

Fine Structure of the Drum Muscles of the Piranha (Serrasalminae, Characidae)

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Summary. The anterior and the posterior drum muscles of the piranha resemble each other in all essential fine structural aspects: myofibrils are slender; sarcomeres are short compared with those of other drum muscles; mitochondria, located in the periphery of the fibers, are numerous and show an irregular internal structure; and the sarcoplasmic reticulum is abundant. Triads appear at the level of the Z lines. The drum muscles have many motor endplates, which, however, lack the characteristic junctional-fold apparatus. No lipid substances could be demonstrated in these muscles. In the posterior drum muscle the fibers depart from their orderly longitudinal arrangement at irregular intervals.

Key words: Drum muscles – Fine structure – Piranha.

Introduction

There exist a number of studies on the morphology and histology of the sound-producing apparatus in fish of various systematic groups (Schneider and Hasler, 1960; Skoglund, 1961; Schneider, 1964; Cohen and Winn, 1967; Markl, 1971). The diversity of the sounds produced is matched by that of the associated structures (for review, see Schneider, 1961; Schaller, 1971; Dorn and Schaller, 1972a,b; Daugherty and Marshall, 1976). In many fish the essential elements of the apparatus comprise the swim bladder and the apposed or attached drum muscles. Here, sound production occurs by contraction of the drum muscles, the swim bladder serving as a resonator.

This structural principle also applies to the sound apparatus of the piranha. Paired drum muscles lie along the two sides of the large swim bladder. Each of these is subdivided by a septum of connective tissue into an anterior and a posterior part. The muscles originate in the trunk musculature and insert on a tendon running beneath the swim bladder.

Without exception, existing electrophysiological recordings from the drum muscles of different fish have shown a high fusion frequency, indicating that they are all fast muscles (Packard, 1960; Tavalga, 1962; Schneider, 1964, Gainer, Kusano and Matheson, 1965; Markl, 1971). The striking capacity of these muscles for rapid contraction is associated with a special fine structure, demonstrated in *Opsanus spec.* by Fawcett and Revel (1961), and by Eichelberg (1976) in *Therapon jarbua*.

The aim of the present investigation was to compare the fine structure of the drum muscles in the piranha with drum muscles of other species, and to determine whether fine structural differences exist between the anterior and posterior muscles.

Materials and Methods¹

The piranhas used in this study measured between 10 and 15 cm in length. They were obtained as young fish from a commercial trader and were kept for about 2 years in an aquarium in the institute. To rule out the possibility of muscle contraction, 0.5 ml Curarin (Asta) was injected into the body cavity. 5 min thereafter the fish were decapitated and dissected in order to expose the drum muscles. All the muscles were first fixed in situ with 1% OsO₄ for 1/2 h, and then dissected out and cut into small pieces about 1 mm². The latter were then fixed further in 1% OsO₄ in 0.1 M phosphate buffer for 1 h. Block contrasting was done overnight at 4° C in a 1% solution of phosphotungstic acid and uranyl acetate in 70% ethanol. The samples were embedded in a 1:1 mixture of styrol and methacrylate.

The ultrathin sections were postcontrasted by immersion in a 0.3% lead citrate solution for 3 min. Electron micrographs were taken on a Siemens Elmiskop I. Both the ultrathin sections and the 1 μm thick ("semithin") sections for phase-contrast microscopy were made with a Reichert ultramicrotome.

In addition, measurements were obtained from some muscle fibers fixed in Bouin's fluid, embedded in paraffin, and stained with hemalum/eosin.

Results

Viewed macroscopically, both drum muscles are more compact than the trunk musculature of the fish. They lack the almost translucent appearance that frequently characterizes the musculature of the back. Moreover, they are considerably more red in color than most regions of the trunk muscle.

Microscopic study of the anterior and posterior drum muscles confirmed that the two areas are similar in all fundamental structural aspects. The fibers of both muscles appear irregularly rounded in cross section. Measurement of longitudinal sections indicated that the average thickness of the fibers in the anterior muscle was 49 μm and that of the posterior muscle, 44 μm ($n = 50$). The difference in fiber thickness was negligible.

One conspicuous peculiarity occurred in the posterior drum muscle of the piranha: the fibers display sudden irregularities over their entire length, giving the appearances of "whorls" (Fig. 1). These tangled regions occur frequently but at irregular intervals and are not associated with a particular part of the muscle. They are also evident in electron micrographs, where they appear as regions of the fiber in which the myofibrils are completely disordered, so that immediately adjacent fibers

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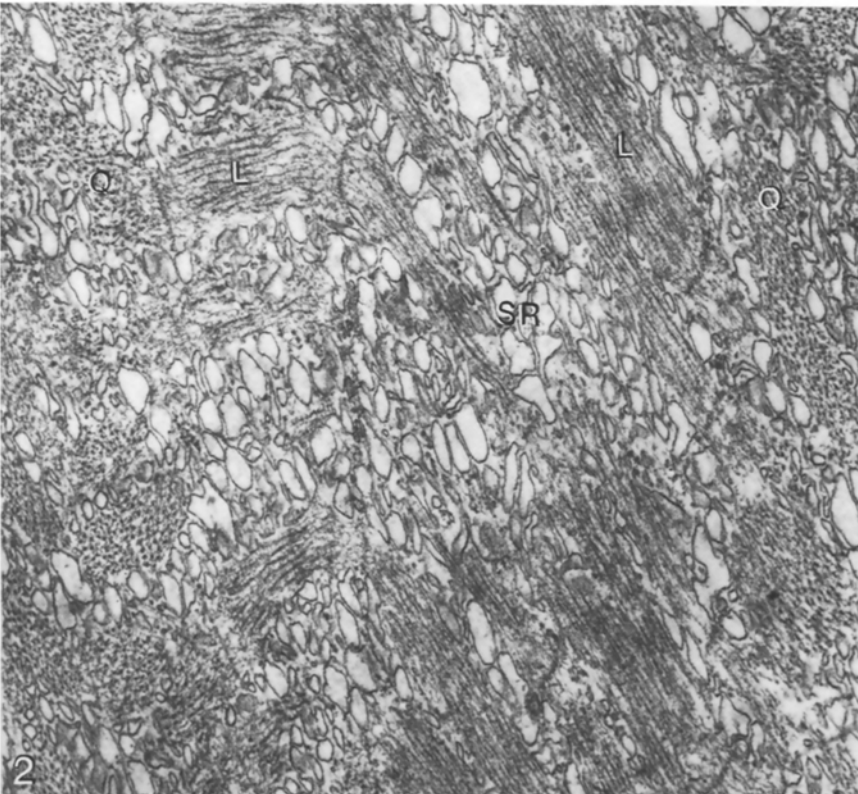
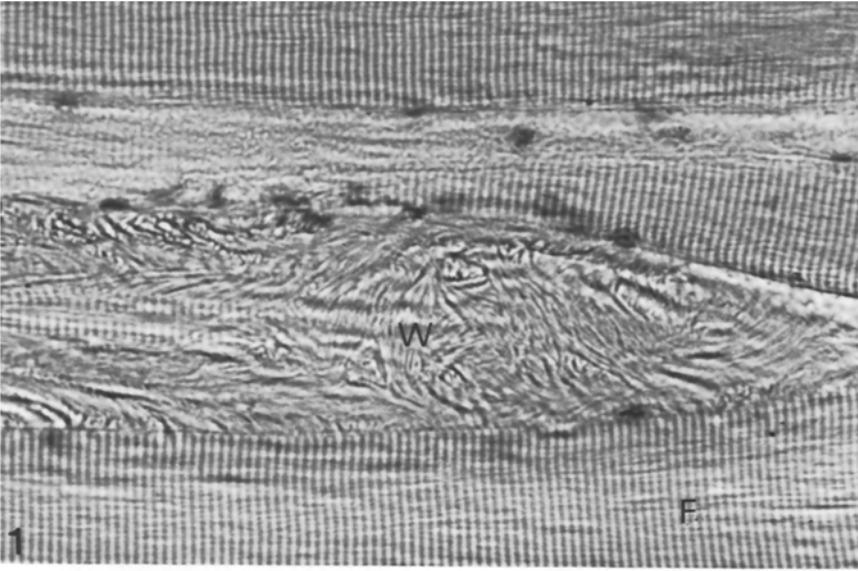


Fig. 1. Longitudinal section through the posterior drum muscle. *F* muscle fiber running parallel to the long axis of the muscle; *W* muscle fiber displaying a "whorl" formation. Bouin; hemalum/eosin, $\times 150$

Fig. 2. Ultrathin section in the region of a fiber "whorl". The myofibrils are oriented both longitudinally (*L*) and transversely (*Q*). *SR* sarcoplasmic reticulum. OsO_4 , $\times 25,000$



Fig. 3. Longitudinal section of the anterior drum muscle. *S* sarcomere; *SR* sarcoplasmic reticulum; *T* triad; *Z* Z line. $\text{OsO}_4 \times 54,000$

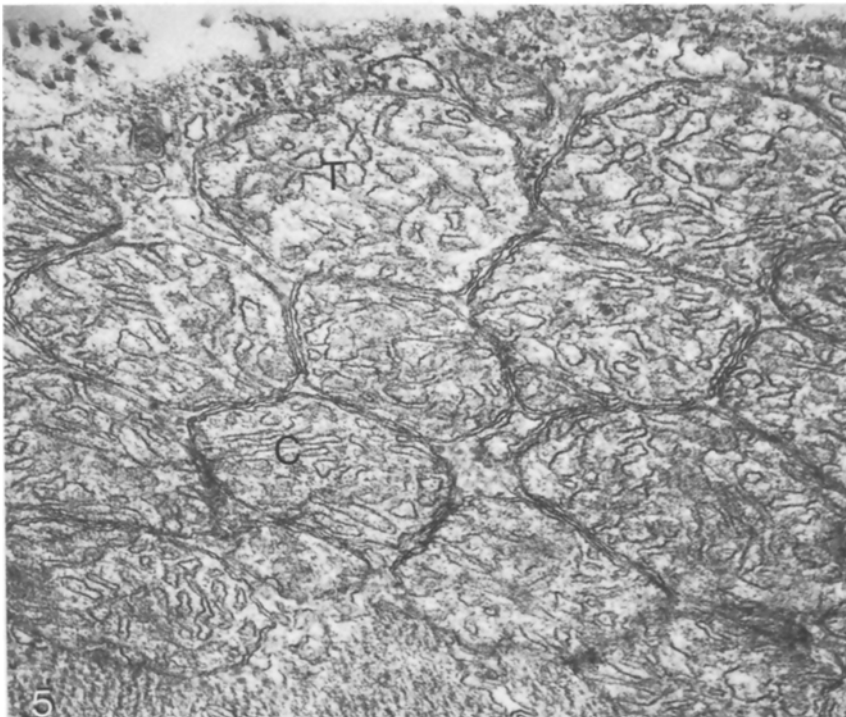
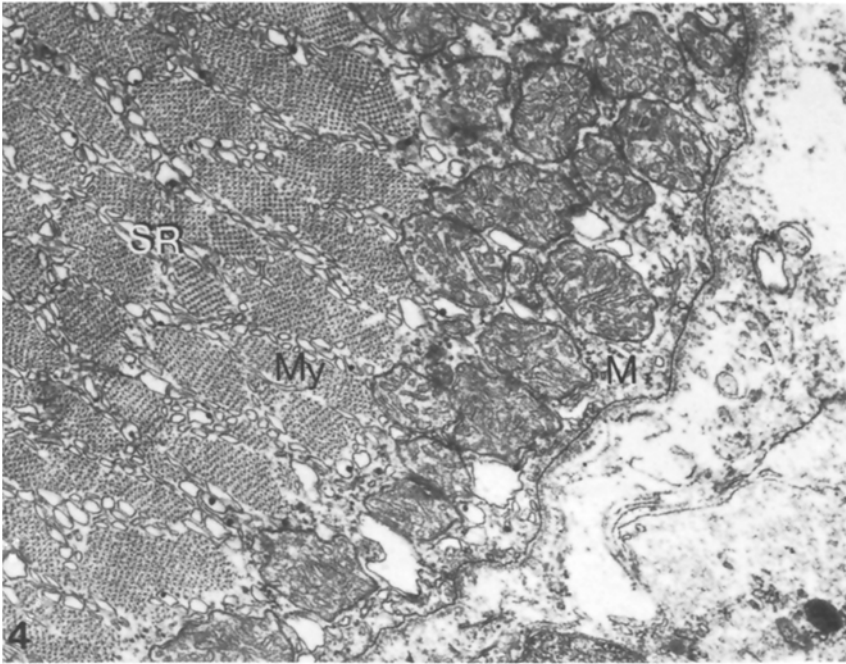


Fig. 4. Cross section of a fiber in the anterior drum muscle showing the peripheral location of the mitochondria (*M*). *My* myofibrils; *SR* sarcoplasmic reticulum. OsO_4 , $\times 32,000$

Fig. 5. Mitochondria in the anterior drum muscle. *C* distinct cristae; *T* tubular structures. OsO_4 $\times 54,000$



Fig. 6. Motor endplate without distinct junctional folds. *M* mitochondria; *ME* motor endplate; *My* myofibrils; *S* synaptic vesicles; *SR* sarcoplasmic reticulum. OsO_4 , $\times 46,000$

may be cut transversely, longitudinally, or at intervening angles (Fig. 2). Apart from the whorls, the myofibrils are regularly oriented along the long axes of the fibers in both drum muscles. These fibrils have the periodic structure typical of striated muscle (Fig. 3).

Compared with drum muscles of other species, the sarcomere length in the piranha drum muscle is short. With the fixation procedure employed, the sarcomeres in the anterior drum muscle measured $1.2\ \mu\text{m}$ in length on the average, and those in the posterior muscle averaged $1.4\ \mu\text{m}$. In both cases the sarcomere width was quite variable, ranging from 0.16 to $0.8\ \mu\text{m}$.

In both drum muscles the mitochondria lie, almost without exception, in the periphery of the fibers (Fig. 4). The rare mitochondria in the interior of the fiber always appeared alone. The mitochondria are irregularly oval in form. No preferred orientation in the long axes was discernible. The internal structure is of the crista type. However, it was common to find mitochondria with inner membranes so vesicular in appearance that they resembled more closely the tubule type (Fig. 5).

Smooth sarcoplasmic reticulum (SR) is extremely abundant in both muscles. Each myofibril is surrounded by a wide zone of this membrane system (Fig. 3). In the periphery of the fiber the SR is even more extensive. The appearance of the SR is almost entirely vesicular, with tubular regions only rarely observed. The structure of the T-system was identical in the two drum muscles. The triads always appear at the level of the Z lines (Fig. 3). The drum muscles of the piranha, then, are of the Z-type. The size of the central vesicle varied widely, but as a rule it was considerably smaller than the flanking SR vesicles. The electron density of the adjacent membranes of the SR and T systems, described as characteristically high in drum muscles of other forms, is not so pronounced in piranhas.

Motor endplates are numerous in both muscles. They are restricted in extent, and invariably lack a well-developed fold apparatus (Fig. 6). The sarcolemma in the subneural region displays very small protrusions. It is characteristic of these endplates that the presynaptic nerve endings contain relatively few vesicles. The mitochondria are also sparse and conspicuously small, with very little internal structure. In contrast, numerous large mitochondria, displaying a dense substructure, are found in the region of the muscle fiber directly surrounding the axon terminal.

Both drum muscles are entirely free of lipid material. On the other hand, glycogen was seen in all regions of the fibers. The energy store evidently consists exclusively of glycogen.

Discussion

The drum muscles of several fish have been examined physiologically (Skoglund, 1961: swim-bladder muscles of *Opsanus tau*; Schneider, 1964: drum muscles of the tigerfish *Therapon jarbua*; Markl, 1971: drum muscles of the piranha). In all cases it was found that the drum muscles are extremely fast with fusion frequencies between 200 and 400 Hz. This physiological property is associated with a characteristic fine structure of the swim-bladder muscles, in which the drum muscles of *Opsanus* and

Therapon correspond in all aspects (Fawcett and Revel, 1961; Eichelberg, 1976). The drum muscles of the piranha, however, differ in some aspects.

Characteristics common to the drum muscles of all three species mentioned above are the extreme thinness of the myofibrils and the very large amount of SR in the fibers. Whereas the sarcomeres of *Opsanus* and *Therapon* measure about 2 μm , those of the piranha are only 1.2 and 1.4 μm , shorter by about one third. These short sarcomeres in the piranha may be associated with the special arrangement of the transverse-tubule system in the drum muscles. In *Opsanus* and *Therapon* the triads are located in the A-I region. The toadfish and tigerfish have two triads per sarcomere, whereas in the piranha, with triads at the level of the Z lines, each sarcomere contains only one triad. However, since these sarcomeres are much shorter, the fiber as a whole may be nearly as well supplied by the excitation-conducting T system as in species with longer sarcomeres.

It has often been claimed that the relative numbers of mitochondria provide an unambiguous criterion by which red and white muscle fibers can be distinguished. For example, Patterson and Goldspink (1972) have shown that in the musculature of the pollock the red and white fibers differ chiefly in the considerably greater abundance of mitochondria in the red fibers. The drum muscles of *Opsanus* and *Therapon* also contain a remarkably high number of mitochondria. Moreover, the mitochondria are very large and have a dense internal structure. Although mitochondria are also numerous in the swim-bladder muscles of the piranha, they are almost entirely restricted to the periphery of the fiber and are much smaller than those in the other two species. Moreover, their internal structure tends to be quite poorly developed.

These findings are not necessarily at variance with the special physiological properties of the piranha drum muscles, because the internal structure of the mitochondria reflects closely the momentary physiological state of the cell. It is plausible that the actual physiological demands on the drum muscles of piranhas kept in aquaria are very moderate; these fish were never heard to produce sounds spontaneously. The typical growling noise could only be elicited by touching the fish. Thus, the swim-bladder muscles are rarely required to work. The mitochondrial fine structure may be correspondingly altered, even though the essential structural elements – the myofibrils, T system and SR – are retained.

Motor endplates are very numerous in the drum muscles of piranhas, as in the *Therapon jarbua*. This, too, is a clear correlate of the speed of these muscles. Cohen and Hess (1967) compared fast and slow fibers in crustaceans and determined that the fast fibers of these animals also have considerably more synapses than slow fibers. On the other hand, it is surprising that motor endplates of the piranha lack junctional folds, for Hess (1965) showed that in *Thamnopsis sirtalis* the fold apparatus in the slow fibers was considerably less well developed than in the fast fibers. The same correlation was found in rats by Padykula and Gauthier (1970) and in tree frog larynx muscles by Eichelberg and Schneider (1973). In both cases fast muscle fibers had endplates with especially well developed junctional folds. In cyclostomes and teleosts, however, the degree of folding of the subneural sarcolemma appears not to be a criterion for the speed of the muscle. In the great variety of muscles studied so far, without exception, the neuromuscular junctions

lack folds (Reger, 1961; Korneliussen, 1973; Davey et al., 1975). The development of the junctional folds in the motor endplates is evidently species-specific, and not characteristic of a fast muscle fiber.

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