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Histochemical Study on Intermediate Muscle in Fishes

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Two types of muscle, dark and ordinary ones, have long been recognized in the skeletal muscle of fishes (Lagler et al., 1977). Ogata and Mori (1964) reported that a third type of muscle was in fishes as well as in other vertebrates. The differences of constituents between the dark and ordinary muscles have been described by Love (1970). However, little is known about the intermediate muscle. This study was performed to examine histochemically the differences among the three types of muscles.

Materials and methods

Five specimens of the carp *Cyprinus carpio* (mean body weight 61.2 g), 5 rainbow trout *Salmo gairdnerii* (mean body weight 21.5 g), 5 *Tilapia zillii* (mean body weight 21.9 g), 4 sardine *Sardinops melanosticta* (mean body weight 2.9 g) and one jack *Trachurus japonicus* (body weight 98 g) were used. The dark, intermediate and ordinary muscles were removed from the central part of the dorsal muscle and frozen by precooled n-hexane (−80°C). The samples were cut in a cryostat and subjected to the histochemical study described below: aldolase, lactic dehydrogenase (LDH), succinic dehydrogenase (SDH), reduced nicotinamide adenine dinucleotide dehydrogenase (NADH-DH), malic dehydrogenase (MDH), PAS and Oil red O reactions. Myoglobin staining took place in the carp only. The enzyme reactions were treated at 25°~30°C in the carp, *Tilapia*, sardine and jack, and at 15°~20°C in the rainbow trout. PAS, Oil red O and myoglobin reactions were stained at room temperature.

Results

The dark muscle had a small diameter in the muscle fiber and abundant sarcoplasm, while the ordinary muscle had a large diameter and scanty sarcoplasm. The intermediate muscle had a slightly smaller diameter and a little more abundant sarcoplasm than the ordinary one. Thus, three types with morphologically different characteristics were observed in the trunk muscle of the five species studied. The intermediate muscles interposed in the above properties between the dark and ordinary ones. A fairly large number of the intermediate muscles were observed in the trunk muscle of the carp, rainbow trout and *Tilapia*, while the sardine and jack had a small number of these muscles.

In all fishes studied, the dark muscle reacted most intensely to all stainings tested. The reactions of all the enzymes were observed in the sarcoplasm beneath the sarcolemma and in the intermyofibrillar sarcoplasm. The intermediate muscle demonstrated more intense reactions to all the stainings than the ordinary muscle, but less intense than the dark muscle (Fig. 1). The sites of reactions were quite similar to the case of the dark muscle (Fig. 2). As a whole, the staining reactions of the intermediate muscles gradually became weak as it transformed towards the ordinary ones. All the staining reactions in the ordinary muscle were weaker than the other muscles. The reactions of the aerobic enzymes (SDH, MDH and NADH-DH) were almost localized in the sarcoplasm beneath the sarcolemma and were absent in the intermyofibrillar sarcoplasm (Fig. 3). The reactions of the glycolytic enzymes (aldolase and LDH) were observed in the sarcoplasm beneath the sarcolemma and the intermyofibrillar sarcoplasm.

Discussion

It is known that many fishes have the intermediate muscles sandwiched between dark and ordinary muscles (Bone, 1966; Johnston et al., 1974). Patterson et al. (1975) described that this muscle showed intermediate activities of the aerobic enzymes between the dark and the ordinary ones. Our results agree with the above mentioned observations.

An interesting finding in the present study was that the localizations of the aerobic enzymes in the intermediate and dark muscles were different from such localizations in the ordinary muscle. In the intermediate and dark muscles, the activities of the aerobic enzymes were observed not only in the sarcoplasm beneath the sarcolemma but also in the intermyofibrillar sarcoplasm. While the ordinary muscle contained aerobic enzymes in the sarcoplasm only beneath the sarcolemma. Because the aerobic enzymes are localized

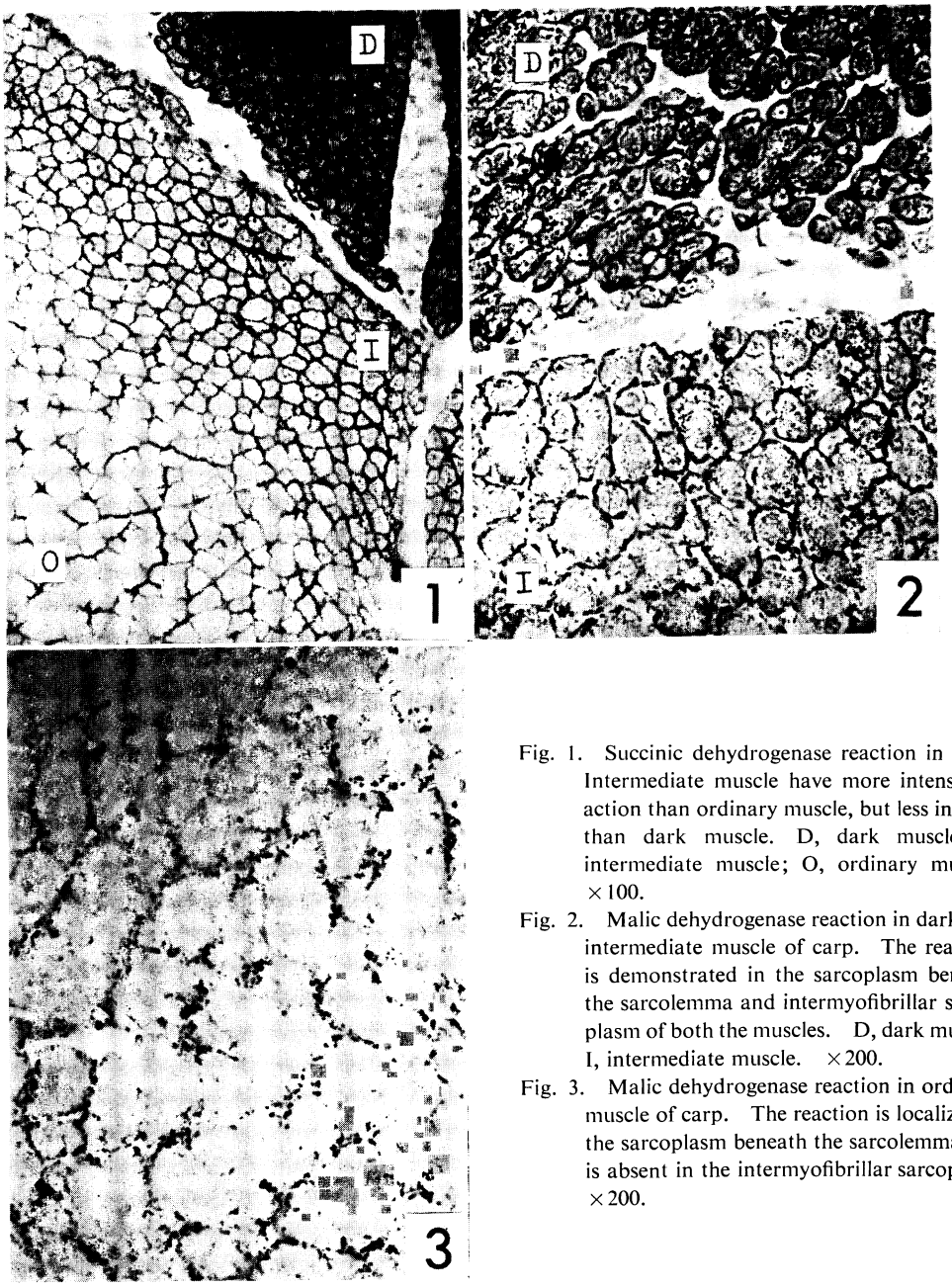

Fig. 1. Succinic dehydrogenase reaction in carp. Intermediate muscle have more intense reaction than ordinary muscle, but less intense than dark muscle. D, dark muscle; I, intermediate muscle; O, ordinary muscle. ×100.

Fig. 2. Malic dehydrogenase reaction in dark and intermediate muscle of carp. The reaction is demonstrated in the sarcoplasm beneath the sarcolemma and intermyofibrillar sarcoplasm of both the muscles. D, dark muscle; I, intermediate muscle. ×200.

Fig. 3. Malic dehydrogenase reaction in ordinary muscle of carp. The reaction is localized in the sarcoplasm beneath the sarcolemma and is absent in the intermyofibrillar sarcoplasm ×200.

in the mitochondria (Lehninger, 1975), it is thought that differences in the site of reactions is due to the localization of the mitochondria. Furthermore, the intermediate muscle had a moderate myoglobin reaction. It is well known that the dark muscles have very high aerobic metabolism and are used in continuous movement (Rayner and Keenan, 1967). Therefore, it is thought that the intermediate muscle also carries out aerobic metabolism moderately and is used in the continuous movement.

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魚類における中間筋の組織化学的研究

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魚類の中間筋を組織化学的に検討した．コイ，ニジマス，ティラピア・ジリ，マイワシおよびマアジにおいて，中間筋の存在が確認された．中間筋の SDH, MDH, NADH-DH, アルドラーゼ, LDH, PAS, オイルレッドOおよびミオグロビン反応は，血合筋と普通筋の中間の活性を示した．好気的代謝に関与する酵素の局在性が，各々の筋肉において異なり，血合筋と中間筋は筋鞘膜下および筋原線維間筋形質に酵素活性があるが，普通筋では酵素活性が筋鞘膜下筋形質だけに存在した．

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