ULTRASTRUCTURE AND ADENOSINE TRIPHOSPHATASE ACTIVITY OF RED AND WHITE MUSCLE FIBERS OF THE CAUDAL REGION OF A FISH, *SALMO GAIRDNERI*

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ABSTRACT

Electron microscopy, together with quantitation using a tracing device linked to a digital computer, reveals that the red and white muscle fibers of *Salmo gairdneri* differ in diameter, organization of myofibrils, dimensions of myofilaments, volumes and surface areas of T system and sarcoplasmic reticulum, morphology of mitochondria, and content of mitochondria, lipid, and glycogen. Biochemical studies show that the ATPase activity of white fibers is three times that of the red fibers. Actomyosin content of red fibers is higher than that of the white fibers. The functional significance of these differences between two fiber types is discussed.

INTRODUCTION

The caudal musculature of *Salmo gairdneri*, the rainbow trout, consists of eight muscles which are capable of delicate organized movements (28). The caudal musculature is derived from the caudal myomeres, each of which consists of two types of muscle fibers: red and white. The two types of fibers form separate portions of the caudal musculature, easily recognized by their color. Lorenzini (26) first found red and white muscle fibers in the elasmobranches among vertebrates. The red color of fish muscle fiber is attributed to the high myoglobin content and highly developed vascular bed of the fibers.

References to electron microscope study of red and white muscle fibers of fish are scanty (4, 6, 12, 13, 30, 31, 39). Most offer descriptions of limited aspects of the cytology of either one or both types of fibers.

Three different suggestions have been put forward to account for the functions of red and white muscle fibers of fish. One expresses the idea that only red muscle fibers are active during slow swimming, whereas white muscle fibers are active during rapid swimming (2, 7, 8, 14, 18, 38, 41). The second suggestion is that the main purpose of the red muscle fibers is not muscular work, but that of carrying out synthetic processes similar to those taking place in the liver (9, 44). The third suggestion is that red muscle fibers are used in rapid swimming and white in slow continuous swimming (5, 6, 10, 15). It is interesting to note that even workers studying the electrophysiological properties of red and white fibers in fish have come to divergent conclusions (5, 8, 18, 38, 41).

The purpose of the present study is to investigate and evaluate ultrastructural differences between red and white muscle fibers of the rainbow trout in relation to swimming behavior, by detailed examination of their ultrastructure and adenosine triphosphatase (ATPase) activity.

MATERIALS AND METHODS

The rainbow trout was chosen as the species to be examined because information about the metabolism

and swimming behavior is available. Fifty specimens averaging about 50 cm fork length were used for electron microscopy.

Microscope Methods

Six fixation procedures were used: (*a*) 3–6% glutaraldehyde, buffered at pH 7.3, with 0.1 M phosphate, for 2 and 24 hr, and after several hours of washing in buffer, postfixation in 1% osmium tetroxide, buffered with 0.1 M phosphate at pH 7.3, for 1.5 hr; (*b*) 3–6% glutaraldehyde in salmonid physiological solution (pH 7.3) (19) for 2 and 24 hr. Muscle bundles were rinsed for several hours in physiological solution and postfixed in 1% osmium tetroxide in the physiological solution for 1.5 hr; (*c*) 5% glutaraldehyde plus 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) (21), for 2 and 24 hr, rinsing in phosphate buffer for several hours, and postfixation in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) for 1–2 hr. Sometimes half strength of this fixative was used; (*d*) 2% potassium permanganate in the physiological solution, for 2 and 24 hr for glycogen (40); (*e*) 1% osmium tetroxide in the physiological solution (pH 7.3) for 1–2 hr; (*f*) 1% osmium tetroxide in 0.1 M cacodylate buffer containing 2 mM $CaCl_2$, pH 7.3–7.4, for 1–2 hr. The figure legends indicate which fixation method was used in each case.

Rest length of muscle bundles was determined by fixing them *in situ*, maintaining their length in the body. Muscle bundles were then tied to toothpicks at their ends so as to hold them at their rest lengths until after washing in buffer and physiological solution. In case of double fixation, first fixation, and buffer and physiological solution rinses were done at room temperature. Just before the second fixation, bundles of about 1 mm in length and 0.5 mm in thickness were cut from a portion of the muscle, midway between the two ends. The postfixation, all single fixations, and dehydration were done at 0°C. After dehydration in absolute ethanol at room temperature, followed by propylene oxide, the material was embedded in Araldite 502. Sections were cut on a Sorvall-Porter-Blum microtome with glass or diamond knives, and stained with 10% uranyl acetate solution in absolute methanol, followed by 0.2% lead citrate solution in distilled water. In the study of glycogen a saturated aqueous solution of lead hydroxide was used. All sections were examined with a Philips EM 100 or 200 or GEM-100B operated at 60 kv, using 25 or 60 μ diameter objective aperture. All electron micrographs for measurements were taken after accurately calibrating the magnification of the microscope used. The relative weights of the red and white muscles in different sections of the body of the fish were determined. Four transverse slices of about 12 mm in thickness each were taken from different sections of the body; the red and white muscle fibers were stripped out carefully from one side of each slice under a dissecting microscope, except for muscles of the caudal peduncle. In the caudal peduncle, stripping involved the superficial flexor muscles only, because the connective tissue separation of fiber types was best developed there. Since it was difficult to avoid contamination of the white muscle fibers in stripping out the red muscle fibers from other caudal muscles, such muscles were excluded from weighing. The stripped muscle was weighed on an analytical balance.

The diameters of red and white muscle fibers were measured from light photomicrographs taken from 1 μ thick sections of Araldite-embedded materials. Since the cross-sectional outlines of muscle fibers are not perfectly circular, the mean of two measurements of the longest and shortest axes of the fiber was taken in each case.

All measurements were taken in red and white fibers fixed by the same method and lying at comparable depths within the block of tissue.

The dimensions of myofilaments were measured on electron micrographs by Micro Compararator Fine scale (6581-M10; Arthur H. Thomas Co., Philadelphia, Pa.).

Six caudal muscles (two superficial flexors, two deep flexors, hypochordal longitudinal, and interradial) were used for the study of ultrastructure of red and white muscle fibers. Since results showed that the ultrastructure did not differ from muscle to muscle, electron micrographs of fibers from only the superficial flexors are used in illustrations.

Biochemical Methods

Live rainbow trout were used for all these experiments. The fish were an average of 32.5 cm in length. All operations were conducted at 0°C and as rapidly as possible. The fish were decapitated, then skinned. Red muscle was carefully excised from the superficial dorsal and ventral flexor muscles, and from the myomeres of the epaxial and hypaxial muscles. White muscle was taken after excision of red muscle. Each excised muscle sample was cut into small pieces which were homogenized in a tissue grinder. The homogenate was slowly stirred with 3 volumes of an extractant made up to 0.6 M KCl, 0.01 M Na_2CO_3, and 0.04 $NaHCO_3$ at pH 8.0 for periods of 5–15 min, and then allowed to stand for 5 hr at 0°C. The mixture was then diluted 10 times with deionized water and the actomyosin was allowed to precipitate overnight. The precipitate was centrifuged in an International Refrigerated Centrifuge (Model PR-6) (International Equipment Company, Needham Heights, Mass.) at 3000 *g* for 10 min. The precipitate was collected and dissolved in 0.6 M KCl and filtered. The connective tissue elements were discarded and actomyosin was

collected and diluted 10 times with deionized water and was allowed to precipitate for 5 hr. The precipitate was centrifuged at 3000 *g* for 10 min and the resulting supernatant was discarded and the precipitate was collected. The precipitate was again dissolved in 0.6 M KCl and centrifuged as before and the precipitate was collected. This step was repeated once more. The precipitate thus obtained was dissolved in 0.6 M KCl and centrifuged at 12,000 *g* in a Beckman/Spinco Centrifuge (Model L) for 20 min. The supernatant solutions of actomyosin were collected for examination. Protein concentrations of actomyosin of red and white muscles were determined by the method of Lowry et al. (27). ATPase activity determinations were made in pH-stat (Radiometer TTT1, Radiometer Co., Copenhagen, Denmark) according to the method of Kay and Brahms (22), by following the proton liberation during ATP hydrolysis. The assays were carried out at pH 8.0 and 10°C, in a medium containing 2×10^{-3} M $MgCl_2$, 0.05 M KCl, and 2×10^{-3} M ATP. The maintenance of a lower temperature in the enzymatic assay was for the cold water fish used. Such fish were kept in an aquarium, at about 10°C. Enzyme was added to the medium and equilibrated for short periods before the enzymatic assay was begun. Rates of dephosphorylation (micromoles of PO_4 per g of enzyme per second) were inferred from the slopes of the titrigraph obtained. ATPase activity measurements were made in each case with two distinct preparations of enzymatically active actomyosin.

OBSERVATIONS

Relative Proportion of Red and White Muscle Fibers

Table I gives information about the relative amounts of red and white muscle fibers in different sections of the body. The mean of ten measurements from ten specimens averaging 22.5 cm in length are given in the table.

TABLE I

Relative Proportion of the Red to the White Muscle Fibers (% by Weight)

	Anterior to the dorsal fin	In region of the dorsal fin	Trunk posterior to the dorsal fin	Caudal peduncle
Red muscle	1.1	4.4	8.4	13.0
White muscle	98.9	95.6	91.6	87.0

Results from the caudal peduncle include superficial flexor muscles only.

TABLE II

Diameter of Red and White Muscle Fibers of the Caudal Musculature (in μ)

Caudal musculature	Red muscle	White muscle
Superficial dorsal flexor	26 (4.1)	60 (17.8)
Superficial ventral flexor	26 (5.2)	56 (23.7)
Hypochordal longitudinal	26 (3.2)	57 (14.1)
Deep dorsal flexor	24 (5.1)	50 (12.7)
Deep ventral flexor	27 (3.2)	52 (18.6)
Interradial	30 (5.3)	61 (20.8)

The mean diameter of white muscle fibers is more than two times that of red muscle fibers. This difference in mean diameter between two types of fiber is found to be statistically significant ($P < 0.005$).

The figure for each muscle is an average of 50 measurements and the standard deviation of each is given in parentheses.

Diameter of Red and White Muscle Fibers

Table II shows different diameters of red and white muscle fibers.

Form of the Myofibrils

The organization of myofibrils of red and white muscle fibers is best understood from examination of transverse and longitudinal sections at low magnification. In transverse sections (Fig. 1) some of the myofibrils of the red muscle fiber are often confluent and form a more or less continuous mass of myofilaments in which isolated areas of sarcoplasm containing sarcoplasmic reticulum, mitochondria, lipid droplets, and glycogen particles are found. Other discrete myofibrillar fascicles average 1 μ across. Since the fusion of myofibrils is not extensive in a well oriented cross-section, the organization of myofibrils of the red muscle fibers does not resemble true *Felderstruktur* (24, 25) which has been demonstrated in tonus fibers by electron microscopy (17, 32, 36). Longitudinal sections of the red muscle fibers show (Fig. 2) that most of the myofibrils may run as independent units. Mitochondria and lipid droplets can be seen between the myofibrils.

The myofibrils of the white muscle fibers are usually distinct. In transverse sections myofibrils are ribbon-shaped and aligned along the radii of

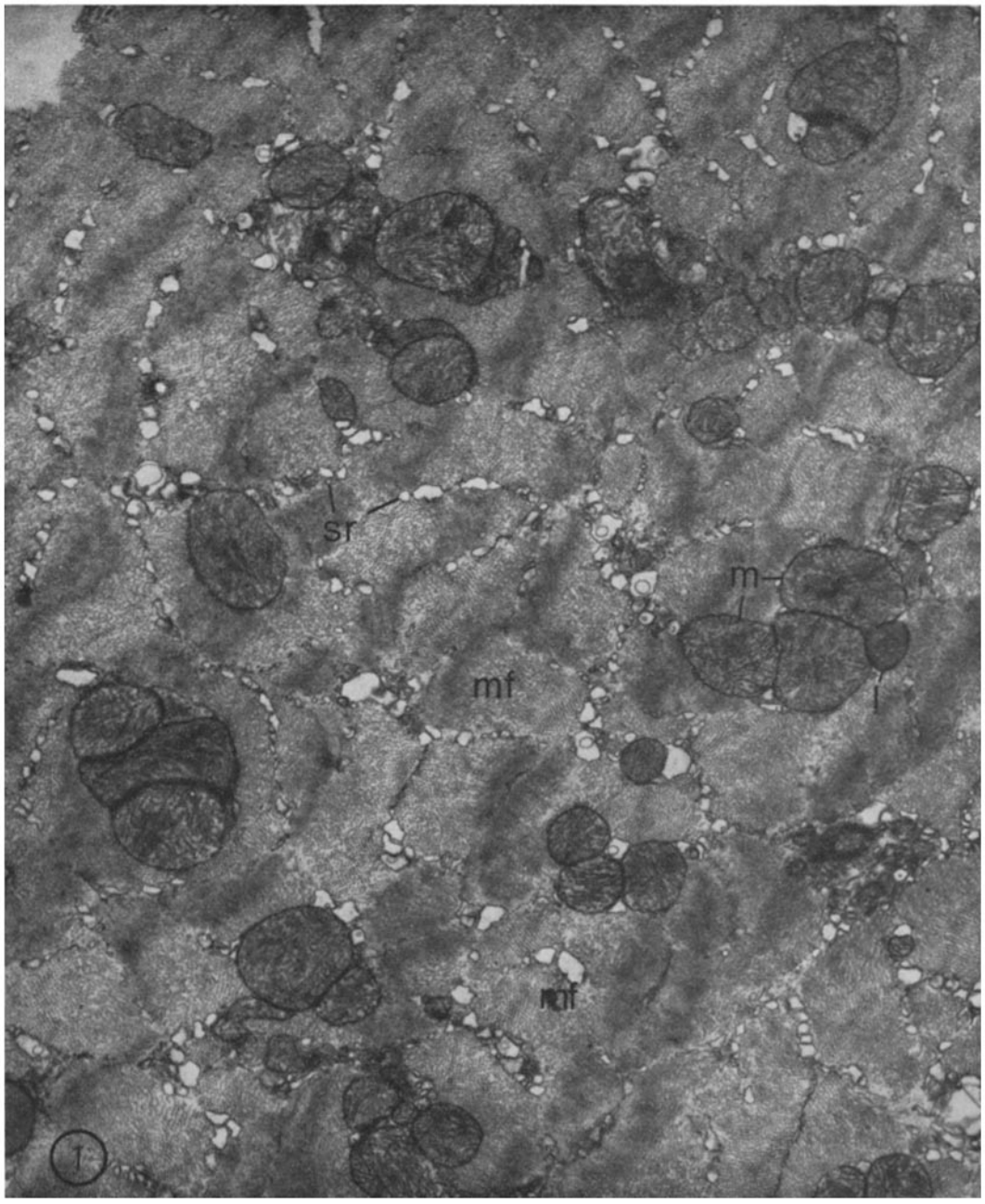

FIGURE 1 Electron micrograph of a portion of a red fiber of the superficial dorsal flexor muscle in transverse section. Some of the myofibrils are confluent, forming a more or less continuous mass of myofilaments (*mf*) with isolated areas of sarcoplasm containing sarcoplasmic reticulum (*sr*), mitochondria (*m*), and lipid droplets (*l*). (Fixation method *a*). × 26,400.

the fiber (Fig. 3). They measure on the average 3 μ in their wider dimension, except for the inner fibrils which are smaller and fill up the central core of sarcoplasm of the fiber. The sarcoplasmic area between the myofibrils is occupied mainly by sarcoplasmic reticulum and glycogen particles. The peripheral zone of sarcoplasm of the fiber is occupied by nuclei, sparse mitochondria, and glycogen

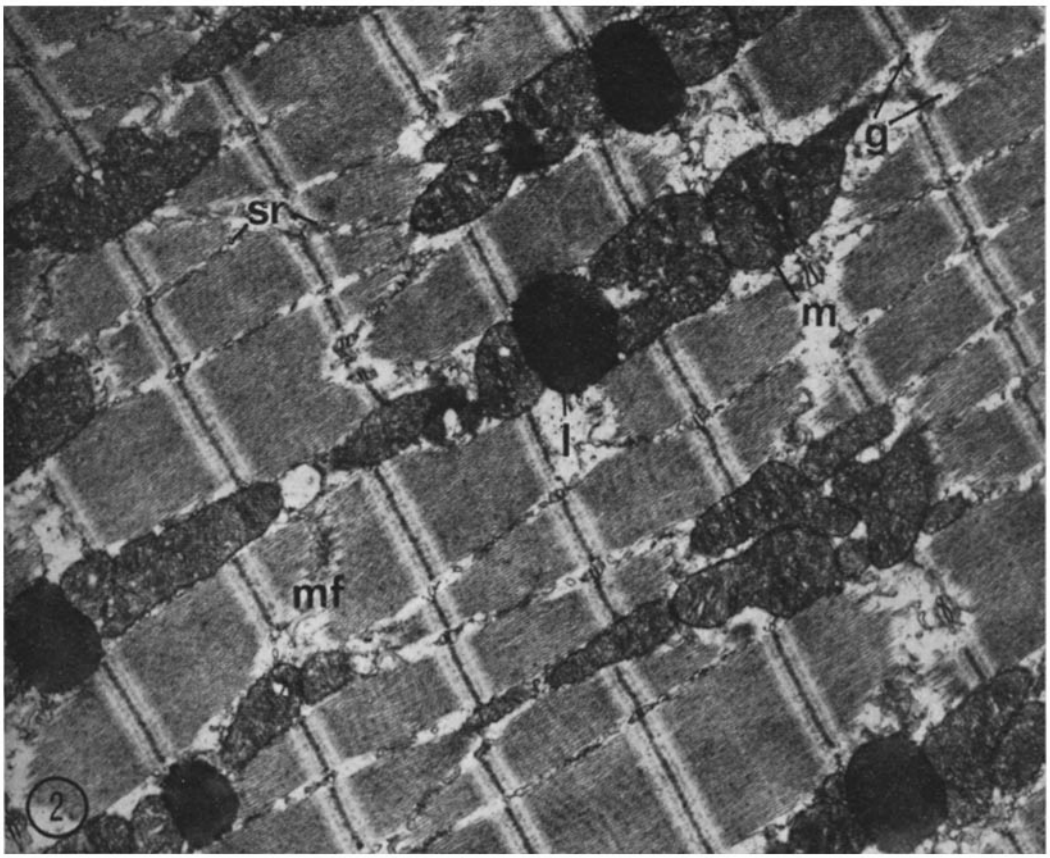

FIGURE 2 Electron micrograph of a portion of a red fiber of the superficial dorsal flexor muscle in longitudinal section. Myofilaments (*mf*) are grouped in independent myofibrils. Note adundance of mitochondria (*m*). *g*, glycogen particles. *l*, lipid droplet. *sr*, sarcoplasmic reticulum. (Fixation method *b*). × 15,600.

particles. In longitudinal section (Fig. 4) the myofibrils are seen to run as independent units throughout the length of the fiber. The organization of the myofibrils of the white muscle fibers is in agreement with that of true *Fibrillenstruktur* (24, 25) which has been demonstrated in twitch fibers by electron microscopy (17, 32, 36).

Arrangement and Dimensions of Actin and Myosin Filaments

The arrangement of myofilaments in red and white muscle fibers is found to be hexagonal. The mean diameters of actin and myosin filaments of red muscle fibers are not the same as those of white muscle fibers under fixation method *b*. The average diameters of actin and myosin filaments of red and white fibers are 80 A and 75 A (SD for red muscle 11.6 and white muscle 11.0) and 180 A and 175 A (SD for red muscle 8.8 and white muscle 9.2), respectively.

SARCOPLASMIC RETICULUM

The Triads

The triads are located in most of the Z line regions of both red and white muscle fibers (Fig. 5). The transverse tubules of white and red muscle fibers are seen to branch and interconnect with one another among the myofibrils (Fig. 3). This interconnection is also found in the sarcoplasm beneath the sarcolemma but outside the myofibrils in white muscle fibers (Fig. 6). A continuous transverse tubule is seen in both fibers, so it is concluded that the transverse tubules are continuous most or all of the way across the fiber at each Z line in both

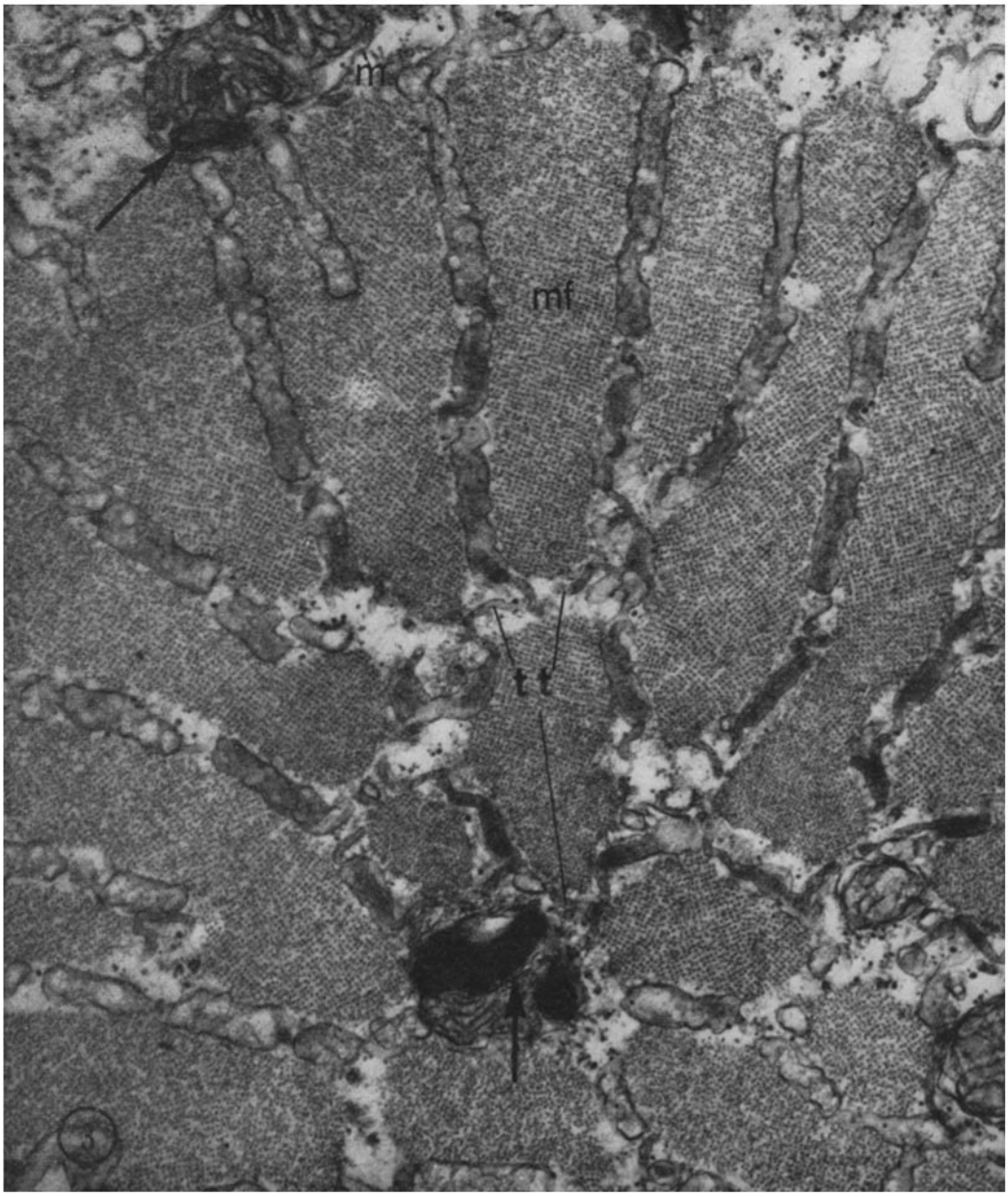

FIGURE 3 A portion of a white fiber of the superficial dorsal flexor muscle in transverse section. Radially arranged myofibrils with T system (*tt*) which shows branching and interconnection among the myofibrils. The arrows show whorls of membranes within the mitochondria (*m*). Two of the whorls (lower) are poorly preserved. *mf*, myofilaments. (Fixation method *b*). × 36,000.

types of fiber. The average dimension of a transverse tubule parallel to the red muscle fiber axis is 240 A, while in the white muscle fiber it is 320 A.

In the red muscle fiber the space between the terminal cisterna and the transverse tubule averages about 100 A. The cisternae average about 0.11 μ in the longitudinal direction. In the white muscle fiber the space between the terminal cisterna and the transverse tubule is about 120 A. The cisternae average about 0.13 μ in the longitudinal direction.

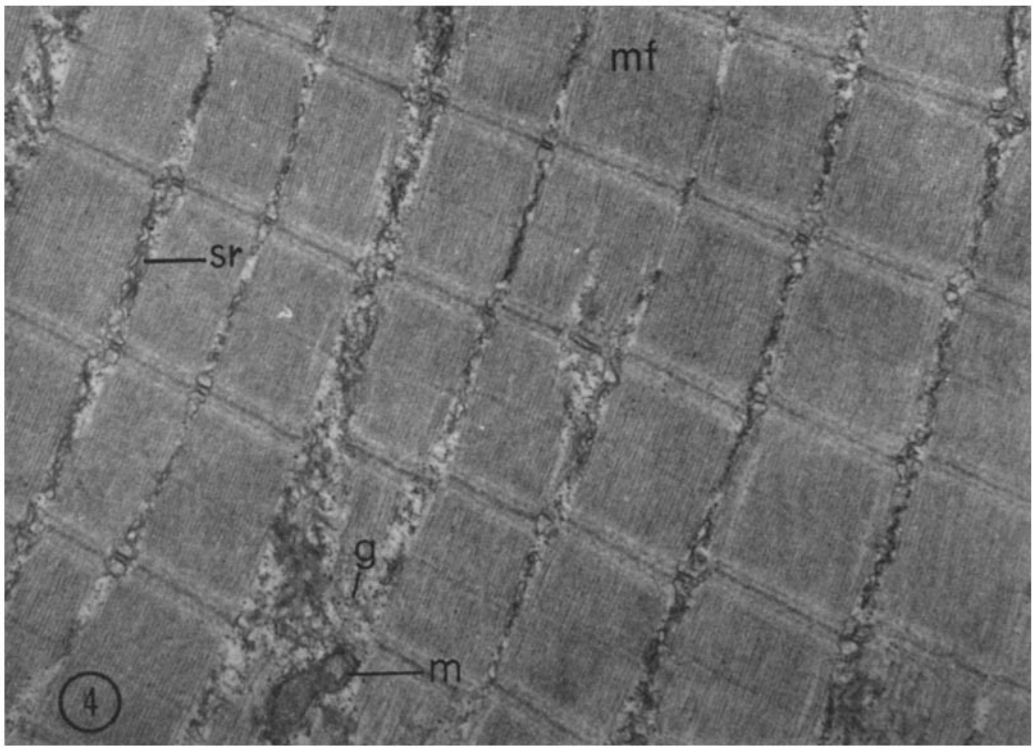

FIGURE 4 A portion of a white fiber of the superficial dorsal flexor muscle in longitudinal section. Myofilaments (*mf*) are grouped in discrete myofibrils well-delineated by sarcoplasmic reticulum (*sr*). Note scarcity of mitochondria (*m*). *g*, glycogen particles. (Fixation method *b*). × 21,600.

In both fibers the terminal cisternae appear not to have lateral continuity at the Z line (Fig. 7) (34).

Longitudinal Reticulum

The relative abundance of sarcoplasmic reticulum in red and white muscle fibers is better understood from examination of low power electron micrographs. Longitudinal sarcotubules of the reticulum are abundant in both red and white fibers (Fig. 5). The longitudinal sarcotubules in both fibers fuse to form continuous collars around the myofilaments near the center of the A band. These collars are found to contain a number of openings which are best seen in face views of the sarcoplasmic reticulum. This type of collar is referred to as a "fenestrated collar" by Peachey (34) in frog muscle.

Volume of Transverse Tubules and Sarcoplasmic Reticulum

Since it has been shown that high and low capacitances of muscle fibers are related to large and small transverse tubule areas respectively (1, 11, 33, 34), and that high and low rates of calcium release and uptake during contraction and relaxation of myofilaments are related to large and small sarcoplasmic reticulum areas respectively (20, 42, 43), it is of interest to estimate the total transverse tubule and sarcoplasmic reticulum volumes and surface areas from electron micrographs of red and white fish muscles.

The volumes of transverse tubules and sarcoplasmic reticulum were estimated from measurements on electron micrographs and were expressed as fractions of the fiber volume. The mean triad width (W_t) was measured on transverse and longitudinal sections. The mean dimension of triad parallel to the fiber axis (L_t) was obtained from longitudinal sections. The mean length (L_A) of interfibrillar space per unit fiber cross-sectional area was measured using a tracing device linked to a digital computer (35). Total number of transverse tubular networks per cm fiber length is $1/S$, where S is the mean sarcomere length. These data are given in

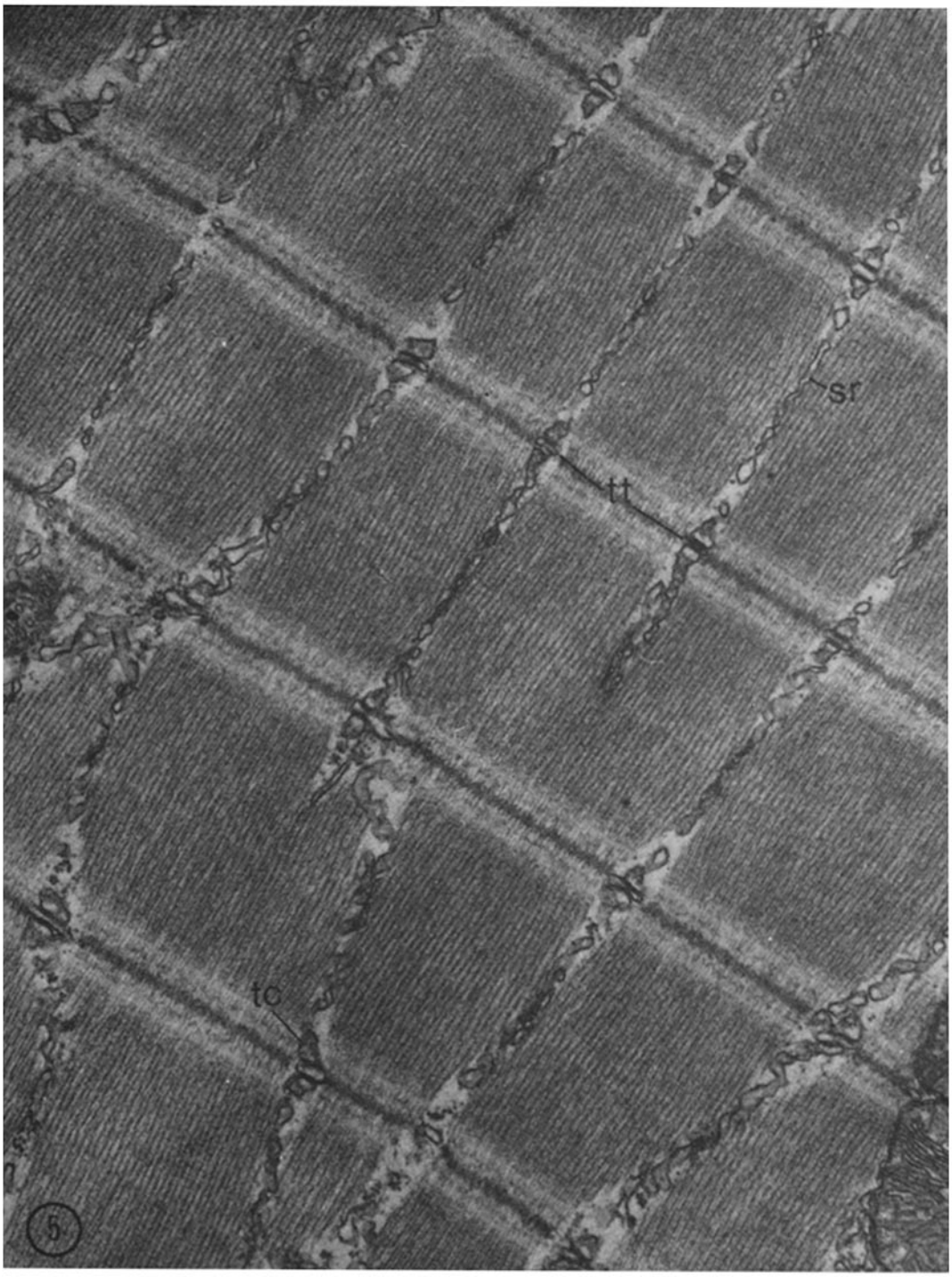

FIGURE 5 Red fiber of the superficial ventral flexor muscle showing the relative abundance of T system (*tt*) and sarcoplasmic reticulum (*sr*). The frequent interruptions in the contact between terminal cisternae (*tc*) and sarcoplasmic reticulum and in the continuity of the latter in red fibers occurred in all fixation procedures used. (Fixation method *c*). × 26,400.

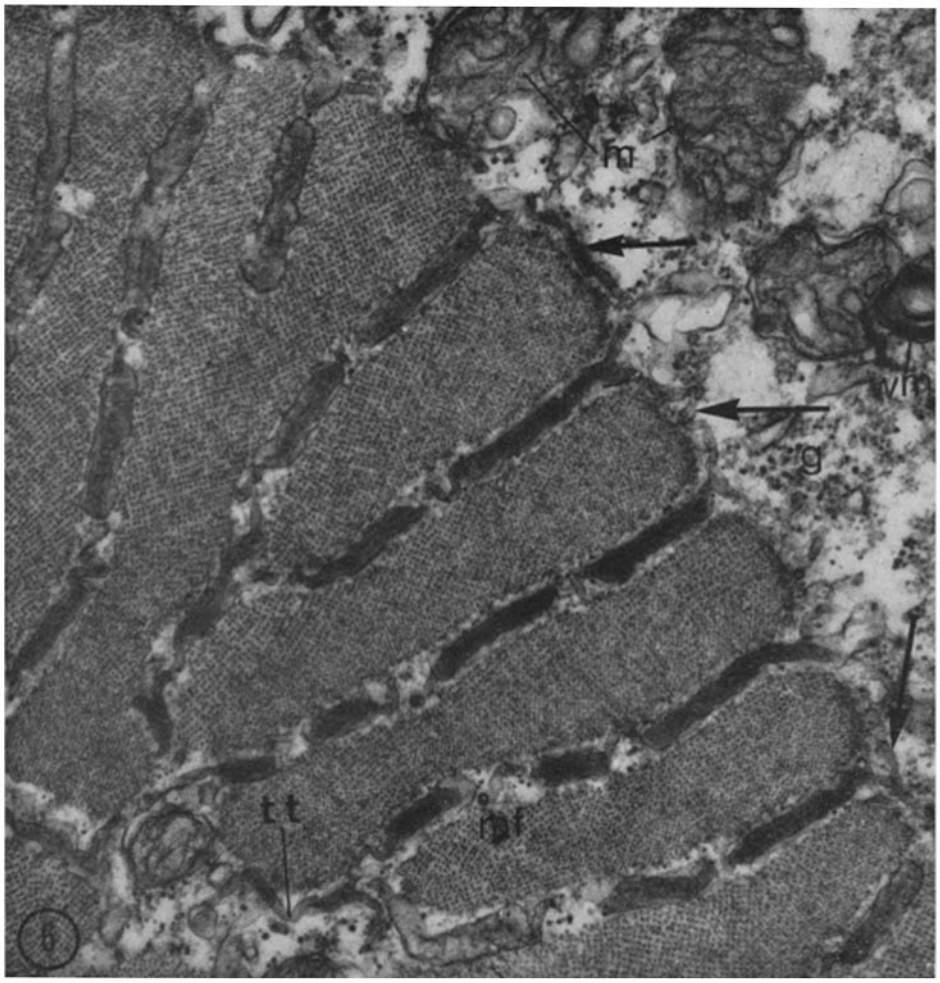

FIGURE 6 This electron micrograph is the right-hand corner of Fig. 3. The arrows show the interconnection of transverse tubules (*tt*) outside the myofibrils. *g*, glycogen particles. *wm*, whorl of membrane. (Fixation method *b*). × 36,000.

table III. The total fractional volume of transverse tubules (ρt) per fiber volume is given by

$$\rho t = W_t \cdot L_t \cdot L_A/S.$$

The surface area to fiber volume ratio for the transverse tubules is

$$\xi = 2L_A\ (W_t + L_t)/S.$$

The volume of terminal cisternae relative to the fiber volume (ρc) can be obtained in the same way, by substituting L_c, the mean longitudinal dimension of the terminal cisternae, for L_t, and by allowing for two terminal cisternae per sarcomere.

$$\rho c = 2W_tL_cL_A/S.$$

The surface area to fiber volume ratio for the terminal cisternae is

$$\xi = 4L_A(W_t + L_c)/S.$$

Since the structure of the longitudinal tubules and fenestrated collar are rather irregular, they were approximated by a simple model consisting of straight tubules running between the two ter-

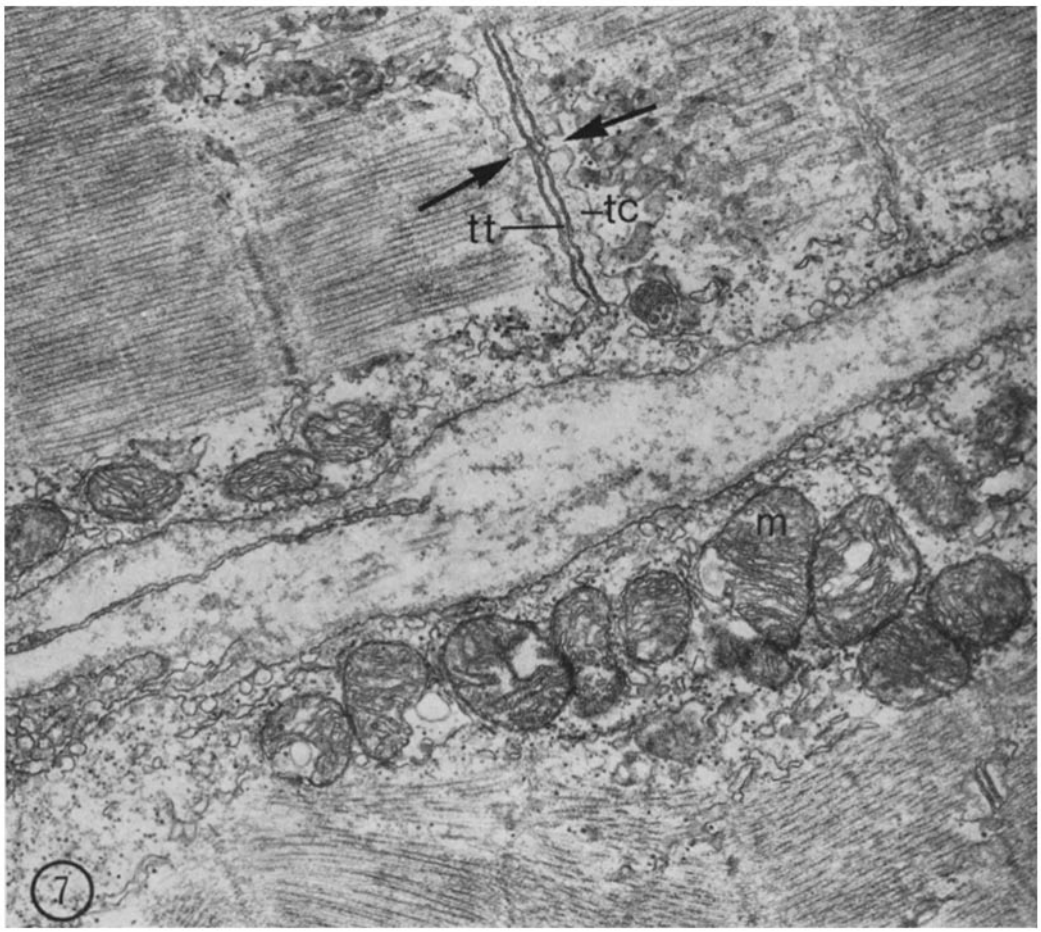

FIGURE 7 White fibers of the superficial ventral flexor muscle. Interruptions of terminal cisternae (*tc*) are shown by big arrows. Note smaller size of mitochondria (*m*) with scanty cristae and absence of lipid droplets. *tt*, transverse tubules. (Fixation method *c*). × 51,600.

minal cisternae. If there are N of these tubules per unit length of interfibrillar space in the transverse plane, and if these tubules have a mean diameter of $2r$, then surface and volume parameters will be given by

$$\rho l = N_l \pi r^2 L_A (S - 2L_c)/S$$
$$\xi = 2N_l \pi r L_A (S - 2L_c)/S.$$

The measured parameters and the calculated volumes and surface areas are given in Tables III and IV. The first two columns give values expressed as volume or surface area per cm^3 of fiber volume. The last three columns are related to a cm^2 of fiber surface, and are calculated by multiplying by (A_F/P_F), using the mean fiber areas (A_F) and fiber perimeters (P_F) measured in cross-sections, as given below Table III.

Z Line

The Z line of red muscle fibers seems to have more dense material than that of the white fiber (Figs. 8, 9). The structure of Z line in both fibers seems to be better explained in terms of interconnection of the opposing sets of actin filaments (23).

Other Organelles and Inclusions of the Red and White Muscle Fibers

NUCLEUS: The nuclei of both types of fibers are situated peripherally beneath the sarcolemma.

TABLE III

Dimensions of Transverse Tubules and Sarcoplasmic Reticulum of Red and White Muscle Fibers

Compartment	Fiber	Mean width (Wt)	Mean dimension in longitudinal direction (Lt or Lc)	Mean diameter (2r)	Length of interfibrillar space/unit fiber area (L_A)	Number/unit interfibrillar distances (N_L)
		μ	μ	μ	μ^{-1}	μ^{-1}
Transverse tubule	White	0.19	0.03	—	1.07	—
	Red	0.15	0.02	—	0.72	—
Terminal cisternae	White	0.19	0.13	—	1.07	—
	Red	0.15	0.11	—	0.72	—
Longitudinal tubule	White	—	—	0.20	1.07	3.63
		—	—	0.12	0.72	5.21

White fiber $A_F = 34 \times 10^2\ \mu^2$; $P_F = 234\ \mu$. White fiber S = 1.3 μ.
Red fiber $A_F = 0.86 \times 10^3\ \mu^2$; $P_F = 116\ \mu$. Red fiber S = 1.5 μ.

TABLE IV

Volumes and Surface Areas of Transverse Tubules and Sarcoplasmic Reticulum of Red and White Muscle Fibers

Compartment	Fiber	Volume of compartment/volume of fiber	Surface area of compartment/volume of fiber	Surface to volume ratio for fibers	Volume per cm² fiber surface	Total area per cm² fiber surface
			cm^{-1}	cm^{-1}	cm	
Transverse tubule	White	0.004	0.36×10^4	1.45×10^{-3}	0.68×10^{-5}	5.26
	Red	0.001	0.16×10^4	0.737×10^{-3}	0.10×10^{-5}	1.26
Terminal cisternae	White	0.040	0.52×10^4	1.45×10^{-3}	0.59×10^{-4}	15.30
	Red	0.015	0.24×10^4	0.737×10^{-3}	0.11×10^{-4}	3.67
Longitudinal tubule	White	0.097	0.19×10^5	1.45×10^{-3}	0.14×10^{-3}	28.39
	Red	0.036	0.12×10^5	0.737×10^{-3}	0.26×10^{-4}	8.90
Total SR	White	0.137	2.42×10^4		0.604×10^{-4}	43.69
	Red	0.051	1.44×10^4		0.37×10^{-4}	12.57

This pattern of distribution of nuclei in white muscle fibers is different from that of the dogfish. By light microscopy Bone (8) showed that the nuclei of the white muscle fibers are scattered throughout the sarcoplasm of the fiber.

MITOCHONDRIA: The ratio of mitochondria in red and white fibers is about 7:3 by number. The electron microscope study shows that most of the mitochondria of the red muscle fibers are larger in size than those of the white muscle fibers and the number of cristae of red muscle mitochondria appears to be higher than that of white muscle fibers (Figs. 7, 10).

Sometimes mitochondria are found to contain whorls of membranes (Figs. 3, 6).

LIPID AND GLYCOGEN: The red muscle fibers are rich in lipid and relatively low in glycogen, whereas white fibers have little lipid and much glycogen. Glycogen content of the white muscle fibers appears to be reduced to a considerable extent after

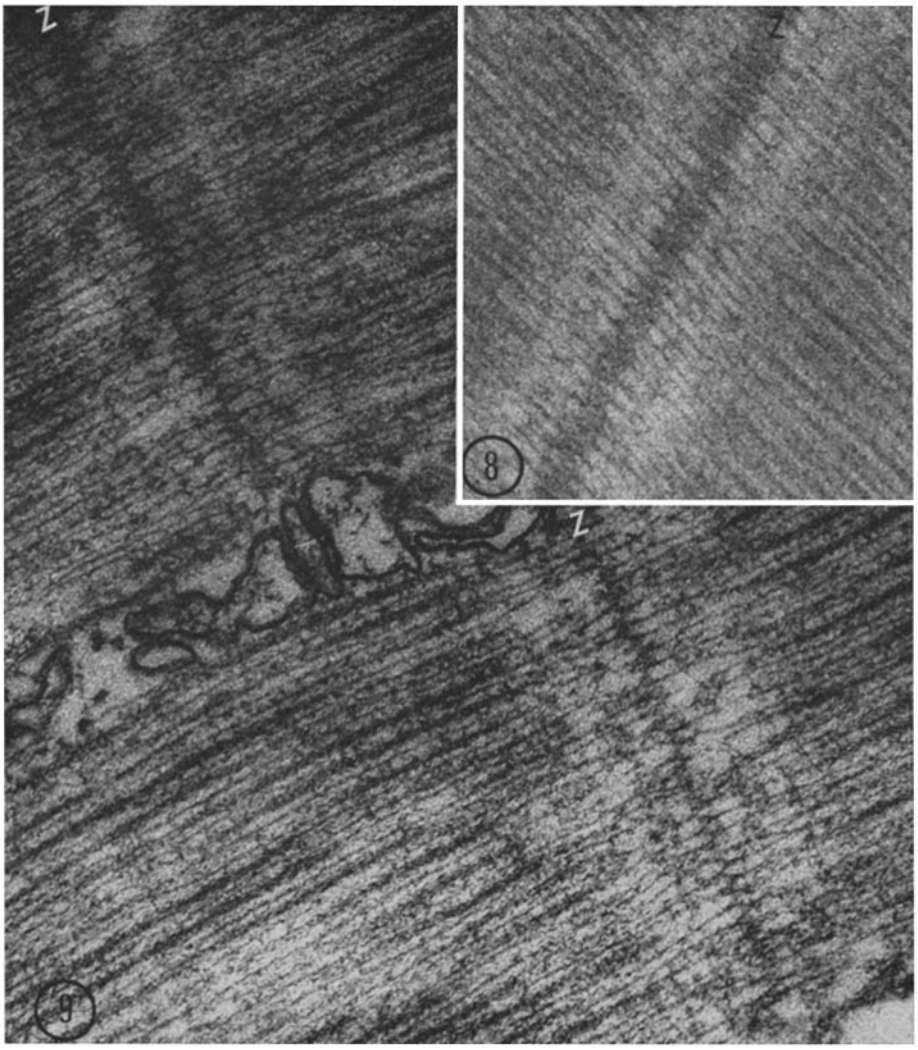

FIGURE 8 The interconnection of opposing sets of actin filaments in the Z line (z) of a red fiber. (Fixation method *a*). × 102,000.

FIGURE 9 The interconnection of opposing sets of actin filaments in the Z line of a white fiber. (Fixation method *a*). × 102,000.

strenuous exercise of 15 min, which is brought about by continuous chasing of the fish in a fish tank. The detailed results of this study will be published elsewhere.

ATPase Activities of Red and White Muscle Fibers

Biochemical analysis of ATPase activity shows that red and white muscle fibers are significantly different. Actomyosin extracts also show differences between the types of fibers (Table V).

It is evident from the data of Table V that actomyosin of white muscle fibers has an average ATPase activity three times that of actomyosin of red fibers, although red muscle actomyosin content is greater than that of white. Gergely et al. (16) found in rabbits that "the ATPase activity of myofibrils from red muscles is considerably lower than that of white myofibrils."

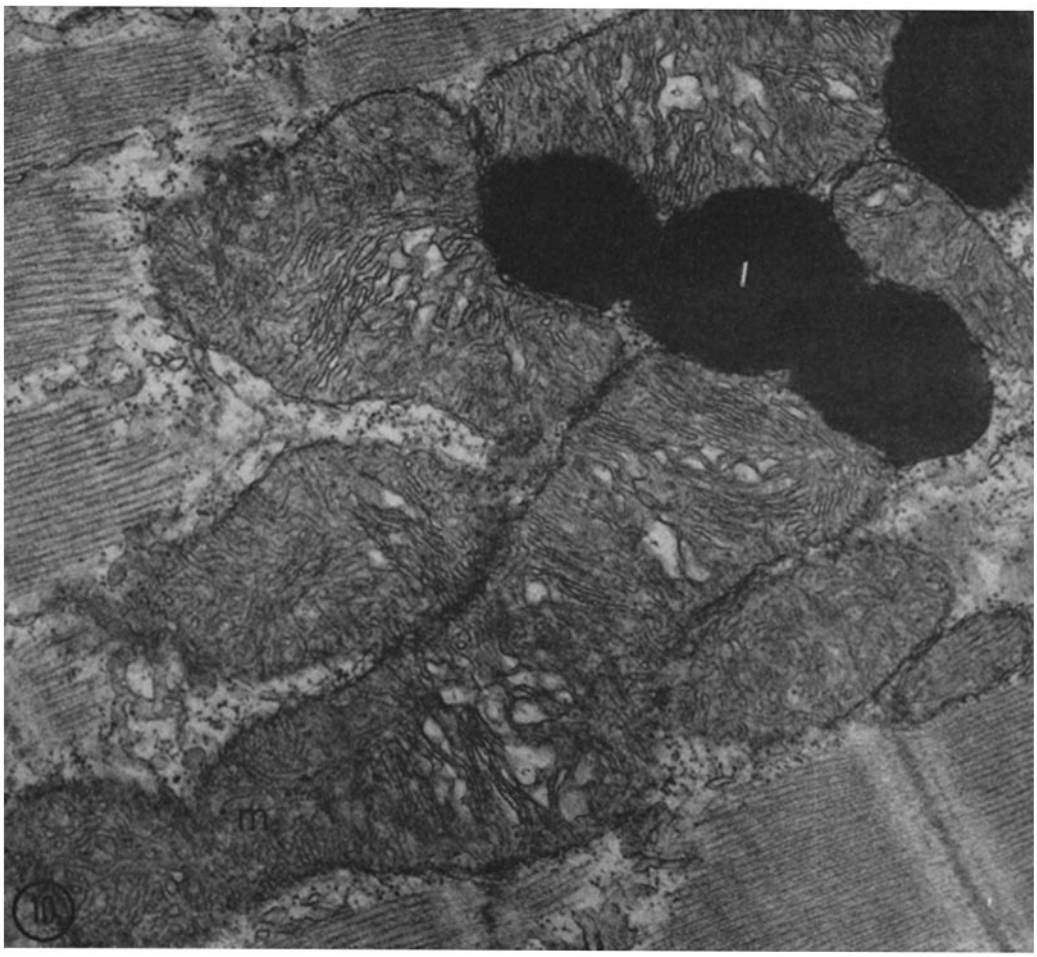

FIGURE 10 The mitochondria (*m*) of red fiber of the superficial ventral flexor muscle showing particularly large number of cristae. Note that the lipid droplets (*l*) are in close association with mitochondria. (Fixation method *c*). × 51,600.

DISCUSSION

Role of the Red and White Muscle Fibers in Relation to Swimming Behavior

The higher concentration of red muscle fibers in the caudal peduncle than in the rest of the body is quite significant, knowing that this region together with the caudal fin acts as a main locomotory organ of this fish. The continuous energy requirement in this region is probably relatively higher than that of the rest of the body and it is probably mainly supplied by the red muscle fibers. The data on lipid, glycogen, and mitochondria of the two types of muscle fibers along with enzymatic activities (29), suggest that these distinctive muscle fibers probably utilize different metabolites for their energy. The red muscle fibers probably utilize fat, and the white muscle fibers utilize glycogen. This will be discussed in detail elsewhere.

Actomyosin of the white muscle fibers has an average of three times the ATPase activity of the actomyosin of the red muscle fibers, which can be compared to the ATPase activity of myosin of rabbit's fast (extensor digitorum longus) and slow (soleus) muscles given by Barany et al. (3). Since it has been widely accepted that the hydrolysis of ATP by actomyosin is directly involved as the energy supply in the process of contraction, and that the rate of contraction of a given type of muscle fiber is proportional to the rate of ATP hydrolysis

TABLE V

Protein Concentrations and ATPase Activities of the Red and White Muscle Fibers

		Actomyosin concentration in extracts (%) of wet wt. tissue	ATPase activity (μmoles of PO_4/g/sec)
Red muscle	Sample 1	0.66	0.75
	Sample 2	0.62	0.72
White muscle	Sample 1	0.55	2.4
	Sample 2	0.59	2.1

by actomyosin, the present results suggest that the rate of contraction of the red muscle fibers is slower than that of the white muscle fibers. This idea conforms with the preliminary report of electrophysiological investigations on the red and white muscle fibers of fish (8, 38).

Rainbow trout belongs to a group called "stayers" which includes fishes which show sustained swimming. It appears that the red muscle fibers with their high lipid content take a role in sustained swimming, while white fibers with their glycogen content are used in strenuous swimming or activities where a burst of speed is necessary. The study of ontogeny of red and white muscle fibers reveals that as the embryo hatches and assumes free swimming activities, red muscle fibers become distinguishable. It appears that the red muscle fibers assume their function in swimming activities of the fish, which requires more energy than its activities before hatching. The details of this study will be published elsewhere.

T System

Previous authors (17, 32, 36), working on the fine structure of physiologically known fast and slow muscle fibers of frog and garter snake, showed clear-cut differences in the quantity of triads and sarcoplasmic reticulum between two types of muscle fibers. The T system and sarcoplasmic reticulum are found to be abundant in fast muscle fibers, whereas in the slow muscle fibers the T system and sarcoplasmic reticulum are poorly developed (32, 36). It has been shown further (1, 33, 34) that frog's slow muscle fibers have a relatively low capacitance and a small transverse tubule area and fast fibers have a high capacitance with a large transverse tubule area.

As seen in Table IV, the transverse tubular system in white muscle fibers occupies 0.4 per cent of the fiber volume whereas in the red muscle fibers it occupies 0.1 per cent. The value for white muscle fiber comes close to the value of 0.3 per cent measured in frog sartorius fibers (34). The terminal cisternae in white muscle fiber occupy 4 per cent of the fiber volume, while in the red muscle fiber they occupy 1.5 per cent. The longitudinal tubules together with fenestrated collar occupy 9.7 per cent of the white muscle fiber volume, while in the red muscle fiber this value is 3.6 per cent. The value for sarcoplasmic reticulum of white muscle fibers comes close to the value of 13 per cent measured in frog sartorius fibers (34). The total volume of the membrane-limited compartments of transverse tubules and sarcoplasmic reticulum in white muscle fiber is 14.1 per cent, while in the red muscle fiber it is 5.2 per cent. These results indicate that the total volume of membrane-limited compartments of the white muscle fiber is about 2.7 times that of the red muscle fiber.

It has been widely accepted that a major part of membrane capacitance of muscle fiber resides in the transverse tubule membranes (37). One could now expect that a twitch fiber would possess higher transverse tubule area than that of a tonic fiber. Peachey (34) estimated the area of transverse tubules of frog sartorius muscle fiber from electron micrographs and found it to be seven times the outer surface area of a 100 μ diameter fiber. Preliminary electrophysiological studies (18) on fish white and red muscle fibers show that the capacitance of white muscle fiber is about 7 $\mu F/cm^2$ and that for the red muscle fiber is about 2 $\mu F/cm^2$. The total areas of the transverse tubules of white and red muscle fibers estimated here are found to be 5.3 cm^2 and 1.3 cm^2 respectively per cm^2 outer surface areas. These values are in agreement approximately with the values obtained from physiological studies (18). Thus it becomes clear that a major part of membrane capacitances of white and red muscle fibers of fish does reside in the transverse tubule membranes. Earlier electron microscope studies (30, 31) suggested that the transverse tubular systems in white and red muscle fibers of fish are similar, so that differences in surface area could not account for the difference in membrane capacitances shown by physiological studies (18). The present estimation of transverse tubular areas in white and red muscle fibers does not support the similarity found by earlier investigators. Mere pres-

ence of transverse tubules in all Z line areas does not necessarily imply that they are similar quantitatively in surface areas. Other factors, such as dimensions of the tubules, fibril size, and fiber surface to volume ratio must be taken into account in estimating relative transverse tubule and fiber surface areas. When we did this, we obtained values in rather good agreement with the capacitance data.

Sarcoplasmic Reticulum

Estimates of volumes and surface areas presented here show that there is more sarcoplasmic reticulum in white fibers than in red fibers. It has been found in recent years that the rate of calcium uptake during contraction of myofilaments probably depends on the specific calcium binding or transporting activity of the membranes of sarcoplasmic reticulum and on the surface area of sarcoplasmic reticulum relative to sarcoplasmic volume. So in a fast muscle it is not unusual to find sarcoplasmic reticulum with a very large surface area, and conversely with the slow muscle. Van der Kloot (43) has calculated changes in calcium ion concentration of a hypothetical 1 μ myofibril as a function of time and of the amount of surrounding sarcoplasmic reticulum, using assumptions about the rate of calcium uptake from his previous experiments (42). In the case where the collar of sarcoplasmic reticulum was assumed to be 0.2 μ thick, a 20% change in calcium concentration took approximately 2.5 times as long as it did when sarcoplasmic reticulum was assumed to be 0.4 μ thick, and almost four times as long as when the collar of sarcoplasmic reticulum was taken to be 1 μ thick. These calculations suggest that an increase in the size of sarcoplasmic reticulum does have a pronounced effect on the rate at which the value of concentration of calcium ion within the myofibril falls. The examination of volumes and surface areas of sarcoplasmic reticulum of the red (5.1 per cent of the fiber volume; 12.6 cm^2 area per cm^2 of outer surface) and white (13.7 per cent of the fiber volume; 43.7 cm^2 area per cm^2 of outer surface) muscle fibers, in the light of Van der Kloot's results, suggests that the rate of fall of the concentration of calcium ion in the myofibril of the red muscle fiber is slower than that of the white muscle fiber.

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