*Acta Medica Okayama*

*Volume* 19, *Issue* 3 1965 *Article* 4

JUNE 1965

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**Abstract**

Using double staining method of succinic dehydrogenase and cholinesterase, the structural differences of motor endplate in the red, the white and the intermediate muscle fibers of the mouse limb muscles were observed. The endplate of the white fiber had a large size and complicated interlacing structure. The endplate of the red fiber had a small size, simple and compact structure. The endplate of the intermediate fiber had a medium size and moderately developed structure.

*PMID: 4221927 [PubMed - indexed for MEDLINE] 

Acta Med. Okayama 19, 149—153 (1965)

# A HISTOCHEMICAL STUDY ON THE STRUCTURAL DIFFERENCES OF MOTOR ENDPLATE IN THE RED, WHITE AND INTERMEDIATE MUSCLE FIBERS OF MOUSE LIMB MUSCLE

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*Received for publication, June 20, 1965*

Recent histochemical studies[1,2,3] revealed that almost all mammalian striated muscles consisted of three types of muscle fibers, namely, the red fiber which has a strong activity of oxidative enzymes, the white fiber a weak activity, and the intermediate fiber, intermediate activity between that of the red and the white fiber.

In this study, the structural differences of motor endplate among these three types of fibers of the mouse limb muscles are demonstrated by the double staining method of succinic dehdrogenase and cholinesterase.

## MATERIALS AND METHODS

*M. triceps brachii*, *M. gastrocnemius* and *M. adductor magnus* of the adult mouse were used for this study. The muscles were immersed in a bath of isopentane cooled with carbon dioxide (about-75°C) for 1 minute. The serial longitudinal sections (10—50 $\mu$) were cut in a cryostat at −20°C and mounted on glass slides. All sections were rapidly thawed by placing a warm finger under the slide, then dried by an electric fan in a cold room at 2°C for 30 minutes.

For the histochemical demonstration of motor endplate in each type of muscle fibers, the double staining method of succinic dehydrogenase and cholinesterase was used. For the demonstration of succinic dehydrogenase, unfixed sections were incubated in the following solutions, 5 ml of 0.2 *M* sodium succinate, 5 ml of 0.2 *M* phosphate buffer at pH 7.6 and 10 ml of 0.1 % Nitro blue tetrazolium. The sections were incubated at room temperature for 10 minutes, then washed well with distilled water (2°C) and fixed in 10 % formalin (2°C) for 10 minutes. Thereafter the sections were washed with distilled water (2°C) and incubated in the following solution for the demonstration of cholinesterase after the thiocholine technique of GOMORI[4]. The stock solution was made up 0.3 g of copper sulfate ($CuSO_4 \cdot 5H_2O$), 0.375 g of glycine, 1.0 g of magnesium chloride ($MgCl_2 \cdot 6H_2O$), 1.75 g of maleic acid, 30 ml of 1 *N* NaOH, and 170 ml

of hot saturated solution of sodium sulfate ($Na_2SO_4$). Before use, 40 mg of acethyl thiocholine iodide were dissolved in a few drops of water and added to 20 ml of the stock solution. The sections were incubated for 15—60 minutes at 37°C, rinsed in three changes of saturated $Na_2SO_4$ solution and immersed in a dilute solution of yellow ammonium sulfide. Then the sections were rinsed in distilled water and mounted in glycerine jelly.

Immediately after mounting, no crystal formation was observed in the section. However, about 30 minutes after mounting, pinkish needle-shaped crystal appeared in the sections, so observation and photography were taken within 30 minutes after mounting.

As the control, the sections stained only with thiocholine technique after GOMORI[4] were observed.

## RESULTS

In the double-stained section, the three types of fibers were distinguishable by their difference of succinic dehydrogenase activity, i. e., the small red fiber showed a strong, the large white fiber a weak, and the intermediate fiber a moderate activity. No marked differences of activity and localization of cholinesterase were observed between the sections double-stained with succinic dehydrogenase and cholinesterase reaction on one hand and the sections stained only with cholinesterase reaction on the other.

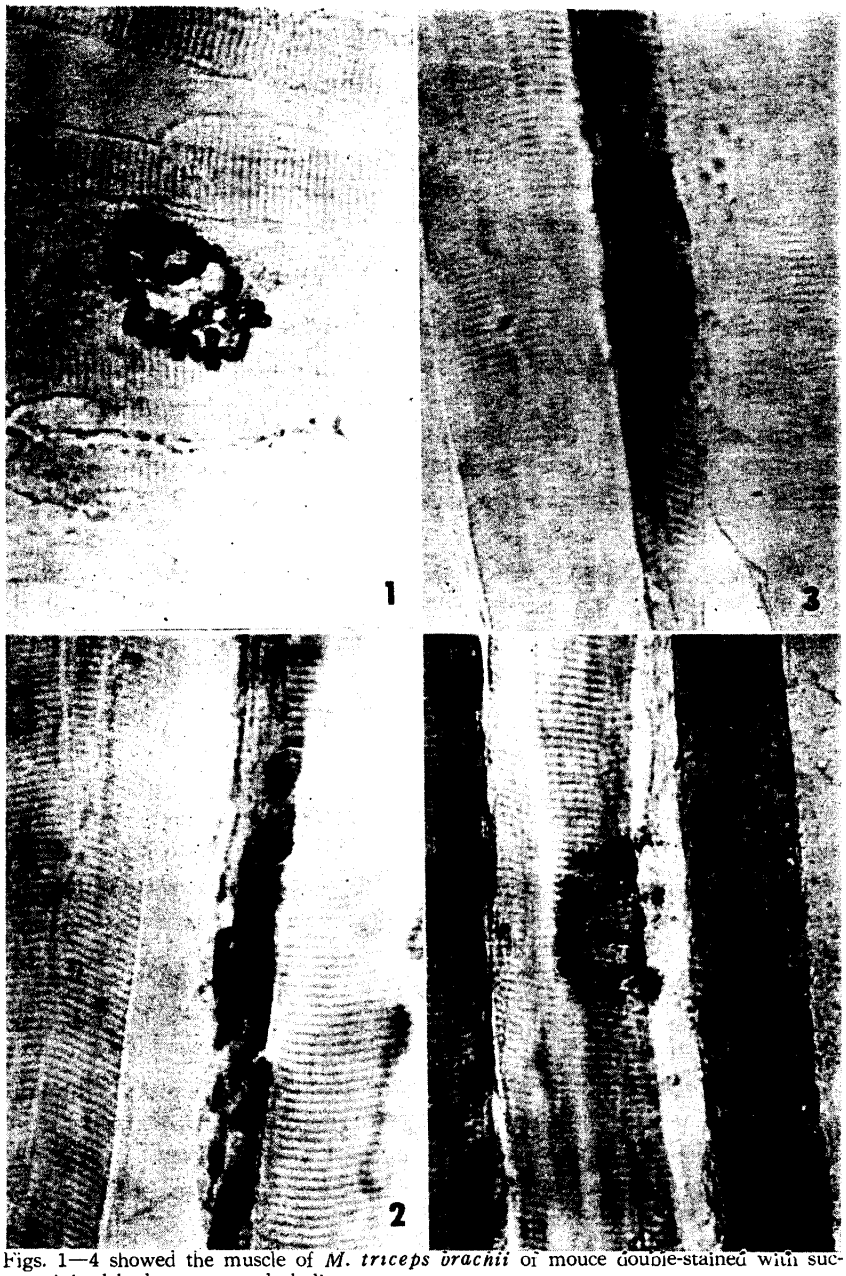
The endplate of each type of fiber is situated in almost the same region, i. e., usually in the middle of the fiber.

The motor endplate of the white fiber had a large size and well-developed strucuture (Fig. 1). The average diameter of the area of the endplate was 23 $\mu$. Its shape was variable; round, oval or irregular. The cholinesterase-stained material, i. e., myoneural apparatus, was well developed and made a complicating interlacing structure.

The endplate of the red fiber was small in size and rather simple in structure (Figs. 2 and 3). Its shape was round, oval or irregular. The average diameter of the area of the endplate was 14 $\mu$. Its shape was round or irregular. The myoneural apparatus was less complicated and rather compacted in form.

The endplate of the intermediate fiber was medium in size and of moderately developed structure (Fig, 4). Its shape was round, oval or irregular. The average diameter of the area of the endplate was 20 $\mu$. The development of subneural apparatus was intermediate between that of the white and the red fibers.

Although differences in the size and shape of motor endplate were observed in eaeh type of fibers, no marked differences of cholinesterase activity were noted.




Figs. 1—4 showed the muscle of *M. triceps brachii* of mouce double-stained with succinic dehydrogenase and cholinesterase.

Fig. 1 The motor endplate of white fiber. Note the endplate has a large size and complicated interlacing structure of myoneural apparatus. The white fiber showsd a weak activity of succinic dehydrogenase. × 1,000

Figs. 2 and 3 The motor endplate of red fiber. Note the endplate has a smaller size and rather simpler and compact structure. The red fiber showsd a strong activity of succinic dehydrogenase. × 1,000

Fig. 4 The motor endplate of intermediate fiber. Note the endplate has a medium size and moderately developed structure. × 1,000

## DISCUSSION

HESS[5] reported that in the extraocular muscles of mammals the fast (white) and slow (red) fibers had different types of motor endplate, i. e., the fast fiber had a single endplate of "en plaque" type, while the slow fiber had many endplates of "en grappe" type. However, in other muscles of mammals it was generally accepted that all muscle fibers had endplates of "en plaque" type, and there have been no reports describing structural differences of endplate in the red, white and intermediate fibers.

From the present study, it was observed that all muscle fibers of limb muscles had a single endplate of "en plaque" type, however, some differences of size and structure were demonstrated in each type of muscle fibers. Namely, it was observed that the white fiber had a larger and better developed motor endplate than the red. This difference might be attributed to the differences in fiber diameter and physiological characteristics. The diameter of the white fiber was two or three times that of the red. OGATA[6] reported from his electron microscopic study that sarcoplasmic reticulum was better developed in the white fiber than in the red fiber. At present it is thought that the sarcoplasmic reticulum may serve to transmit excitatory impulses to the interior of the muscle fiber. If this is true, it correlates well with the present results in the fast-contracting white fiber, which has a well-developed motor endplate and sarcoplasmic reticulum, while the slow-contracting red fiber has a poorly developed endplate and sarcoplasmic reticulum.

OGATA[6] reported that the content of mitochondria and development of sarcoplasmic reticulum of the intermediate fiber was intermediate between that of the red and the white fibers. In this study, it was revealed that the motor endplate of the intermediate fiber was also intermediate in size and structure between that of the red and the white fibers.

## SUMMARY

Using double staining method of succinic dehydrogenase and cholinesterase, the structural differences of motor endplate in the red, the white and the intermediate muscle fibers of the mouse limb muscles were observed.

The endplate of the white fiber had a large size and complicated interlacing structure.

The endplate of the red fiber had a small size, simple and compact structure.

The endplate of the intermediate fiber had a medium size and moderately developed structure.

ACKNOWLEDGEMENT

The author wishes to acknowledge the kind advice given by Prof. S. TANAKA.

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