View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Okayama University Scientific Achievement Repository

Acta Medica Okayama

Volume 19, *Issue* 4 1965 *Article* 2

AUGUST 1965

Histochemical studies on the red, white and intermediate muscle fibers of some skeletal muscles. I. Succinic dehydrogenase activity and physiological function of intercostal muscle fibers

Akira Nishiyama*

*Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

Histochemical studies on the red, white and intermediate muscle fibers of some skeletal muscles. I. Succinic dehydrogenase activity and physiological function of intercostal muscle fibers*

Akira Nishiyama

Abstract

From the histochemical study of the intercostal muscles of cat, the following results were obtained. 1. Three different types of muscle fibers have been clearly distinguished in intercostal muscles by histochemical demonstration of succinic dehydrogenase; namely, the white fibers are stained faintly, while the red fibers deep blue and the intermediate fibers purple or bluish purple. 2. The difference in these stains is due to the degree of the enzyme activity, i. e., the areas of high SDH activity are stained deep blue while those of relatively low SDH activity are stained purple. 3. At oil immersion magnification, the differences among the three types of fibers are clearly distinguishable by the amount, size, distribution pattern and subsarcolemmal precipitation of Nitro-BT formazan particles. 4. Concerning the spatial distribution of these three types of fibers in each intercostal muscle, the muscles in the cranial and caudal parts of thorax (I-IV, VIII-XII) show a higher proportion of red fibers, while those in the middle thorax show a higher proportion of white fibers. 5. The vertebral portion of the first internal intercostal muscle is composed of only two types of fibers, red and intermediate ones, and their diameters are almost the same in size as in soleus muscle. In the middle intercostal muscle (V-VII), an intimate relationship can clearly be observed between the size and the enzyme activity of muscle fibers as in the gastrocnemius muscle. 6. In comparison with the anatomy of thorax and the distribution of muscle fibers, it may be presumed that there is a close relationship between the distribution and the scope of thorax movements, however, no definite relation between the distribution pattern and respiratory participation of muscle fibers. 7. Hence, it appears that the intercostal muscles in the cranial and caudal parts of thorax perform original respiratory movements, while the muscles in the middle thorax mainly perform voluntary respiratory movements, perhaps display their function during forced breathing. The intermediate fibers may usually have some tonus and carry out the role of resisting ribs from falling inside by negative pressure of the thoracic cavity.

---

*PMID: 4223027 [PubMed - indexed for MEDLINE] Copyright ©OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 19, 177—189 (1965)

HISTOCHEMICAL STUDIES ON THE RED, WHITE AND INTERMEDIATE MUSCLE FIBERS OF SOME SKELETAL MUSCLES

I. SUCCINIC DEHYDROGENASE ACTIVITY AND PHYSIOLOGICAL FUNCTION OF INTERCOSTAL MUSCLE FIBERS

Akira NISHIYAMA

Department of Surgery, Okayama University Medical School, Okayama, Japan
(Director: Prof. S. Tanaka)

Received for publication, August 2, 1965

Since the classic work of RANVIER[25], it is well known that the striated muscles of the mammals are composed of two different kinds of muscle; namely, red and white muscles. The former performs slow contraction and the latter does rapid one. DENNY-BROWN[3] showed that the red muscle is concerned with tonic postural adjustment and the white with phasic contraction. In electromyographic field NUMOTO[16] and TOKIZANE[30] indicated that the kinetic motor unit was innervated by the pyramidal system, while the tonic motor unit was controlled by the extrapyramidal system.

Since SELIGMAN and RUTENBERG[28] published the histochemical technique for succinic dehydrogenase using Blue tetrazolium as an electron accepor, histochemical studies of the enzyme system in mammalian striated muscles have been reported by several investigators.[13,33] OGATA[18,19,20] of our laboratory reported that the limb muscle fibers of the vertebrate can be divided into three types from the difference in the activities of their histochemically demonstrable oxidative enzymes.

On the other hand, two different opinions have been presented concerning functional differences of the intercostal muscles. PATON and ZAIMIS[23], studying the effect of neuromuscular blocking agents (D-tubocurarine and decamethonium), considered that the intercostal muscles of cat resembled the diaphragm and slow types of muscle such as soleus, rather than the fast types such as tibialis anterior. However, BISCOE[1] concluded that the intercostal muscles were fast muscle in cat from the observation of isometric contraction response to indirect stimulation. Regarding functional differences between the internal and external intercostal muscles, the opinions are also divided among physiologists.

In the previous paper[21] it has been demonstrated that three different types of muscle fibers are clearly distinguished in the intercostal muscles of mammals

Produced by The Berkeley Electronic Press, 1965

by the difference in oxidative enzyme activities. In the present study detailed structural differences in individual intercostal muscle fibers and spatial distribution of these three types of fibers in each intercostal muscle of cat were studied by the histochemical demonstration of succinic dehydrogenase.

MATERIALS AND METHODS

The intercostal muscle of healthy adult cats served as materials. The muscle was removed immediately after death of the animals anesthetized with pentobarbital sodium 20 mg/kg body weight. Without fixation, cross-sections were made of muscle belly through a whole muscle and cut into 20—40 μ thick in a $-20°C$ cryostat and mounted on the slide glass. For the histochemical demonstration of succinic dehydrogenase, the sections were dried at room temparature for about 5 minutes and incubated for 20—40 minutes at 37°C in substrate solution consisted of M/5 sodium succinate 5 ml, M/10 phosphate buffer, pH 7.4, 5 ml, Nitro-blue tetrazolium (-BT) 5 mg/3 ml, 6 ml and distilled water, 10 ml. Then the sections were mounted with glycerine jelly.

For the calculation of spatial distribution of the muscle fibers, respective numbers of three types of the fibers within a certain lens field were counted under the light microscope and the average was taken of the percentages counted in 12 adult cats. The diameter of the fibers was determined with the aid of micrometer scale.

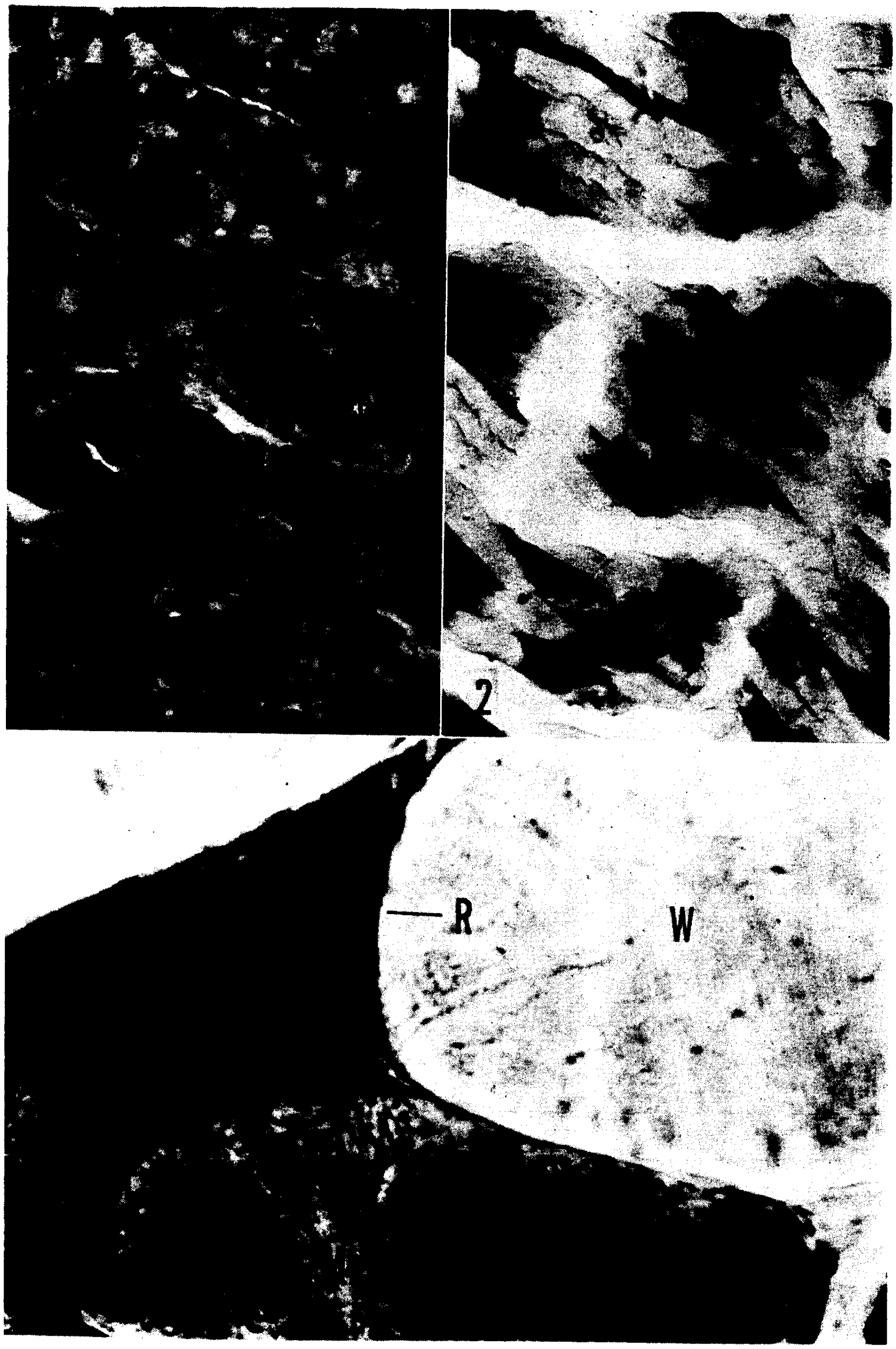
RESULTS

In transverse sections of the intercostal muscle of cat, three types of fibers were clearly distinguished, i. e., generally small red fibers showed a higher SDH activity while large white fibers a lower activity, and the third type of fibers, 'intermediate fibers', showed a moderate activity between the red and white fibers (Fig. 1). Furthermore, a careful observation at oil immersion magnification revealed the following characteristics of these three types of fibers individually, that is, white fibers: Fibers of this type were stained pale showing

Fig. 1 Intercostalis externus of cat, cross-section, succinic dehydrogenase. Note three types of fibers, namely, the small fiber with a higher enzyme activity, the large fiber with a lower activity and the third type of fiber, intermediate fiber, being intermediate in size with a moderate activity. ×125

Fig. 2 Intercostalis externus of cat, longitudinal section, succinic dehydrogenase. ×125

Fig. 3 Intercostalis externus of cat, cross-section, succinic dehydrogenase. Note three types of fibers, namely, the small fiber (R) containing a large number of diformazan granules and with an active subsarcolemmal SDH activity. The large white fiber (W) with a few granules reveals fine streaks in sarcoplasm. The intermediate fiber (M) contains a moderate number of diformazan granules which compose a uniform network. ×1,600


2
R
W

a slight or no enzyme activity and a few granules, containing numerous fine streaks in their sarcoplasm (Fig. 3, W). Red fibers: these were stained deep blue, showing a higher SDH activity with a large number of diformazan granules. These granules could be divided into two groups from their size, larger and smaller ones, and the granules were preferentially distributed toward the periphery of the fibers. Particularly, this fiber showed a strong subsarcolemmal SDH activity with distinct accumulation of large diformazan particles (Fig. 3, R). Intermediate fibers: Fibers of this type were stained purple or bluish purple, showing an intermediate enzyme activity between the red and white fibers. Di-

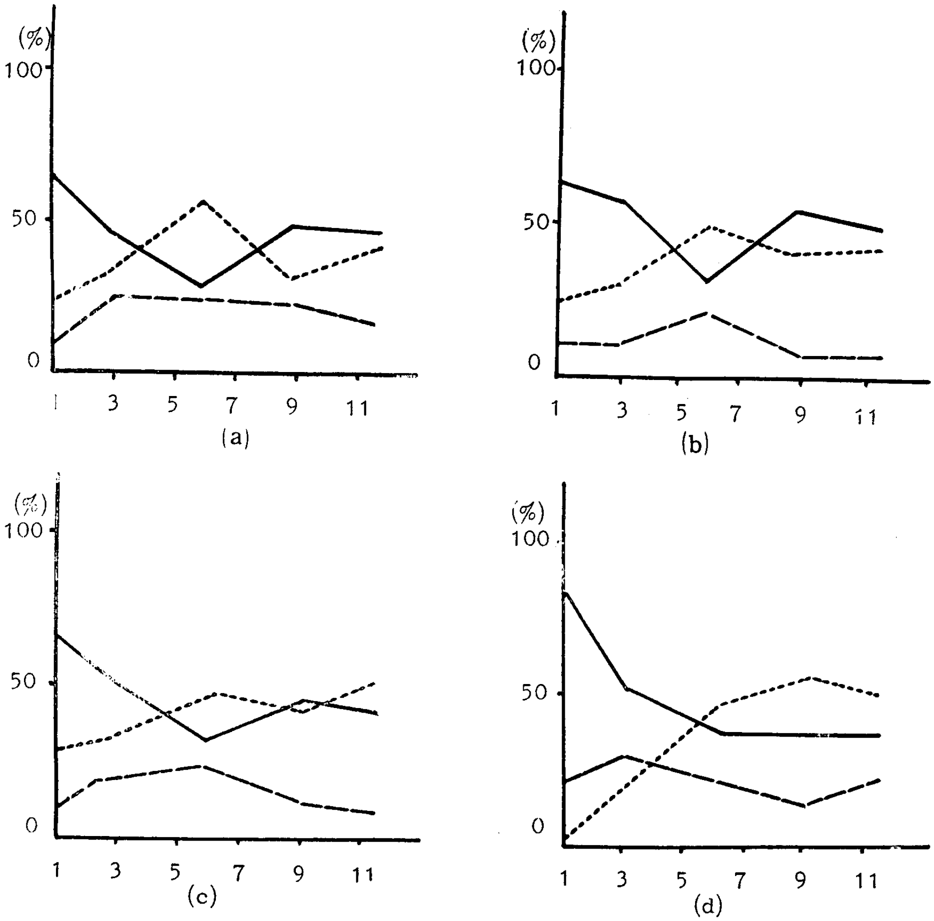


Fig. 4 Spatial distribution of three types of fibers in intercostal muscles of the cat. Ordinate indicates the percentage of three types of fibers and abscissa indicates each intercostal spaces.
(a) sternal region of intercost. ext. (b) vertebral region of intercost. ext.
(c) sternal region of intercost. int. (d) vertebral region of intercost. int.
solid line: red fiber dotted line: white fiber broken line: intermediate fiber

formazan granules were generally smaller in size than those observed in the red fiber, and constituted a uniform network (Fig. 3, M).

It has been further observed that the spatial distribution and the diameter of these three types of fibers vary considerably with respective intercostal muscles. Therefore, the percentages of the fibers contained in a unit area of the muscle were continuously calculated. Anatomically, internal and external intercostal muscles are distributed into two distinct regions, namely, intercartilagineous (sternal) and interosseous (vertebral). As a specimen of each region, parasternal and paravertebral parts of the intercostal muscles were chosen.

As shown in Figures 4 and 5, the muscles in cranial and caudal parts of

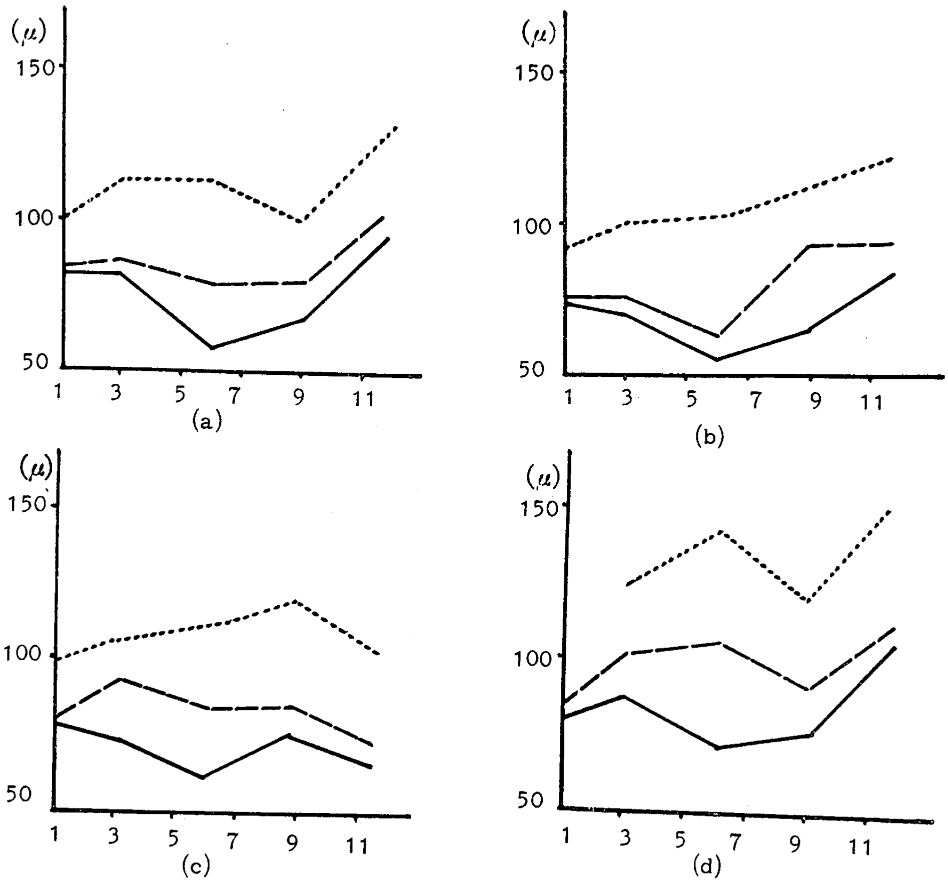
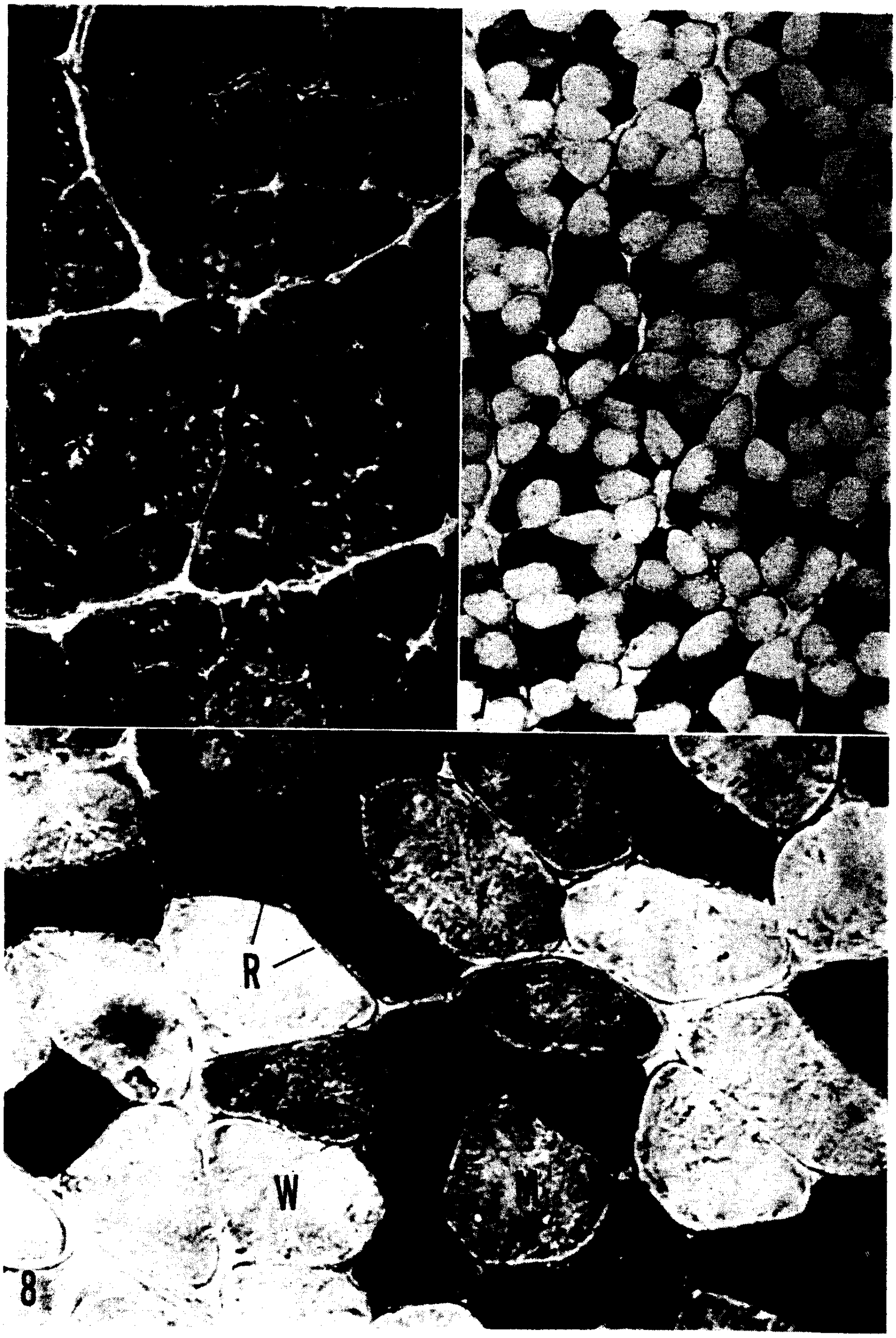


Fig. 5 Diameters of three types of fibers in intercostal muscles of the cat. Ordinate indicates the diameter of three types of fibers and abscissa indicates each intercostal spaces.
(a) sternal region of intercost. ext. (b) vertebral region of intercost. ext.
(c) sternal region of intercost. int. (d) vertebral region of intercost. int.
solid line: red fiber dotted line: white fiber broken line: intermediate fiber


R
W
8

Nishiyama: Histochemical studies on the red, white and intermediate muscle

the thorax (I-IV and VIII-XII intercostal) show a higher ratio of red fibers and lower ratio of white ones. Particularly, the vertebral region of the first internal intercostal muscles is composed of only two types of fibers, red and intermediate, as soleus muscle (Fig. 4, d). Their diameters are almost the same (Fig. 6). While the muscles in the middle thorax (V-VII intercostal) show a higher proportion of white fibers. In this region, an intimate relationship between the size and the enzyme activity of muscle fibers can clearly be recognized, i.e., the fiber with a low enzyme activity has a larger diameter, while that with a high enzyme activity a smaller diameter, and that with an intermediate degree of the activity an intermediate diameter (Figs. 7, 8). However, there is no great difference in the distribution pattern of the intermediate fibers in respective intercostal muscles, and as for the size and distribution of three types of fibers there is no marked difference between the internal and external intercostal muscles or sternal and vertebral portions of the same intercostal spaces.

DISCUSSION

1. Heterogeneity of the fibers

WACHSTEIN and MEISEL[33] first stated that succinic dehydrogenase showed different activities depending upon different muscles of mammals. The relationship between this enzyme activity and the size of the fiber was first reported by NACHLAS and PADYKULA[13]. Concerning the other oxidative enzymes, similar relationship has been reported by several workers[4,5], i. e., small fibers with high oxidative enzymes are stained intensely, while large fibers with a low activity of these enzymes stained faintly.

In the present investigation, hues of the staining varied when Nitro-BT was used as a hydogen acceptor. The red fibers were stained deep blue, while intermediate fibers purple or bluish purple, and the white fibers faintly. Generally, the observation of both red and blue stainings with the methods employing many ditetrazoles gives rise to some confusion. SELIGMAN and RUTENBERG[28] explained that the areas with a low enzyme activity are only able to reduce one half of ditetrazole and to yield a monoformazan which will be expected to be colored red. However, BURTNER *et al.*[2] stated that this red compound is a monotetrazole as an impurity in commercial ditetrazoles. PEARSE[24] also indicated that

Fig. 6 The vertebral portion of the first intercostalis internus of cat, cross-section, succinic dehydrogenase. One type of fiber (R) shows a higher enzyme activity while the other (M) a lower activity ×330

Fig. 7 The VIth intercostalis externus of cat, cross-section, succinic dehydrogenase. Three types of fibers are clearly distinguished by their diameters and the enzyme activities. ×125

Fig. 8 The VIth intercostalis externus of cat, cross-section, succinic dehydrogenase. Note three types of fibers (R, W, M). ×400 both red and purple compounds were consistently observed when using Nitro-BT. However, in this experiment no red stain in tissue sections has been noted with our samples of pure Nitro-BT, in agreement with a study reported by NACHLAS *et al.*[14]. Therefore, it may be assumed that the difference in stainability of these muscle fibers is attributable to the degree of enzyme activity, i. e., the areas with a higher SDH activity are stained deep blue, while those with a relatively low SDH activity are stained purple.

It is well known that mitochondria contain many enzymes of TCA cycle. According to GREEN[9] mitochondria contain a complex electron transfer system, and one of these is the succinic dehydrogenase system. ODA[17] has demonstrated, using the electron microscope, that cytochemical reaction of the succinic dehydrogenase system is limited in mitochondria. NOVIKOFF *et al.*[15] also stated that Nitro-BT formazan was deposited inside the mitochondria. Thus, it is accepted by most workers that this enzyme demonstrated by the tetrazolium technique gives an accurate picture of mitochondria. Hence, it is obvious that the red muscle fibers which participate in tonic contraction are rich in mitochondria, and supplied with abundant energy by the oxidative metabolism. Whereas the white muscle fibers which participate only in phasic movement have large amounts of glycogen and phosphorylase[4,7,26] and supplied with energy for contraction by anearobic glycolysis.

On the other hand, recent electron microscopic studies have revealed differences between the red and the white muscles with regard to the amount and the distribution pattern of mitochondria and sarcoplasmic reticulm. As already mentioned in this study on the cat intercostal muscles, three types of muscle fibers were clearly distinguished, especially the differences between the red and intermediate fibers were distinct by not only the size and enzyme activity of the fibers but also by the amounts, distribution pattern and subsarcolemmal precipitation of Nitro-BT formazan particles. Similar observations have been reported by STEIN and PADYKULA[29] in limb muscle of rat. Recently OGATA[22] has described similar findings in limb muscles of mice using the electron microscope. That is, in the red fiber generally there are distinctly outlined accumulations of large mitochondria beneath the sarcolemma and in intrafibrillar space, while in the intermediate fiber usually mitochondria are sporadically dispersed in the subsarcolemmal space, and in the intrafibrillar space a pair of mitochondria are usually observed on both sides of Z-line. Sarcoplasmic reticulm is considerably well developed in the white fibers but that of the red and intermediate fibers poorly.

It is well recognized that the muscle tonus can be classified into plastic and contractile tonus, and that the red fibers participate in plastic tonus, while the white fibers in contractile tonus. However, the muscle which performs a powerful contraction and requires contractile tonus contains denser sarcosomes in the red fiber (for example, diaphragm), while the muscle which performs isotonic contraction contains small amount of sarcosomes in it. Furthermore, sarcoplasmic reticulm presumed to be a transmitter of the excitatory impulses to the muscle fiber, as described by OGATA[22], is well developed in the white fiber but less in the red and the intermediate fibers. Thus, it may be assumed that the intermediate fiber is a slow contracting fiber and has a function similar to the red fiber. As described in KONDO's report[10], the white, intermediate and red muscle fibers might be associated with phasic contraction, plastic tonus and contractile tonus, respectively.

2. Distribution of the fibers

Regarding the spatial distribution of the three types of fibers, OGATA[18] stated that generally the muscle located near the body surface is rich in the white fiber, while deep muscle is rich in the red and intermediate fibers. TSUKAMOTO and MORI[32] pointed out using the limb and jaw muscles of rat, rabbit and cat, that the white fibers are situated rather at the periphery and the red fibers rather at the central part of the muscle. However, the length of the intercostal muscles of cat is generally short in the direction of muscle fiber, and there is no striking difference in the distribution of each type of fibers between the periphery and central parts of the muscle.

In this study, it has been demonstrated that the intercostal muscles in cranial and caudal parts of the thorax show a higher proportion of red fibers, while that in the middle thorax a higher proportion of white fibers. These results suggest some functional differences under different situations of the intercostal muscles in thorax.

The movement of thorax during quiet breathing are managed by the cooperation of ribs, sternum, vertebra and diaphragm. In the case of man, the thorax enlarges downwards by contraction of diaphragm, forwards by elevating ribs but not backwards. However, as several workers have already pointed out, there is no concrete evidence that the physiological functions of respiratory muscles of man and those of the quadrupedal animals are exactly identical.

Considering anatomical relation of the thorax, the cranial part of the intercostal muscles is covered with many accessory respiratory muscles, namely, M. scalenus, M. transversus costarum and M. serratus anterior. Furthermore, in this region the intercostal spaces are relatively narrower than that in other regions. The action of intercostal muscles is closely related with the angle formed by the direction of muscle and rib[6]. The angles in the first and second intercostal muscles are extremely small. On the other hand, the muscles in the middle thorax are considerably well developed and both the angle and the intercostal space are wide. The muscles in the lower thorax, resembling those in the upper ones, are poorly developed. Consequently, in comparison with the anatomy of thorax described above and the spatial distribution of muscle fibers, it appears that there is a close relationship between the distribution pattern and the scope of thorax movements.

Though the function of the intercostal muscles in respiration has been the subject of contention[12,27], these muscles are generally considered to have the following functions, namely, the external intercostal muscles and intercartilagineous portion of the internal intercostal muscle are engaged in inspiratory enlargement of the thoracic cavity and the interosseous portion of the internal intercostal muscle in its exspiratory diminution.

According to GESSEL's experiment[8], using electromyography on dog, the inspiratory action potentials of external intercostal muscles are confined mostly to the anterior half or two-thirds of the chest and dorsal aspects, approximately from the insertion of M. serratus anterior to the back, while the muscles below the VIth intercostal space show occasionally the opposite function, that is, the exspiratory participation. The intercartilagineous portion of the internal intercostal muscles in the Ist to Vth intercostal spaces actively participate in inspiration, while those below the VIth intercortal spaces mostly exspiration. TOKIZANE *et al*[31]. have also reported similar observations about man.

Thus, the action of intercostal muscles is considerably complicated. Concerning this problem, KOTANI[11] postulates that the internal and external intercostal muscles are each functionally consisted of an elaborate mixture of both inspiratory and exspiratory fibers, which are distributed with a density of being able to manage harmonious respiratory movement. In the present study, there was no essential difference in the distribution pattern of muscle fibers between the internal and external intercostal muscles. Hence, we cannot find any definite relation between the distribution and respiratory participation of different types of muscle fibers.

All these observations lead us to suggest that the intercostal muscles in the cranial and caudal parts of thorax, showing a higher proportion of red fibers, perform the tonic contraction, perhaps original involuntary respiratory movements. On the contrary, the muscles in the middle thorax, showing a higher proportion of white fibers, may perform mainly the phasic contraction, perhaps voluntary respiratory movements, for instance, they may display their function with assistance of many other accessory respiratory muscles during forced breathing. While, intermediate fibers, correlating the plastic tonus, show a neary regular proportion in each intercostal muscle. So it may be considered that this fiber usually has some tonus and carries out the role in defense of the ribs from caving in by negative pressure of the thoracic cavity. This hypothesis may give a clue to explain the fact that the respiratory action is endowed with

Nishiyama: Histochemical studies on the red, white and intermediate muscle

the dual character of automaticity of the heart and the voluntary character of the skeletal muscle.

SUMMARY

From the histochemical study of the intercostal muscles of cat, the following results were obtained.

1. Three different types of muscle fibers have been clearly distinguished in intercostal muscles by histochemical demonstration of succinic dehydrogenase; namely, the white fibers are stained faintly, while the red fibers deep blue and the intermediate fibers purple or bluish purple.

2. The difference in these stains is due to the degree of the enzyme activity, i. e., the areas of high SDH activity are stained deep blue while those of relatively low SDH activity are stained purple.

3. At oil immersion magnification, the differences among the three types of fibers are clearly distinguishable by the amount, size, distribution pattern and subsarcolemmal precipitation of Nitro-BT formazan particles.

4. Concerning the spatial distribution of these three types of fibers in each intercostal muscle, the muscles in the cranial and caudal parts of thorax (I-IV, VIII-XII) show a higher proportion of red fibers, while those in the middle thorax show a higher proportion of white fibers.

5. The vertebral portion of the first internal intercostal muscle is composed of only two types of fibers, red and intermediate ones, and their diameters are almost the same in size as in soleus muscle. In the middle intercostal muscle (V-VII), an intimate relationship can clearly be observed between the size and the enzyme activity of muscle fibers as in the gastrocnemius muscle.

6. In comparison with the anatomy of thorax and the distribution of muscle fibers, it may be presumed that there is a close relationship between the distribution and the scope of thorax movements, however, no definite relation between the distribution pattern and respiratory participation of muscle fibers.

7. Hence, it appears that the intercostal muscles in the cranial and caudal parts of thorax perform original respiratory movements, while the muscles in the middle thorax mainly perform voluntary respiratory movements, perhaps display their function during forced breathing. The intermediate fibers may usually have some tonus and carry out the role of resisting ribs from falling inside by negative pressure of the thoracic cavity.

ACKNOWLEDGEMENT

Grateful acknowledgement is made to Prof. S. TANAKA for his guidance and painstaking proofreading of this manuscript. The author also expresses his gratitude to Dr. T. OGATA and Dr. T. KAWASHIMA for their valuable instructions and advices during the investigation.

Produced by The Berkeley Electronic Press, 1965

REFERENCES

1. BISCOE, T. J.: The isometric contraction characteristics of cat intercostal muscle. *J. Physiol.* 164, 189, 1962
2. BURTNER, H. J., BAHN, R. C. & LONGLEY, J. B.: Observations on the reduction of tetrazolium salts in biological system. *J. Histochem. Cytochem.* 4, 428, 1956
3. DENNY-BROWN, D. E.: The histological features of striped muscle in relation to its functional activity. *Proc. of Royal soc. of London* 104B, 371, 1929
4. DUBOWITZ, V. & PEARSE, A. G. E.: A comparative study of oxidative enzyme and phosphorylase activity of skeletal muscle. *Histochemie* 2, 105, 1960
5. FENNELL, R. A. & WEST, W. T.: Oxidative and hydrolytic enzymes of homozygous dystrophic and heterozygous muscles of the house mouse. *J. Histochem. Cytochem.* 11, 374, 1963
6. FUKUDA, K.: Human physiology. Nanzando, Tokyo, 1948 (in Japanese)
7. GEORGE, J. C. & NAIK, R. M.: Relative distribution and chemical nature of the fuel store of the two types of fibers in the pectralis major muscle of the pigeon. *Nature* 181, 709, 1958
8. GESSEL, R.: Individuality of breathing. *Amer. J. Physiol.* 115, 168, 1936
9. GREEN, D. E.: Enzymes in metabolic sequences in Chemical Pathway of Metabolism, edited by Greenberg, D. M., Academie Press, New York. 1954
10. KONDO, M.: Histological studies on correlation between function and structure of skeletal muscle. Part 1. Histological studies on correlation between function and structure of striated muscle fiber (in Japanese). *Okayama Iggakai Zasshi* 71, 3569, 1959
11. KOTANI, S.: On the participation of the intercostal muscles in the normal breathing (in Japanese). *J. Physiol. Soc.*, Japan 21, 146, 1959
12. MARTIN, H. N. & HARTWELL, E M.: On the respiratory function of the internal intercostal muscles. *J. Physiol.* 2, 24, 1879
13. NACHMIAS, V. T. & PADYKULA, H. A.: A histochemical study of normal and denervated red and white muscles of the rat. *J. Biophys. Biochem. Cytol.* 4, 47, 1958
14. NACHLAS, M. M. & SELIGMAN, A. M.: Cytochemical demonstration of succinic dehydrogenase by the use of a new *p*-nitrophenyl substituted ditetrazole. *J. Histochem. Cytochem.* 5, 420, 1957
15. NOVIKOFF, A. B., SHIN, W. Y. & DRUCKER, J.: Mitochondrial localization of oxidative enzymes: Staining results with two tetrazolium salts. *J. Biophys. Biochem. Cytol.* 9, 47, 1961
16. NUMOTO, M.: Electromyographycal studies on the regulation of the contraction of muscles in the central nervous system (in Japanese). *Okayama Iggakai Zasshi* 64, 1610, 1952
17. ODA, T.: Cytochemical and biochemical studies on the terminal electron transport system. II. Cytochemical demonstration of the succinic dehydrogenase system and cytochrome oxidase in mitochondria with the electron microscope (in Japanese). *Symposia of the Society for Cellular Chemistry* 8, 1958
18. OGATA, T.: A histochemical study of the red and white muscle fibers. Part I. Activity of the succinoxydase system in muscle fibers. *Acta Medicinae Okayama* 12, 216, 1958
19. OGATA, T.: A histochemical study of the red and white muscle fibers. Part II. Activity of cytochrome oxidase system in muscle fibers. *Acta Medicinae Okayama* 12, 228, 1958
20. OGATA, T.: A histcchemical study of the red and white muscle fibers. Part III. Activity of diphosphopyridine nucleotide and triphosphopyridine nucleotide diaphorase in muscle fibers. *Acta Medicinae Okayama* 12, 233, 1958
21. OGATA, T., KAWASHIMA, T. & NISHIYAMA, A.: Histochemical demonstration of three types of muscle fibers of the intercostal muscles. *Acta Medicinae Okayama* 17, 257, 1964

22. OGATA, T.: An electron microscope study on the red, white and intermediate fibers of mouse. *Acta Medicinae Okayama* 18, 271, 1964
23. PATON, W. D. M. & ZAIMIS, E. J.: The action of D-tubocurarine and of decamethonium on respiratory and other muscles in the cat. *J. Physiol.* 112, 311, 1951
24. PEARSE, A. G. E.: Histochemistry theoretical and applied. 2nd edition, Little, Brown and Company, Boston. 1961
25. RANVIER, L. A.: De quelques faits relatifs à lhistologie et à la physiologie des muscles stries. *Arch. Physiol. Nor. Pathologique* 6, 1, 1874
26. SAITO, K.: Histochemical studies on the skeletal muscle and functional differentiation of the anterior column cells. Part 1. Histochemical studies on the skeletal muscle fibers (in Japanese). *Okayama Iggakai Zasshi* 71, 4377, 1959
27. SCHARPEY-SCHAFER, E. & MACDONALD, A. D.: The action of the intercostal muscles. *J. Physiol.* 60, 25, 1925
28. SELIGMAN, A. M. & RUTENBERG, A. M.: The histochemical demonstration of succinic dehydrogenase. *Science* 113, 317, 1951
29. STEIN, J. M. & PADYKULA, H. A.: Histochemical classification of individual skeletal muscle fibers of the rat. *Amer. J. Anat.* 110, 103, 1962
30. TOKIZANE, T. & TSUYAMA, N.: Clinical electromyography, 2nd edition, Kyodo-isho Publishing Co., Tokyo, 1952 (in Japanese)
31. TOKIZANE, T., KAWAMATA, K. & TOKIZANE, H.: Electromyographic studies on the human respiratory muscle. *Jap. J. Physiol.* 2, 232, 1952
32. TSUKAMOTO, S. & MORI, M.· Succinic dehydrogenase activity of skeletal muscle and its electrophysiological function. Proceedings of the Second Annual General Meeting of Japanese Histochemical Association. 26—30, Nov. 1961
33. WACHSTEIN, M & MEISEL, E.: The distribution of histochemically demonstrable succinic dehydrogenase and of mitochondria in tongue and skeletal muscle. *J. Biophys. Biochem. Cytol.* 1, 483, 1955