseter muscle in the vole. Glycogen granules are found in the interfibrillar sarcoplasm. g, glycogen granule; m, mitochondrion; sr, sarcoplasmic reticulum; t, transverse tubule. Bar = 1 μm.

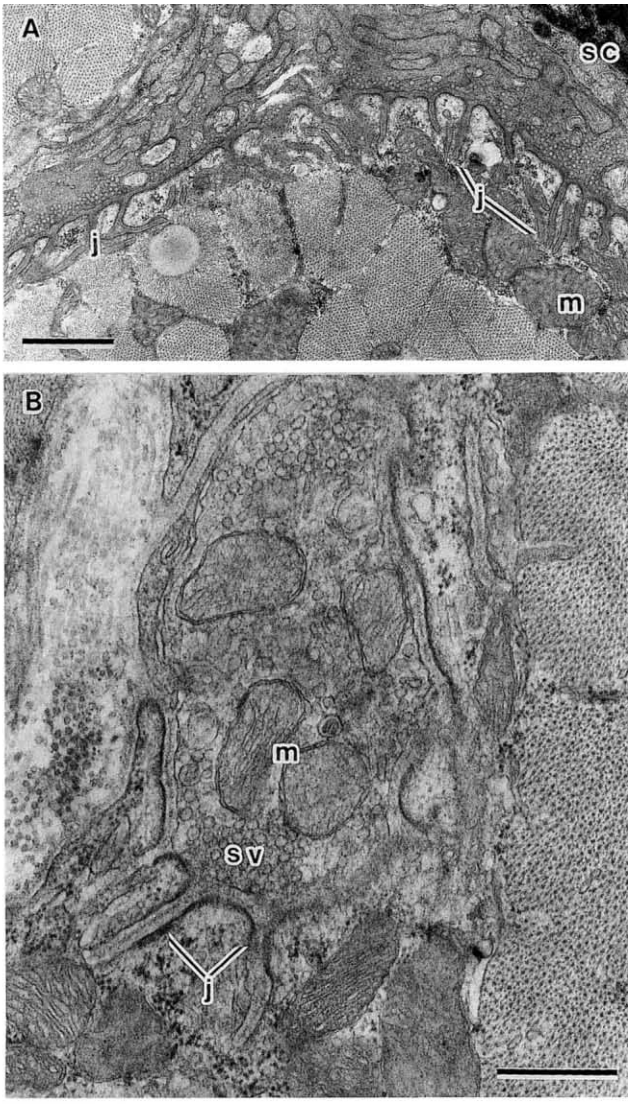


Fig. 5. Neuromuscular junction of the masseter muscle fibers in the vole. (**A**) The invaginated sarcoplasmic surface at the neuromuscular junction of the fiber is illustrated in this low-power electron micrograph. (**B**) In the terminal axoplasm, moderate numbers of synaptic vesicles and mitochondria occur, and the junctional folds are relatively long, unbranched and straight. j, junctional fold; m, mitochondrion; sc, Schwann's cell; sv, synaptic vesicle. Bar = 1 μm.

In addition to the histochemical evidence, FO fibers in the vole are considerably smaller in diameters than FOG ones in the mouse (present study), rat (Rokx *et al.*, 1984) and guinea pig (Suzuki, 1977), and FG fibers in the mouse (present study) and rat (Rokx *et al.*, 1984). This fact, as pointed out by Hill (1965), seems to imply that FO fibers are favorable for rapid diffusion of oxygen and nutrient essential for muscle contraction. The ultrastructural features of the vole masseter muscle fibers, i.e. well developed sarcoplasmic reticula, transverse tubules and neuromuscular junctions, seem to directly related to fast muscle contraction as proposed for other mammals (Ohtsu and Uchida, 1979; Schmalbruch, 1979; Flucher, 1992). On the other hand, densely packed mitochondria, and numerous lipid droplets deposited around mitochondria may be closely associated to sustaining muscle contraction (Gauthier, 1969).

The above histochemical and ultrastructural findings emphasize strongly the following view that the FO fibers are specialized for fast and sustained contraction in which supplies of lipid droplets may be exhausted quickly. Accordingly, the masseter muscles of the vole can masticate fast and enduringly. However, the masseter muscles of the vole have a markedly less store of glycogen granules than those of the mouse (present study) and rat (Rokx *et al.*, 1984), and are less dependent on anaerobic glycolysis as SO fibers of the goat (present study), cattle and sheep (Suzuki, 1977) which are typical herbivores. For this reason, the vole masseters do not appear to engage in powerful or sudden activity for biting or gnawing as the mouse and rat which are adaptive for omnivorous food habit. Thus, such structural properties of masseter muscles in the vole, having an intermediate profile between omnivorous rodents and herbivorous ruminants, must be considered in relation to the functional differentiation of this masticatory muscle required for its own characteristic properties of herbivorous food habit.

Now, several isoforms of myosin heavy chain have been identified in mammalian muscle fibers (Bar and Pette, 1988; Hämäläinen and Pette, 1993; Schiaffino and Reggiani, 1994). According to Hermanson *et al.* (1991), the pectoral muscle of a microchiropteran species, which is known to be markedly well-developed on account of the adaptation for flight, and to be composed of only FO fiber (Powers *et al.*, 1991), comprises rat IIa myosin heavy chain alone. This study has also indicated the functional involvement of this myosin isoform for flying. It remains to be elucidated whether FO fibers of the vole masseter muscle are equipped with such nature of myosin molecule, as the result of its masticatory adaptation.

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