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THE UNIVERSITY OF ALBERTA

FUNCTIONAL MORPHOLOGY OF THE CAUDAL REGION OF A TELEOSTEAN FISH, SALMO GAIRDNERI

by

ASISH CHANDRA NAG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Functional morphology of the caudal region of a teleostean fish, Salmo gairdneri, submitted by Asish Chandra Nag in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The caudal skeleton of Salmo gairdneri consists of a pair of massive uroneurals in addition to other usual caudal skeletal elements. The uroneurals appear to stabilize three loose epurals, and with two other uroneurals, to stiffen the upturned terminal portion of the vertebral column which supports the abduction and adduction of the lobes of the caudal fin and flexion of the caudal peduncle and fin.

The caudal region consists of 7 muscles: 1. superficial dorsal flexor muscle, 2. superficial ventral flexor muscle, 3. carinals, 4. interradials, 5. deep dorsal flexor muscle, 6. deep ventral flexor muscle, and 7. hypochordal longitudinal muscle. These muscles are polyneuronally innervated by branches and subbranches of 10 spinal nerves. The functions of the muscles in relation to swimming behavior of the fish are discussed. The caudal fin is innervated by numerous nerve fibers, some of which end in connective tissue without any association with effectors, and are presumably sensory fibers. The probable function of these nerve fibers is proprioceptive.

The caudal muscles consist of two types of muscle fibers: red muscle fiber, and white muscle fiber. The red muscle fiber differs from the white muscle fiber in respect of diameter, sarcomere length, organization of myofibrils, arrangement of myofilaments, transverse tubules and sarcoplasmic reticulum volumes, Z line, and M line. The functional significance of these

structural differences is discussed, except for the Z line. The red muscle fibers have higher content of lipid droplets and mitochondria than the white muscle fibers. The white muscle fibers are richer in glycogen content. ATPase activity of the white muscle fibers is 2.7 times that of the red muscle fibers. Oxidative enzymes are strongly reactive in the red muscle fibers and weakly reactive in the white muscle fibers. Phosphorylase is strongly reactive in the white muscle fibers and weakly reactive in the red muscle fibers. The functional significance of these differences in enzymatic activities between two types of fibers are discussed in relation to roles of both muscle fibers in swimming activities. Most of the characteristics of fast muscle fiber, as they are found in higher vertebrates, are found in the white muscle fiber of this fish, while most of the characteristics of slow muscle fiber as they are observed in higher vertebrates are found in the red muscle fiber of this fish.

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INTRODUCTION

It has long been observed that the caudal peduncle and fin act as a main locomotory organ in most fish. The caudal peduncle and fin consist of a well-organized skeleton and muscles which are capable of delicate organized movements. The caudal skeleton (Regan, 1910; Totton, 1914; Barrington, 1937; Gosline, 1960; and others) and musculature (Greene and Greene, 1913; Schmalhausen, 1913; Grenholm, 1923; Baerends and Baerends, 1950; and Fierstine and Walters, 1968) of teleostean fishes have been the subjects of taxonomical and gross morphological studies for a long time. Nursall (1962 and 1963 a, b) first treated the caudal skeleton and musculature from the point of view of function in an elaborate way on the macroscopic level. He showed in Hoplopagrus guntheri how different caudal muscles take part in the propulsion of the caudal fin and thereby help in swimming of the fish. His work also included the phylogeny of the caudal musculature of fish, illustrating a gradual evolution of specialized effectors to actuate an organ of increasingly complex function.

The caudal musculature is derived from the caudal myomeres and each of the myomeres consists of two types of muscle fiber. The two types often form separate portions of the myomere, and, where they do, may be recognized at once by their color. Lorenzini (1678) first found red and white muscle fibers in the elasmobranchs among vertebrates. Kafuku (1950) showed that in addition to the superficial lateral strip of red muscle, there is also a deep-lying red portion of the myomere in the scombroid fishes.

Various suggestions have been put forward to account for the presence of two different types of muscle fiber within the fish myomere. There have been three major suggestions. The first one expresses the idea that only red muscle fibers are active during slow swimming, whereas white muscle fibers are active during rapid swimming. The first proponents of this idea were perhaps Arloing and Lavocat (1875). Later workers to come to the same conclusion were Chevrel, 1913; Boddeke et al., 1959; Barets, 1961; George, 1962; Bone, 1966; and Rayner and Keenan, 1967.

The second suggestion expressed the idea that the main purpose of red muscle fibers was not muscular work, but that they functioned as an organ, carrying out similar synthetic processes to those taking place in the liver (Braekkan, 1956; Wittenburger and Diaciuc, 1965).

The third suggestion expressed an idea just opposite to the first one, namely that the red muscle fibers were used in rapid swimming and the white in slow continuous swimming (Gerebtzoff, 1956; Buttkus, 1963; Bergman, 1964b, 1967). Buttkus (1963) supposed that the red muscle "is designed for rapid acceleration".

In recent years many investigators have undertaken detailed studies of the metabolism of red and white muscle fibers of fish. Black et al. (1962) investigated the changes in glycogen, pyruvate, and lactate in the muscle of 1-1/2 year old rainbow trout during and following muscular activity. They found that muscle glycogen was depleted 50 per cent or more in the first 2 minutes of severe activity. Correlated with this rapid depletion of glycogen was a

sudden accumulation of muscle pyruvate and lactate. These authors did not mention whether their muscle samples consisted of red or white muscle fibers or both. Bilinski (1963) found in rainbow trout that fatty acid oxidation was more active in red than in white muscle. Connor et al. (1964) found that after sustained swimming of three species of salmonid fishes there was no significant diminution of muscle glycogen.

Gordon (1968) showed that the metabolic rates in minced preparations of unstimulated red tuna muscle were over six times as great as rates in white muscle. Stevens (1968) worked on the effect of exercise on the distribution of blood to various organs in rainbow trout and concluded that it is unlikely that much blood is diverted away from the visceral organs and to the muscle during exercise in fish. Lindsey (1968), investigating the temperature of the red and white muscles in recently caught marlin and other large tropical fish, suggested that, although active species which produce much heat may have well-developed red muscle, the heat need not arise primarily in the red muscle, at least in the marlin under conditions of the test.

Electron microscopic study on the red and white muscle fibers of fish is very scanty. Fawcett and Revel (1961), following Skoglund's investigation (1959) on the neuromuscular mechanism of sound producing swim bladder muscle of Opsanus sp., carried out an electron microscopic study of the sarcoplasmic reticulum of the muscle of O. tau, and O. beta. They believed that the unusual physiological properties (lag between stimulation and response is

very short) of this muscle may be directly related to the extraordinary development of the sarcoplasmic reticulum. They did not mention whether this muscle was red or white or mixed. Reger (1961), working on the extrinsic eye muscles of Fundulus heteroclitus found the absence of subsynaptic sarcolemmal infoldings in these muscles. Again, we do not know whether this muscle was red or white or mixed. Nakajima (1962) working on the red and white muscle fibers of Ophiocephalus argus by means of electron microscope and Nishihara working similarly with Carassius auratus both came to the conclusion that the internal structure of both muscle fibers is similar to that of the fast fibers of the frog. Franzini-Armstrong and Porter (1964) first demonstrated clearly that sarcolemmal invaginations constitute the T system in muscle fibers. They worked on the red and white muscle fibers of Mollienesia sp. Bergman (1967) showed discrete myofibrillar bundles, abundance of sarcoplasmic reticulum, and absence of subsynaptic sarcolemmal infoldings in the neuromuscular junction of the red muscle fibers of the dorsal fin of Hippocampus hudsonius. He considered (1964 b) the red muscle fibers of the dorsal fin of this fish to be twitch fibers.

The survey of literature indicates clearly that our knowledge of the detailed structural and functional characteristics of the caudal peduncle and fin of the fish in relation to swimming behavior is very poor. A survey of literature also indicates that previous studies on red and white muscle fibers are sketchy and unorganized.

There remains yet a great dispute concerning organizations, structures, and functions of these two muscle fibers. Three different suggestions are put forward to account for the functions of red and white muscle fibers. Support for one or another is based mostly on histological studies. It is interesting to note that Tekuchi (1959), Bergman (1964 b), Bone (1966), and Rayner and Keenan (1967) did not come to the same conclusion from their preliminary electrophysiological investigations on these muscle fibers. Bergman found that the red muscle fibers show mechanical properties of fast fibers of amphibia. Bergman did not work on the white muscle fibers while others worked on the white fibers and found them to be similar to fast fibers of amphibia.

The extensive studies of metabolism of muscle fibers of rainbow trout by Black et al. (1962) give us information which is obtained from the studies of mixed samples of muscles. He did not treat muscle samples, separating red and white muscle fibers.

The scanty electron microscopic studies so far done on these two muscle fibers of fish could not show us any ultrastructural differences which are characteristics of fast and slow muscle fibers of amphibia and other higher vertebrates.

The purpose of the present study is to determine and evaluate the functional significance of the caudal structures of Salmo gairdneri, the rainbow trout, in relation to swimming behavior, by detailed examination of the organization of the caudal skeleton, musculature with an emphasis on the red and white muscle fibers, and innervation from the gross to the ultrastructural level (except for the skeleton).

MATERIALS AND METHODS

S. gairdneri was chosen as the species to be examined because it was easily available from the Calgary trout hatchery, and the Department of Zoology is equipped to maintain this species. Information about the metabolism and swimming behavior of this species is also available.

Methods of gross morphology

Ninety-two specimens of average 24 cm and six specimens between 45 cm and 50 cm fork length were dissected to study the caudal skeleton, musculature, and innervation. Both alizarin stained and unstained specimens were dissected for the study of the caudal skeleton. (A dissecting microscope was used in tracing myosepta and nerves of the caudal musculature). The Sihler's chloral hydrate-Ehrlich's acid hematoxylin technique (Freihofer, 1966) for staining the nerves of the whole caudal fin was used. Both unstained and stained specimens were dissected (with the help of the dissecting microscope) to trace the details of innervation of the caudal fin.

The relative weights of the red and white muscles in different sections of the body of the fish were determined. Four slices of about 12 mm 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. 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 are excluded from weighing.

The muscle fibers were weighed carefully on an analytical balance. The mean of ten measurements from ten specimens averaging 22.5 cm length are given in Table I.

Histological method

The pattern of innervation of the caudal musculature was chiefly studied by silver impregnation techniques mainly on thick frozen sections and teased preparations (Palmgren, 1948; and Holmes, 1943). Methylene blue preparations of the muscles were made but they did not give satisfactory results.

Certain nerves of the caudal fin that end in the connective tissue between the fin rays appeared to be sensory nerves and they were dissected out, and whole-mount preparations by Holmes' silver impregnation technique were prepared. These silver impregnated materials were compared with the materials stained with the Sihler technique.

Mitochondria were studied in sections of materials embedded in Esterwax 1960 (Steedman, 1960) after fixing in Regaud's fluid and stained by means of Regaud's ferric haematin technique for mitochondria. The results of this preparation were checked against the results of electron microscopic work in order to get a fair estimate of mitochondria content in red and white muscle fibers.

The diameters of the red and white muscle fibers were measured from light photomicrographs taken from fresh frozen sections. 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 is taken in each case. The diameters of the muscle fibers are given in Table II.

The study of organization of muscle fibers throughout the length of the body of embryos, sac fry, and early free swimming specimens of S. gairdneri was made from the preparations of the material fixed in formol-calcium (Baker, 1944) and stained with Sudan 4 for lipid and Mallory's triple stain for the organization of myomeric musculature.

Histochemical methods

Frozen sections of gelatin embedded muscles fixed in formol-calcium (Baker, 1944) were used for oil red "O" and Sudan 4 for lipid. Esterwax 1960-sections of material fixed in ice-cold Rossman's fluid (Macmanus and Mowry, 1960) were used for the Periodic Acid-Schiff technique for glycogen. Muscle samples for the latter technique were taken from unexercised and exercised fish. The fish were fed liver and pellets for 4 weeks after their arrival from the Calgary trout hatchery before the experiments were started. The fish were exercised by continuous chasing in a tank.

Rapid-frozen fresh muscle blocks (up to 3 mm cube) were cut at 5-10 μ on a cryostat, at -20°C, and mounted directly on coverslips. The following procedures were followed for the study of enzymes:

1. succinate dehydrogenase (Pearse, 1957, 1961)
2. lactate dehydrogenase (Hess, Scarpelli, and Pearse, 1958)
3. nicotinamide adenine dinucleotide (NADH) diaphorase (Nachlas, Walker and Seligman, 1958)

4. phosphorylase (Takeuchi and Kuriaka, 1955; Takeuchi, 1956)

Biochemical methods

Live S. gairdneri were used for all these experiments. The fish were of average 32.5 cm 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 M $NaHCO_3$ at pH 8.0 for periods of 5 and 15 minutes, and then allowed to stand for 5 hours at 0°C. The mixture was then diluted 10 times with deionized water and was allowed to precipitate overnight. Actomyosins of red and white muscles were precipitated out and the precipitate was centrifuged in an International Refrigerated Centrifuge (Model PR-6) at 3,000 g for 10 minutes. 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 hours. The precipitate was centrifuged at 3,000 g for 10 minutes 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 minutes. 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. (1951). ATPase activity determinations were made in pH-stat (Radiometer TTT1) according to the method of Kay and Brahms (1963), by following the proton liberation during ATP hydrolysis. The assays were carried out at pH 8 and 25°C, in a medium containing 0.1 M $MgCl_2$, 0.5 M KCl, 5 X 10^{-3} M ATP, and 5 X 10^{-4} M glutathione. 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 titrigraph obtained. ATPase activity measurements were made in each case with two distinct preparations of enzymatically active actomyosin.

Electron microscopy

Six fixation procedures were used: 1. fixed in 3-6 per cent glutaraldehyde, buffered at pH 7.3 with 0.1 M phosphate, for 24 hours and after several hours of washing in buffer, post-fixed in 1 per cent osmium tetroxide, buffered with 0.1 M phosphate at pH 7.3, for 1.5 hours; 2. fixed for 24 hours in 3-6 per cent glutaraldehyde in salmonid physiological solution (pH 7.3) (Hoar and Hickman, 1967) containing NaCl 7.40 gm, KCl 0.36 gm, $CaCl_2$ 0.17 gm, $NaHCO_3$ 0.31 gm, Na_2HPO_4 1.6 gm, and NaH_2PO_4 0.4 gm/per liter. Muscle bundles were rinsed for several hours in physiological solution and post-fixed in

1 per cent osmium tetroxide in the physiological solution; 3. fixed in 5 per cent glutaraldehyde and 4 per cent paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) (Karnovsky, 1965), were rinsed in phosphate buffer for several hours, and were post-fixed in 1 per cent osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) for 1 to 2 hours. Sometimes half strength of this fixative was used; 4. fixed in 2 per cent potassium permanganate in distilled water for 24 hours for Revel's electron microscopic method (1964) for glycogen; 5. fixed in 1 per cent osmium tetroxide in the physiological solution (pH 7.3); 6. fixed in 1 per cent osmium tetroxide in 0.1 M cacodylate buffer containing 2 mM calcium chloride, pH 7.3 to 7.4.

Muscle bundles were tied to toothpicks at their ends so as to hold them at their slack lengths until after washing in buffer and physiological solution.

The specimens of developmental stages were treated with first three fixation procedures mentioned.

All fixations and dehydrations were at 0°C. After dehydration in absolute ethanol followed by propylene oxide, the material was embedded in Araldite 502. Sections (500-600A) were cut on a Porter-Blum microtome by glass or diamond knives, and stained with 10 per cent uranyl acetate solution in absolute methanol, followed by 0.2 per cent lead citrate solution in distilled water. In the study of glycogen a saturated aqueous solution of lead hydroxide was used, and thereafter examined with Philips EM 100 and 200 operated at 60 kv using objective apertures of 25 and 40 μ diameters respectively.

Swimming behavior

Swimming behavior was studied in young adult S. gairdneri in aquarium and fish tank. Swimming was induced in sac fry by disturbances such as switching on a light above the trough, banging lightly on the trough and by creating a current in the water. Free swimming fry would swim at feeding.

Terminology

The terminology of Nybelin (1963) for caudal skeleton, and of Nursall (1963 a) for caudal musculature has been followed. Lund (1967) rejects Nybelin's terminology of the teleostean caudal skeleton on the basis that Nybelin's characterization of the boundary between the ural and pre-ural regions as the point at which the caudal vessels bifurcate, varies greatly in the teleosts and is modified by function. He defines hypurals as all haemal spines supporting caudal fin rays, and ural centra as all centra supporting such spines. It is to be noted that Lund gives no evidence of variation in the point of bifurcation of the caudal vessels. My own experience (1967) with the teleostean caudal skeletons and blood vessels and that of investigators who have examined large numbers of teleost caudal skeletons (Gosline, 1965; Monod, 1967; Patterson, 1968) is that this point is constant. It is quite clear that Nybelin's terminology is based on a sound reference point.

RESULTS

The caudal skeleton

The caudal skeleton of S. gairdneri (Fig. 1) consists of 2 ural centra, 1 cartilaginous urostyle, 3 pairs of uroneurals, 3 epurals, 6 hypurals, 18 branched fin rays, 4 unbranched fin rays, and 22 procurrent rays. The anterior uroneural on each side bears a high crest, between which the front ends of the epurals are sandwiched. The results of the present study are consistent with those of Norden (1961).

The caudal musculature

The caudal musculature (Figs. 2-4) on each side consists of 2 layers: superficial and deep. The superficial layer consists of 4 muscles: superficial dorsal flexor muscle, superficial ventral flexor muscle, carinals (supra- and infra-carinal muscles), interradials.

The superficial dorsal flexor muscle is attached by two tendons and one aponeurosis to the branched caudal fin rays of the epichordal lobe of caudal fin. This muscle is connected to the hypochordal longitudinal muscle by a bundle of deeper muscle fibers, which is not found in other fishes so far reported. The superficial ventral flexor muscle is attached by six tendons and one aponeurosis to the branched and unbranched caudal fin rays of the hypochordal lobe of caudal fin. The supra- and infra-carinal muscles extend from the bases of the dorsal and anal fins to the anterior procurrent fin rays, between the dorsal and ventral margins of the superficial dorsal and ventral flexor muscles of each side. The attachments of these muscles are accomplished by three tendon slips - two above and one below. The

interradial muscles of the branched caudal fin rays of two lobes of the caudal fin combine to form a single band of muscle fibers at the base of caudal fin and are restricted therein. These superficial muscles are composed of two types of muscle fibers: red and white, which lie side by side as separate bundles and are easily separable for examination or analysis. The red muscle fiber content of the two superficial flexor muscles appears to be the highest of all caudal muscles. These two muscles appear to act primarily to flex the caudal peduncle and fin. The flexion of the caudal fin rays of the epichordal lobe by the superficial flexor muscle is partially done through the intervention of the hypochordal longitudinal muscle. The dorsal and ventral most parts of these muscles serve also to prevent adduction of the fin rays during flexion of the caudal peduncle and fin. The contraction of supra- and infra-carinal muscles appears to move the dorsal and ventral caudal bases vertically upward and downward, respectively, to some extent.

The interradial muscles chiefly adduct the fin rays. The contraction of the fused band of interradial muscles appear to flex the caudal fin independently of other caudal muscles. They appear to be involved in fanning of the caudal fin when the fish is hovering or resting in the water without progression.

The deep layer consists of 3 muscles: deep dorsal flexor muscle, deep ventral flexor muscle, and hypochordal longitudinal muscle. The deep dorsal flexor muscle is a broad band of muscle lying ventral to, and parallel with, the superficial dorsal flexor. It is attached, on the one hand, to the vertebrae and their neural spines by muscle

fibers and, on the other hand, to the branched caudal fin rays of the epichordal lobe by four tendons. The deep ventral flexor muscle lies over the basal portion of the hypochordal longitudinal muscle. This muscle is divided into lateral and medial bundles. The lateral bundle is attached anteriorly to haemal spines by muscle fibers, while posteriorly it is attached to the branched caudal fin rays of the hypochordal lobe by four tendons. The medial bundle is attached anteriorly to vertebrae, haemal spines, and hypurals by muscle fibers while posteriorly attached to the unbranched and branched caudal fin rays of the hypochordal lobe by five tendons. The hypochordal longitudinal muscle appears from beneath the medial bundle of the deep ventral flexor to insert on hypural 1 by muscle fibers and on the branched caudal fin rays of the epichordal lobe by four tendons. When the superficial flexors are removed, the hypochordal longitudinal muscle is prominent. These three muscles are composed of red and white muscle fibers which lie side by side as separate bundles and are separable for examination. The red muscle fiber content of the hypochordal longitudinal muscle appears to be higher than that of the two deep flexor muscles.

The dorsal rays of the caudal fin are flexed by the deep dorsal flexor muscle. Its dorsalmost tendon slips also abduct fin rays. The chief function of two bundles of the deep ventral flexor appears to be flexion of the caudal peduncle and fin and, secondarily, it causes abduction of the caudal fin rays. The hypochordal longitudinal muscle adducts and flexes the caudal rays on which it remains attached.

Relative proportion of the red and white muscle fibers

Table I gives information about the relative concentration of the red and white muscle fibers in different sections of the body (see also Fig. 5).

Table I Relative proportion of the red to the white muscle fibers (per cent by weight). Results from the caudal peduncle include superficial flexor muscles only.

	Anterior to the dorsal fin	Middle of the body	Posterior part of the trunk	Caudal peduncle
Red muscle	1.1	4.4	8.4	> 13.0
White muscle	98.9	95.6	91.6	87.0

The measurement in Table I shows that the red muscle is > 13.0 per cent of the total mass of the superficial flexor muscles which are the only muscles from which a measurement was made in the caudal peduncle. The proportion of red muscle decreases in successive anterior sections of the body.

Diameter of red and white muscle fibers

The diameters of the red and white muscle fibers are different from one another. Table II shows different diameters of red and white muscles.

Table II Diameter of red and white muscle fibers of the caudal musculature (in μ). The figure for each muscle is an average of 20 measurements and the standard deviation of each is given in parentheses.

Caudal musculature	Red muscle	White muscle
Superficial dorsal flexor	3.7 (1.09)	5.8 (2.58)
Superficial ventral flexor	4.0 (1.30)	5.58(2.37)
Hypochordal longitudinal	3.75(0.81)	5.7 (1.41)
Deep dorsal flexor	3.5 (0.74)	5.0 (1.27)
Deep ventral flexor	3.8 (0.80)	5.2 (1.86)
Interradial	3.3 (0.71)	6.0 (2.78)

The data given in Table II show that the mean diameter of white muscle fibers is about 1.5 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 distributions of diameters of the red and white muscle fibers are given in Figs. 6 and 7.

Sarcomere length of red and white muscle fibers

Five consecutive sarcomeres of ten randomly chosen fibers from each of the caudal muscles were measured on electronmicrographs. Both muscle fibers are processed simultaneously under the same preparative procedures. The sarcomere length of red muscle fibers is different from that of white muscle fibers (Table III).

Table III Difference in sarcomere length between red and white muscle fibers of the caudal musculature (in μ). Standard deviation in parentheses.

Caudal musculature	Red muscle	White muscle
Superficial dorsal flexor	1.5 (0.07)	1.29 (0.03)
Superficial ventral flexor	1.6 (0.05)	1.32 (0.05)
Hypochordal longitudinal	1.54(0.07)	1.29 (0.02)
Deep dorsal flexor	1.58(0.07)	1.28 (0.03)
Deep ventral flexor	1.59(0.11)	1.33 (0.17)
Interradial	1.47(0.07)	1.29 (0.02)

The data in Table III show that the mean sarcomere length of the red muscle fibers is about 1.2 times that of the white muscle fibers. This difference in mean sarcomere length between two types of fiber is found to be statistically significant ($P < 0.005$). The distributions of sarcomere lengths of red and white muscle fibers are given in Figs. 8 and 9.

Mitochondria content of red and white muscle fibers of the caudal musculature

Approximately 70 per cent of mitochondria of the caudal musculature are located in the red muscle fibers and 30 per cent in the white muscle fibers (Figs. 10-15). The mitochondria content of the superficial flexor and hypochordal longitudinal muscles appears to be greater than that of the deep flexor and interradial muscles. The electron microscopic study shows that the 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 mitochondria of the red muscle fibers appears to be higher than that of the white muscle fibers (Figs. 16-20, 30, 71, 82).

Enzymatic activities of red and white muscle fibers of the caudal musculature

Biochemical analysis of ATPase activity of red and white muscle fibers shows that red and white muscle fibers are significantly different. Actomyosin extracts also show differences between the types of fibers. Table IV summarizes ATPase activities and protein concentrations of red and white muscle fibers.

Table IV Protein concentrations and ATPase activities of the red and white muscle fibers.

	Actomyosin concentration in extracts (per cent) of wet wt. tissue	ATPase activity (μ moles of PO_4/g/sec)
Red muscle	0.66	0.75
	0.62	0.58
White muscle	0.55	2.4
	0.59	1.3

It is evident from the data of Table IV that actomyosin of white muscle fibers have an average ATPase activity 2.7 times that of actomyosin of red muscle fibers. This result is in agreement with that of Gergely et al. (1965) who worked on red and white muscle fibers of rabbit and found that "the ATPase activity of myofibrils from red muscles is considerably lower than that of white myofibrils". However, actomyosin content of the red muscle fibers is higher than that of the white muscle fibers. The higher actomyosin content of the red muscle fibers is reflected in the morphology of actin and myosin filaments whose dimensions are given in page 33, Table VII.

Histochemical study (Figs. 21-28) shows that the red muscle fibers reacted strongly for all the oxidative enzymes studied. White muscle fibers show weak reaction for oxidative enzymes. The reactivity of red muscle fibers for phosphorylase is weak, whereas, in white muscle fibers it is very strong. The correlations of different enzyme activities with red and white muscle fibers are shown in Table V.

Table V Correlations of different enzyme activities with the red and white muscle fibers.

Muscle	Succinic dehydro-genase	Lactate dehydro-genase	NADH diaphor-ase	Phos-phorylase	Relation of oxidative enzymes to phosphorylase
Red	S	S	S	W	R
White	W	W	W	S	R

S = strong reaction

W = weak reaction

R = reciprocal

Fat and glycogen content of the red and white muscle fibers of the caudal musculature

The red muscle fibers are rich in lipid content whereas the white muscle fibers have negligible amounts (Figs. 10-13, 29). The lipid droplets are found to be intimately associated with the mitochondria (Figs. 16-18).

Closer examination of the electronmicrographs indicates that the white muscle fibers are richer in glycogen content (Figs. 12, 13). This observation is in substantial agreement with that for pigeon pectoralis major muscle given by George and Naik (1958). Glycogen content of the white muscle fibers in rainbow trout appears to be reduced to a considerable extent after strenuous exercise of 15 minutes, which is brought about by continuous chasing of the fish in a fish tank (Figs. 30, 31). These results agree with those of Bone (1966) on dogfish muscle fibers.

Innervation of the caudal musculature and fin

The caudal musculature is innervated by a nerve plexus involving spinal nerves 52-61 (Fig. 32). The gross distribution of the spinal nerves in the caudal musculature and fin is given in Table VI.

Table VI The gross distribution of the spinal nerves in the caudal musculature and fin.

Muscle	Spinal nerves 52	53	54	55	56	57	58	59	60	61
Superficial dorsal flexor	X	X	X	X						
Deep dorsal flexor				X	X	X	X	X	X	X
Superficial ventral flexor	X	X	X	X						
Deep ventral flexor	X	X	X		X					
Hypochordal longitudinal					X	X	X	X	X	
Interradial				X	X	X	X	X	X	
Inter ray connective tissue			X	X	X	X	X	X		

These nerves, after emerging from the spiral cord, run with their branches and subbranches on the myosepta of the myomeres in the form of bundles (Figs. 33, 34). The branches of nerves of different roots often unite together and run on the same myoseptum. The nerve bundles supply branches to the adjoining muscle fibers of both sides (Figs. 35-39). A red or a white muscle fiber receives several motor terminations of different axons (Figs. 36, 38, 44). These terminations are more or less club-shaped (Figs. 37, 39, 40-43). The presence of polyneuronal innervation in both fibers agrees with Barets (1961). Shenk and Davidson (1966), and Hudson (1969), while working on fast-acting muscles of teleosts, found polyneuronal innervation. The former authors did not mention whether the muscle was red or white, while Hudson stated that he worked with white muscle.

Spinal nerves 54-59, with their large number of branches and subbranches, innervate the caudal fin in a complicated way (Figs. 45, 46). The nerves running between each pair of fin rays give off branches to the connective tissue between the rays. The branches shown by arrows in Figs. 45, 47, 48, 49 seem to be sensory fibers since they cannot be traced to effectors, while others are seen to be associated with chromatophores. The branches ending in the connective tissue between fin rays without any association with chromatophores possess the characteristic endings shown in Figs. 50, 51.

Ultrastructural characteristics of the red and white muscle fibers of the caudal musculature

Besides mitochondria, lipid, etc., certain functional structures have been investigated to distinguish the red fibers from the white.

This investigation includes the organization of myofibrils, dimensions and arrangement of actin and myosin filaments, T system, sacroplasmic reticulum, Z line, M band, and other organelles and associated structures of the muscle cell.

Organization of the myofibril

The organization of myofibrils of red and white muscle fibers is better understood from examination of transverse and longitudinal sections at low magnification. In transverse sections (Figs. 52, 54) the myofibrils of the red muscle fiber are fused together to 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. This continuous mass of myofilaments fills most of the fiber except for a peripheral zone of sarcoplasm containing nuclei, lipid droplets, mitochondria and glycogen particles. This organization of myofibrils of the red muscle fibers resembles that of Krüger's Felderstruktur (1933, 1952) which has been demonstrated in tonus fibers by electron microscopy (Hess, 1960; Peachey and Huxley, 1962; Page, 1965; Fawcett and McNutt, 1969). Longitudinal sections of the red muscle fibers sometimes show (Fig. 55) that the myofibrils run as independent fascicles for a distance. Closer examination reveals that these facicles vary greatly in width. They are not independent throughout their length, but are often confluent, which causes irregularities in fibrillar width. If a transverse section passes through an area of independent fibrils of red muscle fibers one will get a picture of discrete myofibrillar fascicles averaging 1 μ across. This may be the cause for which

previous investigators (Nakajima, 1962; Franzini-Armstrong and Porter, 1964; and Nishihara, 1966 and 1967) could not find Felderstruktur in red muscle fibers.

The myofibrils of the white muscle fibers are usually in distinct unbranched and branched circumscribed fascicles. In transverse sections myofibrils are seen to be situated along the radii of the fiber (Figs. 53, 56). They measure on the average 3μ across, 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 particles. In longitudinal section (Fig. 57) the myofibrils are seen to run as independent fascicles throughout the length of the fiber unlike those of red muscle fiber. The organization of the myofibrils of the white muscle fibers is in agreement with those of Krüger's Fibrillenstruktur (1933 and 1952) which has been demonstrated in twitch fibers by electron microscopy (Hess, 1960; Peachey and Huxley, 1962; and Page, 1965). In twitch fibers the myofibril units are discrete unbranched and branched fascicles well delineated by sarcoplasmic elements.

With the assumption that red fibers are slow, an attempt was made to fix rapidly the muscle of a cruising fish. It was hoped that contracted sarcomeres could be demonstrated. The results show that some individual sarcomeres or even parts of half sarcomeres of red muscle fibers are contracted (Fig. 58). In order to know whether tonic property of the red muscle fiber produces a contracted state

of sarcomeres of the myofibrils or the fixation produces it, both red and white muscle fibers were fixed and processed simultaneously with an assumption that if the contracted state of myofibrils occurs owing to fixation, this will occur in both fibers. None of the sarcomeres of white muscle fiber is contracted. This result in red muscle fiber may be due to graded contraction which is usually seen in slow muscle fiber or may be due to different response to the fixative (i.e., glutaraldehyde) from that of white muscle fiber. If this result in red muscle fiber has appeared due to graded contraction it raises a basic question as it is raised by the result of Hoyle's (1967) experiment on "ribs-skin muscle fiber of garter snake". Does the graded contraction of a slow muscle fiber involve partial, but equal, activations of each sarcomere, or are individual sarcomeres activated to a varying extent? In order to explain these findings Hoyle (1967) proposed that "the fundamental unit in excitation-contraction coupling is a single cisternal element of the SR ..... . Either the cisternal elements or units (C.Us) have a range of thresholds or the amount of calcium released by each is directly proportional to the local potential difference (or current flow), both possibilities may occur. In a graded contraction only a portion of the C.Us are excited, perhaps to different extents, resulting in a very heterogenous set of microanatomical changes - but graded tension. A conventional 'all or none' twitch would result from a rapid, nearly synchronous firing of all the C.Us."

Dimensions of actin and myosin filaments

The average diameters of actin and myosin filaments of red muscle fibers differ from those of white muscle fibers. The average diameters of actin and myosin filaments of white muscle fibers are the same as those of skeletal muscle fibers of rabbit, frog, and blowfly given by previous investigators (Hanson and Huxley, 1955; Huxley, 1960; and Huxley and Hanson, 1960). Table VII gives comparative data concerning diameters of actin and myosin filaments.

Table VII Diameters of actin and myosin filaments.

Organism		Actin	Myosin
Fish	Red muscle fiber	88A	182A
	White muscle fiber	74A	138A
Blowfly Frog Rabbit		50-70A	100-150A

Arrangement of actin and myosin filaments

The arrangement of actin and myosin filaments in the red muscle fibers reveals that in addition to the usual occurrence of hexagonal arrangement (i.e., one myosin surrounded by a regular hexagonal orbit of actins) (Fig. 59), there is an occasional occurrence of a different arrangement in which one myosin is surrounded by an irregular orbit of actins averaging 8 in number (Figs. 60, 61). It is to be noted that Page (1965) observed that the hexagonal arrangement of myofilaments in slow muscle fiber was not invariable throughout. Hoyle (1967, 1968) noted that invertebrates (arthropods) often have an irregular arrangement of actin and myosin filaments. This irregular arrangement is evident in early free swimming specimens of rainbow trout (Figs. 62, 63) when red muscle fibers are first distinguishable. The arrangement of myofilaments in white muscle fibers is found invariably to be hexagonal (Fig. 64). An attempt was made to examine whether or not any distortion in hexagonal arrangements of myofilaments of the red and white muscle fibers could be effected as an artifact of fixation. In order to study this effect both kinds of fibers were fixed by osmium tetroxide only instead of double fixation by glutaraldehyde-osmium method. Both fibers were processed simultaneously under the same post-fixation procedures and examined by electron microscope. The results show that the hexagonal arrangement of myofilaments of both fibers is not distorted by single fixation procedure (Figs. 65, 66). They are found in the same state of arrangement as they are in double fixation. It appears to be clear that the occurrence of an irregular arrangement of myofilaments of red muscle fibers is most

probably not an artifact of fixation.

The triads

The transverse tubules and terminal cisternae form triads in the red and white muscle fibers of rainbow trout like those of other vertebrates (Porter and Palade, 1957; Franzini-Armstrong and Porter, 1964; Peachey, 1965 b; Page, 1965; and others). Collectively the transverse tubules constitute the T system of skeletal muscle. The triads are located in the sarcoplasm of most of the Z band regions of both fibers (Figs. 67, 68). The transverse tubules of white muscle fibers are seen to branch and interconnect with one another among the myofibrils (Fig. 69). This interconnection is also found in the sarcoplasm below the sarcolemma and outside the myofibrils (Fig. 70). It has been observed that the transverse tubules run continuously as far as the triad stays in the plane of the section. As a continuous tubule is seen in both fibers whenever the plane of the section is favorable, it is concluded that the transverse tubules are continuous most or all of the way across the fiber at each Z line in both types of fiber. The average dimension of a transverse tubule parallel to the red muscle fiber axis is 240A, while in the white muscle fiber it is 320A.

The terminal cisternae are larger dilated sacs in both fibers (Figs. 71, 72). In the red muscle fiber they are on the average 100A distant from the transverse tubules and extend for about 0.13μ in the longitudinal direction, whereas in the white muscle fiber they are on the average 120A distant from the transverse tubules and extend

for about 0.3μ in the longitudinal direction. In both fibers they are usually interrupted at the Z line level (Figs. 20, 73, 76, 77). It is possible that they may not have complete lateral continuity. This idea agrees substantially with that of Peachey (1965 b). Occasionally terminal cisternae are found to be continuous from one sarcomere to the next as reported before (Porter and Palade, 1957; Peachey, 1965 b; Page, 1965) and they are seen to contain dense diffuse granular material (Figs. 71, 73-75). In early free swimming specimens the terminal cisternae in the red muscle fibers are connected to the outer membrane of the nuclear envelope (Figs. 76, 77) as reported before in frog muscle by Peachey (1965 b). Furthermore the terminal cisternae are found to contain circular and elongated openings which are not so far reported in other animals (Figs. 76, 77). The faces of the terminal cisternae are connected to the transverse tubules in both fibers by dense structures (Figs. 72, 78-80). This resembles the relationship found in the bat (Revel, 1962); in the frog (Peachey, 1965 b); (Franzini-Armstrong, 1968, 1969); and in the newt (Kelly, 1967, 1969). Recently much emphasis has been given to the study of these dense structures, considering them to provide tight-junctional contacts between the two membrane systems. It is possible that these dense structures bridge the gaps of 100A and 120A between the two membrane systems in red and white muscle fibers respectively. Occasionally this gap in the red muscle fiber is occupied by dense material (Figs. 81, 82).

Since it has been shown that high and low capacitances of muscle fibers are related to large and small transverse tubule areas

respectively (Peachey, 1965 a, b), and that high and low rates of calcium uptake and release during contraction and relaxation of myofilaments are related to large and small sarcoplasmic reticulum areas respectively (Van der Kloot, 1966 and 1969), it is of interest to estimate the approximate total transverse tubule and terminal cisternae volumes from electronmicrographs.

As the average lengths of red and white muscle fibers are 1 cm in both fibers and sarcomere lengths are 1.5 μ in red and 1.3 μ in white muscle fibers, the total number of sarcomeres in a red muscle fiber is 6,666 and in a white muscle fiber 7,692. As two surfaces of transverse tubules are abutting on the terminal cisternae of a sarcomere, the total number of transverse tubules in a fiber is equal to the total number of sarcomeres in a fiber. Considering the average diameter of transverse tubules, their height or extension throughout the width of fibers, average diameters of both fibers, and total number of sarcomere per fiber, the approximate total transverse tubule volume of a white muscle fiber of volume 0.24×10^{-6} cm^3 can be estimated as follows:

$\pi r^2 h$ multiplied by the total number of sarcomeres per fiber.

$\pi = 3.1416$; $r = 0.000016$ cm; $h = 0.00055$ cm;

Total number of sarcomeres = 7,692.

Total transverse tubule volume of a white muscle fiber:

$3.1416 \times (0.000016)^2 \times 0.00055 \times 7{,}692 = 0.34 \times 10^{-8}$ cm^3

The approximate transverse tubule volume of a red muscle fiber of volume 0.10×10^{-6} cm^3 can be estimated as follows:

$$3.1416 \times (0.000012)^2 \times 0.00036 \times 6{,}666 = 0.11 \times 10^{-8} \text{ } cm^3$$

It appears from the foregoing estimates that the total transverse tubule volume of a white muscle fiber is more than 3 times that of a red muscle fiber. If we consider the approximate total volume of terminal cisternae of triads of white and red fibers we will have to include two terminal cisternae per sarcomere, one from each end. Considering the diameters of two terminal cisternae per fiber, their extensions throughout the width of fibers, and total number of sacromeres per fiber, the approximate total terminal cisternae volume of a white muscle fiber of volume 0.24×10^{-6} cm^3 is as follows:

$$3.1416 \times (0.00003)^2 \times 0.00055 \times 7{,}692 = 0.12 \times 10^{-7} \text{ } cm^3$$

The approximate total terminal cisternae volume of a red muscle fiber of volume 0.10×10^{-6} cm^3 is as follows:

$$3.1416 \times (0.000013)^2 \times 0.00036 \times 6{,}666 = 0.13 \times 10^{-8} \text{ } cm^3$$

The above estimate indicates that the total terminal cisternae volume of a white muscle fiber is more than 9 times that of a red muscle fiber.

The function of the transverse tubules of the triads is now widely accepted as to provide for spread of electrical activity from the surface of the muscle into its interior (Huxley and Taylor, 1958; Peachey and Porter, 1959; and Gage and Eisenberg, 1967), while the

terminal cisternae and contiguous longitudinal sarcotubules of the reticulum are probably involved in release and recapture of calcium ions in the contraction-relaxation cycle (Costantin, Franzini-Armstrong, and Podolsky, 1965; Ebashi and Lipmann, 1962; and Hasselbach, 1964).

Sarcoplasmic reticulum

The relative abundance of sarcoplasmic reticulum in red and white muscle fibers is better understood from examination of low power electronmicrographs. Examination of the profiles of longitudinal sarcotubules of the reticulum reveals that this element is equally abundant in both red and white fibers (Figs. 67, 68). The different fixation procedures tried reveal that the contact between terminal cisternae and longitudinal sarcotubules and the continuity of the latter are not consistently preserved in different parts of sections of red muscle fibers, while they are in white muscle fibers under the same preparative procedures (Figs. 71, 72, 83, 84). The study of red muscle fibers of early free swimming specimens also shows poor preservation of the contact of terminal cisternae with the longitudinal sarcotubules, and the continuity of the latter (Fig. 87). This result suggests that the membranous compartments of the sarcoplasmic reticulum of red and white muscle fibers do not respond identically to identical fixation procedures.

The longitudinal sarcotubules in both fibers fuse to form continuous collars around the myofilaments near the center of the A band of myofibrils. These collars are found to contain a number

of openings which are best seen in face views of the sarcoplasmic reticulum (Figs. 85, 88). This type of collar is referred to as a "Fenestrated collar" by Peachey (1965 b) in frog muscle. It is of interest to see that the longitudinal sarcotubules of the red muscle fibers of early free swimming specimens occasionally lie in close contact with one another without fusion near the center of the A band (Fig. 86). This might be expected in a developmental stage where "Fenestrated collars" may not appear in all myofibrils.

The measurement of diameters of longitudinal sarcotubules from electronmicrographs of transverse sections of fibers indicates that the average diameter of this element in red muscle fibers is 0.08 μ and in white muscle fibers is 0.12 μ. It is less easy to estimate longitudinal sarcotubule volume because of their complex structural forms. In order to estimate the volume, one must take into account average diameter and length of the longitudinal sarcotubules, fiber length, total number of sarcomeres, population of sarcotubules around each myofibril, and the average number of myofibrils. The consideration of these points gives the following approximate total longitudinal sarcotubular volume of a white muscle fiber of volume 0.24×10^{-6} cm^3:

$\pi r^2 h$ multiplied by numbers of longitudinal sarcotubules per fibril, multiplied by total number of sarcomeres per fiber, multiplied by total number of fibrils per fiber.

r = 0.000006 cm; h = 0.000117 cm;

No. of longitudinal sarcotubules = 20;

No. of sarcomeres = 7,692; No. of fibrils = 40.

The total longitudinal sarcotubular volume of a white muscle fiber:

$3.1416 \times (0.000006)^2 \times 0.000117 \times 20 \times 7{,}692 \times 40 = 0.27 \times 10^{-7}$ cm^3

The total longitudinal sarcorubular volume of a red muscle fiber:

$3.1416 \times (0.000004)^2 \times 0.000101 \times 20 \times 6{,}666 \times 40 = 0.82 \times 10^{-7}$ cm^3

The above estimate shows that the total longitudinal sarcotubular volume of a white muscle fiber is about 3 times that of a red muscle fiber.

It appears from these preliminary estimates that the total volumes of membrane-bound compartments measured in red and white muscle fibers are different from one another. They are higher in white muscle fibers than in red muscle fibers. These estimates could be in error to an extent of 15-25 per cent because of the irregular form of the structures and of the assumptions made in the estimate. Nonetheless it gives us an idea that the same degree of relative abundance of transverse tubules and sarcoplasmic reticulum in two kinds of fibers does not involve the same extent of surface areas. The relative surface areas of transverse tubules appear to contribute to the relative capacitances of red and white muscle fibers. Similarly the relative surface areas of sarcoplasmic reticulum appear to contribute to the relative rate of calcium uptake and release in a muscle fiber.

Z line

The Z line of red and white muscle fibers appears to be similar except in the presence of a somewhat greater density of extra fibrillar

component in the Z line of the red muscle fiber (Figs. 89, 90). The structure of Z line of the red and white muscle fibers seem to be better explained in terms of interconnection of the opposing sets of actin filaments by thin filaments traversing the Z line (Knappies and Carlsen, 1962). The results of the present study are not consistent with the view of Nishihara (1967) who reported the presence of a zig-zag arrangement of linkage between actin filaments from adjacent sarcomeres of the red and white muscle fibers of the goldfish, Carassius auratus.

M band

The thickened central segments of the myosin filaments comprising the conspicuous M band average 940A along the longitudinal direction of the red muscle fibers, while in the white muscle fibers they average 583A. The M band of the red muscle fiber contains more electron dense component than that of the white muscle fiber (Figs. 91, 92). Four to six thin lines can often be resolved within the M band of red and white muscle fibers. Occasionally, the lines at the two edges of M band are not resolved and only the three central lines are seen. The structural basis for these periodic linear markings is probably the bridges occurring in lateral register at specific sites along the M band shown in Figs. 93, 94.

Other organelles and associated structures of the red and white muscle fibers

Sarcolemma

The term sarcolemma refers to the plasma membrane of the muscle cell. With electron microscopy, the plasma membranes of the red and white muscle fibers are found to be associated with an extracellular coating which has been widely accepted as a protein-polysaccharide coating corresponding to the basal lamina or basement membrane of epithelial cells. Besides this coating, fine loose collagen fibrils are found outside the sarcolemma of both fibers (Figs. 20, 95).

Nucleus

The nuclei of both types of fibers are situated peripherally beneath the sarcolemma (Figs. 15, 96). This pattern of distribution of nuclei in white muscle fibers is different from that of the dogfish (Bone, 1966). By light microscopy Bone showed that the nuclei of the white muscle fibers are scattered throughout the sarcoplasm of the fiber.

Pinocytosis vesicles

The sarcolemma of red and white muscle fibers are seen to contain small invaginations resembling the pinocytosis vesicles (Figs. 20, 97, 98) seen at the surface of capillary endothelial cells (Bruns and Palade, 1968), smooth and cardiac muscle cells (Fawcett and McNutt, 1969). The inner and outer surfaces of the wall of this vesicle are smooth in nature. Some of the vesicles are budded off from the

sarcolemma and lie in the sarcoplasm below the sarcolemma. The physiological significance of this type of vesiculation of the plasmalemma is still not fully established for any cell type. The work of Bruns and Palade (1968) indicates that the smooth-surfaced vesicle of endothelium is involved in transcellular transport.

Fibroblast

Fibroblasts are frequently found in association with loose collagen fibrils outside the sarcolemma of the red muscle fibers (Fig. 95). They can be easily identified by their greater quantity of rough-surfaced endoplasmic reticulum in the cytoplasm (Kelly and Zacks, 1969). The significance of the frequent occurrence of fibroblasts in this region can be understood, considering the function of this cell. Since it is known that collagen fibrils are synthesized by fibroblast, it is possible that they may help in regeneration of the collagen fibril layer of the sarcolemma when this layer is injured. A possible reason for their more frequent occurrence among red muscle fibers rather than white fibers may be because the red fibers are usually external to the white. The external fibers may be more subject to injury than the internal. Another possible significance for their frequent occurrence among the red muscle fibers could be that they help in formation of collagen fibrils outside the sarcolemma of new muscle fibers which probably appear from myosatellite cells as discussed in the subsequent paragraph.

Myosatellite cell

Myosatellite cells are frequently found to be associated with the sarcolemma of red muscle fibers (Fig. 99). Since these cells are found to be undifferentiated muscle cells, they can be identified by their scant rough-surfaced endoplasmic reticulum and abundant free ribosomes in the cytoplasm (Kelly and Zacks, 1969). In addition to these characteristics they can be identified by their situation beneath the protein-polysacchride coating of the sarcolemma of the muscle fiber and their separation from the muscle fiber by an intercell-space (Mauro, 1961; and Moss and Leblond, 1970). The significance of the frequent occurrence of myosatellite cells among red muscle fibers of young adult fish is probably that they are precursors of red muscle fibers (Moss and Leblond, 1970). Another possible significance for the frequent occurrence of myosatellite cells with red muscle fibers may be that they are involved in regeneration of red muscle fibers (Mauro, 1961) which may be more subject to injury than are the deeper lying white fibers.

Ontogeny of red and white muscle fibers

Light microscopic search in embryos, sac fry, and early free swimming specimens of S. gairdneri showed that red muscle fibers are easily distinguishable only in early free swimming specimens. The three stages were then studied by electron microscope. Four-week old embryos have differentiated characteristic white muscle fibers (Fig. 100) but there is no indication of the presence of red muscle fibers. A considerable number of undifferentiated cells and a number

of cells containing myofilaments are found in this stage (Fig. 101).

The undifferentiated cells are found to be of two types. One type of cell is found to contain abundant free ribosomes and scanty rough-surfaced endoplasmic reticulum in the cytoplasm (Fig. 102). This type of cell is considered to be undifferentiated muscle cell (myoblast) by Hay (1963); Price et al. (1964); Przbylski and Blumberg (1966); Fischman (1967); and Kelly and Zacks (1969). Another type of cell is found to contain abundant rough-surfaced endoplasmic reticulum and few free ribosomes in the cytoplasm (Fig. 103). This type of cell is considered to be fibroblast by Przbylski and Blumberg (1966); and Kelly and Zacks (1969). The cells containing myofilaments are found in clusters together with the undifferentiated muscle cells (Fig. 104). Fibroblasts are largely found in association with collagen fibrils (Fig. 104); sometimes they are found between muscle cells.

Examination of clusters of undifferentiated and differentiated muscle cells reveals that they are usually closely associated with one another, leaving interspaces of approximately 30-60A between apposed plasma membranes (Fig. 105). In some cases the plasma membranes of two adjacent muscle cells are not clearly discernible at the region of contact between cells. Lipid droplets and mitochondria are clearly visible in this population of cells. Mitotic figures and paired nuclei in a cell are occasionally found in the population of undifferentiated cells (Figs. 106, 107). The cells with myofilaments which are in close association in a group shown in Fig. 101 are considered to be primary generation of myotubes as

in rat intercostal muscle (Kelly and Zacks, 1969). The myotubes are frequently found to be separated from their adjacent groups of myotubes by pseudopodia of undifferentiated cells (Fig. 108). Sometimes a myotube is found to be embraced by pseudopodia of an undifferentiated cell shown in Fig. 108. This type of pseudopodial projection from undifferentiated cells is found in interspaces between undifferentiated cells too. It has been found occasionally that the plasma membranes of an undifferentiated cell lying betwen two myotubes is not discernible at the regions of contact with the myotubes shown in Fig. 109. Sometimes the pseudopodium of an undifferentiated cell occupies a depression in the wall of another undifferentiated cell and the plasma membranes of these two cells are not clearly discernible at the region of contact (Fig. 110).

In the sac fry stage, well-differentiated myotubes with a greater content of scattered myofilaments are found (Fig. 111). It has been difficult to determine the exact positions of nuclei in myotubes because of the problems of sampling and interpretation of positions of nuclei in various sectional planes in electronmicrographs. The structures of these myotubes are not exactly the same as those of rat intercostal muscle (Kelly and Zacks, 1969). The large myotubes of rat muscle consist of centrally placed glycogen particles and peripherally placed myofilaments. The large myotubes of sac fry stage are not provided with rich glycogen content. Moreover, the glycogen particles are scattered throughout the bodies of large myotubes. Myofilaments are not consistently found at the peripheral zones of myotubes either.

The cytoplasm of certain myotubes of a group are found to be confluent with one another through gaps produced by discontinuities of apposed plasma membranes (Fig. 111). It is difficult to determine whether these gaps are the results of fusions between certain localities of muscle cells or whether they are artifacts of sectioning. In association with these myotubes, a few muscle cells with traces of organization of band patterns of muscle fibers are seen (Fig. 112). The nuclei of these muscle cells are peripherally placed. This population of muscle cells may be called the primary generation of muscle fibers. The easily recognizable characteristics of the red muscle fibers, i.e., fused mass of myofilaments, large number of mitochondria, and high content of lipid droplets have not yet appeared in this primary generation of muscle fibers. The proportion of undifferentiated cells and cells with few myofilaments are found to decline considerably with increasing age.

The consistent hexagonal arrangements of actin and myosin filaments in white muscle fibers of embryos and sac fry are clearly discernible (Figs. 113, 114). The dimensions of actin and myosin filaments of white muscle fibers of embryos are smaller than those of sac fry and young adult individuals. The dimensions of actin and myosin filaments of white muscle fibers of embryo and sac fry average 52A & 118A and 53A & 128A respectively. In young adults, dimensions of actin and myosin filaments are 74A and 138A respectively. The dimensions of myofilaments appear to increase as development proceeds.

Study of early free swimming specimens reveals the presence of muscle fibers with easily recognizable characteristics of red fibers (Fig. 115). This population of red muscle fibers is found in

association with some muscle cells of different stages of development (Fig. 116). A number of fibroblasts are found in association with collagen fibrils outside the zone of red muscle fibers shown in Fig. 116. It is interesting to note that the beginning of free swimming coincides with the time at which red muscle fibers are recognizable.

Swimming behavior

S. gairdneri cruises in the water with varying speed. It shows motivated feeding response when food is given at regular intervals and during this time it propels itself at a higher speed.

The fish is found to carry on independent movement of different parts of the caudal fin without any observable lateral movement of the trunk while pottering about in an aquarium. While resting, the caudal fin exhibits irregular movements which are usually restricted to the median part of the fin without observable lateral movement of the trunk. This was reported by Nursall (1963 a).

It is interesting to observe the locomotion of specimens in early stages of development. After hatching, the sac fry remain quiescent, moving by vigorous tail beating only if disturbed. This movement, generally, covers only a few inches and is confined strictly to the bottom of the trough. About 2 days after hatching the fry start to seek out darkened areas, particles of debris, etc., and congregate in large groups. This act is accomplished by vigorous tail beating and is still confined to the bottom of the trough. About eight days after hatching and the sac size has decreased noticeably the bulk of the fry then seem to want to maintain a more suitable position in

the trough. This is accomplished by a very slow motion of the tail. They still remain on or very close to the bottom of the trough. The fry remain on the bottom of the trough until the bulk of the sac is absorbed. When 5 per cent to 8 per cent of the fish in the trough come up from the bottom and are free swimming feeding of these specimens is started. This feeding activity seems to stimulate a response from the fish resting on the bottom of the trough and they will make occasional sorties. After a few days they all leave the bottom of the trough and become free swimming.

DISCUSSION

(See Summary, p. 63 ff., for an abbreviated statement of differences between red and white fibers).

Functional significance of the caudal skeleton and musculature

The first pair of massive uroneurals with their high crests appear to stabilize three loose epurals, which are held rigidly between them. The first uroneurals, together with two other uroneurals which follow them, appear to stiffen the upturned terminal portion of the vertebral column which supports the abduction and adduction of the lobes of the caudal fin and flexion of the caudal peduncle and fin. The hypurals fan at the end. This serves as a wide surface for the attachment of a large mass of flexor and hypochordal longitudinal muscles essential for caudal propulsion.

The well-developed superficial flexor muscles appear to be involved mainly in the bending of the caudal peduncle during steady swimming. The positions and attachments of the deep flexor muscles appear to reinforce the superficial flexor muscles in their action. The muscular connection of the hypochordal longitudinal muscle to the superficial dorsal flexor, and attachments to the fin rays, suggest that the hypochordal longitudinal muscle plays a double role: a) it initiates lateral sweep of the caudal fin, and b) adducts dorsal fin rays. The anatomical position of the interradial muscles indicate primarily their involvement in adduction of the fin rays, i.e., contraction of the caudal fin. Secondarily, the occasional independent movement of different parts of the caudal fin is probably done by the interradials with the intervention of deep flexor muscles.

The myomeric nature of organization of trunk musculature is changed in the caudal region. Two layers of caudal musculature are

seen. Examination of the anatomical positions of caudal musculature indicates that caudally the epaxial and hypaxial myomeric musculature is separated into superficial and deep layers. The superficial layer becomes the superficial dorsal and ventral flexor muscles and the deep layer becomes the deep dorsal and ventral flexor muscles, and hypochordal longitudinal muscle. The interradial muscles are derived from the superficial layers of the posteriormost sections of the epaxial and hypaxial myomeric musculature. The supra- and infra-carinal muscles are derived from the dorsal and ventralmost sections of the epaxial and hypaxial muscles respectively.

The changes of myomeric muscles into two layers of muscles of complex organization in the caudal region does indicate that it is an active locomotory center where delicate organized movements are present.

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 the rest of the body and it is probably supplied by the red muscle fibers mainly. The data of reciprocal relationship of phosphorylase and oxidative enzymes together with the data of lipid, glycogen, and mitochondria of the two types of muscle fibers, show that these distinctive muscle fibers utilize different metabolites for their energy. The red muscle

fibers utilize fat and the latter is oxidized via the citric acid cycle. The white muscle fibers utilize glycogen via anaerobic glycolysis. These findings confirm the suggestion of Bone (1966). The two different kinds of metabolite appear to be related to two different types of swimming. The study of Boddeke et al. (1959) shows that S. gairdneri belongs to a group called "stayers" which includes fishes who show sustained swimming. It appears that the red muscle fibers with their high lipid content take a role in sustained swimming. Experimental investigations show the rapid decline in glycogen content of white muscle fibers following a strenuous exercise. This finding together with the information obtained from the study of enzymes shows that the white muscle fibers with their glycogen content are involved greatly in strenuous swimming or activities where a burst of speed is necessary.

The biochemical analyses show clearly that actomyosin of the white muscle fibers has an average of 2.7 times the ATPase activity of actomyosin of the red muscle fibers. It is interesting to note that this result agrees well with ATPase activity of myosin of rabbit's fast (extensor digitorum longus) and slow (soleus) muscles given by Barany et al. (1965). ATPase activity of fast muscle of rabbit is 2-3 times that of slow muscle. The physiological significance of the different ATPase activities of these two muscles is revealed by considering the physiological data on their contraction time. The contraction time of the soleus muscle is 2.5 times longer than that of the extensor digitorum longus.

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 the rate of contraction of a given type of muscle fiber is proportional to the rate of ATP hydrolysis by actomyosin (Barany, et al., 1965; Gergely et al., 1965; and others), 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 the fish (Bone, 1966; Rayner and Keenan, 1967; and Hudson, 1969).

Functional significance of ontogeny of red and white muscle fibers

Light microscopic and electron miscroscopic studies show that white muscle fibers appear first in the early stage of development of the fish, before swimming begins. As the embryo hatches out and assumes free swimming activities, red muscle fiber becomes 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.

A brief comment may be made on the problem of formation of multinucleated muscle cells. Although it is now well known that during development striated muscle cells increase their numbers of nuclei by fusion with neighboring cells, a clear-cut picture of fusion is not yet recorded unequivocally either in the present or in previous studies due to the problems of sampling and interpretation of membranes passing through various sectional planes in electronmicrographs. The present study indicates that the clustered muscle cells may develop

in one of two ways. Some of them may fuse with one another and form multinucleated muscle cells, while others are separated and may form independent muscle cells with multiple nuclei by amitotic divisions. This idea is in agreement with that of Kelly and Zacks (1969).

Pattern of innervation of musculature in relation to swimming behavior

The presence of the same pattern of innervation (polyneuronal) in the red and white muscle fibers at first suggests that the fibers are involved in the same type of function. But the little information so far accumulated in experimental studies (Takeuchi, 1959; Barets, 1961; Rayner and Keenan, 1967; and Hudson, 1969) suggests that the red and white muscle fibers correspond to some extent to the slow and fast motor system respectively of the elasmobranchs (Bone, 1966) and Amphibia (Kuffler and Vaughan Williams, 1953) although the patterns of innervation of the latter two groups of animals are different from that of the teleosts. The absence of characteristic innervation for twitch muscle fibers in S. gairdneri like other teleosts except catfish (Barets, 1961), suggests that the functional demand for twitch (mononeuronal) innervation in teleosts is probably met with the existing innervation which is called "slow motor system". The latter term seems to me inappropriate at least for S. gairdneri because the latter together with other salmonids and the carp possess high sustained swimming power (Boddeke et al., 1959) which cannot be called slow swimming. The polyneuronal innervation of S. gairdneri is capable of giving rise to highly active sustained swimming which is different from slow swimming as it is found in "crawler" (Boddeke et al., 1959), so this type of motor system in S. gairdneri may reasonably be called

the "sustained motor system".

Neural control of the caudal fin

The distribution of numerous nerves in the caudal fin reflects the control of complicated organized movements. The connective tissue between the epichordal and hypochordal lobes and between the six and seven inner fin rays of epichordal and hypochordal lobes of the caudal fin possesses characteristic innervation which shows nerve fibers ending in the connective tissue below the skin. These nerve fibers cannot be traced to effectors. It seems that they are sensory fibers. The distribution of these nerve fibers appear to be suited to signal the spatiotemporal patterns of pressure changes arising in the course of active (voluntary) and passive (involuntary) deformation of the fin during cruising and hovering.

Organization of the myofibril

The present study has emphasized that red muscle fibers differ from white muscle fibers in that the red muscle fibers consist predominantly of a continuous mass of myofilaments. The white muscle fibers on the other hand consist of myofilaments grouped in discrete myofibrils.

The functional implication of the association of myofilaments in a single more or less coherent bundle are not fully understood. However, in view of the function of this pattern of organization in the slow "tonus muscle" of Amphibia, which yield a sustained contraction in response to repetitive stimulation, a reasonable inference seems to be that as the myofilaments are organized into

a bigger confluent mass, filling up most of the fiber, they probably take a longer time to bring all myofilaments of the bigger mass into interactions on molecular level simultaneously than the myofilaments of smaller discrete bundles constituting a fiber.

The significance of the presence of a large number of irregularly arranged actin filaments around a myosin filament is not yet well understood. There is no reason to believe that this would affect the basic mechanism of contraction, but it might affect the total force developed, as more actins can be linked to each myosin. Also it might affect the speed at which links are formed, as many myosin cross-bridges have several actin filaments with which they might interact. This idea is in agreement with that of Davies (Personal communication, 1970) whose molecular theory of muscle contraction, Davies (1963), is well known. It is not unusual to see the occurrence of a large number of irregularly arranged actin filaments around a myosin filament in a red muscle fiber, as this may be expected in a muscle which is not considered to be particularly fast. It is interesting to note a recent finding of Hoyle and McNeill (1968) who worked on the levator muscle of the eyestalk of a crab, Podophthalmus vigil (Weber). They stated: "A most significant finding has been that the slower white fibers have a uniquely high ratio of thin to thick filaments, and that in them the thin filaments are not in orbits around the thick ones. They have a fairly fast contraction speed, in view of their sarcomere length, but are characterized by a very slow relaxation rate". The muscles of the insect gut, which are not known to be particularly fast, have

also large numbers of actin filaments around each myosin filament (Davies; Personal communication, 1970).

T system

Previous authors (Peachey and Huxley, 1962; Hess, 1965; Page, 1965), 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 is completely absent (Peachey and Huxley, 1962) or rarely present (Page, 1965), and sarcoplasmic reticulum is poorly developed. It has been shown further by Peachey (1965 a and b) 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.

Although the present study indicates that the relative abundance of transverse tubules of both fibers is the same, the volume is greater in white fibers. This result suggests that the similarity between the red and white muscle fibers concerning T system appears to be superficial, and that they differ from each other in respect to transverse tubule surface areas which are considered to contribute to capacitance of muscle fibers.

Sarcoplasmic reticulum

Closer examination of the relative abundance of sarcoplasmic reticulum in the red and white muscle fibers indicates that it is the same in both fibers, but the over-all results of estimates of volumes of longitudinal sarcotubules of the reticulum of the red and white muscle fibers reveals that the volume of longitudinal sarcotubules of the red muscle fibers is less than that of the white muscle 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 (1969) 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. He obtained assumptions about the rate of calcium uptake from his previous experiment (Van der Kloot, 1966). In the case where the collar of sarcoplasmic reticulum was assumed to be 0.2μ thick, a 20 per cent 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 4 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 myofribril falls. The examination of volumes of sarcoplasmic reticulum of the red and white muscle fibers, in the light of Van der Kloot's results, suggests that the rate of fall of the value of concentration of calcium ion from the myofibril of the red muscle fiber is slower than that of the white muscle fibers. It is widely presumed, during relaxation that the value of concentration of calcium ion decreases in the myofibril and reaccumulates within the sarcoplasmic reticulum.

M line

It appears from recent work on the myosin filament of striated muscle (Pepe, 1966, 1967) that the exact nature of the specialization of the central region of the myosin filaments and of the intermyosin cross-bridges is still not well understood. Pepe, by his antibody-staining technique, came to the conclusion that the M band is formed by a protein, other than myosin, actin or tropomyosin, which is weakly bound to the center of the myosin filament. No such central thickening is present on filaments reconstituted in vitro from purified myosin (Carney and Brown, 1966; Huxley, 1963). This region is found to possess special enzymatic properties not commonly attributed to myosin. Barrnett and Palade (1959), with their electronhistochemical studies on skeletal and cardiac muscles, demonstrated the presence of cholinesterase in the M band, whereas, Karnovsky and Hug (1963), working on cardiac muscle, suggested that the so-called M band enzyme is an organophosphate-resistant esterase, and not, as previously had been suggested by Barrnett and Palade, a cholinesterase. Its role

in cardiac metabolism and physiology, and the reasons for its precise localization at the level of M band, are at present a mystery. The presence of M band and its morphological difference between the red and white muscle fibers suggest that although the myosin filaments of both fibers possess specialization in the central region, the functional implications of this may not be the same.

Z line

The morphological similarity of the Z lines in the red and white muscle fibers shows that the insertion of the I band filaments in the Z lines of both fibers is similar. The Z lines probably give bases for the organized disposition to the actin filaments, which are held in place during the stresses exerted in contraction of the fibers.

SUMMARY

1. The caudal skeleton of S. gairdneri consists of 2 ural centra, 1 carilaginous urostyle, 3 pairs of uroneurals, 3 epurals, 6 hypurals, 18 branched fin rays, 4 unbranched fin rays, and 22 procurrent rays.

2. The caudal musculature consists of 7 muscles: 1. superficial dorsal flexor muscle, 2. superficial ventral flexor muscle, 3. carinals, 4. interradials, 5. deep dorsal flexor muscle, 6. deep ventral flexor muscle, and 7. hypochordal longitudinal muscle, all of which are derived from posterior myomeric musculature.

3. The caudal muscles consist of two types of muscle fibers: 1. red, and 2. white. The relative proportion of the red muscle fibers is greater in the caudal peduncle than in the rest of the body.

4. The red and white muscle fibers of caudal muscles are polyneuronally innervated by branches and subbranches of 10 spinal nerves.

5. The caudal fin is innervated by numerous nerve fibers, some of which ending in connective tissue without any association with effectors, are presumably sensory fibers.

6. The diameter of the white muscle fiber is greater than that of the red muscle fiber. The sarcomere length of the white muscle fiber is smaller than that of the red muscle fiber.

7. The red muscle fibers have a higher content of lipid droplets and mitochondria than the white muscle fibers. The white muscle fibers are richer in glycogen content.

8. ATPase activity of the white muscle fibers is 2.7 times that of the red muscle fibers. Oxidative enzymes are strongly reactive in the red muscle fibers and weakly reactive in the white muscle fibers. Phosphorylase is strongly reactive in the white muscle fibers and weakly reactive in the red muscle fibers.

9. A red muscle fiber consists of a fused mass of myofilaments with scattered interspaces of sarcoplasm. The white muscle fibers consist of discrete myofibrillar bundles well delineated by sarcoplasmic reticulum.

10. Some of the red muscle fibers possess an irregular arrangement of a large number of actin filaments around a myosin filament in addition to the usual hexagonal arrangement of myofilaments which is found in the white muscle fibers.

11. The dimensions of actin and myosin filaments of the red muscle fibers are greater than those of the white muscle fibers. Correspondingly the protein content (actomyosin) of the red muscle fibers is greater than that of the white muscle fibers.

12. The relative abundance of transverse tubules in both fibers is the same, i.e., in both fibers the transverse tubules are present in most Z line regions, but the total transverse tubule volumes of a white muscle fiber is greater than that of a red muscle fiber; the same is the case with the sarcoplasmic reticulum of these two fibers.

13. The Z line and M line of the red muscle fiber have higher content of electron dense components than those of the white muscle fiber.

14. Ontogenically the red muscle fibers appear later than the white muscle fibers. The red muscle fiber becomes distinguishable only in the early free swimming stage.

15. Most of the characteristics of fast muscle fibers as they are found in higher vertebrates are found in the white muscle fibers of this fish, while most of the characteristics of slow muscle fibers as they are found in higher vertebrates are found in the red muscle fibers of this fish.

LITERATURE CITED

Arloing, S. and A. Lavocat. 1875. Recherches sur l'anatomie et la physiologie des muscles striés pales et foncés. Mém. Acad. Sci. Inscript. Toulouse, 7: 177-194.

Baerends, G.P. and J.M. Baerends-Van roon. 1950. An introduction to the study of the ethology of cichlid fishes. Behavior Suppl., 1: 1-243.

Baker, J.R. 1944. The structure and chemical composition of the golgi element. Quart J. Microsc. Sci., 85: 1-71.

Barany, M., M. Barany, T. Reckard, and V. Volpe. 1965. Myosin of fast and slow muscles of the rabbit. Arch. Biochem. Biophys., 109: 185-191.

Barets, A. 1961. Contribution a l'etude des systèms moteurs 'lent et 'rapide' du muscle lateral des teleostéens. Archs Anat. microsc. Morph. exp., 50: 91-187.

Barrington, E.J.W. 1937. The structure and development of the tail in plaice (Pleuronectes platessa) and the cod (Gadus morrhua). Quart J. Microsc. Sci., Lond. New Series, 79: 447-469.

Barrnett, R.J. and G.E. Palade. 1959. Enzymatic activity in the M band. J. Biophys. Biochem. Cytol., 6: 163-169.

Bergman, R.A. 1964 a. The structure of the dorsal fin musculature of the marine teleosts, Hippocampus hudsonius and H. zosterae. Bull. Johns Hopkins Hosp., 114: 325-343.

_____ 1964 b. Mechanical properties of the dorsal fin musculature of the marine teleost, Hippocampus hudsonius. Bull. Johns Hopkins Hosp., 114: 344-353.

Bergman, R.A. 1967. Motor nerve endings of twitch muscle fibers in Hippocampus hudsonius. J. Cell Biol., 32: 751-757.

Bilinski, E. 1963. Utilization of lipids by fish. I. Fatty acid oxidation by tissue slices from dark and white muscle of rainbow trout (Salmo gairdneri). Can. J. Biochem. Physiol., 41: 107-112.

Black, E.C., A.R. Connor, K-C. Lam and W-G. Chia. 1962. Changes in glycogen, pyruvate, and lactate in rainbow trout (Salmo gairdneri) during and following muscular activity. J. Fish. Res. Bd. Canada, 19: 409-436.

Boddeke, R., E.J. Slijper and Van der Stelt. 1959. Histological characteristics of the body-musculature of fishes in connexion with their mode of life. Proc. K. ned. Akad. Wet., 62: 576-588.

Bone, Q. 1966. On the function of the two types of myotomal muscle fiber in elasmobranch fish. J. mar. biol. Ass. U.K., 46: 321-349.

Braekkan, O.R. 1956. Function of the red muscle in fish. Nature, Lond., 178: 747-748.

Bruns, R.R. and G.E. Palade. 1968. Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. J. Cell Biol., 37: 277-299.

Buttkus, H. 1963. Red and white muscle of fish in relation to rigor mortis. J. Fish. Res. Bd. Canada, 20: 45-58.

Carney, J.A. and A.L. Brown. 1966. An electron microscopic study of canine cardiac myosin and some of its aggregates. J. Cell Biol., 28: 375-389.

Chevrel, R. 1913. Essai sur la morpholgie et al physiologie du muscle láteral chez les poissons osseux. Archs Zool. exp. gén., 52: 473-607.

Connor, A.R., C.H. Elling, E.C. Black, G.B. Collins, J.R. Gauley and E. Trevor-Smith. 1964. Changes in glycogen and lactate levels in migrating salmonid fishes ascending experimental endless fishways. J. Fish. Res. Bd. Canada, 21: 255-290.

Costantin, L.L., C. Franzini-Armstrong, and R.J. Podolsky. 1965. Localization of calcium accumulating structures in striated muscle. Science, 147: 158-159.

Davies, R.E. 1963. A molecular theory of muscle contraction: calcium dependent contractions with hydrogen bond formation plus ATP-dependent extensions of part of the myosin-actin cross-bridges. Nature, 199: 1068-1074.

Dubowitz, V. and A.G.E. Pearse. 1961. A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. Histochemie, 2: 105-117.

Ebashi, S. and F. Lipmann. 1962. Adonosine triphosphate-linked concentration of calcium ions in a particulate fraction of rabbit muscle. J. Cell Biol., 14: 389-400.

Fawcett, Don W. and Scott N. McNutt. 1969. The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. J. Cell Biol., 42(1): 1-45.

Fawcett, D.W. and J.P. Revel. 1961. The sarcoplasmic reticulum of a fast-acting fish muscle. J. Biophys. and Biochem. Cytol., 10: 89-110.

Fischman, D.A. 1967. An electron microscope study of myofibril formation in embryonic chick skeletal muscle. J. Cell Biol., 32: 557-577.

Franzini-Armstrong, C. 1968. The triadic junction in frog muscle fibers. J. Cell Biol., 39: 6a (Abstr.).

_____ 1969. Experimental studies of the triadic junction in muscle fibers. J. Cell Biol., 43: 89 (Abstr.).

Franzini-Armstrong, C. and K.R. Porter. 1964. Sarcolemmal invaginations constituting the T system in fish muscle fibers. J. Cell Biol., 22: 675-695.

Freihofer, W.C. 1966. The Sihler technique of staining nerves for systematic study especially of fishes. Copeia, (3): 470-475.

Gage, P.W. and R.S. Eisenberg. 1967. Action potentials without contraction in frog skeletal muscle fibers with disrupted transverse tubules. Science, 158: 1702-1703.

George, J.C. 1962. A histophysiological study of the red and white muscles of the mackerel. Am. Midl. Nat., 68: 487-494.

George, J.C. and R.M. Naik. 1958. Relative distribution and chemical nature of the fuel store of the two types of fibers in the pectoralis major muscle of the pigeon. Nature, 181: 709-710.

Gerebtzoff, M.A. 1956. Contribution a la morphologie comparée des appareils cholinesterasiques myo-neural et musculo-tendineux des vertebrés. Ann Histochem., 1: 145.

Gergely, J., D. Pragay, A.F. Scholz, J.C. Seidel, F.A. Sreter and M.M. Thompson. 1965. Comparative studies on white and red muscle. Molecular Biology of Muscular contraction. Ed. by

S. Ebashi, F. Oosawa, T. Sekine, and Y. Tonowasa. Igaku Shoin Ltd., Tokyo, 145-159.

Gordon, M.S. 1968. Oxygen consumption of red and white muscles from tuna fishes. Science, 159: 87-90.

Gosline, W.A. 1960. Contributions toward a classification of modern isospondylous fish. Bull. Brit. Museum Zool., 6: 327-365.

_____ 1965. Teleostean phylogeny. Copeia, 2: 186-194.

Green, C.W. and C.H. Green. 1913. The skeletal musculature of the salmon. Bull. U.S. Bur. Fish., 33(796): 25-59.

Grenholm, A. 1923. Studien uber die Flossenmuskulatur der Teleostier. Uppsala Univ. Arsskr. Mat. Naturvet., 2: 1-296.

Hanson, J. and H.E. Huxley. 1955. The structural basis of contraction in striated muscle. In Fibrous proteins and their biological significance. Symposia of the society of experimental biology. Cambridge Univ. Press, Cambridge, No. 9, 228-264.

Hasselbach, W. 1964. Relaxation and the sarcotubular calcium pump. Fed. Proc. 23: 909-912.

Hay, E.D. 1963. The fine structure of differentiating muscle in salamander tail. Z. Zellforsch. mikrosk. Anat., 59: 6-34.

Hess, A. 1960. The structure of extrafusal muscle fibers in the frog and their innervation studied by the cholinesterase technique. Amer. J. Anat. 107: 129-151.

Hess, R., D.G. Scarpelli, and A.G.E. Pearse. 1958. Cytochemical localization of oxidative enzymes. II. Pyridine nucleotide-linked dehydrogenases. J. Biophys. Biochem. Cytol., 4: 753-760.

Hoar, W.S. and C.P. Hickman. 1967. A laboratory companion for general and comparative physiology. Prentice-Hall, Inc., Englewood Cliffs, N.J., 296 pp.

Holmes, W. 1943. Silver staining of nerve axons in paraffin sections. Anat. Rec., 86: 157.

Hoyle, G. 1967. Diversity of striated muscle. Am. Zoologist, 7(3): 435-449.

Hoyle, G. and Patricia Ann McNeill. 1968. Correlated physiological and ultrastructural studies on specialized muscles. Ib. Ultrastructure of white and pink fibers of the levator of the eyestalk of Podophthalmus vigil (Weber). J. Exp. Zool., 167: 487-522.

Hudson, R.C.L. 1969. Polyneuronal innervation of the fast muscles of the marine teleost Cottus scorpius L. J. Exp. Biol., 50: 47-67.

Huxley, A.F. and R.E. Taylor. 1958. Local activation of striated muscle fibers. J. Physiol. Lond., 144: 426-441.

Huxley, H.E. 1960. Muscle cells. In The cell: Biochemistry Physiology, Morphology. J. Brachet and A.E. Mirsky, Eds. Academic Press, Inc., New York, 4: 365-481.

_____ 1963. Electron microscopic studies on the structure of natural and synthetic protein filaments from striated muscle. J. Mol. Biol. 7: 281-308.

Huxley, H.E. and J. Hanson. 1960. The molecular basis of contraction in cross striated muscle. In Structure and Function of muscle. G.H. Bourne, Ed. Academic Press, Inc., New York, 1: 183-227.

Kafuku, T. 1950. 'Red muscles' in fishes. I. Comparative anatomy of the scombroid fishes of Japan. Jap. J. Ichthyol., 1: 89-100.

Karnovsky, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol., 27: 137A (Abstr.).

Karnovsky, M.J. and K. Hug. 1963. The nature of the M band enzyme in rat ventricular muscle. J. Cell Biol. 19: 255-260.

Kay, C.M. and J. Brahms. 1963. The influence of ethylene glycol on the enzymatic adenosine triphosphatase activity and molecular conformation of fibrous muscle proteins. J. Biol. Chem., 238(9): 2945-2949.

Kelly, A.M. and S.I. Zacks. 1969. The histogenesis of rat intercostal muscle. J. Cell Biol., 42(1): 135-153.

Kelly, D.E. 1967. Fine structural analysis of muscle triad junctions. J. Cell Biol., 35: 66A (Abstr.).

_____ 1969. Skeletal muscle triad junction fine structure; new observation regarding dimples of the sarcoplasmic reticulum terminal cisternae. J. Cell Biol., 43: 156 (Abstr.).

Knappeis, G.G. and F. Carlsen. 1962. The ultrastructure of the Z disc in skeletal muscle. J. Cell Biol., 13: 323-334.

Krüger, P. 1952. Tetanus and Tonus der quergestreiften Skelettmuskel der Wirbeltiere und des Menschen, Liepzig, Acad. Verlag, Geest und Portig.

Krüger, P., F. Duspiva, and F. Fürlinger. 1933. Tetanus and Tonus der skeletmuskein des Frosches, ein histologische reizphysiologische und chemische untersuchung. Pflügers Archiv f.d. ges. Physiol., 231: 750-786.

Kuffler, S.W. and E.M. Waughan Williams. 1953. Properties of the 'slow' skeletal muscle fibers of the frog. J. Physiol., 121: 318-340.

Lindsey, C.C. 1968. Temperature of red and white muscle in recently caught marlin and other large tropical fish. J. Fish. Res. Bd. Canada, 25(9): 1987-1992.

Lorenzini, S. 1678. 'Osservazioni intorno alle Torpedini'. Onofri, Firenze.

Lowry, D.H., N.J. Rosebrough, A. Lewis Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Lund, R. 1967. An analysis of the propulsive mechanisms of fishes, with reference to some fossil actinopterygians. Ann. Carneg. Musc., Pittsburgh, 39: 195-218.

Macmanus, J.F.A. and R.W. Mowry. 1960. Staining methods. Histologic and histochemical. p. 423. Paul B. Hoeber, Inc., N.Y.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol., 9: 493-495.

Monod, T. 1967. Le complexe urophore des Téléostéens: typologie et évolution (note préliminaire). Colloques int. Cent. natn. Rech. scient., Paris, 163: 111-131.

Moss, F.P. and C.P. Leblond. 1970. Nature of dividing nuclei in skeletal muscle of growing rats. J. Cell Biol., 44(2): 459-462.

Nachlas, M.M., D.G. Walker and A.M. Seligman. 1958. A histochemical method for the demonstration of diphosphopyridine nucleotide diaphorase. J. Biophys. Biochem. Cytol., 4: 29-40.

Nag, A.C. 1967. Functional morphology of the caudal region of certain clupeiform and perciform fishes with reference to the taxonomy. J. Morph., 123(4): 529-558.

Nakajima, Y. 1962. Fine structure of red and white muscle fibers and their neuromuscular junction of the snake fish. Fifth International Congress for electron microscopy, 2: U10.

Nishihara, H. 1966. Some observations on the relationship between structure and function in fish red and white muscle. Sixth International Congress for electron microscopy, 2: 693.

_____ 1967. Studies on the fine structure of red and white fin muscle of the fish (Carassius auratus). Ex. Arch. Hist. Jap., 28(4): 425-447.

Norden, C.R. 1961. Comparative osteology of representative salmonid fishes, with particular reference to the grayling (Thymallus arcticus) and its phylogeny. J. Fish. Res. Bd. Canada, 18(5): 679-791.

Nursall, J.R. 1962. Swimming and the origin of paired appendages. Am. Zoologist, 2: 127-141.

_____ 1963 a. The caudal musculature of Hoplopagrus guntheri Gill (Perciformes: Lutjanidae). Can. J. Zool., 41: 865-880.

_____ 1963 b. The hypurapophysis, an important element of the caudal skeleton. Copeia, (2): 458-459.

Nybelin, O. 1963. Zur Morphologie und Terminologie des Schwanzskeletters der Actinopterygier. Agassiz. Arkiv. Zool., (2)15(35): 485-516.

Page, S.G. 1965. A comparison of the fine structures of frog slow and twitch muscle fibers. J. Cell Biol., 26: 477-497.

Palmgren, A. 1948. A rapid method for selective silver staining of nerve fibers and nerve endings in mounted paraffin sections. Acta Zool., Stockl., 29: 377-392.

Patterson, C. 1968. The caudal skeleton in lower Liassic pholidophorid fishes. Bull. Br. Mus. nat. Hist. (Geol.), 16(5): 201-239.

Peachey, L.D. 1965 a. Transverse tubules in excitation-contraction coupling. Fed. Proc., 27: 1124-1134.

_____ 1965 b. The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. J. Cell Biol., 25(3): 209-231.

Peachey, L.D. and A.F. Huxley. 1962. Structural identification of twitch and slow striated muscle of the frog. J. Cell Biol., 13: 177-181.

Peachey, L.D. and K.R. Porter. 1959. Intracellular impulse conduction in muscle cells. Science, 129: 721.

Pearse, A.G.E. 1957. Intracellular localization of dehydrogenase systems using monotetrazolium salts and metal chelation of their formazans. J. Histochem. Cytochem., 5: 515-527.

_____ 1961. Histochemistry. Theoretical and Applied, J.A. Churchill, Ltd., London, p. 910.

Pepe, F.A. 1966. Some aspects of the structural organization of the myofibril as revealed by antibody-staining methods. J. Cell Biol., 28: 505-525.

_____ 1967. The myosin filament. I. Structural organization from antibody staining observed in electron microscopy. J. Cell Biol., 27: 203-225.

Przbylski, R.J. and J.E. Blumberg. 1966. Ultrastructural aspects of myogenesis in the chick. Lab. Invest., 15: 836-863.

Rayner, M.D. and M.J. Keenan. 1967. Role of red and white muscles in the swimming of the skipjack tuna. Nature, 214(5086): 392-393.

Regan, C.T. 1910. The caudal fin of the Elpidae and of some other teleostean fishes. Ann. Mag. Nat. Hist., Ser. 8(5): 354-358.

Reger, J.F. 1961. The fine structure of neuromuscular junctions and the sarcoplasmic reticulum of extrinsic eye muscles of Fundulus heterochitus. J. Biophys. Biochem. Cytol., 10: 111-121.

Revel, J.P. 1962. The sarcoplasmic reticulum of the bat cricothyroid muscle. J. Cell Biol., 12: 571-588.

_____ 1964. Electron microscopy of glycogen. J. Cell Biol., 12: 104-114.

Sabatini, D.D., K.G. Bensch, and R.J. Barrnett. 1963. Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol., 17: 19-58.

Schmalhausen, J.J. 1913. Zur Morphologie der unpaaren Flossen und inbesonders der Schwanzflosse der Fische. Z. Wiss. Zool., 104(1): 1-80.

Shenk, W.D. and M. Davidson. 1966. A study of the terminal innervation of a fast-acting fish muscle. Biol. Bull., 130(1): 135-140.

Skoglund, C.R. 1959. Neuromuscular mechanism of sound production in Opsanus tau. Biol. Bull., 117: 438-443.

Steedman, H.F. 1960. Section cutting in microscopy. Blackwell Scientific publications, Oxford, 172.

Stevens, E.D. 1968. The effect of exercise on the distribution of blood to various organs in rainbow trout. Comp. Biochem. Physiol., 25: 615-625.

Takeuchi, A. 1959. Neuromuscular transmission of fish skeletal muscles investigated with intracellular microelectrode. J. Cell. Comp. Physiol., 54: 210-211.

Takeuchi, T. 1959. Histochemical demonstration of phosphorylase. J. Histochem. Cytochem., 4: 84.

Takeuchi, T. and H. Kuriaka. 1955. Histochemical detection of phosphorylase in animal tissues. J. Histochem. Cytochem., 3: 153-160.

Totton, A.K. 1914. The structure and development of the caudal skeleton of the teleostean fish Pleuragramma antarcticum. Proc. Zool. Soc. Lond., 251-262.

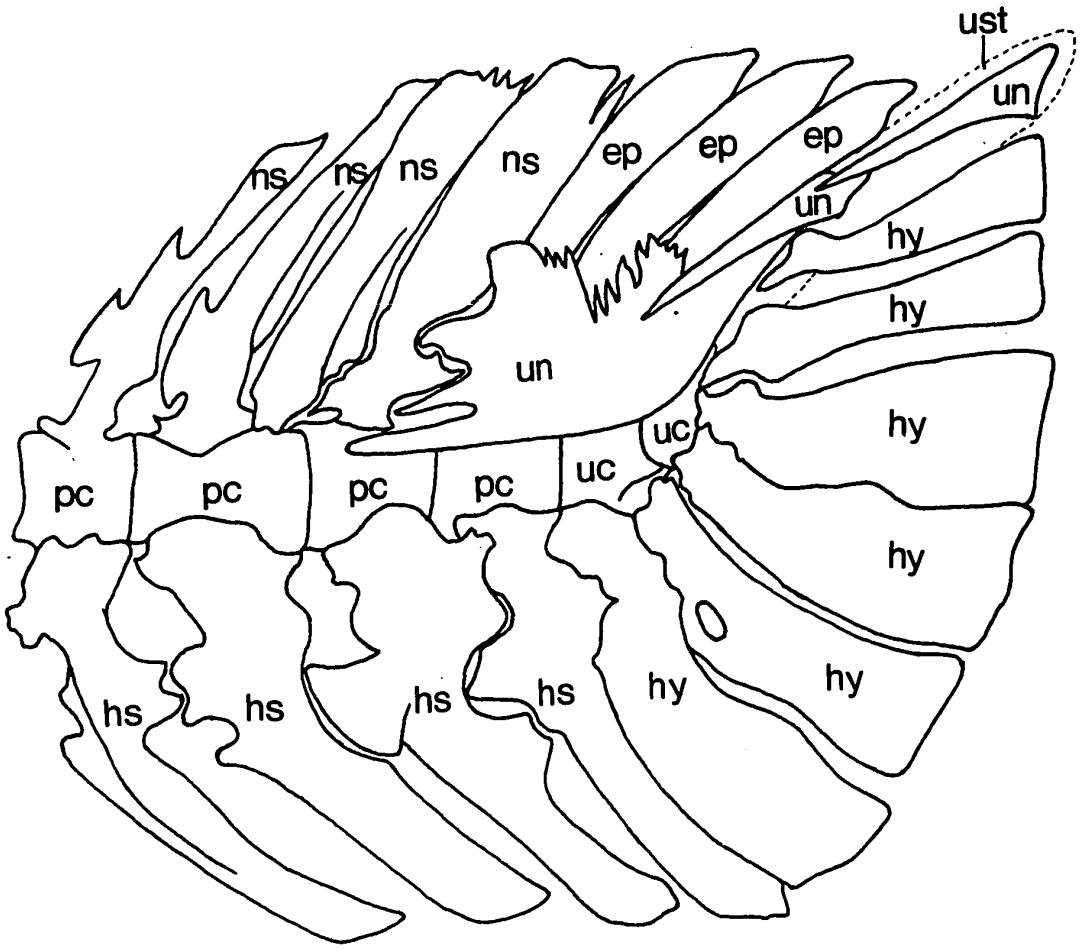
Van der Kloot, W.G. 1966. The exchange of radioactive cations by somatic and cardiac muscles of the crayfish. Comp. Biochem. Physiol., 17: 1019-1043.

_____ 1969. Calculated effects of SR thickness on sarcoplasmic calcium concentrations. Addendum to Mendelson, M. 1969. J. Cell Biol., 42: 562-563.

Wittenberger, C. and I.V. Diaciuc. 1965. Effort metabolism of lateral muscles in carp. J. Fish. Res. Bd. Canada, 22(1): 1397-1406.

Fig. 1 The caudal skeleton of S. gairdneri, about 6X.

ep., epural; hy., hypural; hs., haemal spine; ns., neural spine; pc., preural centrum; uc., ural centrum; un., uroneural; ust., urostyle.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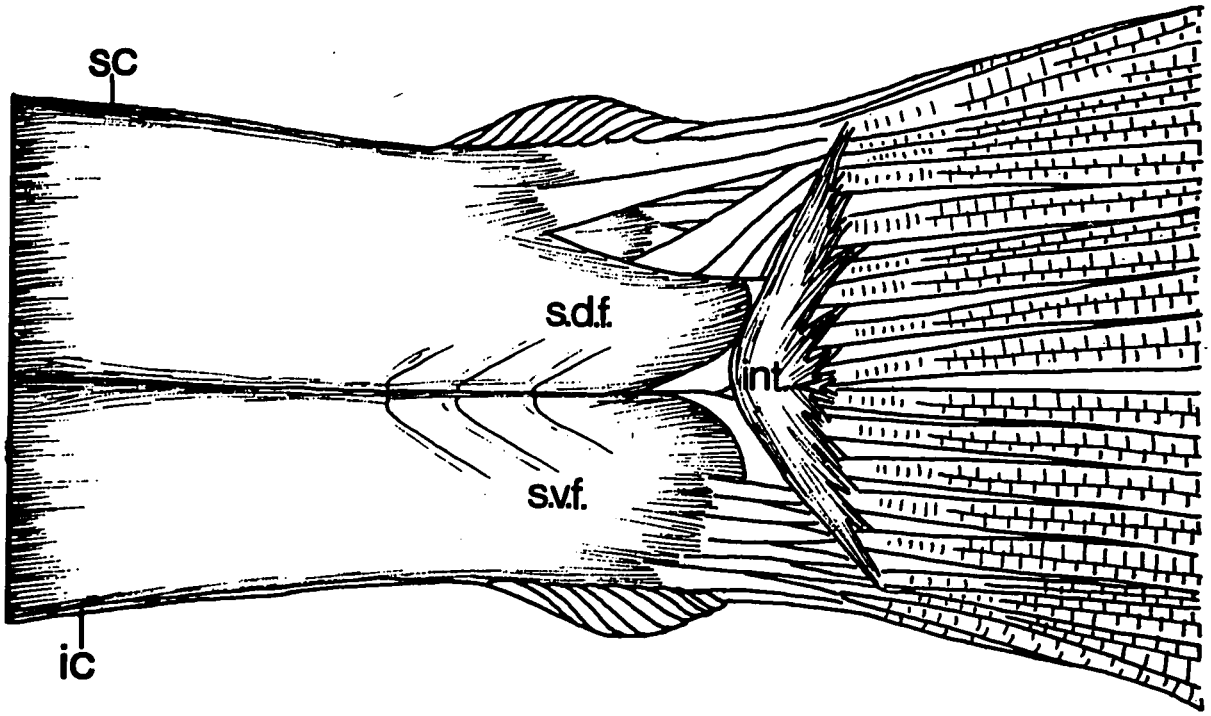
hy
hy

Fig. 2 (upper) The superficial layer of the caudal musculature of S. gairdneri, about 3X.

ic., infra-carinal muscle; int., interradial muscle; sc., supra-carinal muscle; sdf., superficial dorsal flexor muscle; svf., superficial ventral flexor muscle.

Fig. 3 (lower) The deep and carinal caudal musculature of S. gairdneri, about 3X.

db. 1., lateral bundle of deep ventral flexor muscle; d.d.f., deep dorsal flexor muscle; hc.1., hypochordal longitudinal muscle; vb. 2., medial bundle of deep ventral flexor muscle.


SC
s.d.f.
int.
s.v.f.
ic

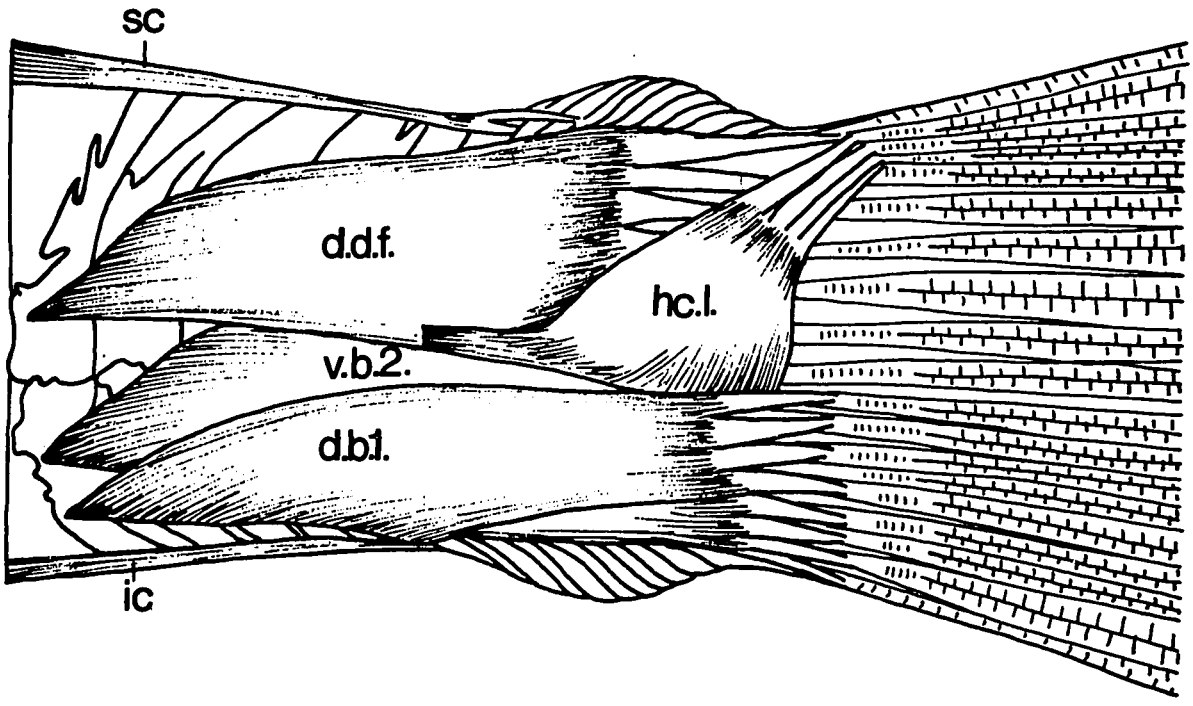

SC
d.d.f.
hc.l.
v.b.2.
d.b.l.
ic

Fig. 4 (upper) The attachments of hypochordal longitudinal muscle, about 4X.

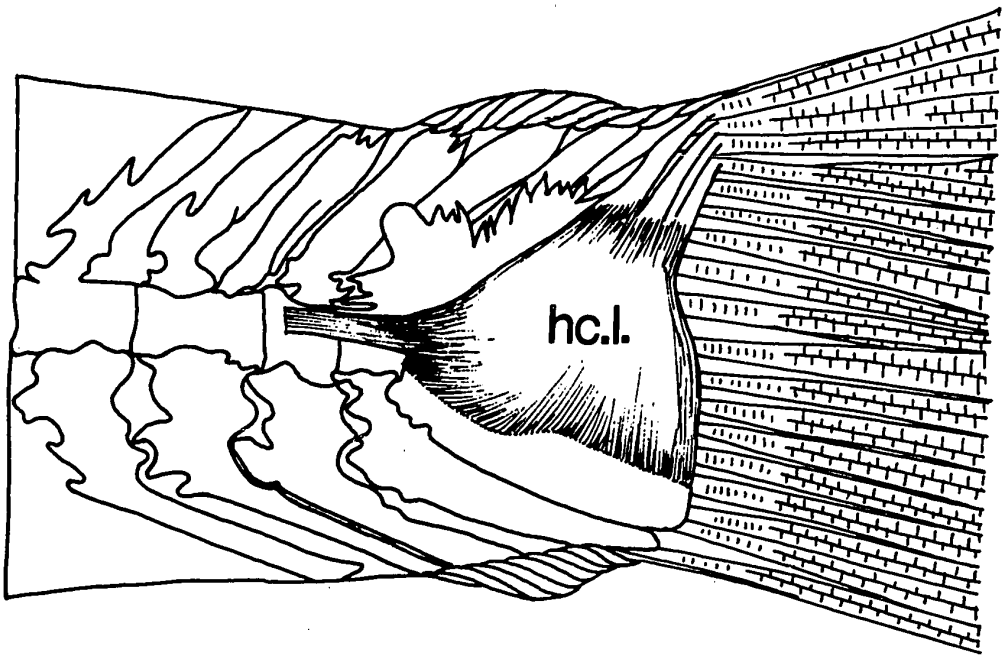
hc.l., hypochordal longitudinal muscle.

Fig. 5 (lower) Sections across the body at different levels to show relative proportion of red and white muscle fibers of S. gairdneri.

black = red muscle fibers

unshaded = white muscle fibers

stippled = vertebral column


hc.l.

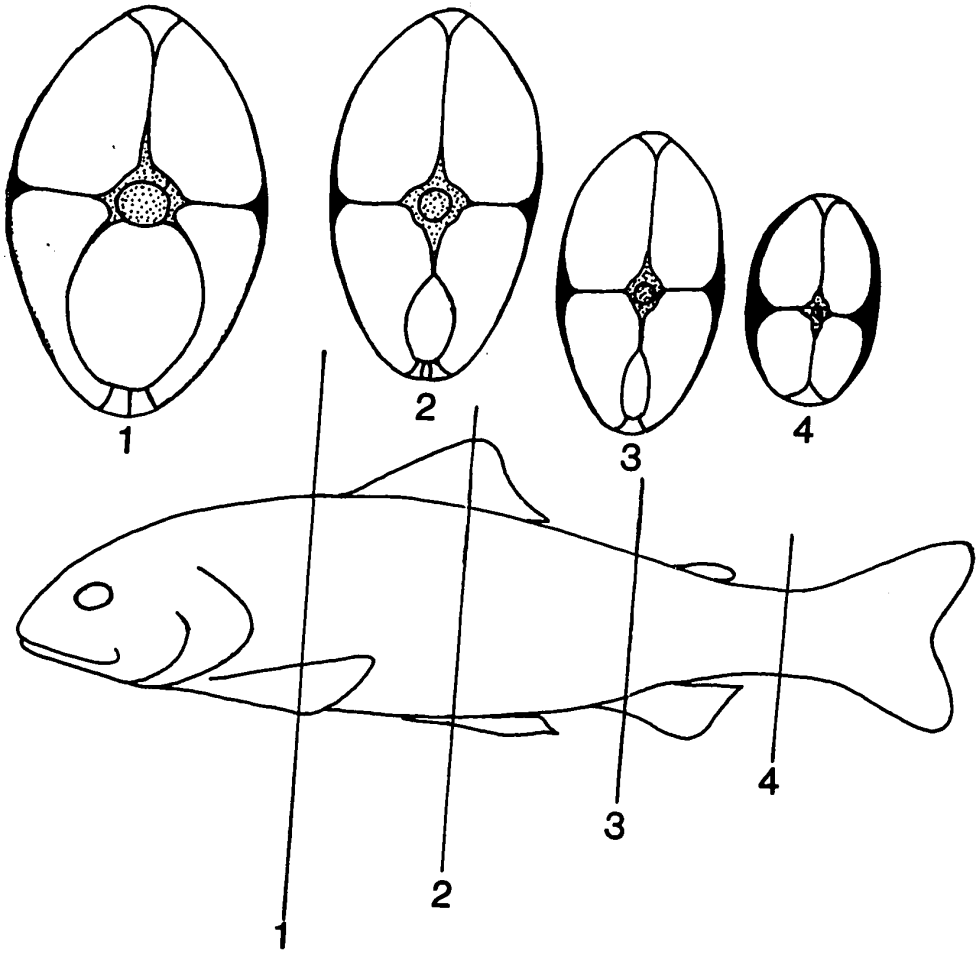
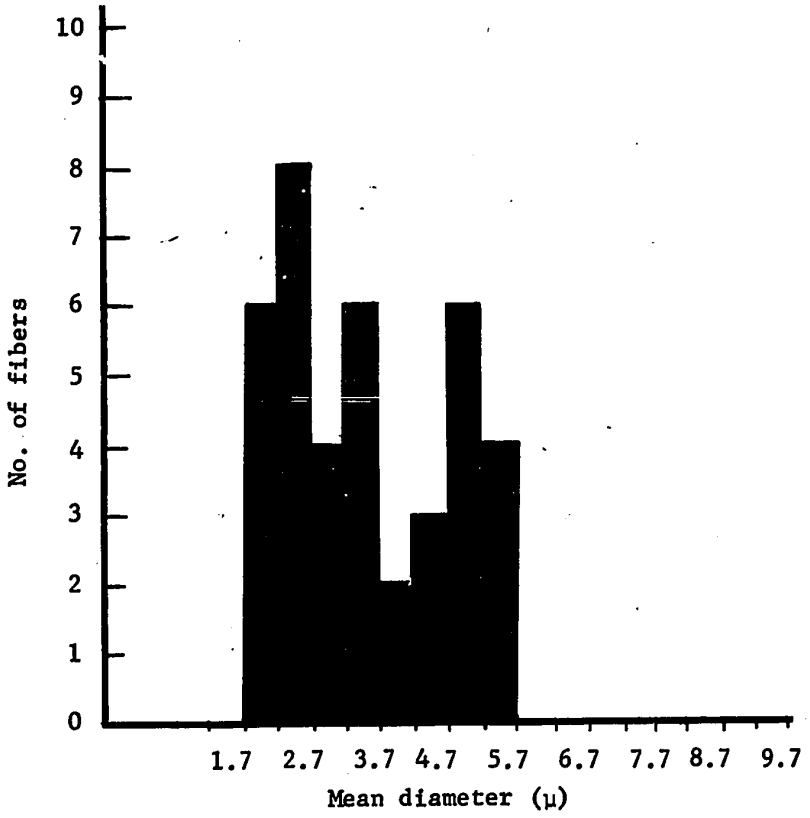
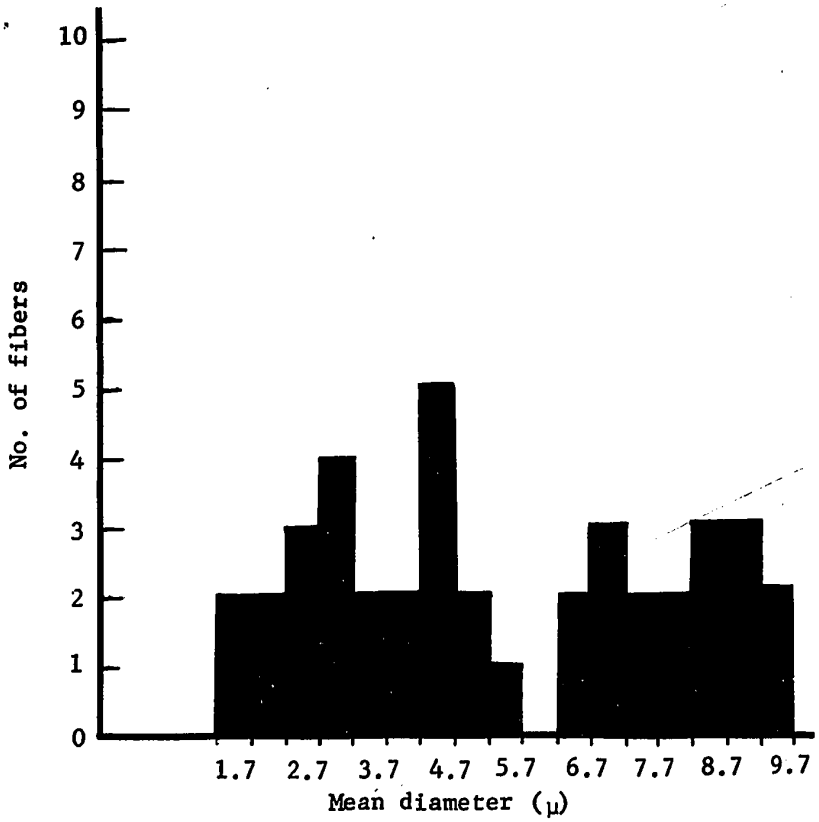
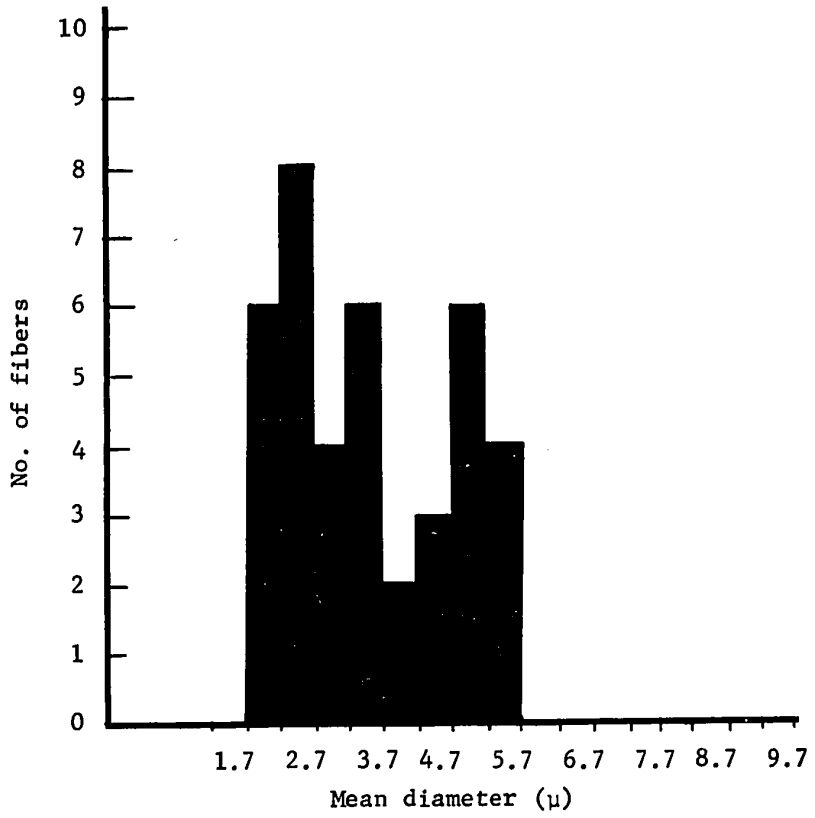

1
2
3
4
1
2
3
4

Fig. 6 Distribution of diameter of red muscle fibers of the superficial flexor muscles.

Fig. 7 Distribution of diameter of white muscle fibers of the superficial flexor muscles.


No. of fibers
0
1
2
3
4
5
6
7
8
9
10
1.7
2.7
3.7
4.7
5.7
6.7
7.7
8.7
9.7
Mean diameter (μ)


No. of fibers
0
1
2
3
4
5
6
7
8
9
10
1.7
2.7
3.7
4.7
5.7
6.7
7.7
8.7
9.7
Mean diameter (μ)


No. of fibers
10
9
8
7
6
5
4
3
2
1
0
1.7 2.7 3.7 4.7 5.7 6.7 7.7 8.7 9.7
Mean diameter (μ)

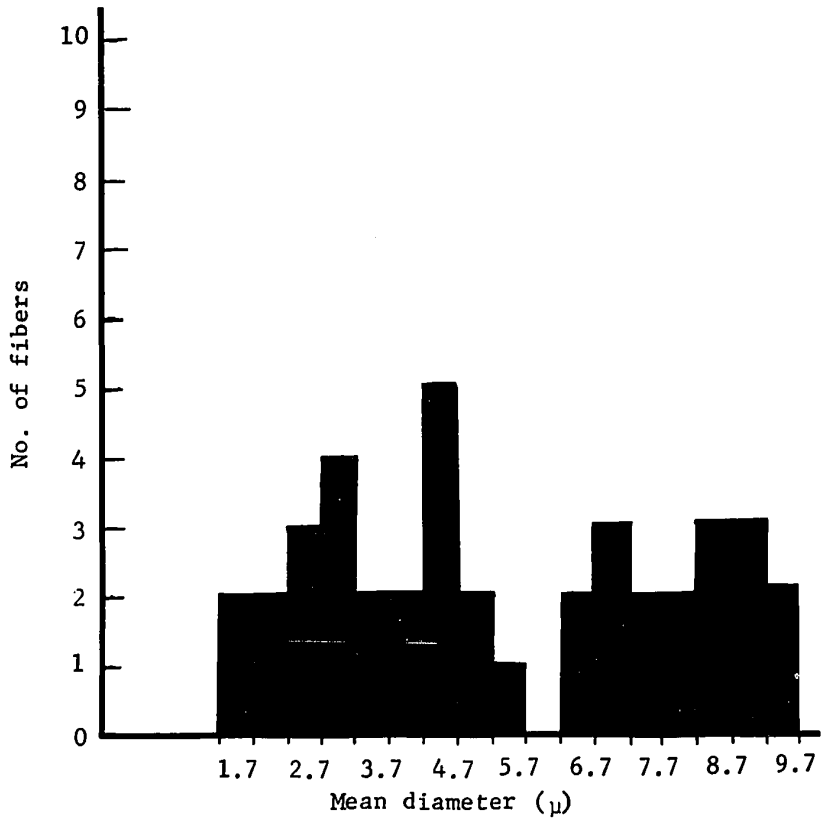
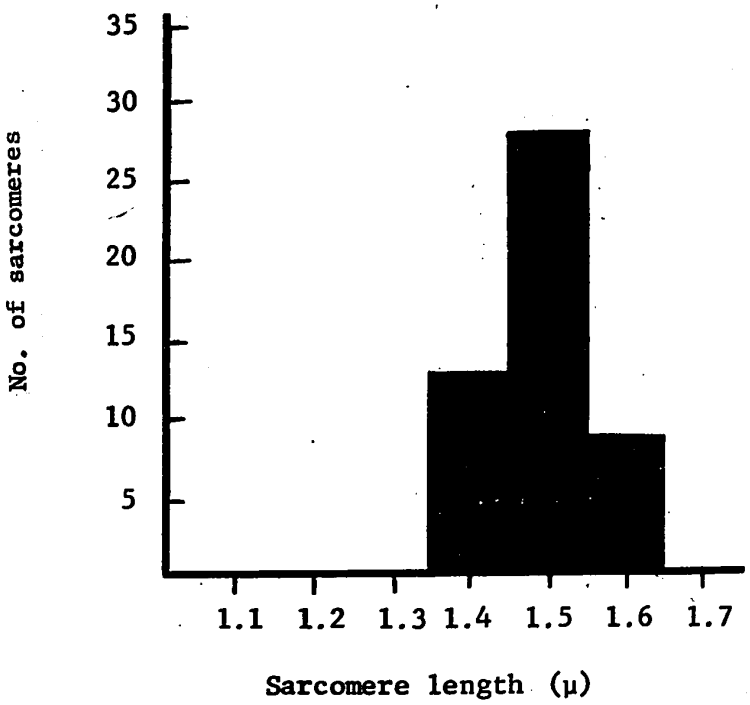
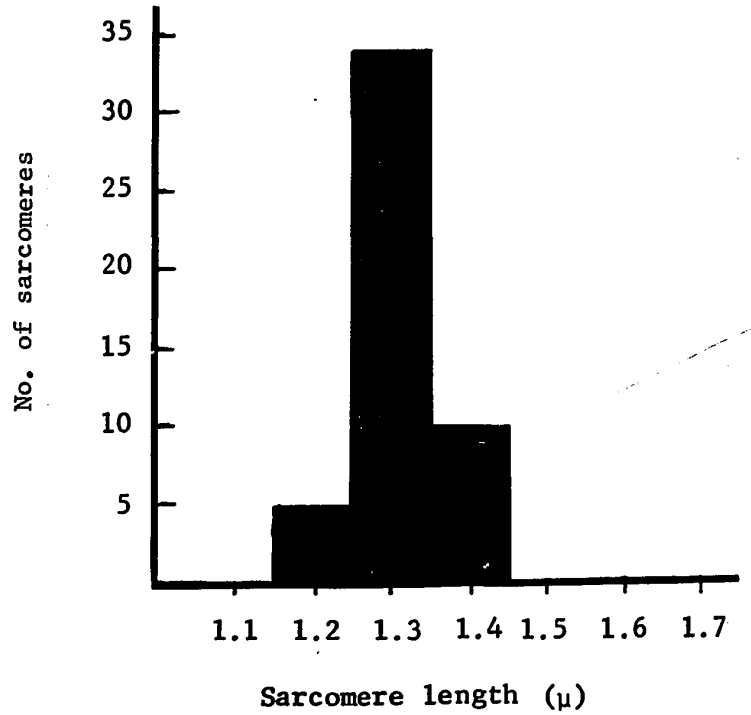
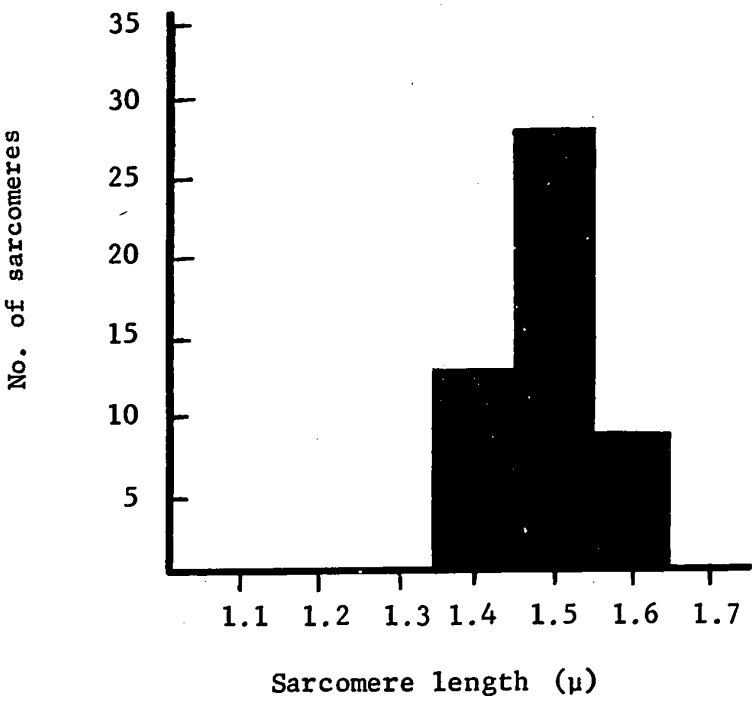

No. of fibers
10
9
8
7
6
5
4
3
2
1
0
1.7 2.7 3.7 4.7 5.7 6.7 7.7 8.7 9.7
Mean diameter (μ)

Fig. 8 Distribution of sarcomere lengths of red muscle fibers of the superficial dorsal flexor muscle.

Fig. 9 Distribution of sarcomere lengths of white muscle fibers of the superficial dorsal flexor muscle.


No. of sarcomeres
35
30
25
20
15
10
5
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Sarcomere length (μ)


No. of sarcomeres
35
30
25
20
15
10
5
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Sarcomere length (μ)


35
30
25
20
15
10
5
No. of sarcomeres
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Sarcomere length (μ)

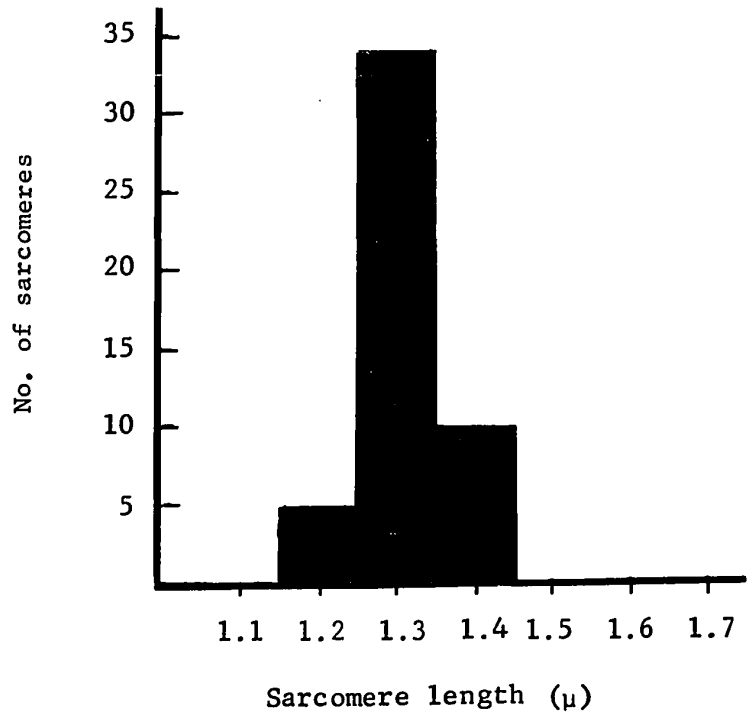

35
30
25
20
15
10
5
No. of sarcomeres
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Sarcomere length (μ)

Fig. 10 Red muscle fiber of superficial dorsal flexor muscle showing abundance of mitochondria. Note the rich lipid content.

L., lipid droplet; M., mitochondrion.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.


L
M
G

Fig. 11 White muscle fiber of superficial dorsal flexor muscle showing relative scarcity of mitochondria. Note the absence of lipid droplets and presence of glycogen.

G., glycogen particles; M., mitochondria.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 18,200X.

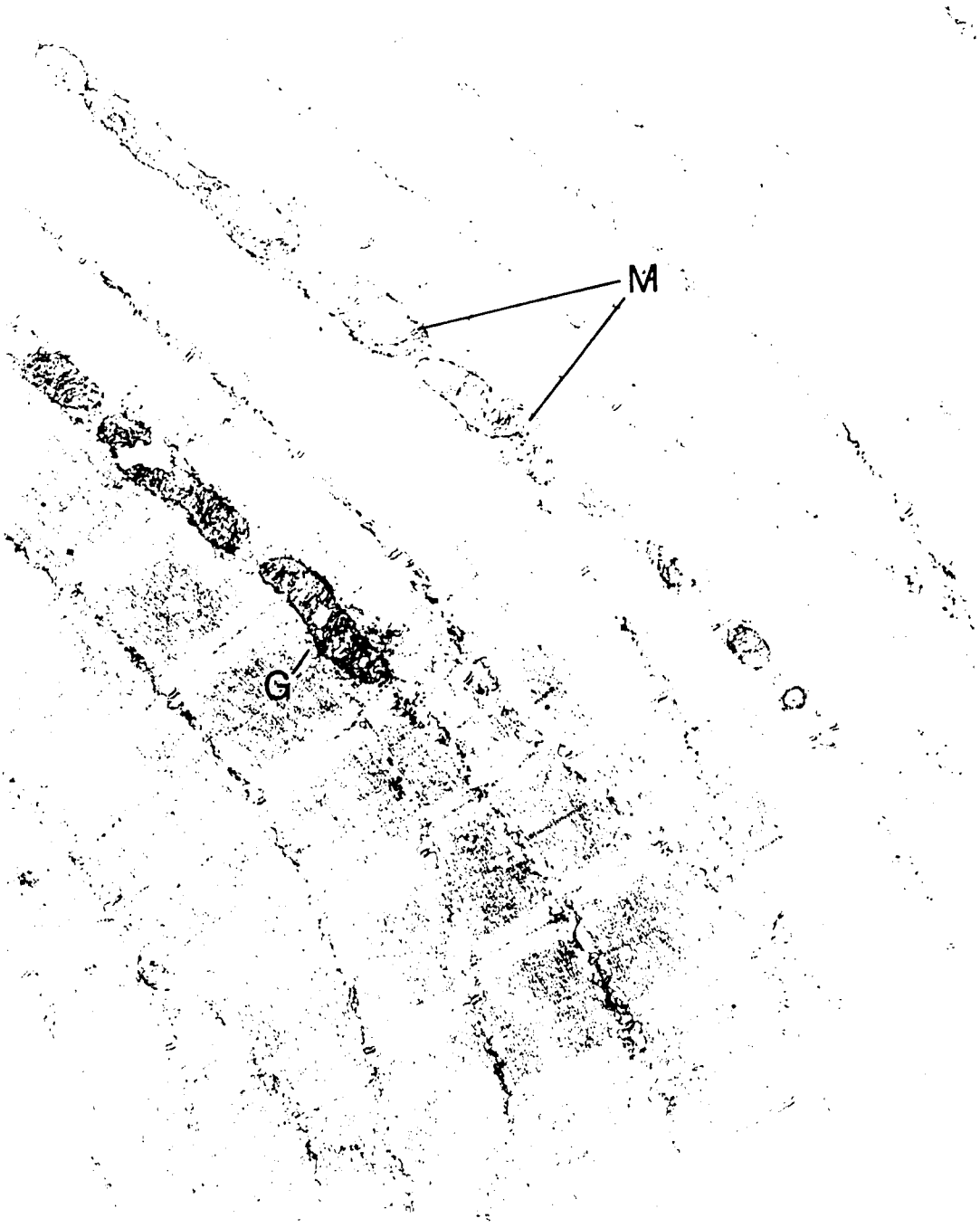

M
G

Fig. 12 Red muscle fiber of superficial ventral flexor muscle showing abundance of mitochondria. Note the rich lipid content.

G., glycogen particles; L., lipid droplet; M., mitochondrion.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.


G
L
M

Fig. 13 White muscle fiber of superficial ventral flexor muscle showing relative scarcity of mitochondria. Note the absence of lipid droplets and presence of glycogen.

G., glycogen particles; M., mitochondrion.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 18,200X.

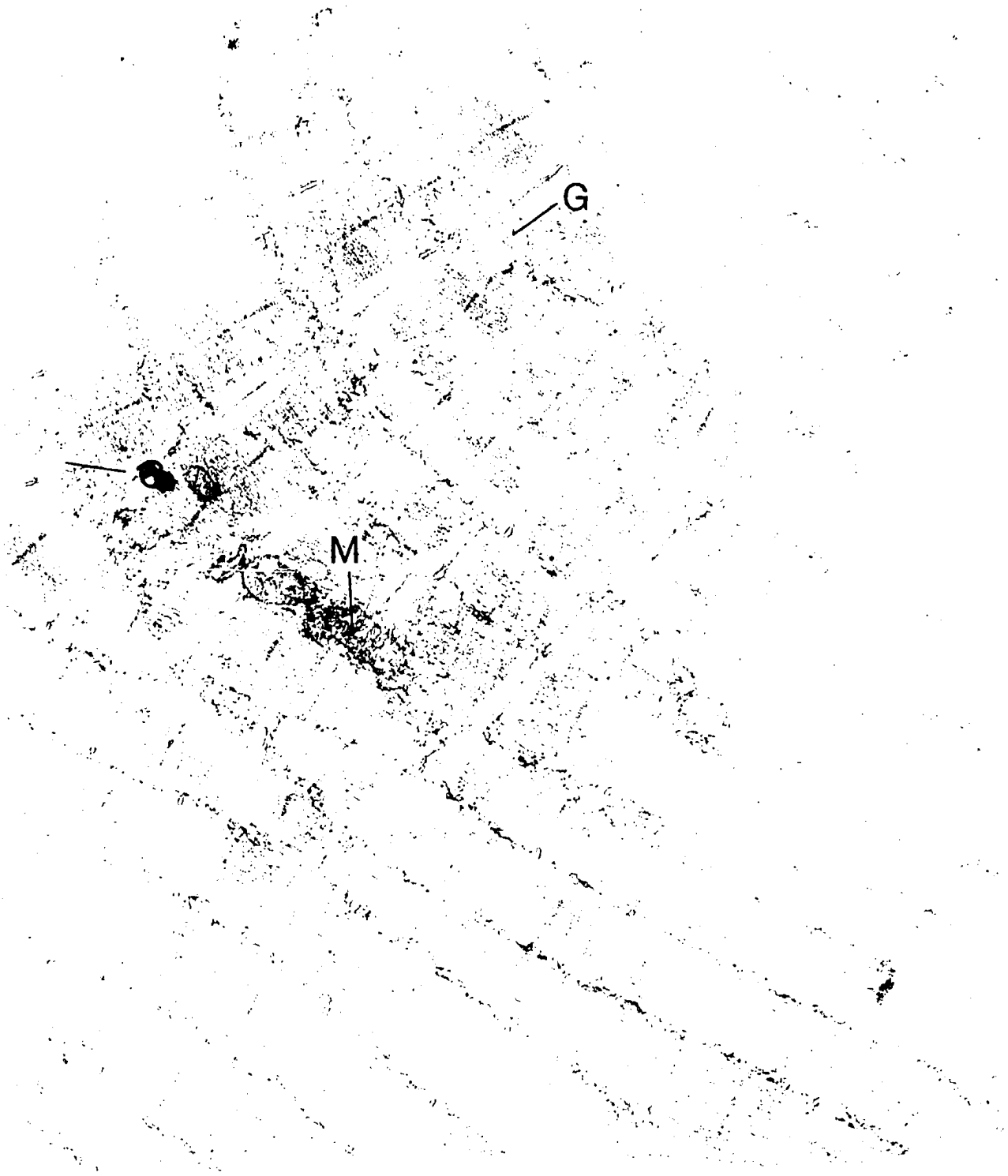

G
M

Fig. 14 Red muscle fiber of deep dorsal flexor muscle showing abundance of mitochondria.

G., glycogen particles; L., lipid droplet; M., mitochondrion.

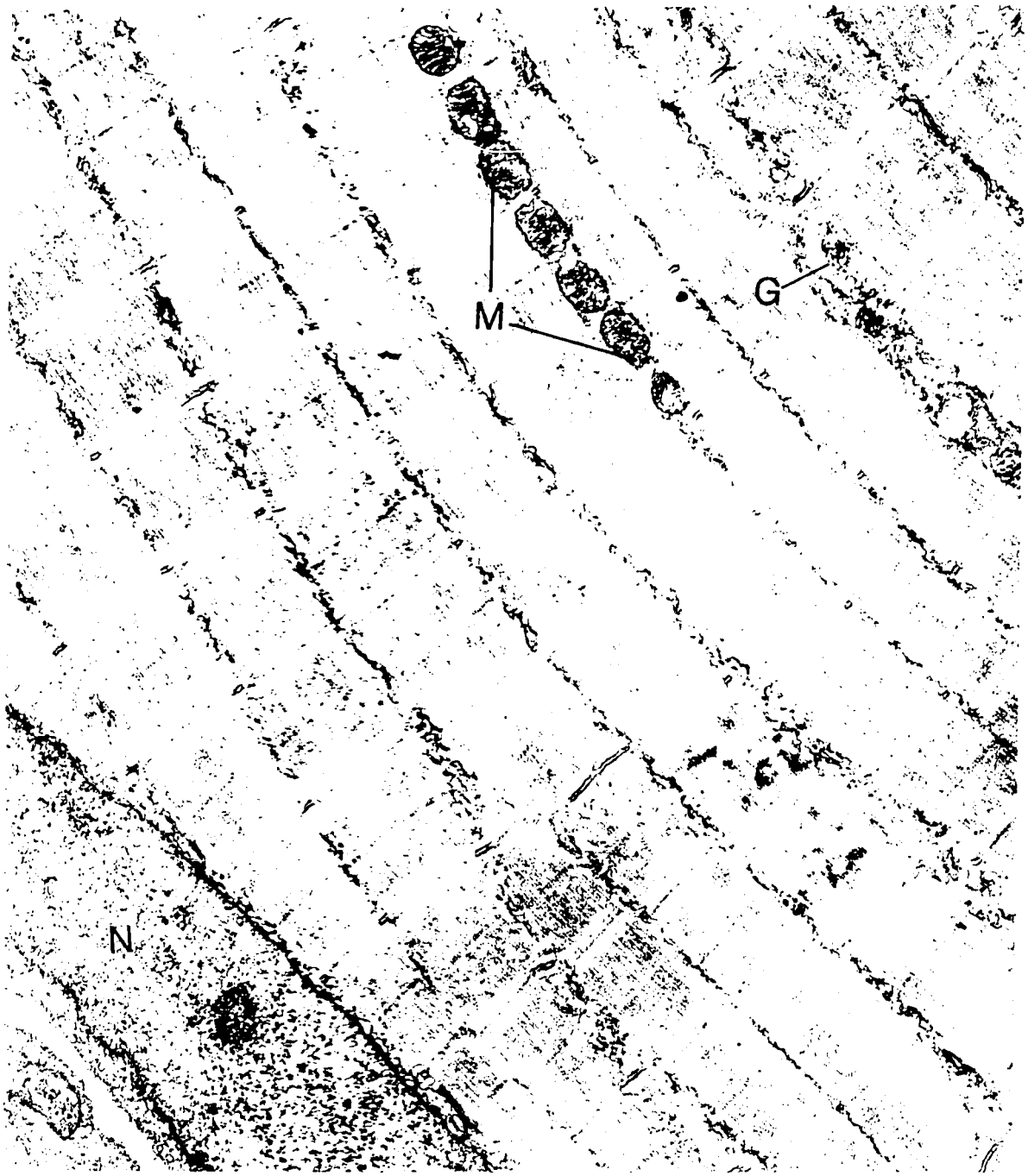
Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.


G
M

Fig. 15 White muscle fiber of deep dorsal flexor muscle showing relative scarcity of mitochondria. Note the absence of lipid droplets and presence of glycogen. A nucleus is situated peripherally at the extreme left corner.

G., glycogen particles; M., mitochondria; N., nucleus.

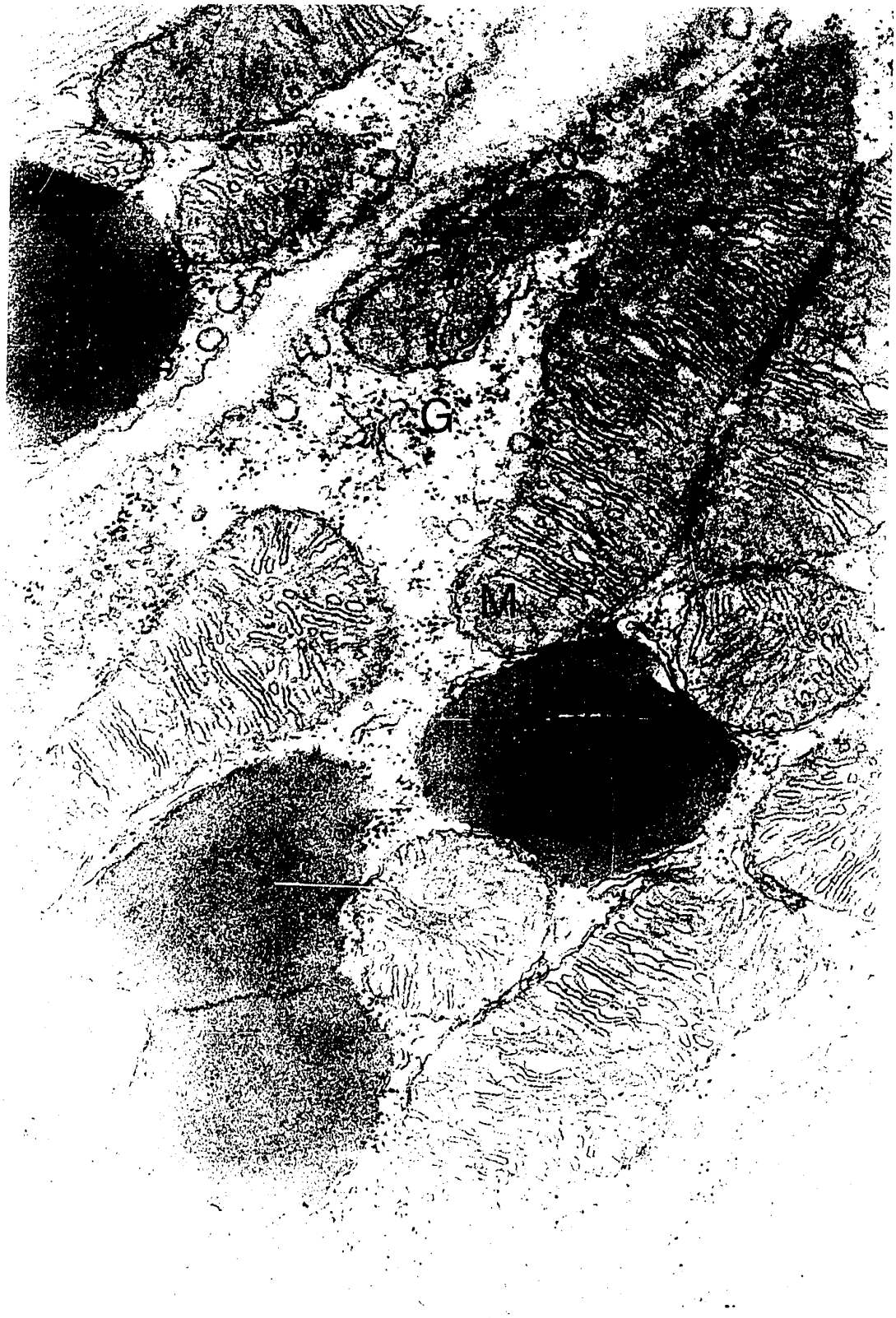
Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 18,200X.


M
G
N

Figs. 16-19 The mitochondria of red muscle fibers (superficial dorsal and ventral flexor muscles) showing particularly large numbers of cristae. Note most of the lipid droplets are in close association with mitochondria.

G., glycogen particles; L., lipid droplet; M., mitochondrion.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 68,800X.


G
M

M


G

Fig. 20 The mitochondria of the white muscle fibers (superficial dorsal flexor muscle) showing particularly scanty cristae. Note smaller size of mitochondria and absence of lipid droplets. The small arrows show pinocytosis vesicles on the sarcolemma of two white muscle fibers. Some of the vesicles are budded off the sarcolemma. Loose collagen fibrils are lying outside the sarcolemma. The big arrows show interruptions of terminal cisternae.

C., collagen fibrils; G., glycogen particles; M., mitochondrion; S., sarcolemma.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate; and Lead citrate. 68,800X.


C
S
M
C'

Fig. 21 (upper left) Succinate dehydrogenase activity in red muscle fibers (superficial dorsal flexor muscle).

Fig. 22 (upper right) Succinate dehydrogenase activity in white muscle fibers (superficial dorsal flexor muscle).

Fig. 23 (lower left) NADH diaphorase activity in red muscle fibers (superficial ventral flexor muscle).

Fig. 24 (lower right) NADH diaphorase activity in white muscle fibers (superficial ventral flexor muscle).

Frozen sections, Pearse's medium for Succinate dehydrogenase. Nachlas, Walker and Seligman's medium for NADH diaphorase. 3,950X.

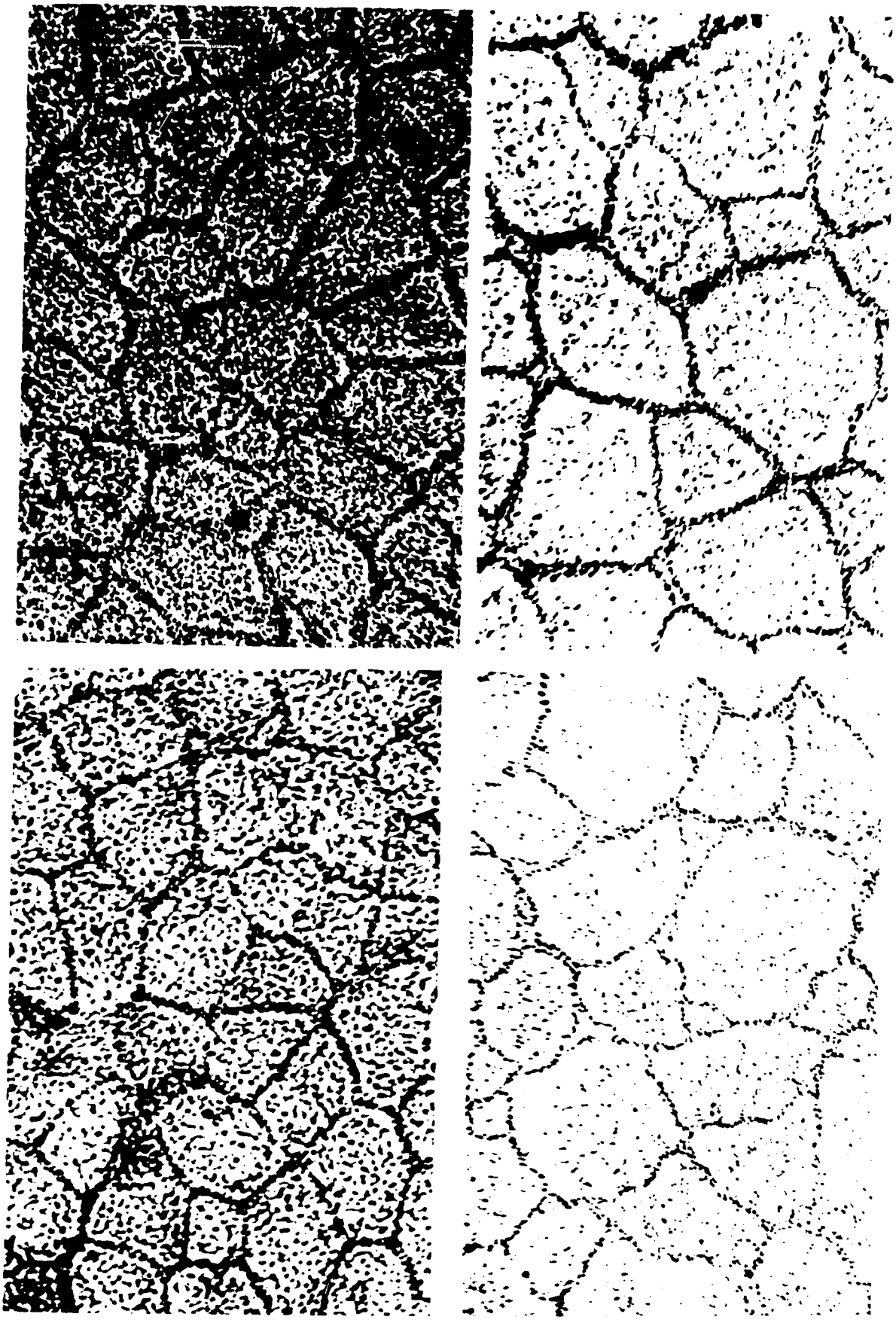


Fig. 25 (upper right) Lactate dehydrogenase activity in red muscle fibers (deep dorsal flexor muscle).

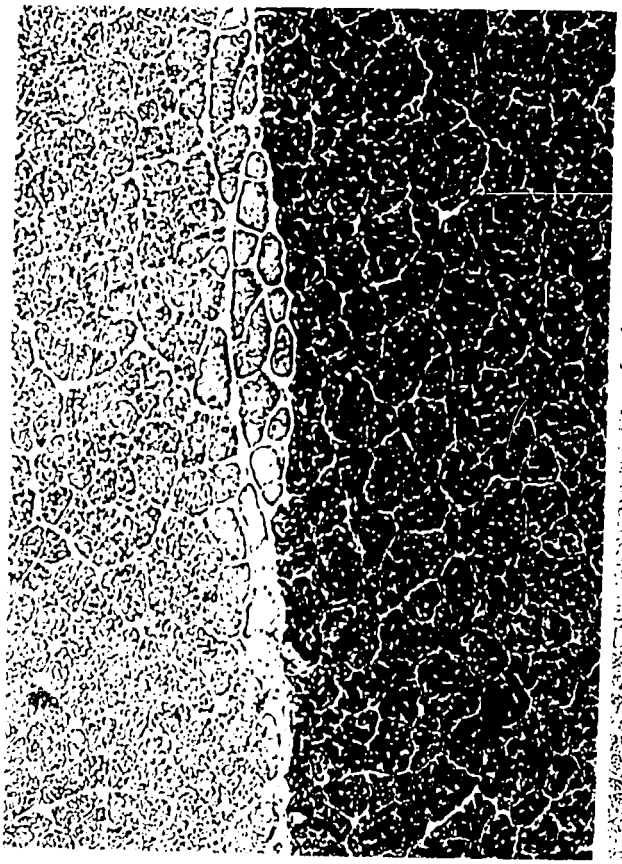
Fig. 26 (upper left) Lactate dehydrogenase activity in white muscle fibers (deep dorsal flexor muscle).

Frozen sections; Hess, Scarpelli and Pearse's medium for Lactate dehydrogenase. 4,000X.

Fig. 27 (lower right) Phosphorylase activity in red (left side) and white (right side) muscle fibers (deep ventral flexor muscle).

Fig. 28 (lower left) Phosphorylase activity in red (left side) and white (right side) muscle fibers (hypochordal longitudinal muscle).

Frozen sections; Takeuchi and Kuriaka's medium for Phosphorylase. 1,400X.



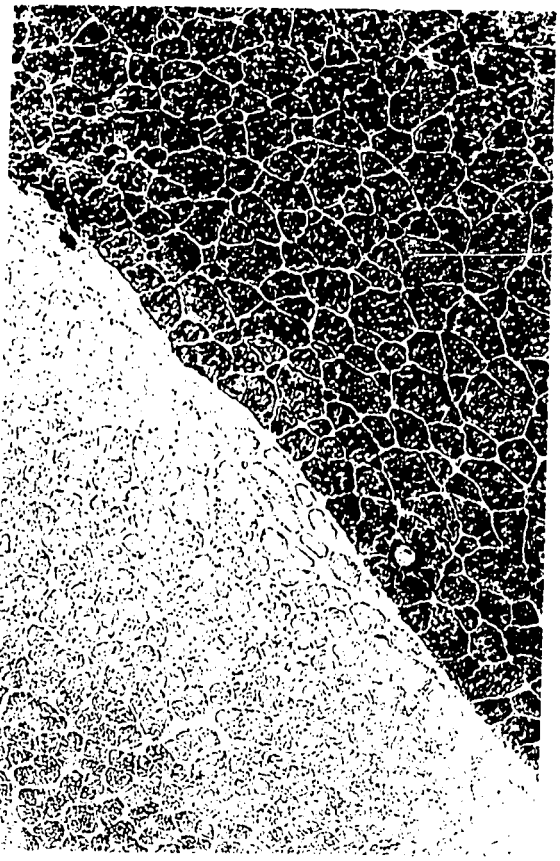


Fig. 29 The rich lipid content of red muscle fiber (hypochordal longitudinal muscle). Note the close association of lipid droplets with mitochondria.

L., lipid droplet; M., mitochondria.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 36,000X.


L
L

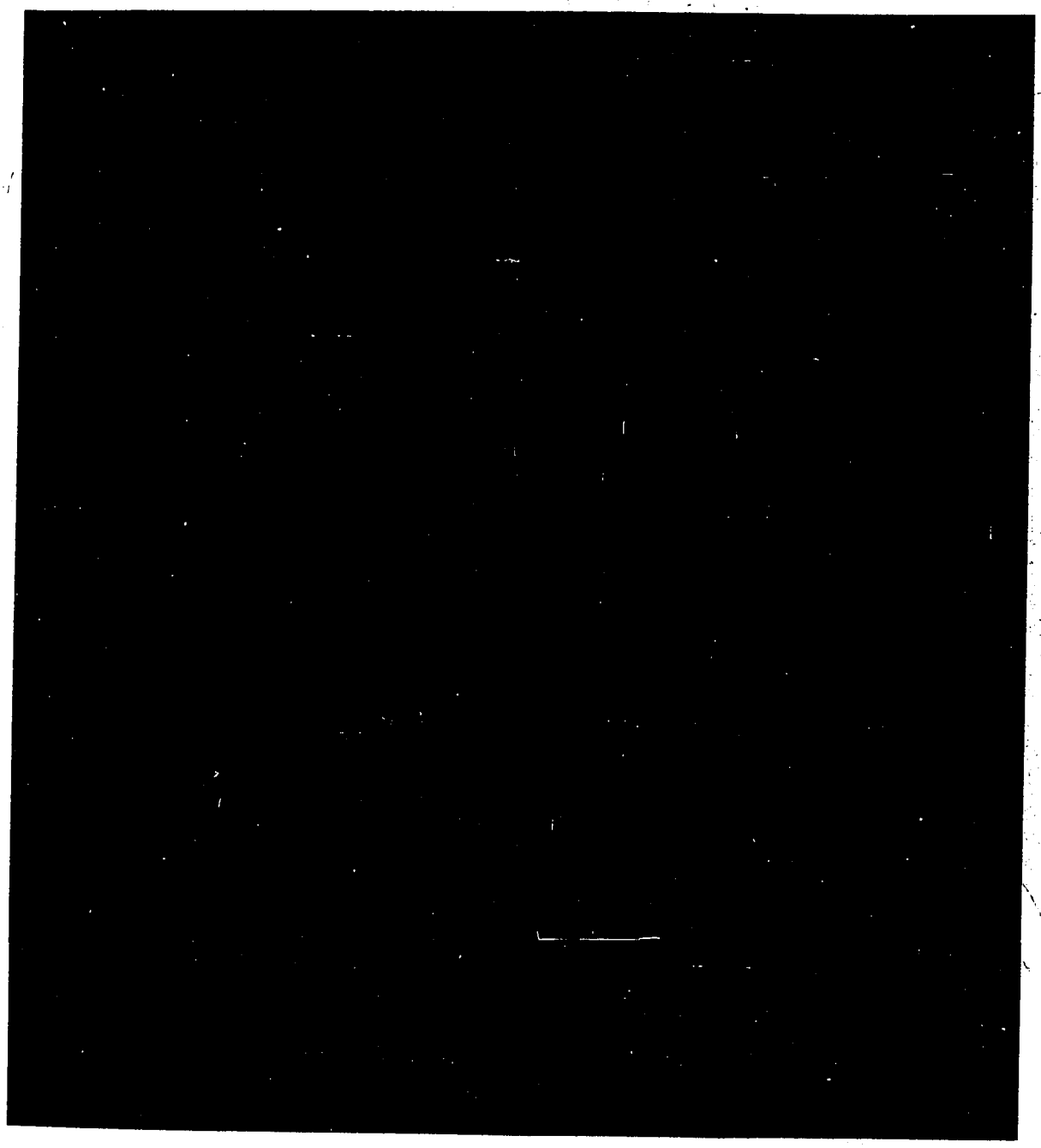


Fig. 30 Glycogen content of white muscle fiber (superficial ventral flexor muscle) before exercise. Note the scanty cristae of mitochondria.

G., glycogen particles; M., mitochondria.

Potassium permanganate in distilled water, Araldite, Uranyl acetate, and Lead citrate. 51,600X.

Fig. 31 Glycogen content of white muscle fiber (superficial ventral muscle) after a strenuous exercise of 15 minutes.

G., glycogen particles.

Potassium permanganate in distilled water, Araldite, Uranyl acetate, and Lead citrate. 51,600X.

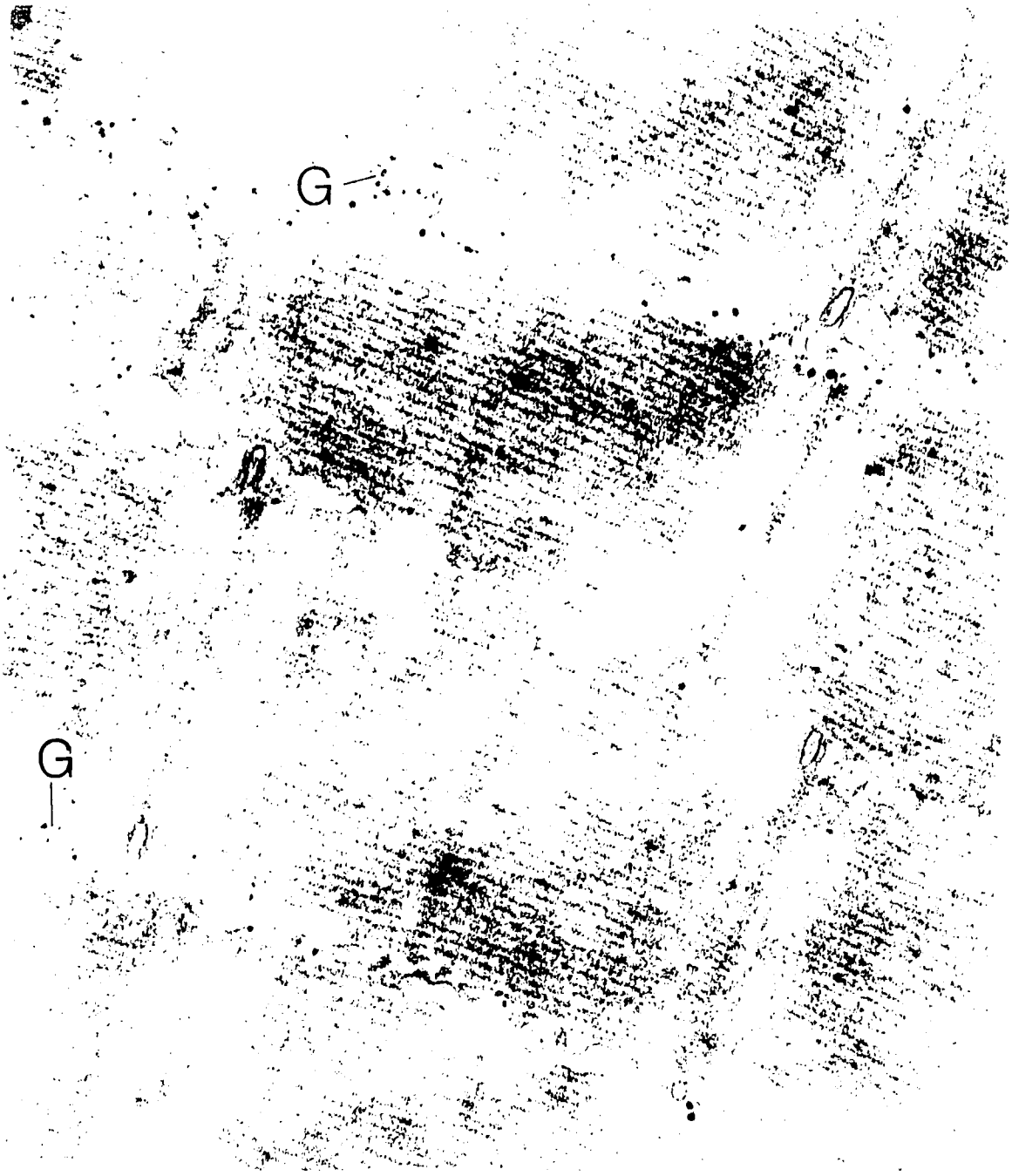
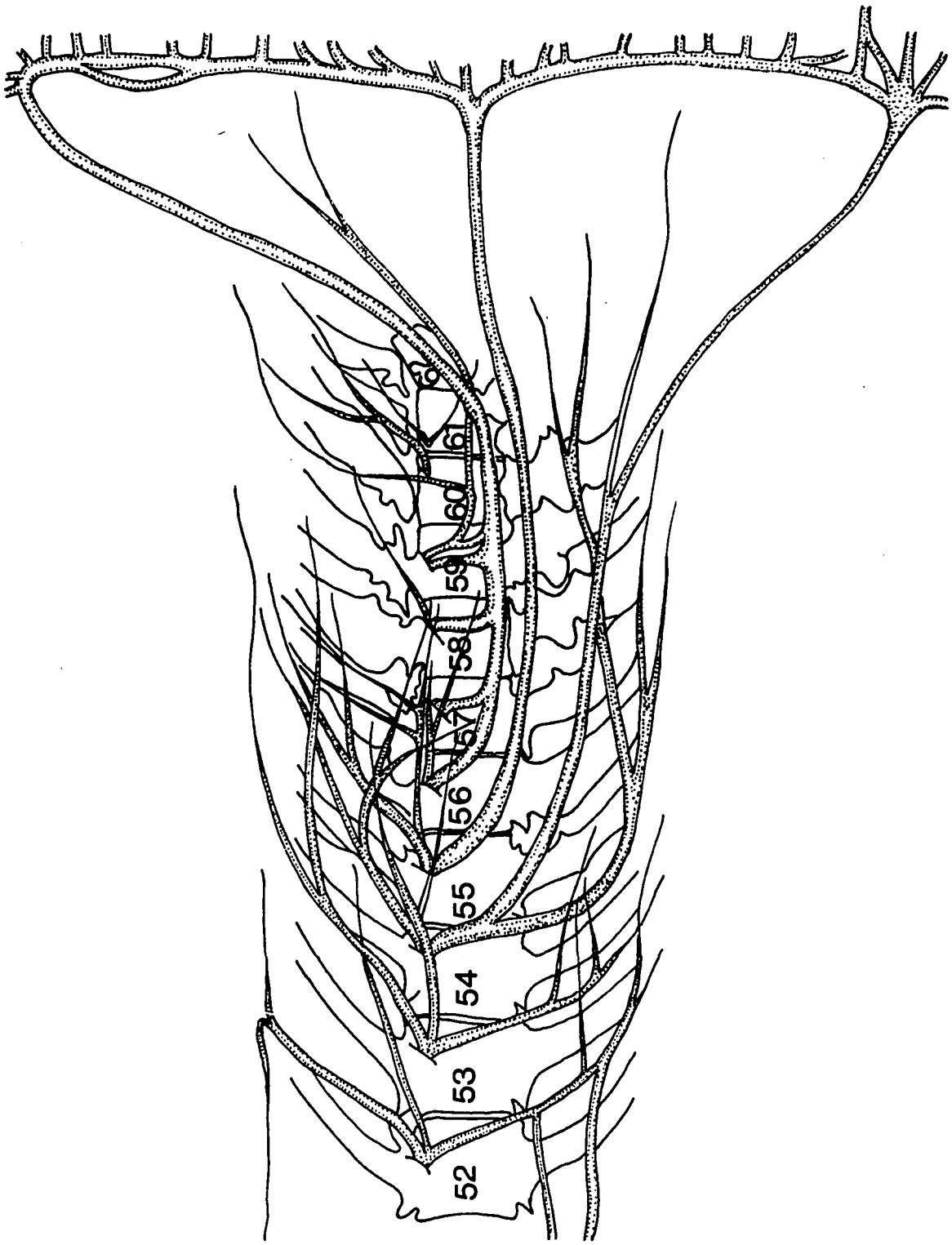

G
G

Fig. 32 The caudal innervation of S. gairdneri, about 8X.


62
61
60
59
58
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56
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54
53
52

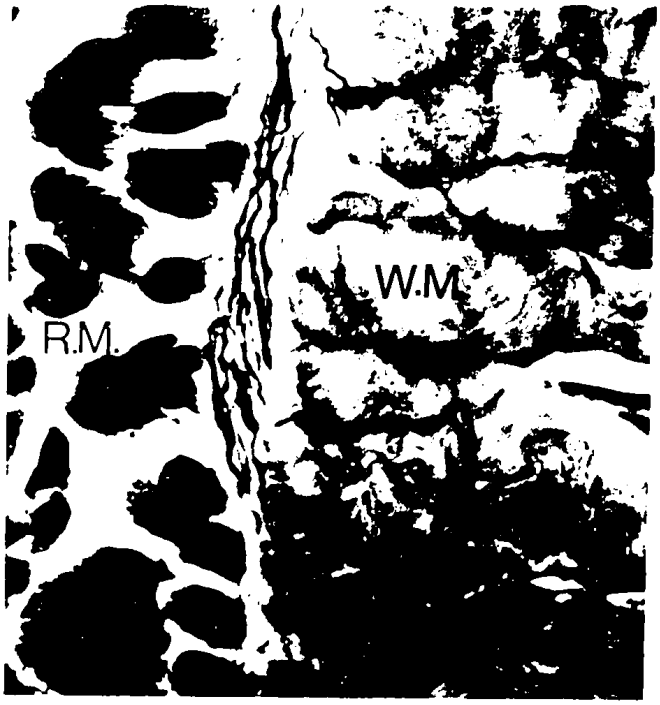
Fig. 33 (upper left) A bundle of nerve fibers running along a myoseptum, supplying branches to adjacent red and white muscle fibers.

Fig. 34 (upper right) A bundle of nerve fibers running along a myoseptum between red and white muscle fibers (superficial ventral flexor muscle). Side branches are shown by the red muscle fibers.

Fig. 35 (lower left)
Fig. 36 (lower right) The courses of branches of nerve bundles of one side of red muscle fibers (superficial flexor muscles) are shown. The same type of branches are present on the other side of the fibers. A muscle fiber is seen to be innervated by nerve fibers on either side which can be better understood in Fig. 44.

MS., myoseptum; R.M., red muscle fibers; W.M., white muscle fibers.

Frozen sections, Palmgren silver impregnation technique. 3,500X.


R.M.
W.M.


R.M.
MS.

Fig. 37 (upper left) The courses of side branches of nerve bundles are shown at one end of red muscle fibers (deep dorsal flexor muscle). The arrows show club-shaped structures which seem to be nerve endings.

Fig. 38 (upper right)
Fig. 39 (lower left) The courses of side branches of nerve bundles are shown in white muscle fibers (superficial flexor muscles). The arrow in lower left figure shows club-shaped structures which seem to be a nerve ending.

Fig. 40 (lower right) White muscle fibers (deep dorsal flexor muscle). An arrow shows a club-shaped structure which seems to be a nerve ending.

Frozen section, Palmgren silver impregnation technique. 3,500X.

Fig. 41 (upper) Red muscle fibers (superficial dorsal flexor muscle). Club-shaped structures shown by two arrows seem to be motor endings. 3,500X.

Fig. 42 (middle) White muscle fibers (superficial ventral flexor muscle). Club-shaped structure shown by an arrow seems to be a motor ending. 4,000X.

Fig. 43 (bottom) White muscle fibers (deep dorsal flexor muscle). Club-shaped structures seem to be motor endings. 3,500X.

Frozen sections, Holmes' silver impregnation technique.

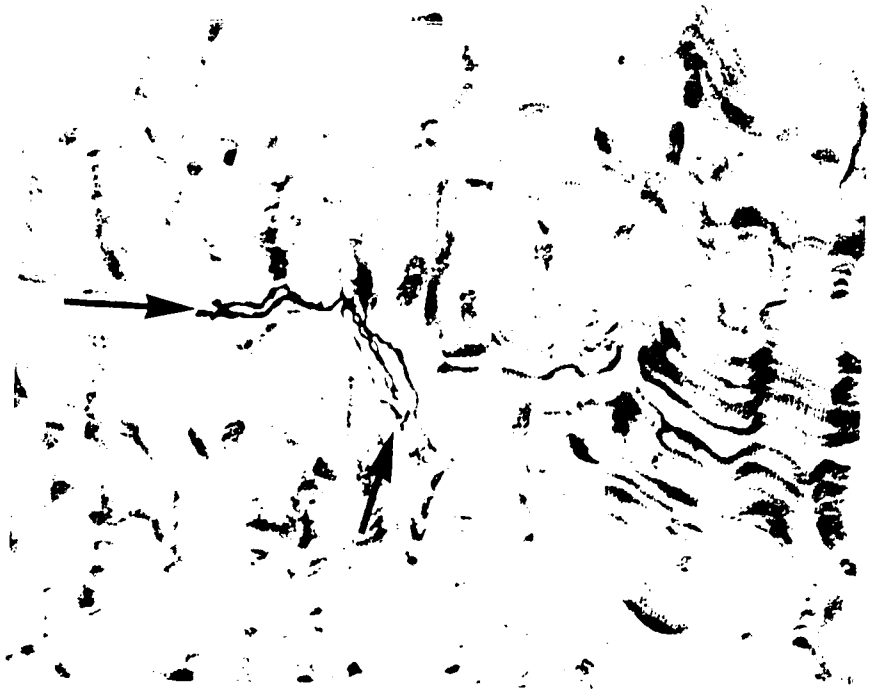


Fig. 44 Schematic diagram of innervation of the red and white muscle fibers.

ms., myoseptum; rm., red muscle fibers; s., skin; wm., white muscle fibers.

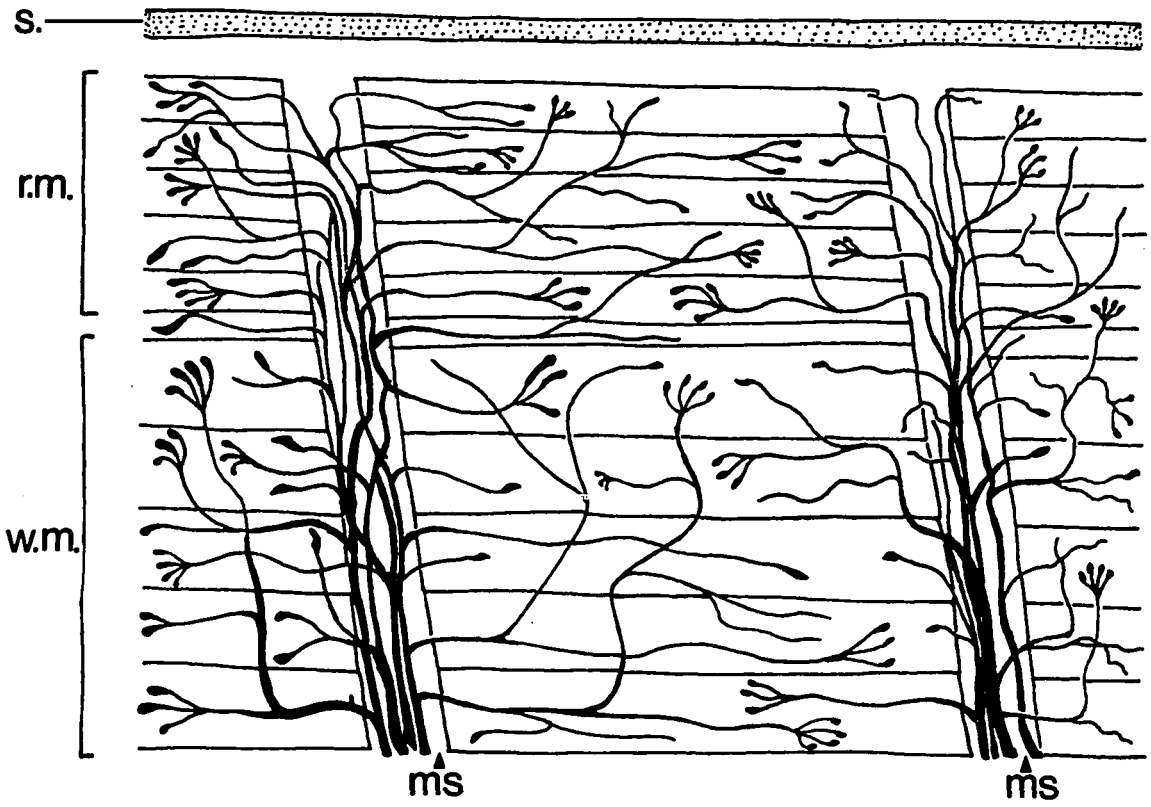

s.
r.m.
w.m.
ms
ms

Fig. 45 Innervation of the caudal fin. The arrows show cut ends of bundles of nerve fibers that run to the connective tissue between fin rays (f.r.) and remain without any association with chromatophores. The remaining nerve fibers in the diagram are associated with chromatophores. About 10X.

f.r., position of fin ray.

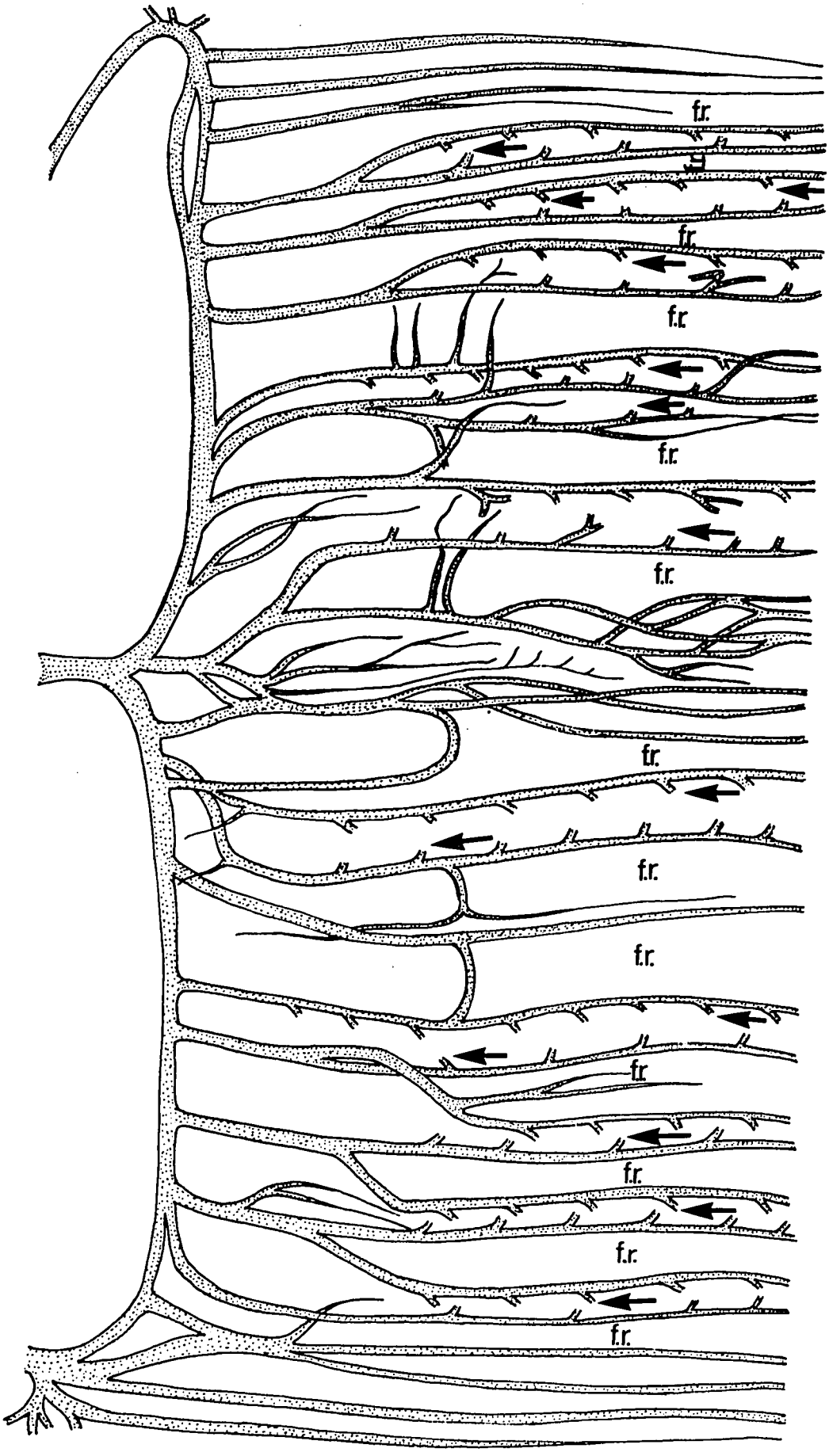

f.r.
f.r.
f.r.
f.r.
f.r.
f.r.
f.r.
f.r.
f.r.
f.r.
f.r.
f.r.
f.r.

Fig. 46 (upper left) Innervation of the caudal fin. The nerve fibers are running in the connective tissue between fin rays. 20X.

Fig. 47 (upper right)
Fig. 48 (lower left)
Fig. 49 (lower right) The nerve fibers are running in the connective tissue between different fin rays. Side branches of the nerves are shown by arrows. These side branches seem to be sensory fibers. 50X.

Whole-mount preparations with Sihler's chloral hydrate - Ehrlich's acid hematoxylene technique for peripheral nerve fibers.

Fig. 50 (upper). Nerve fibers with characteristic endings
Fig. 51 (lower). found in the connective tissue between fin rays. They seem to be sensory fibers.

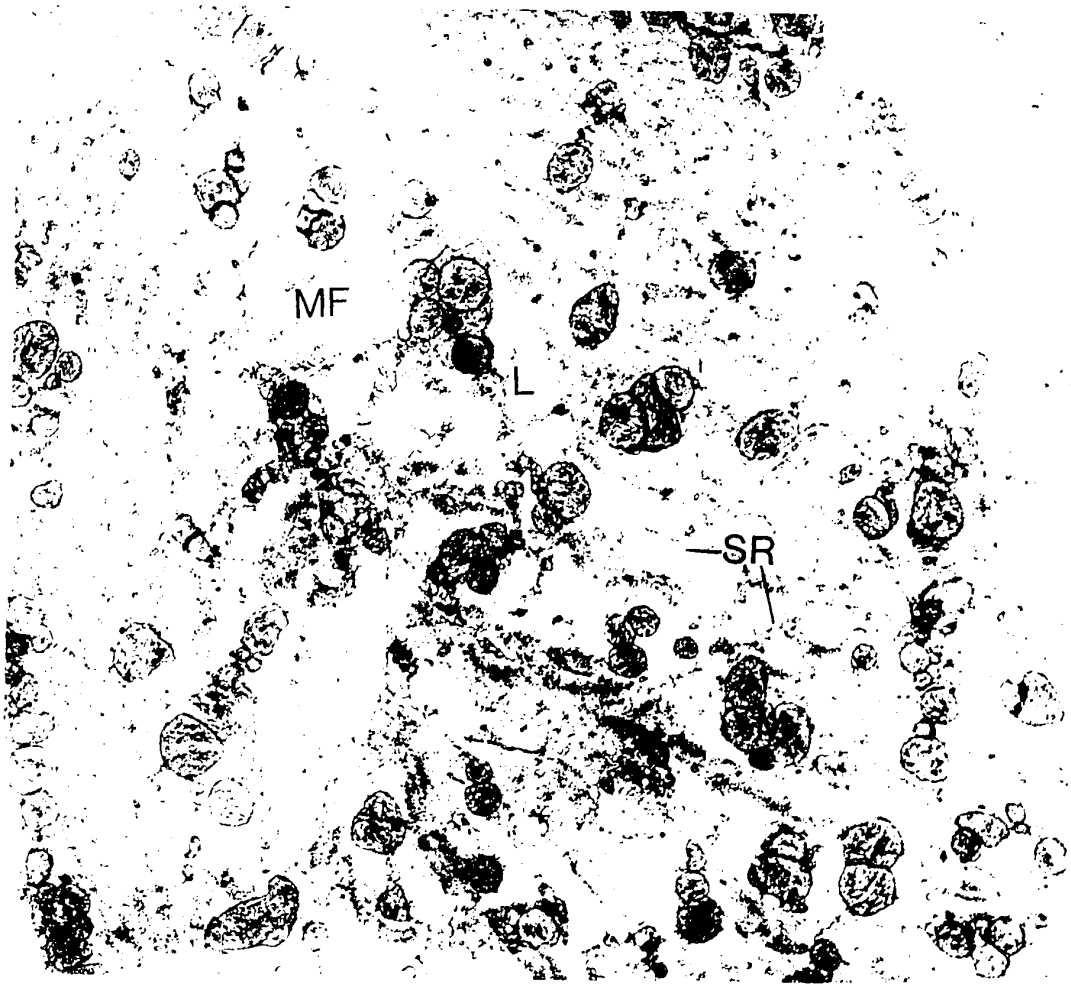
Whole-mount preparations with Holmes' silver impregnation technique. 500X.

Fig. 52 (upper) Low power electronmicrograph of a transverse section of a red muscle fiber (superficial dorsal flexor muscle). Myofilaments are not grouped in discrete myofibrils, but form a single large bundle interrupted by numerous mitochondria, lipid droplets, sarcoplasmic reticulum.

Fig. 53 (lower) Low power electronmicrograph of a transverse section of white muscle fibers (superficial dorsal flexor muscle). Myofilaments are grouped in discrete myofibrillar bundles well delineated by sarcoplasmic reticulum.

L., lipid droplet; M., mitochondria; MF., myofilaments; SR., sarcoplasmic reticulum.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 9,000X.


MF
L
SR


MF

Fig. 54 Higher power electronmicrograph of a transverse section of a red muscle fiber (superficial dorsal flexor muscle) showing a fused mass of myofilaments interrupted by lipid droplets, mitochondria, and sarcoplasmic reticulum.

L., lipid droplet; M., mitochondria; MF., myofilaments; SR., sarcoplasmic reticulum.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 28,000X.

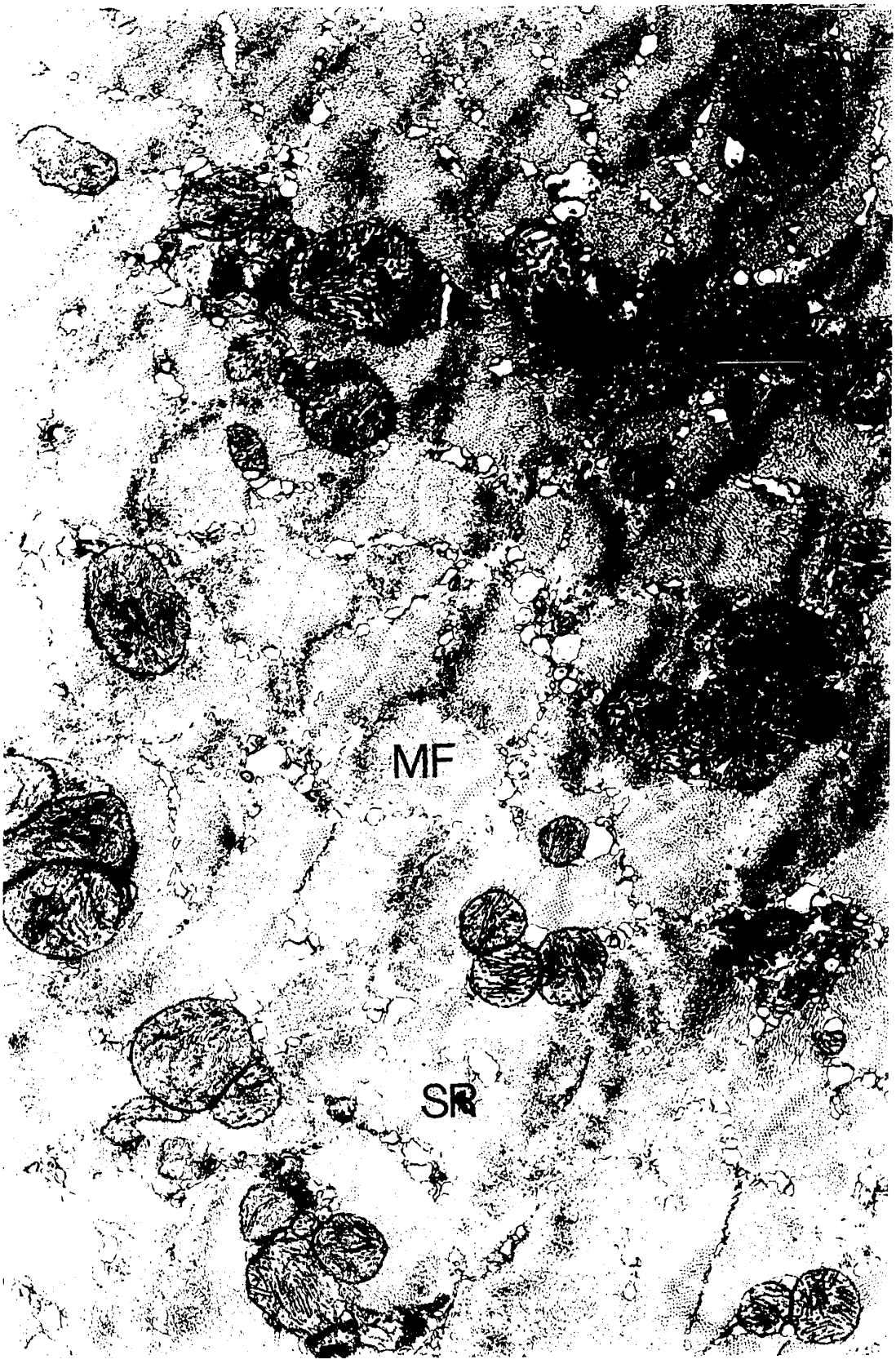

MF
SR

Fig. 55 Longitudinal section (slightly oblique) of red muscle fiber (hypochordal longitudinal muscle). Myofilaments are not grouped in independent myofibrils throughout their length but they are often confluent, forming irregularities in fibrillar width. Note abundance of mitochondria.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 35,200X.

Fig. 56 High power electronmicrograph of a transverse section of white muscle fibers (superficial dorsal flexor muscle). Myofilaments are grouped in discrete myofibrils, well-delineated by sarcoplasmic reticulum.

M., mitochondrion; MF., myofilaments;
SR., sarcoplasmic reticulum.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 28,000X.

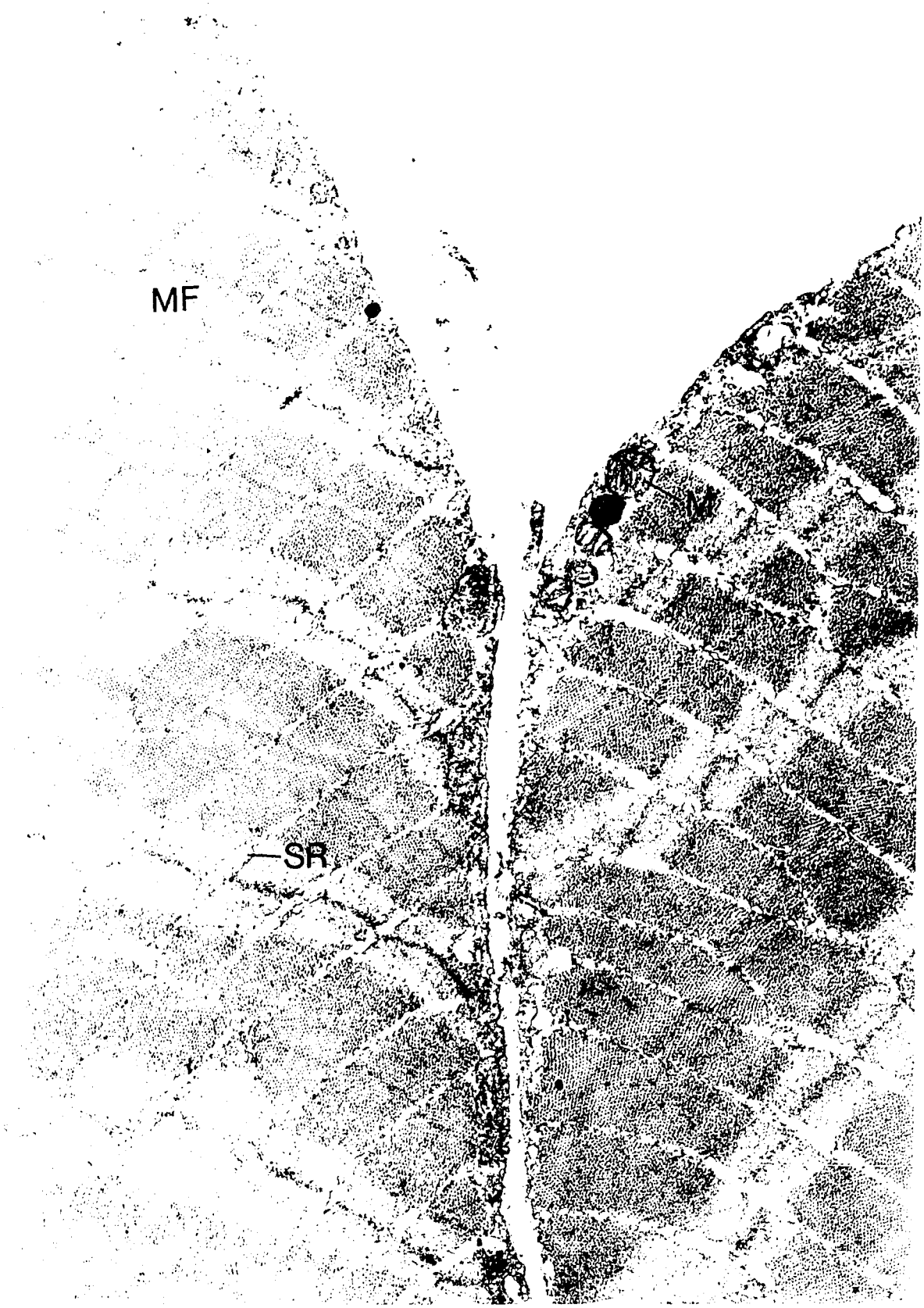

MF
SR

Fig. 57 Longitudinal section of white muscle fibers (hypochordal longitudinal muscle). Myofibrils are running as independent bundles. Note scarcity of mitochondria and lipid droplet.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 35,200X.

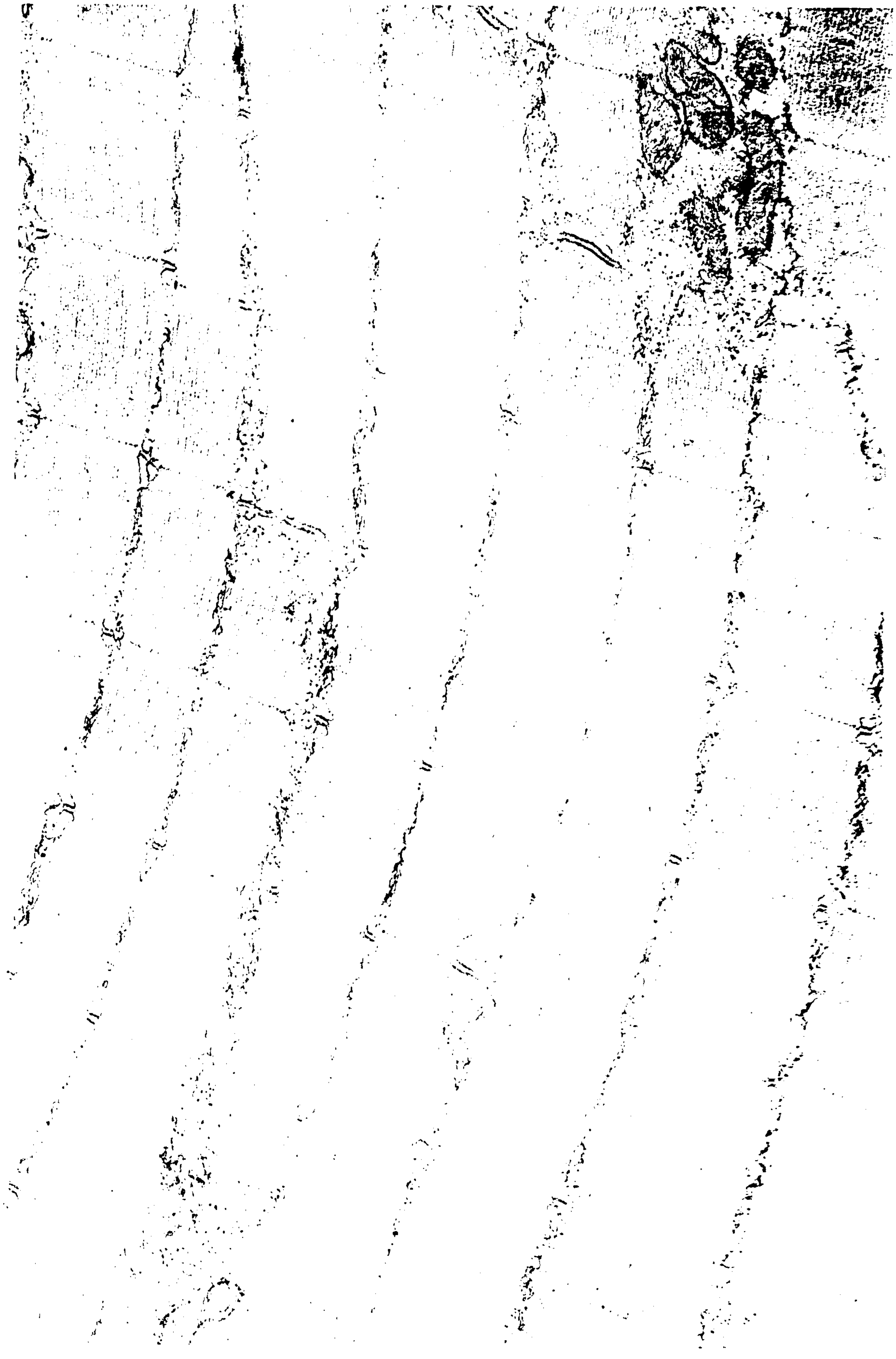
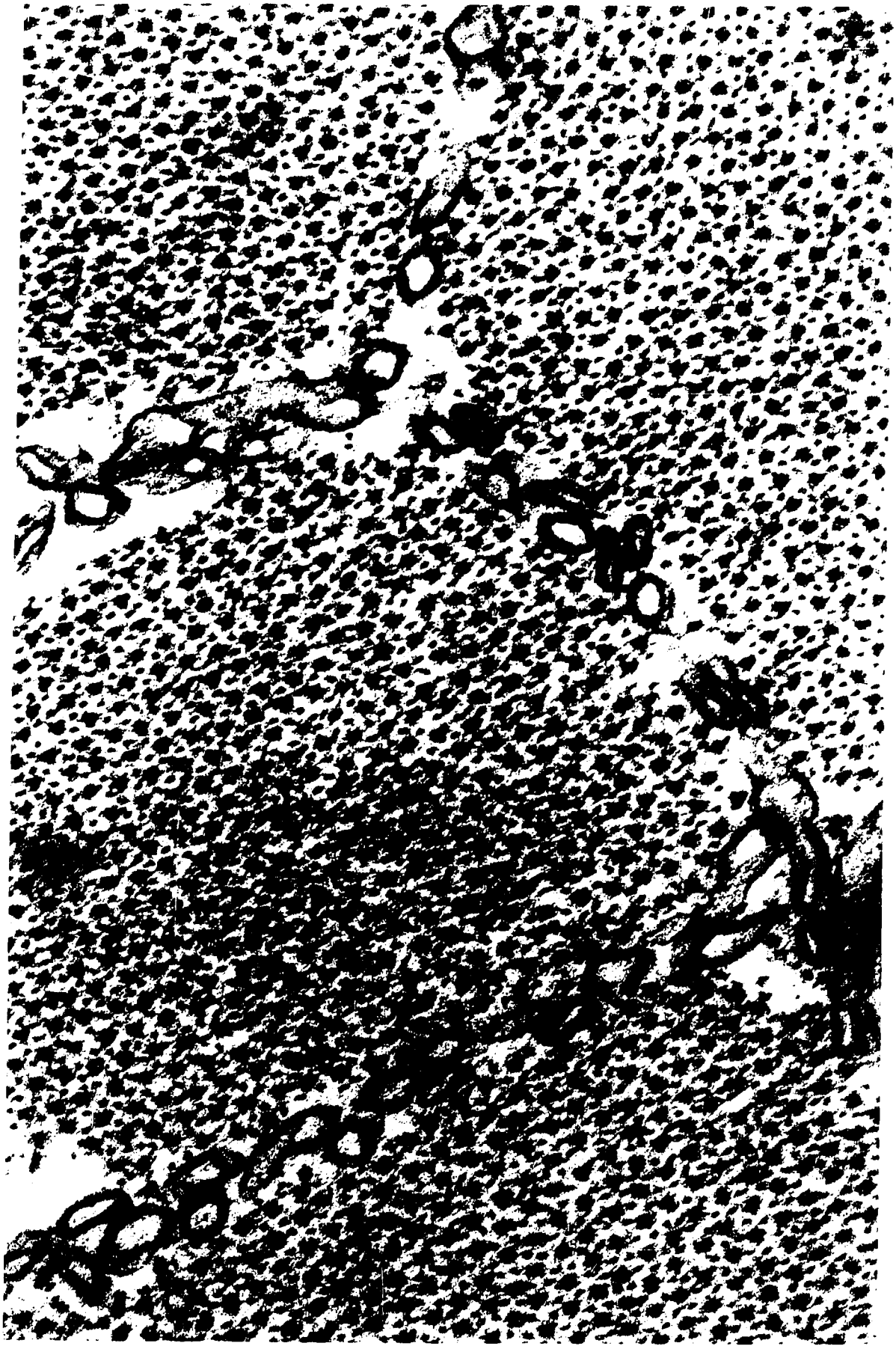


Fig. 58 Longitudinal section of red muscle fibers (superficial dorsal flexor muscle) of a cruising fish fixed quickly. Note the uneven nature of contractions of individual sarcomeres. Some are shortened, others extended.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 24,500X.

Fig. 59 Hexagonal arrangement of myofilaments of red muscle fiber. One myosin is surrounded by a regular hexagonal orbit of actins.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 160,000X.



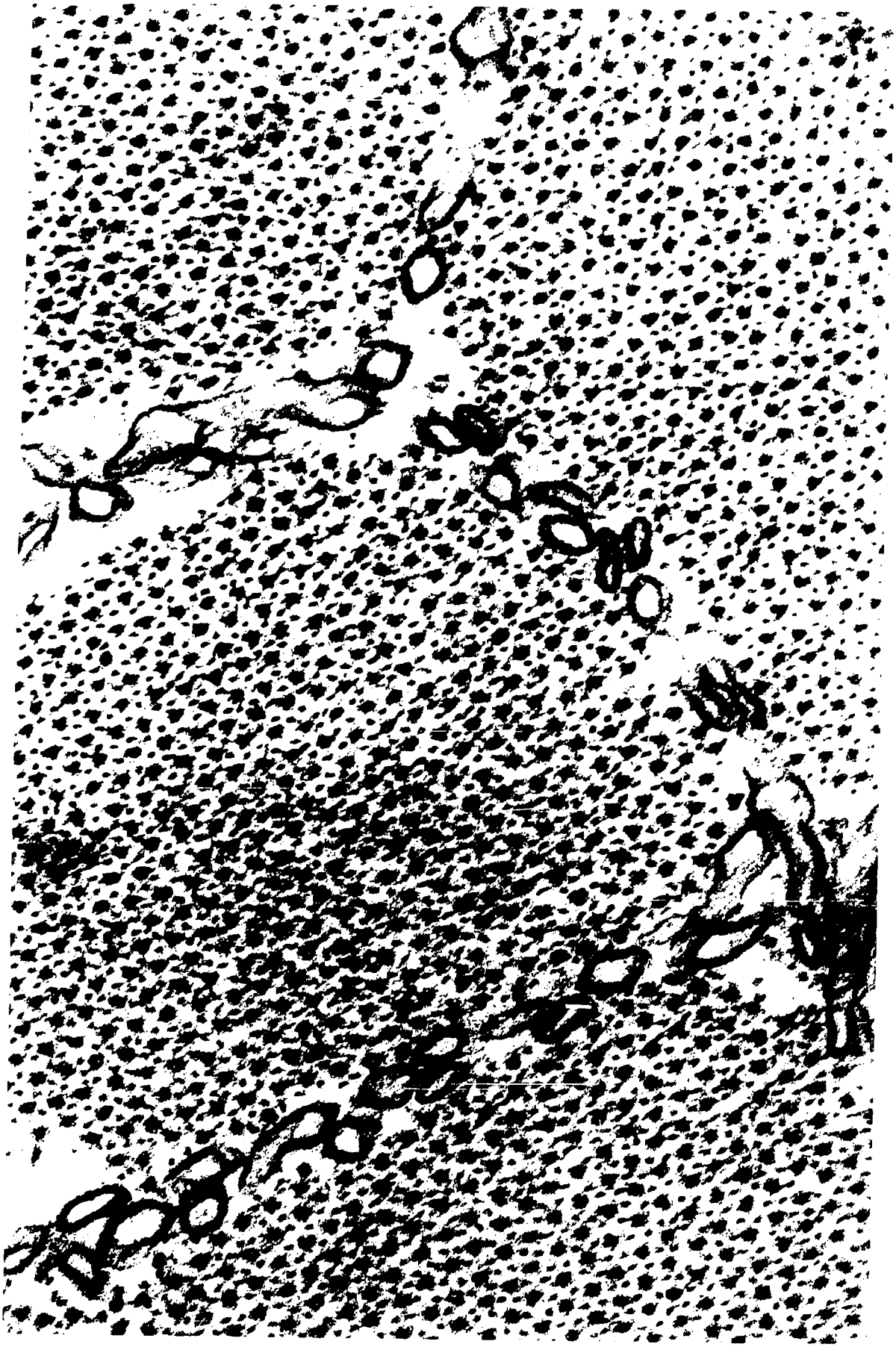
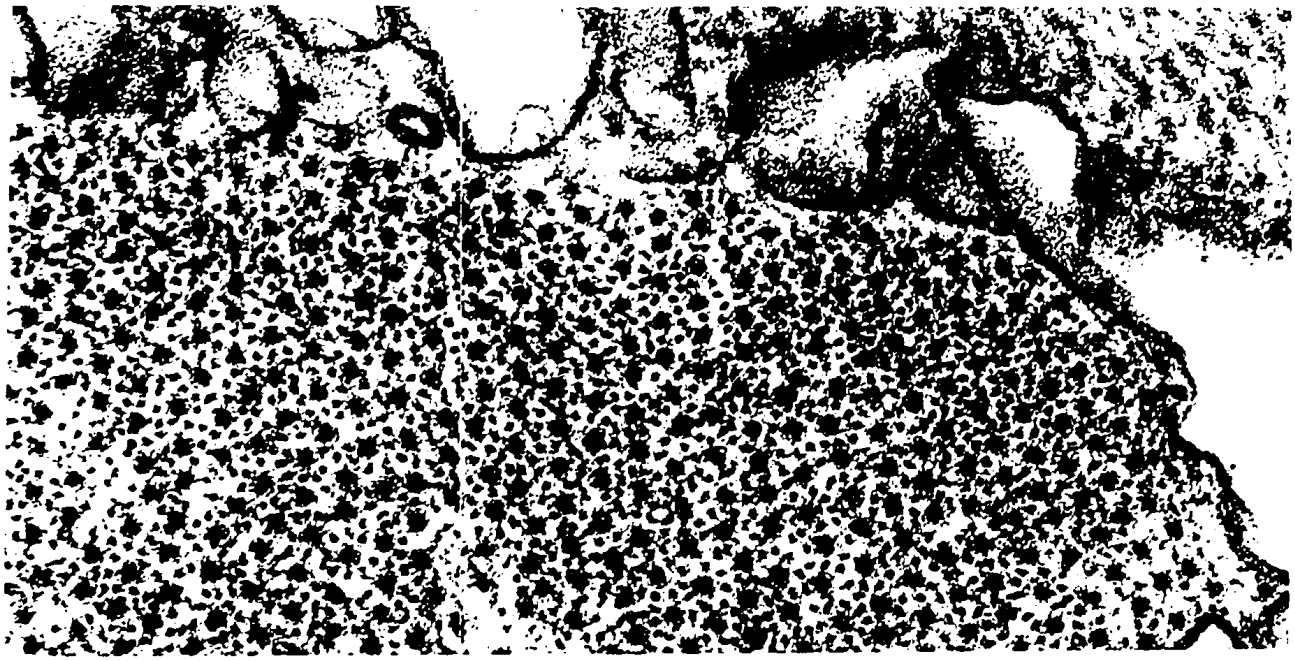


Fig. 60 (upper) Irregular arrangement of actin and myosin filaments of red muscle fiber of a young adult specimen. One myosin is surrounded by an irregular orbit of actins averaging eight in number. 112,000X.

Fig. 61 (lower) A part of the above electronmicrograph under higher magnification. 194,000X.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate.



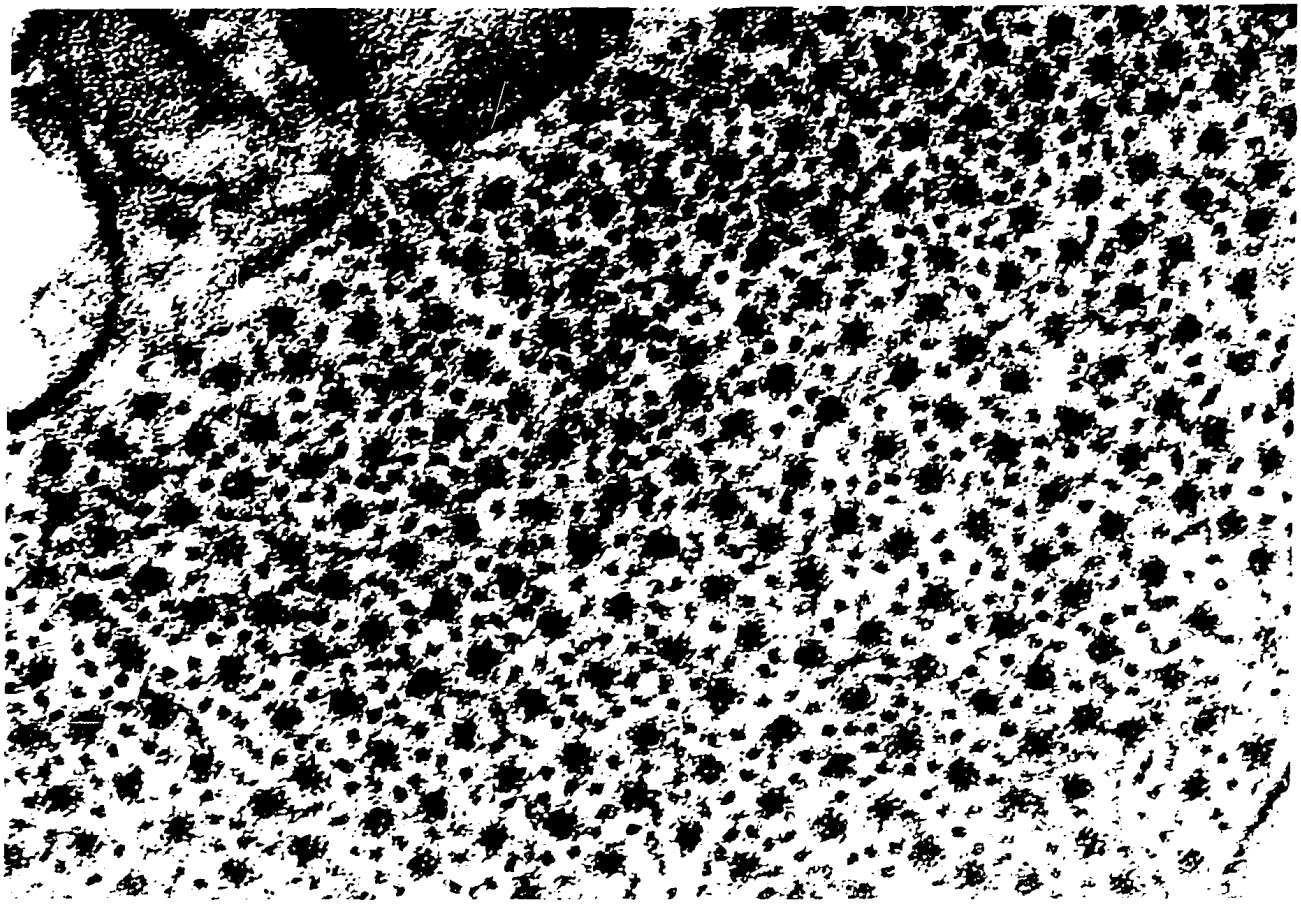


Fig. 62 (upper)
Fig. 63 (lower)

An irregular (on the left side) and a regular hexagonal (on the right side) arrangement of actin and myosin filaments are shown side by side in two different myofibrils of an early free swimming specimen. The myofibril in Fig. 63 (lower) belongs to the preparation of Fig. 62. This myofibril is situated next to the right myofibril of Fig. 62. This myofibril shows the hexagonal arrangement of myofilaments.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 160,000X.

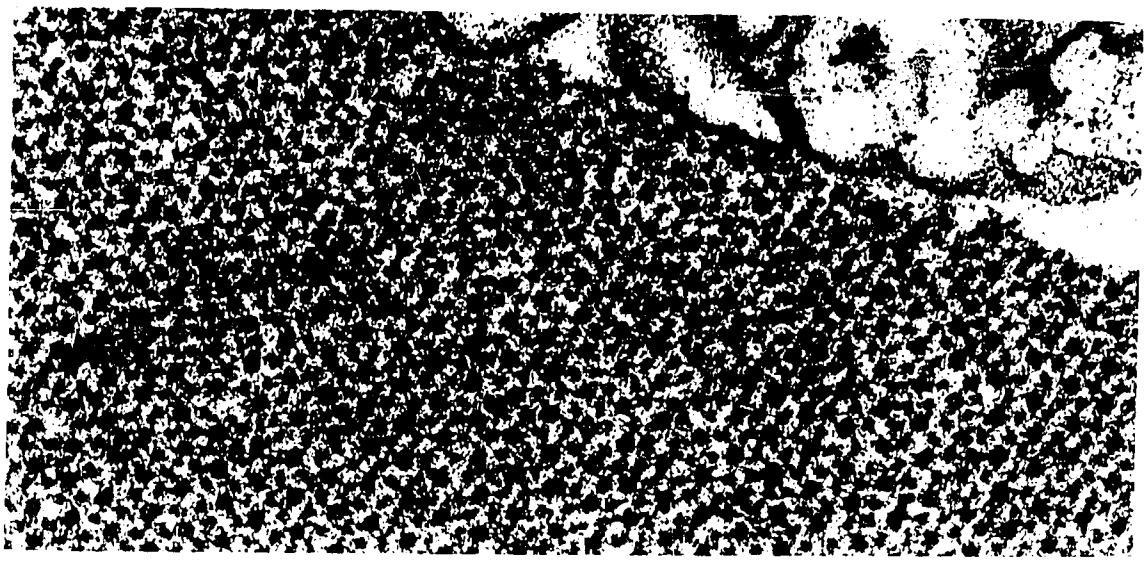
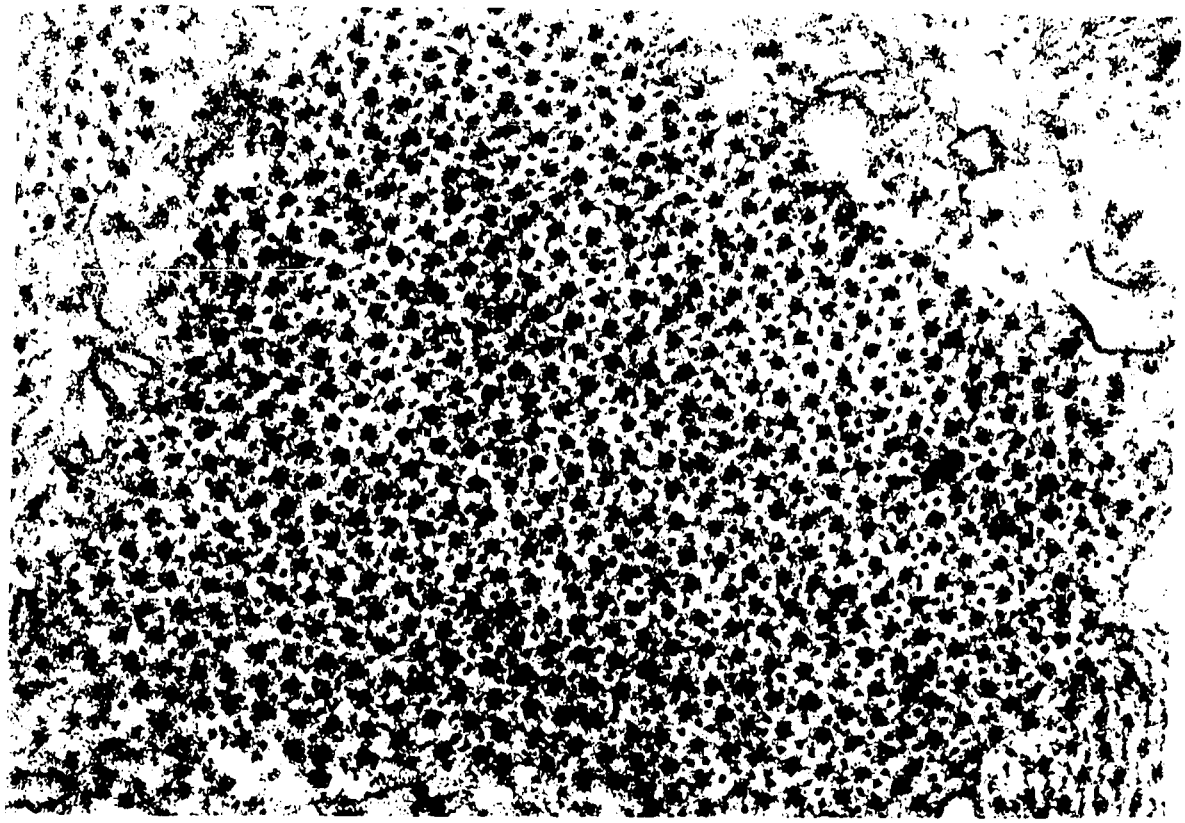


Fig. 64 (upper) Hexagonal arrangement of myofilaments of white muscle fibers.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 160,000X.

Fig. 65 (lower) Hexagonal arrangement of myofilaments of white muscle fiber is not changed and no irregular arrangement of myofilaments appear as an artifact owing to single fixation method which is used in this preparation. The muscle is fixed in Osmium tetroxide dissolved in salmonid physiological solution. Post-fixation procedures are the same as in Fig. 64. 160,000X.



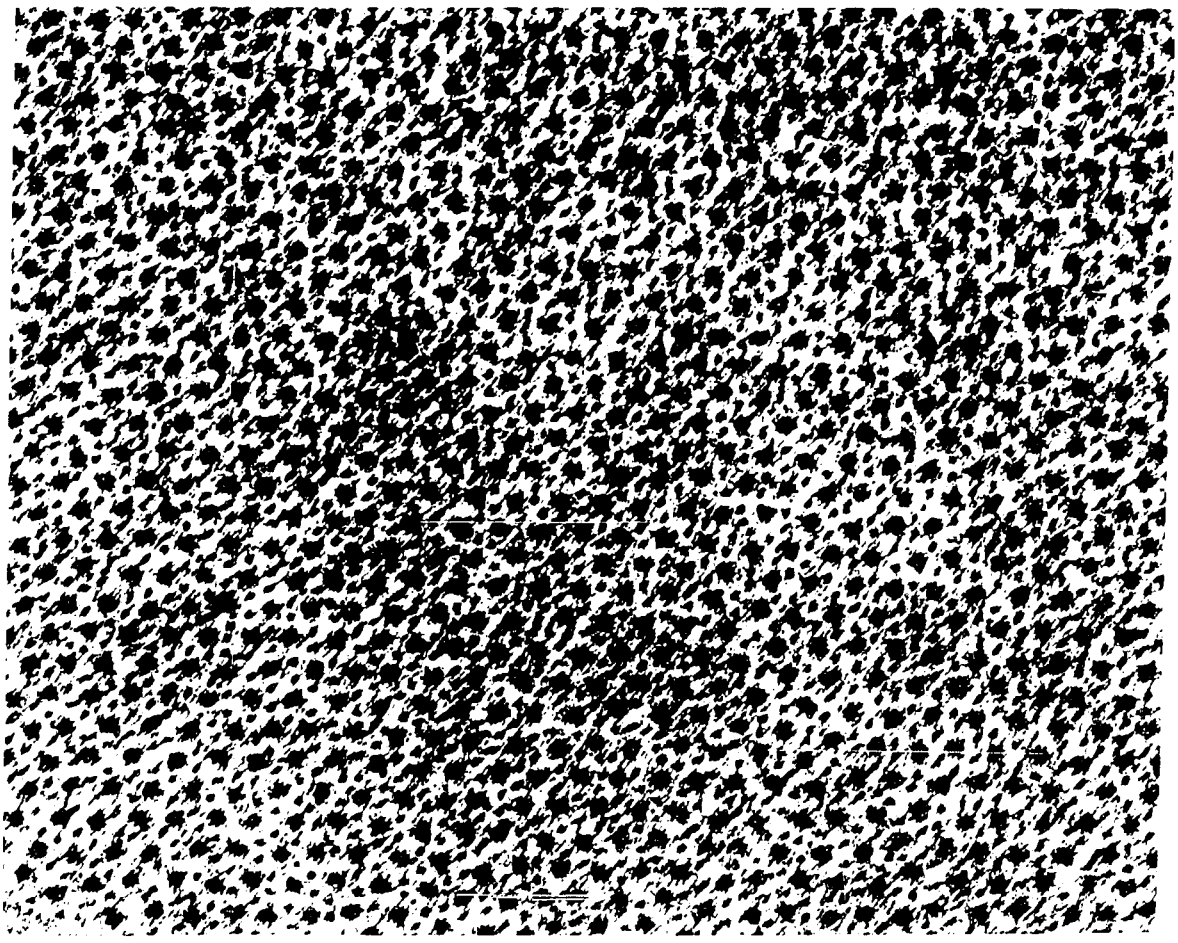


Fig. 66 The hexagonal arrangement of myofilaments of red muscle fiber is not changed and no irregular arrangement of myofilaments appears as an artifact owing to single fixation method which is used in this preparation. The muscle is fixed in Osmium tetroxide dissolved in salmonid physiolgical solution. Post-fixation procedures are the same as in other figures. 160,000X.

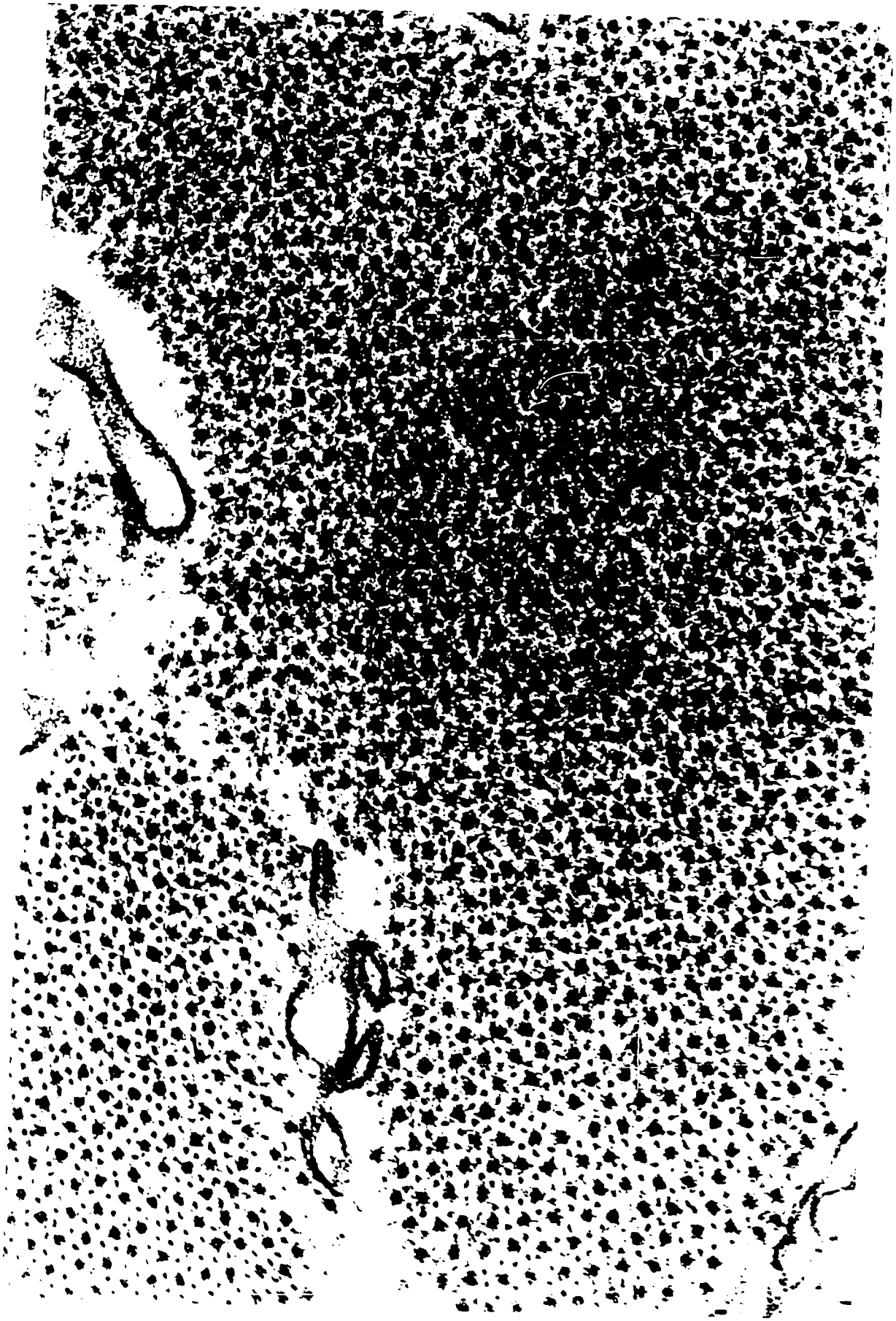


Fig. 67 Low power electronmicrograph of a longitudinal section of red muscle fiber (superficial dorsal flexor muscle) showing the relative abundance of the T system and sarcoplasmic reticulum.

Fig. 68 Low power electronmicrograph of a longitudinal section of white muscle fiber (superficial dorsal flexor muscle) showing the relative abundance of the T system and sarcoplasmic reticulum.

LC., terminal cisternae; LT., longitudinal sarcotubule; TT., transverse tubule.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 24,500X.


TT
LC
LT

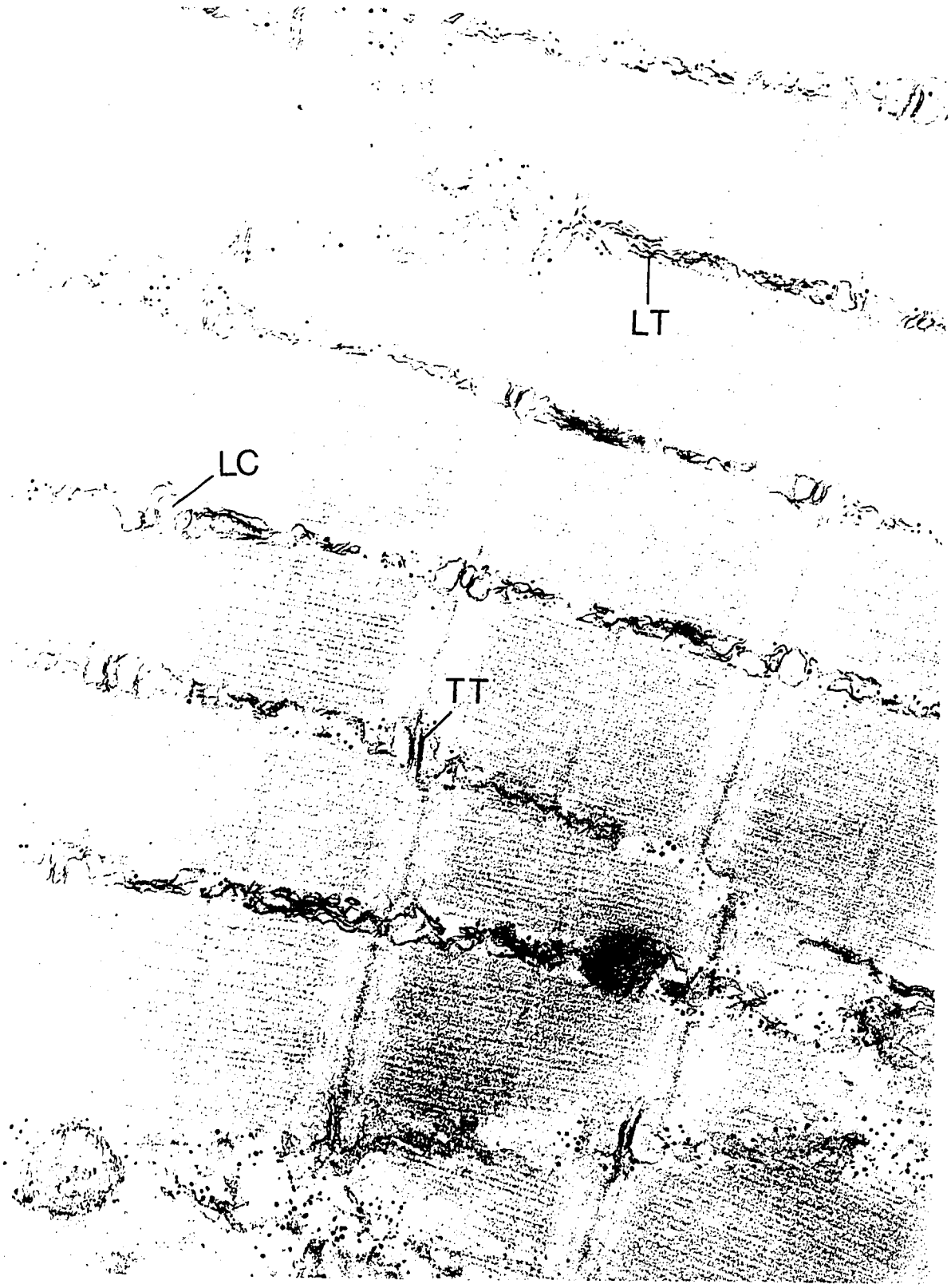

LT
LC
TT

Fig. 69 Transverse section of a white muscle fiber (superficial ventral flexor muscle) showing branching and interconnection of the T system among the myofibrils.

ML., myofibril; TT., transverse tubule.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 28,000X.

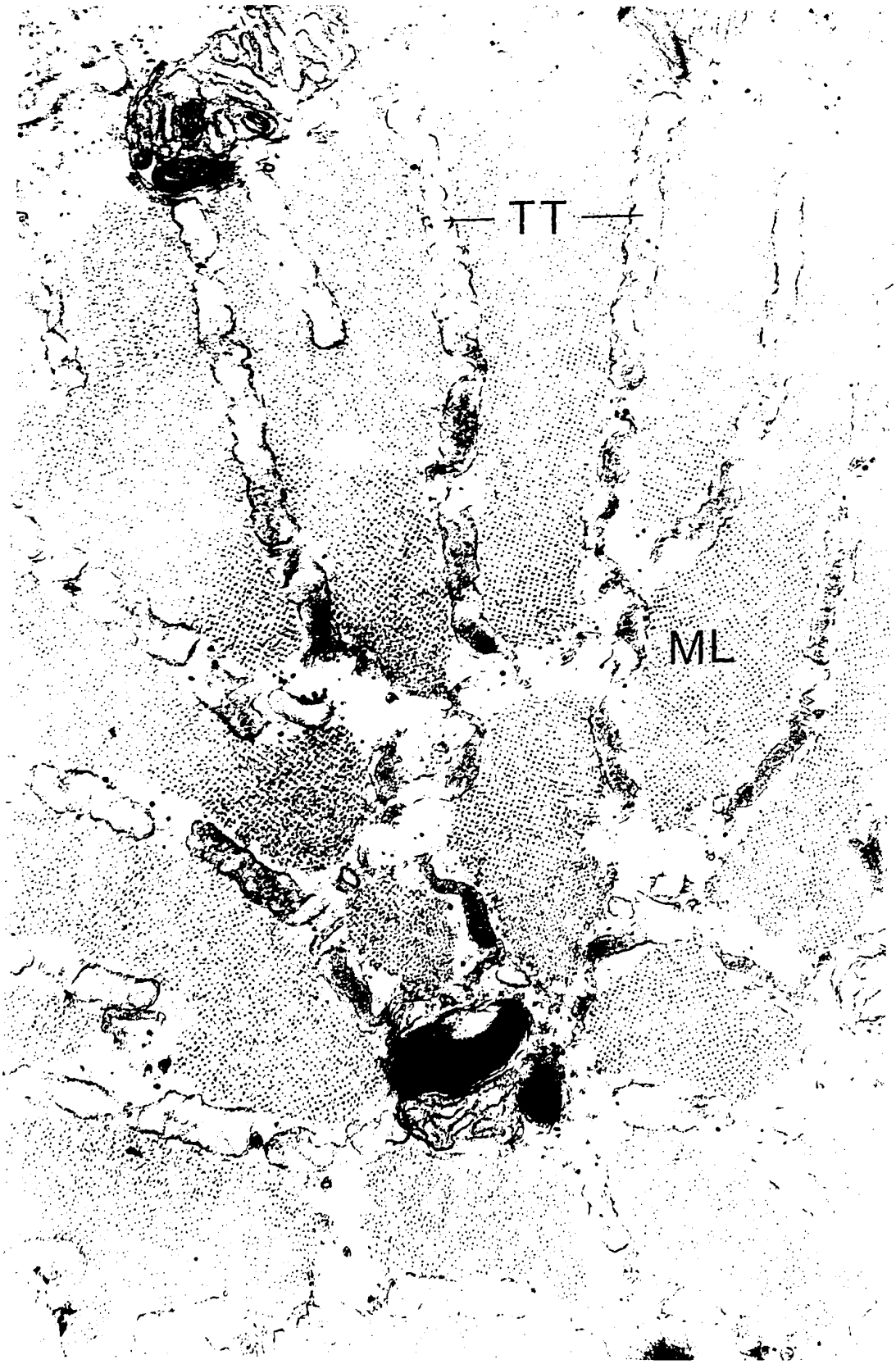

TT
ML

Fig. 70 This electronmicrograph is the right-hand corner of the previous preparation (Fig. 69). The arrows show the interconnection of transverse tubules outside the myofibrils.

G., glycogen particles.

Preparative procedures and magnification are the same as in Fig. 69.

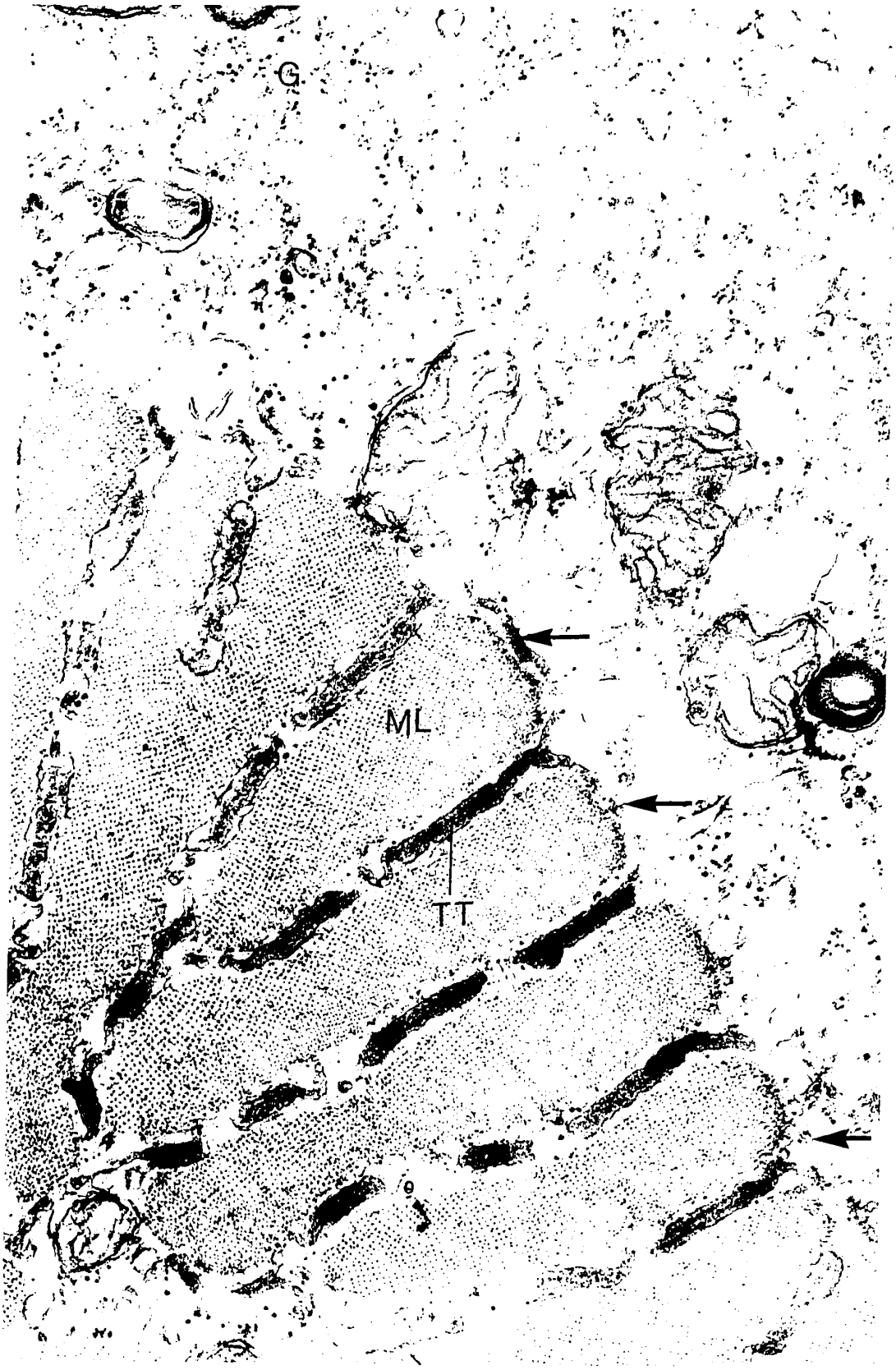
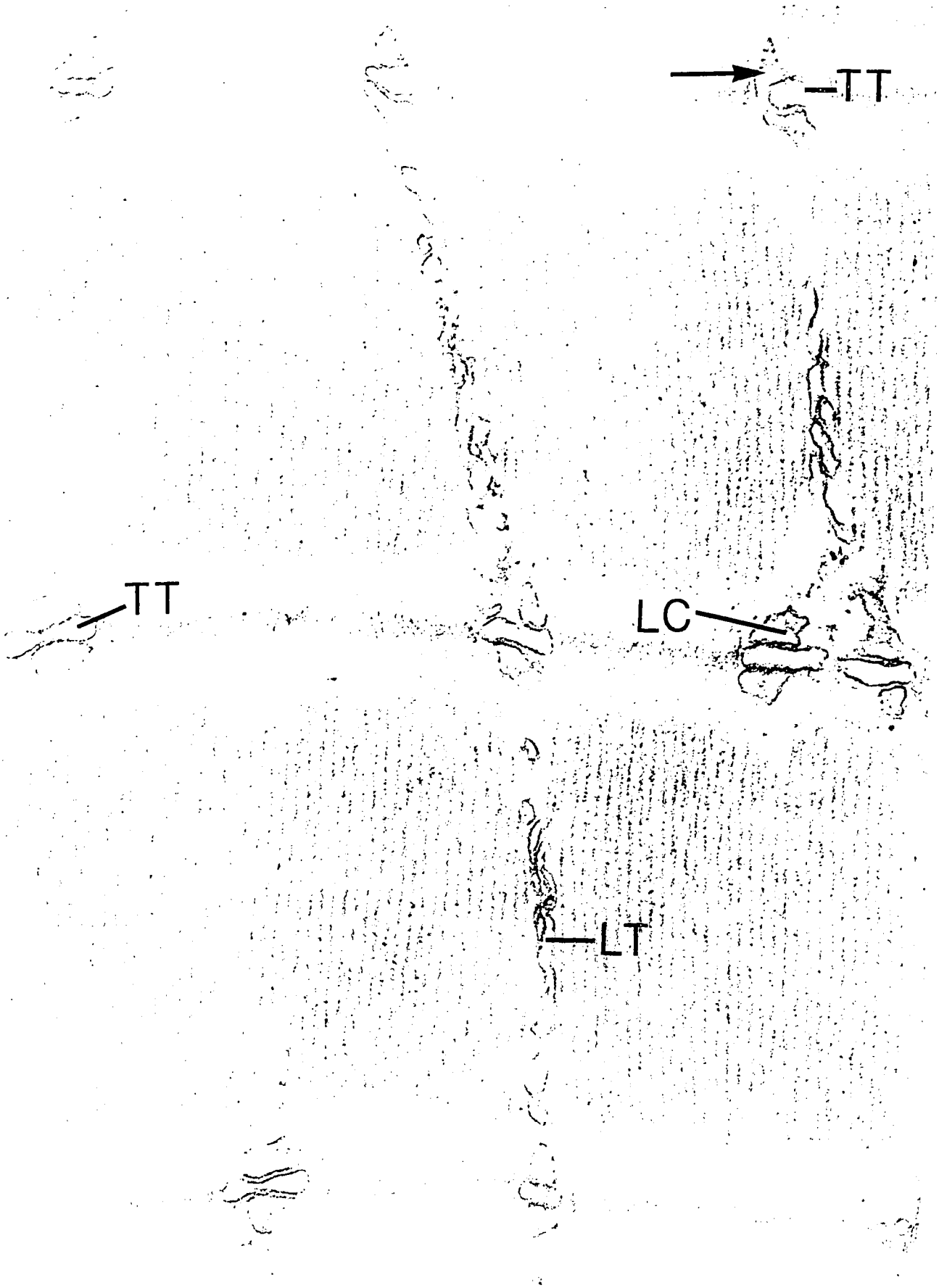

G
ML
TT

Fig. 71 Longitudinal section of red muscle fiber (deep dorsal flexor muscle) showing T system and sarcoplasmic reticulum at a higher magnification. The arrow shows a terminal cisterna which has been continuous from one sarcomere to the next. The contact of longitudinal sarcotubules of the reticulum with the terminal cisternae is not preserved. 92,800X.

Fig. 72 Longitudinal section of white muscle fiber (deep dorsal flexor muscle) showing T system and sarcoplasmic reticulum at a higher magnification. The contact of longitudinal sarcotubules of reticulum with the terminal cisternae is preserved with the same fixation procedures as it is in Fig. 71. The arrows show dense structures between faces of terminal cisternae and transverse tubules. Note scanty cristae of mitochondria and rich glycogen content of myofibrils. 68,800X.

G., glycogen particles; LC., terminal cisternae; LT., longitudinal sarcotubule; M., mitochondrion; TT., transverse tubule.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate.


TT
TT
LC
LT


M
I
G
LT
LC
TT

Fig. 73 T system and sarcoplasmic reticulum of red muscle fiber (deep ventral flexor muscle). The arrows show interruptions of terminal cisternae. Note the circular profile of the transverse tubule and accompanying terminal cisternae which are continuous from one sarcomere to the next.

LC., terminal cisternae; TT., transverse tubule.

Glutaraldehyde in salmonid physiological solution - osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 72,000X.

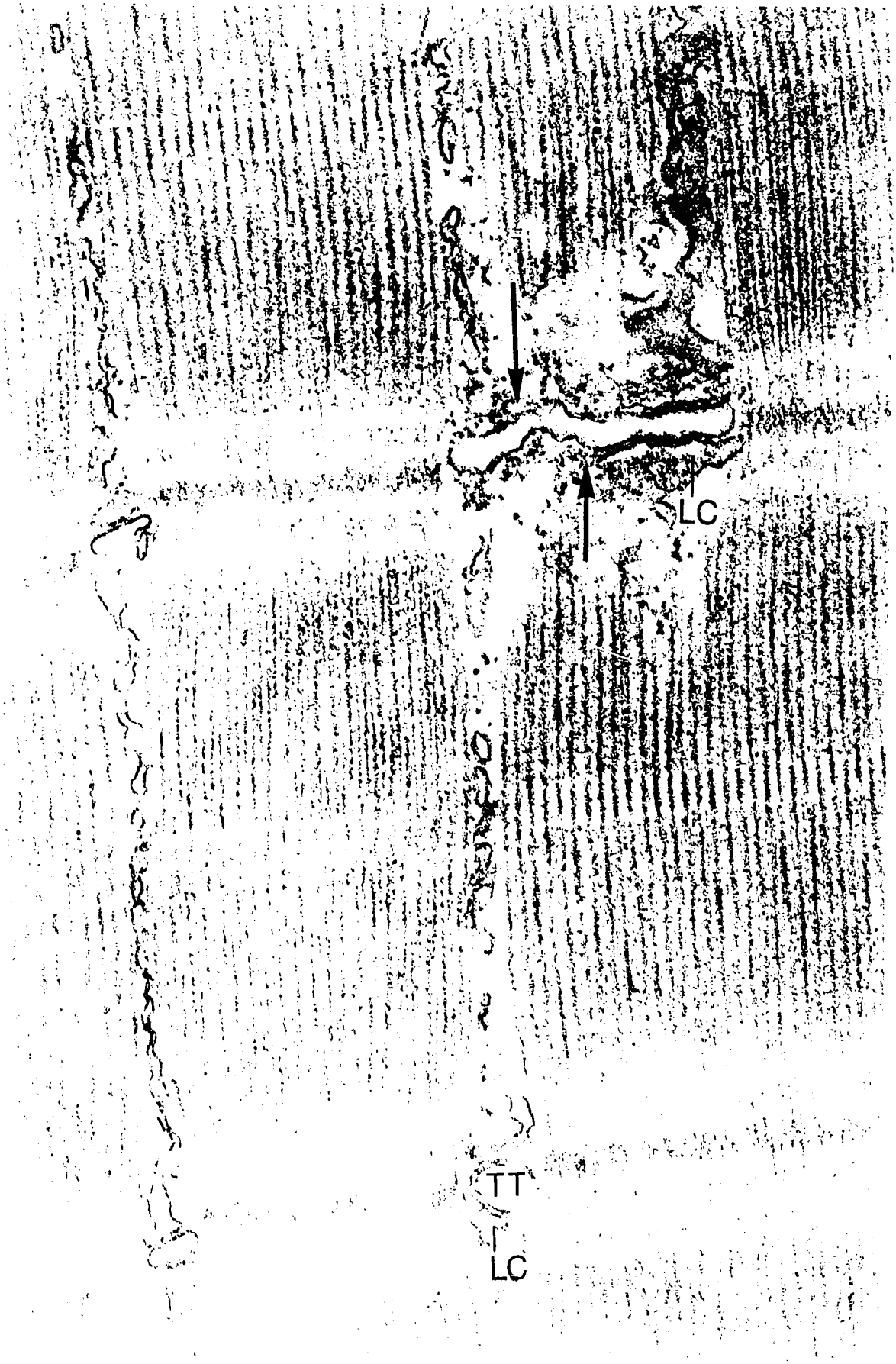
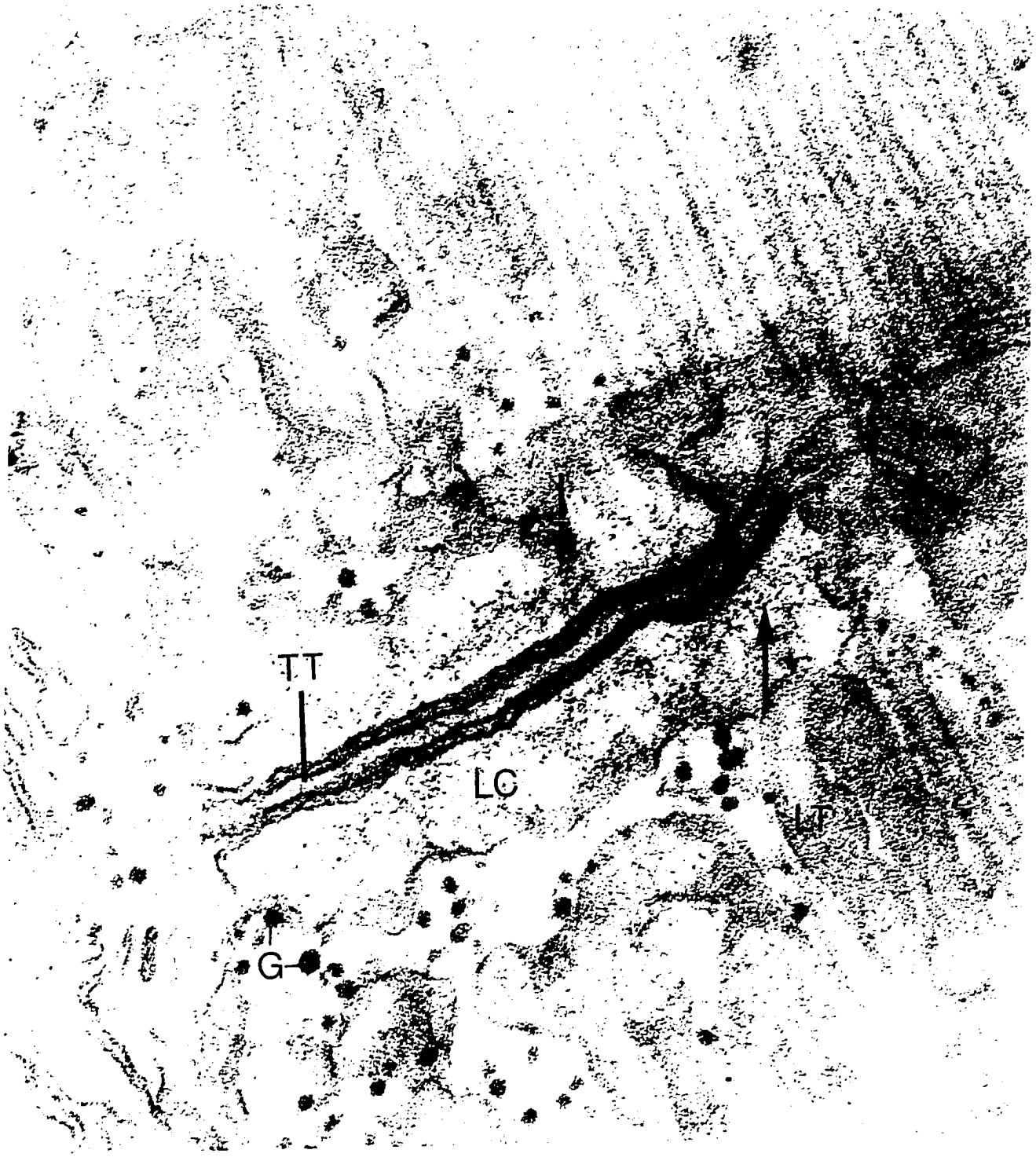

LC
TT
LC

Fig. 74 The arrows show dense diffuse granular material
Fig. 75 in the terminal cisternae of white muscle fibers (deep flexor muscles) at a high magnification.

G., glycogen particles; LC., terminal cisternae; LT., longitudinal sarcotubule; TT., transverse tubule.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 124,800X.


TT
LC
LP
G

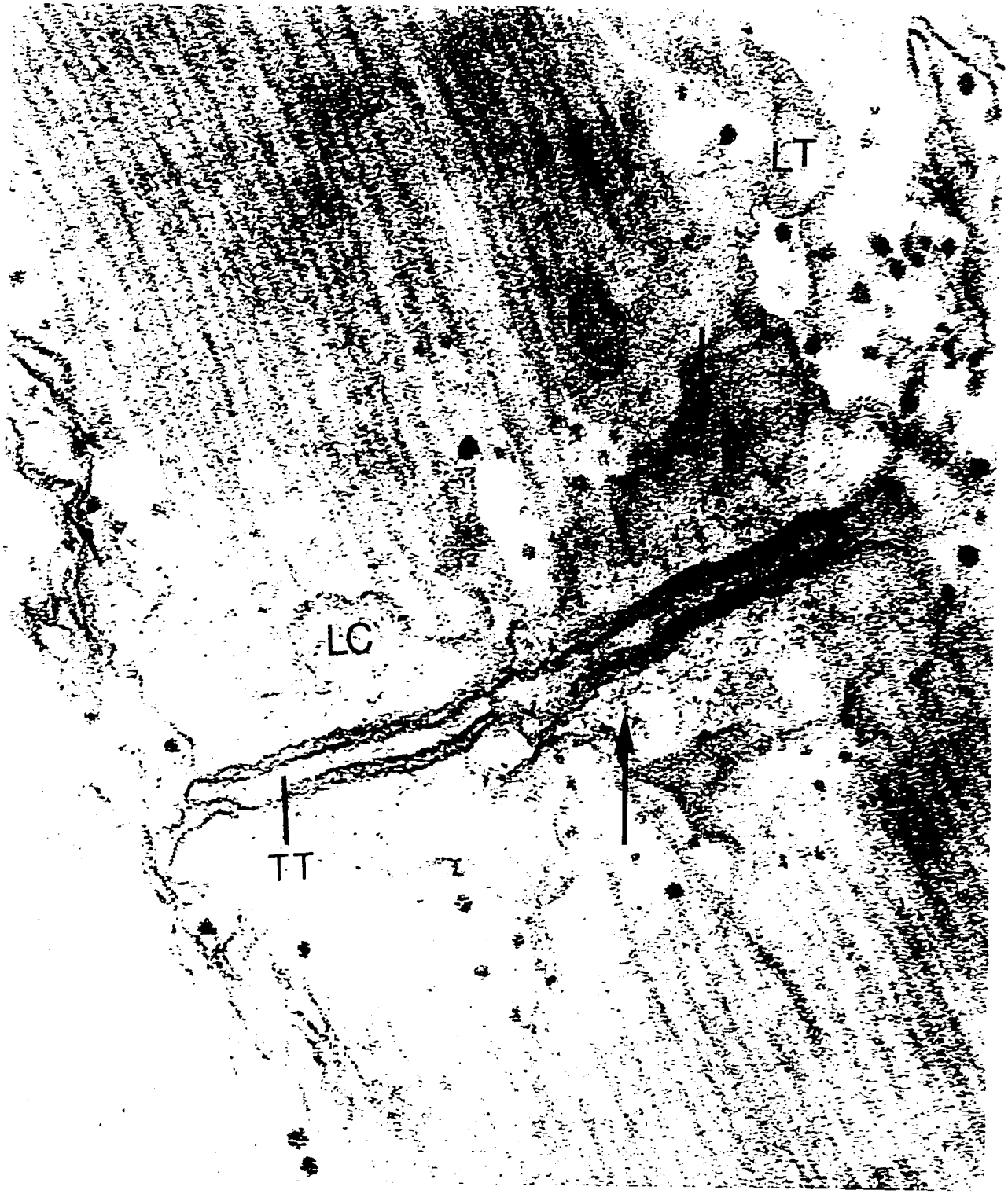
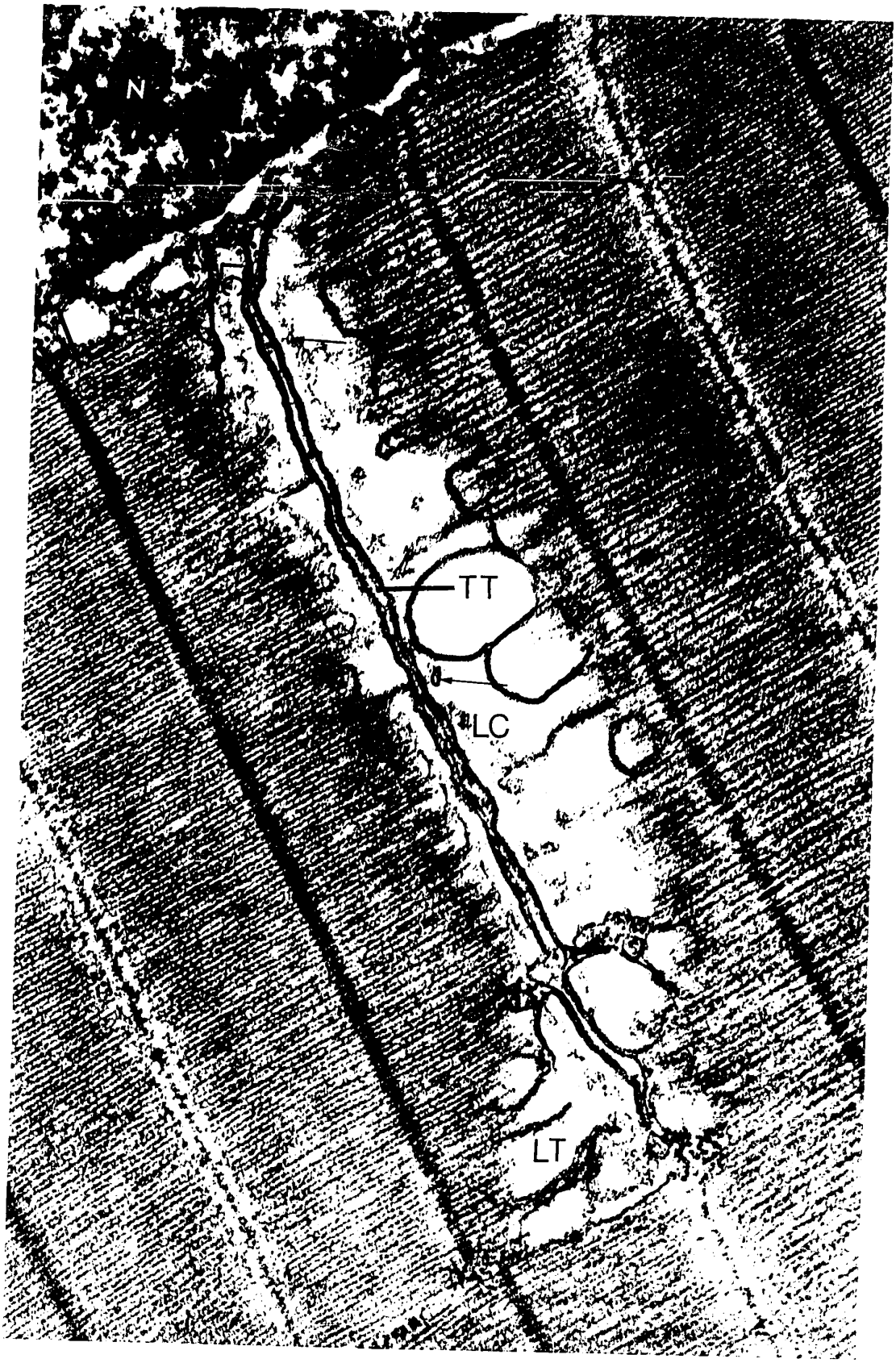

LT
LC
TT

Fig. 76 The red muscle fibers (superficial flexor muscles)
Fig. 77 of an early free swimming specimen show the connection of terminal cisternae with the outer membranes of nuclear envelopes by big arrows. The openings of terminal cisternae are shown by small arrows. The interruptions of terminal cisternae are shown by arrowheads.

LC., terminal cisternae; LT., longitudinal sarcotubule; OE., outer membrane of nuclear envelope; TT., transverse tubule.

Glutaraldehyde-paraformaldehyde, Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 68,800X.


N
TT
LC
LT

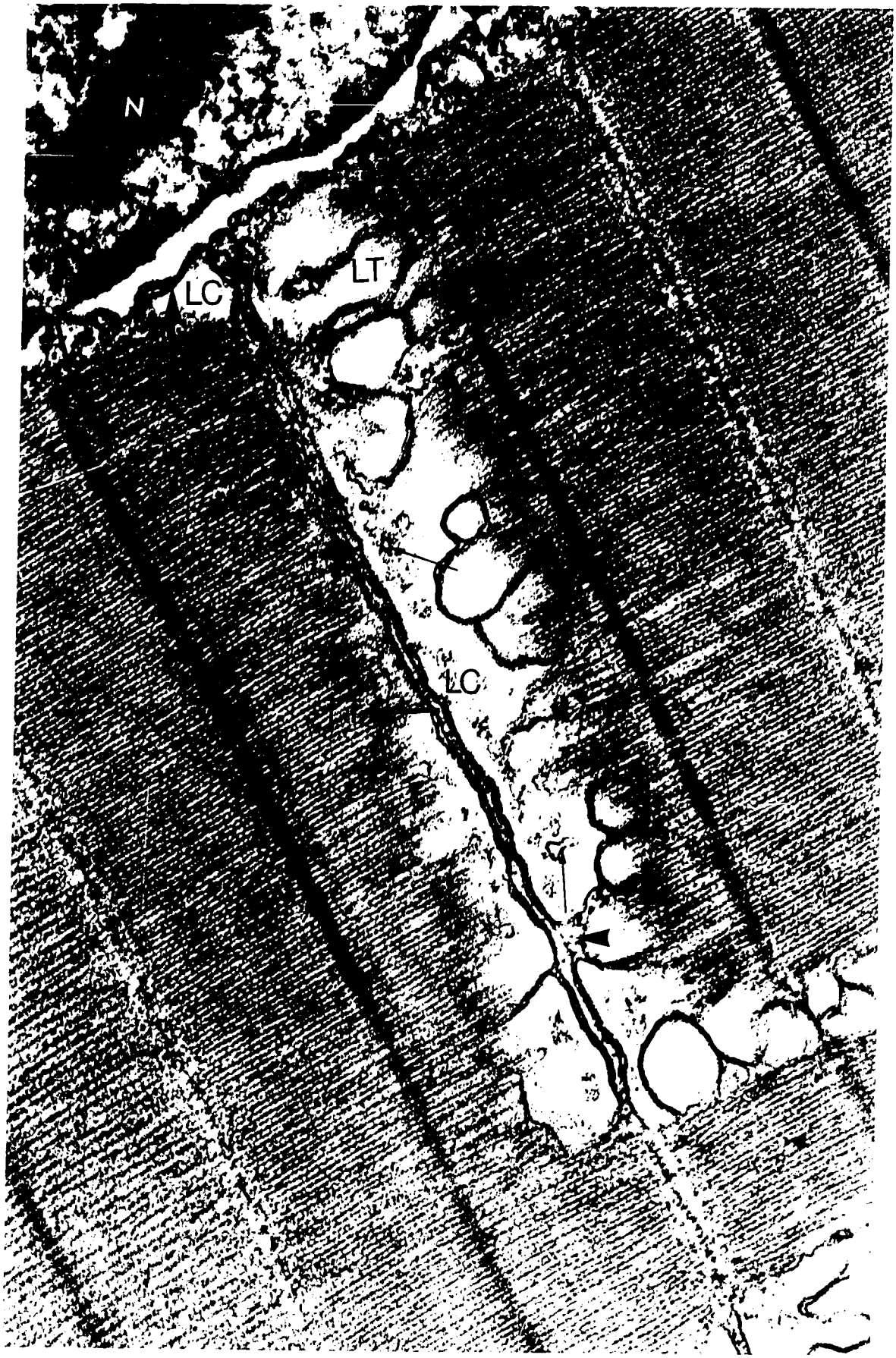

N
LC
LT
LC

Fig. 78 The arrows show dense structures between the faces
Fig. 79 of terminal cisternae and transverse tubules of red
Fig. 80 and white muscle fibers. Note the rich glycogen content of white muscle fibers in Figs. 79, 80 (hypochordal longitudinal and interradial muscles, respectively). Fig. 78 shows red muscle fiber (hypochordal longitudinal muscle).

G., glycogen particles; LC., terminal cisternae; TT., transverse tubule.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. Figs. 78, 79: 68,800X; Fig. 80: 60,000X.

LC
G


G
TT
LC

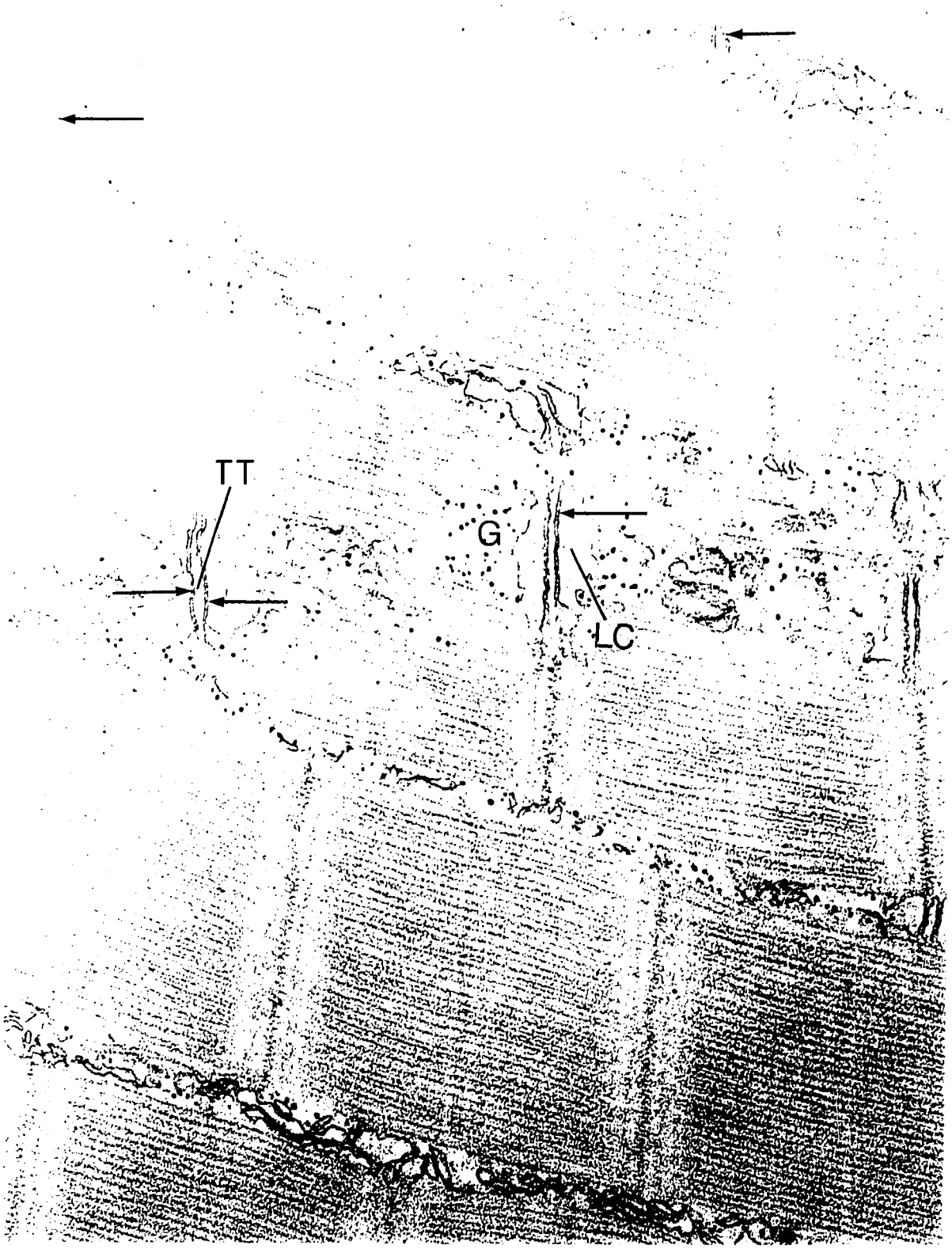
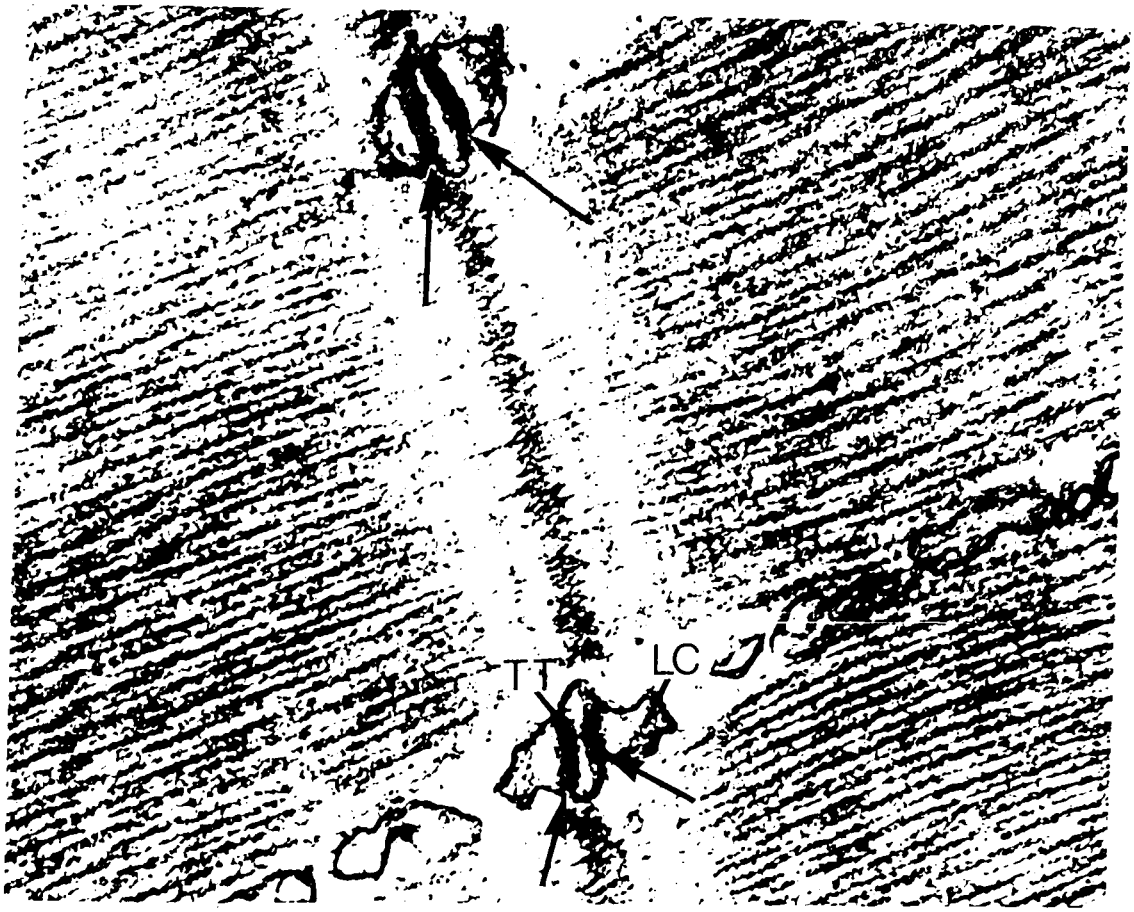

TT
G
LC

Fig. 81 (upper)
Fig. 82 (lower)

The arrows show dense materials in the gap between terminal cisternae and transverse tubules of red muscle fibers (deep dorsal flexor and interradial muscles, respectively) at a higher magnification.

LC., terminal cisternae; TT., transverse tubule.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate.
124,800X.


TT
LC

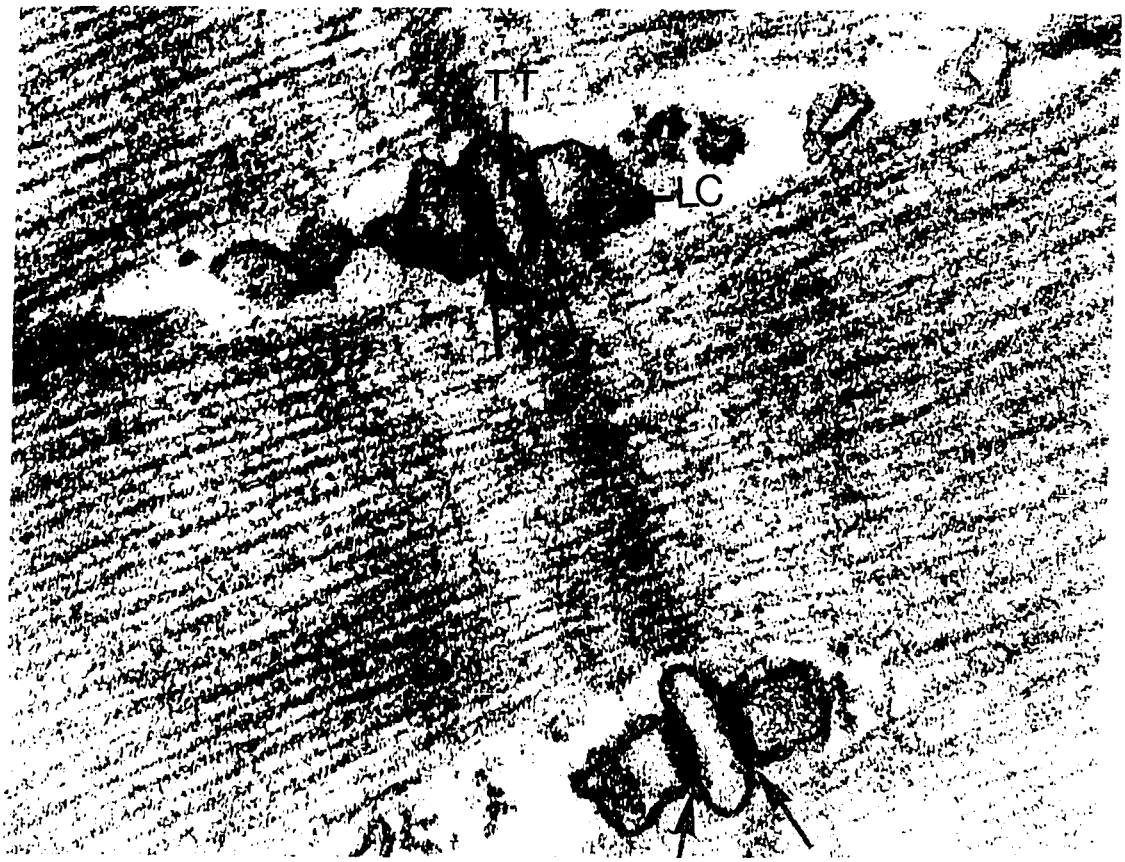

TT
LC

Fig. 83 This figure shows the contact between terminal cisternae and longitudinal sarcotubules of reticulum and the continuity of the latter in the inter- and intra-fibrillar spaces of white muscle fiber (superficial ventral flexor muscle). Note scanty cristae of mitochondria.

LC., terminal cisternae; LT., longitudinal sarcotubule; M., mitochondria; TT., transverse tubule.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 68,000X.

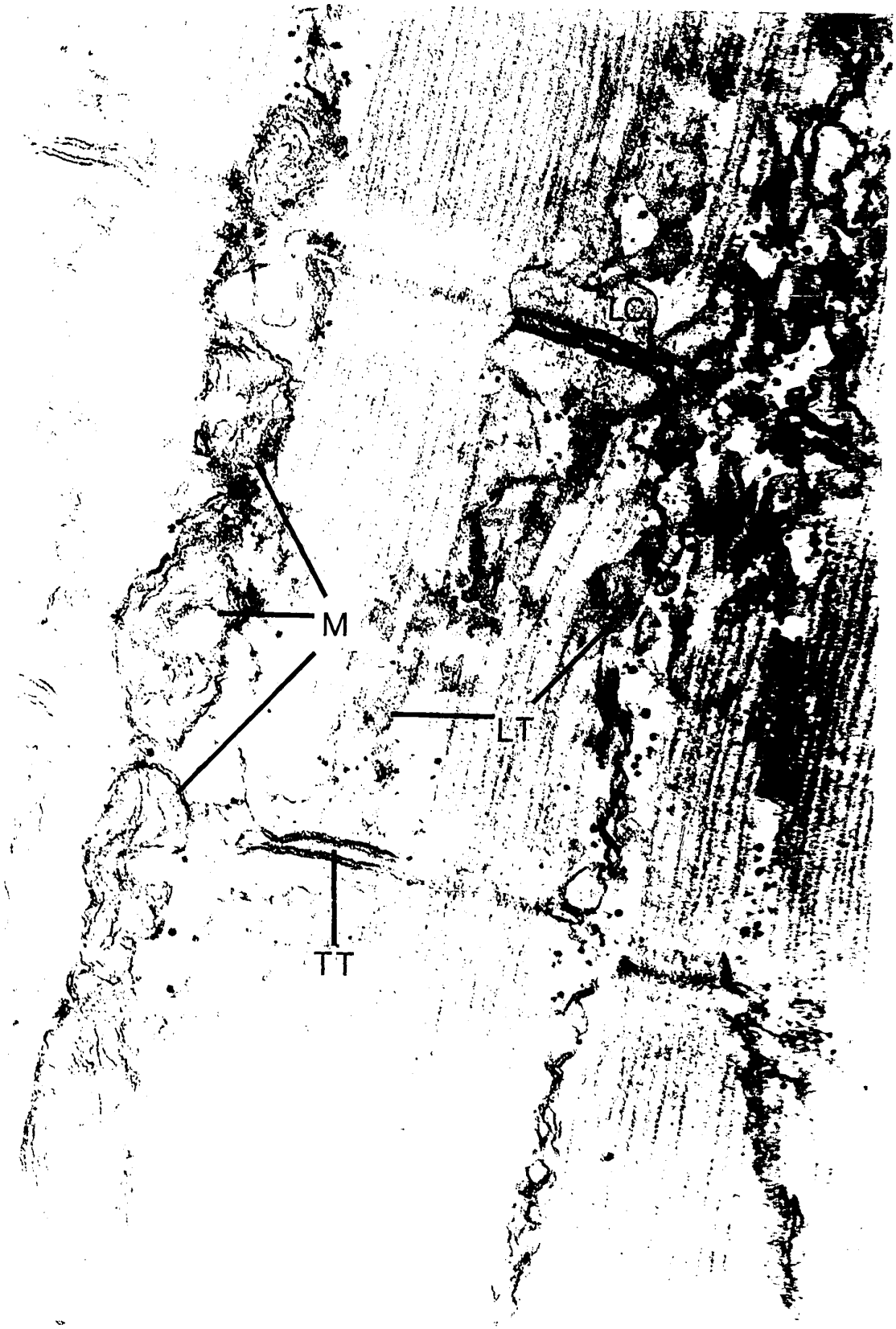

LC
M
LT
TT

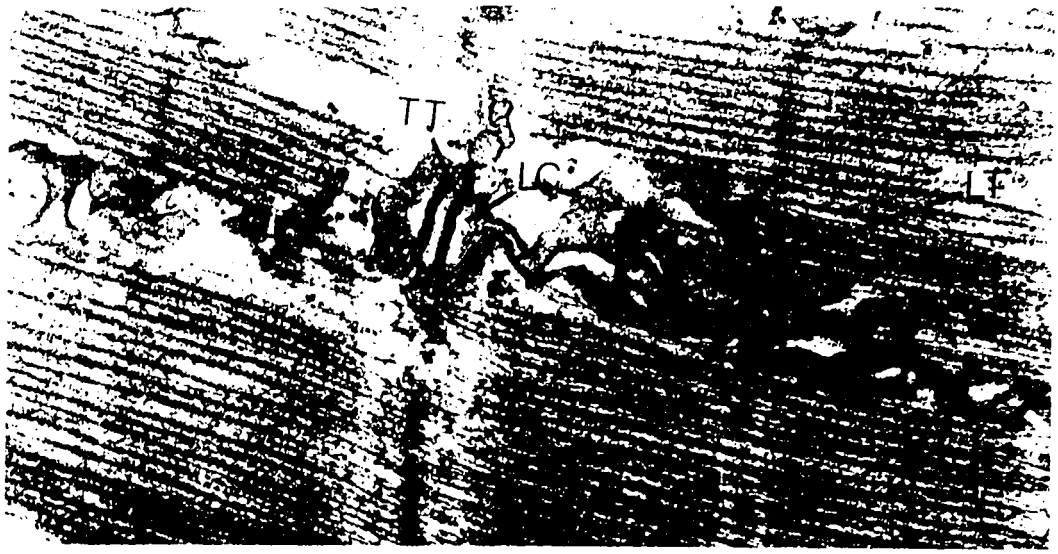
Fig. 84 (upper) The courses of longitudinal sarcotubules of reticulum of red muscle fiber (superficial dorsal flexor). This electronmicrograph shows a part of a myofibril where the contact between terminal cisternae and longitudinal sarcotubule is better preserved.

Fig. 85 (middle) The arrows show "Fenestrated collar" of longitudinal sarcotubules of reticulum of red muscle fiber (superficial ventral flexor muscle).

Fig. 86 (lower) The courses of longitudinal sarcotubules of reticulum of red muscle fiber of early free swimming specimen. Note that the longitudinal sarcotubules of each half of a sarcomere are in close association with one another near the middle of A band. No "Fenestrated collar" is seen.

FC., fenestrated collar; LC., terminal cisternae; LT., longitudinal sarcotubule; TT., transverse tubule.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 51,600X.


TT
LC
LT


LT
LC
LT
LC
TT
TT
FC
FC

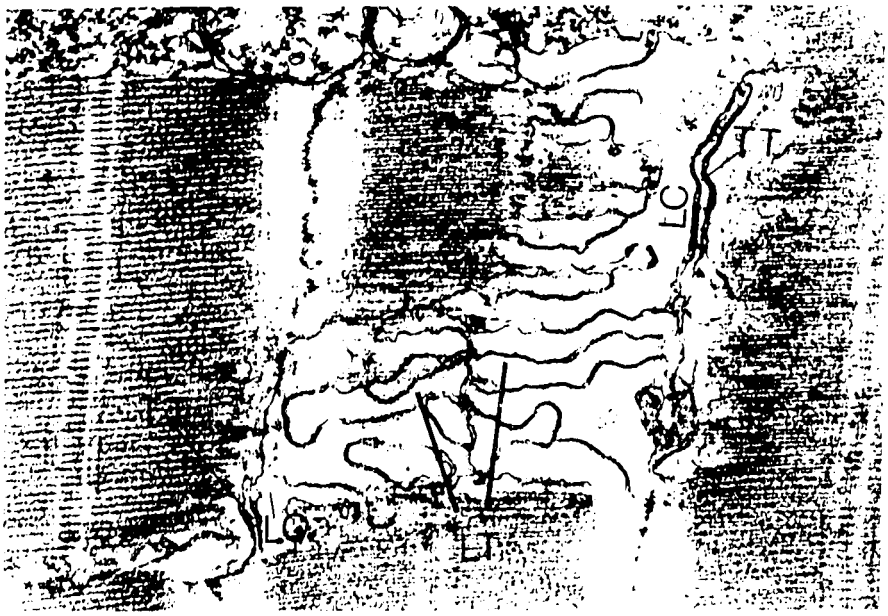

TT
LC
LC
LT

Fig. 87 Longitudinal section of red muscle fiber (superficial flexor muscle of early free swimming specimen). The arrows show that the contact betwen terminal cisternae and longitudinal sarcotubule and the continuity of the latter is not preserved.

LC., terminal cisternae; LT., longitudinal sarcotubule; TT., transverse tubule.

Glutaraldehyde-paraformaldehyde, Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 68,800X.

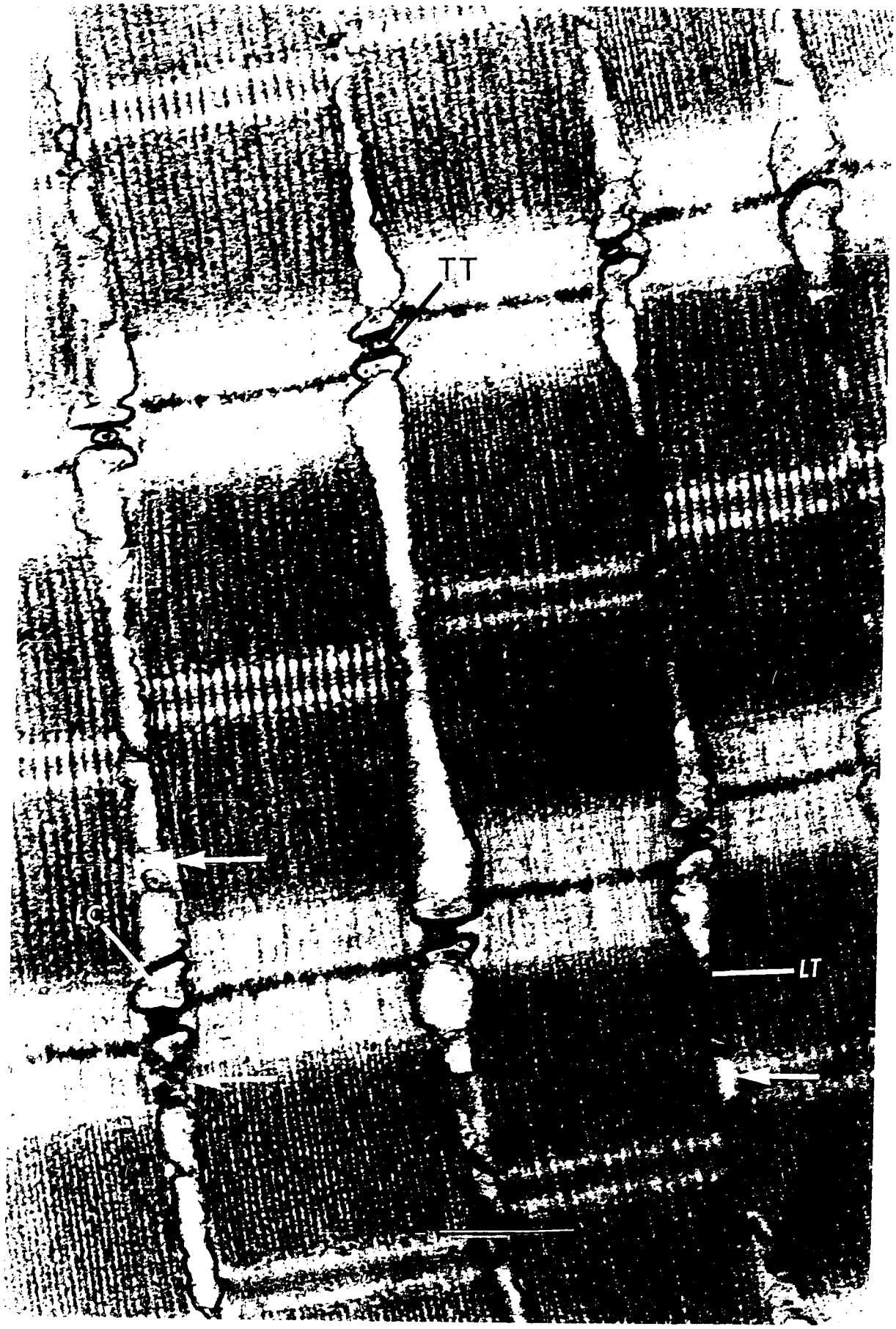

TT
LT

Fig. 88 The arrows show the openings of the "Fenestrated collar" of longitudinal sarcotubules of reticulum of white muscle fiber (superficial ventral flexor muscle). Interruptions of terminal cisternae are shown by arrowheads. Note rich glycogen content.

G., glycogen particles; LC., terminal cisternae; LT., longitudinal sarcotubule; TT., transverse tubule.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 51,600X.


TT
LC
LT
G

Fig. 89 The interconnection of opposing sets of actin
Fig. 90 filaments in the Z line of red (Fig. 89) and white (Fig. 90) muscles (superficial dorsal flexor muscle) are shown. Note the presence of greater density of extra-fibrillar material in Z line of red muscle (Fig. 89).

Z., Z line.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 102,200X.

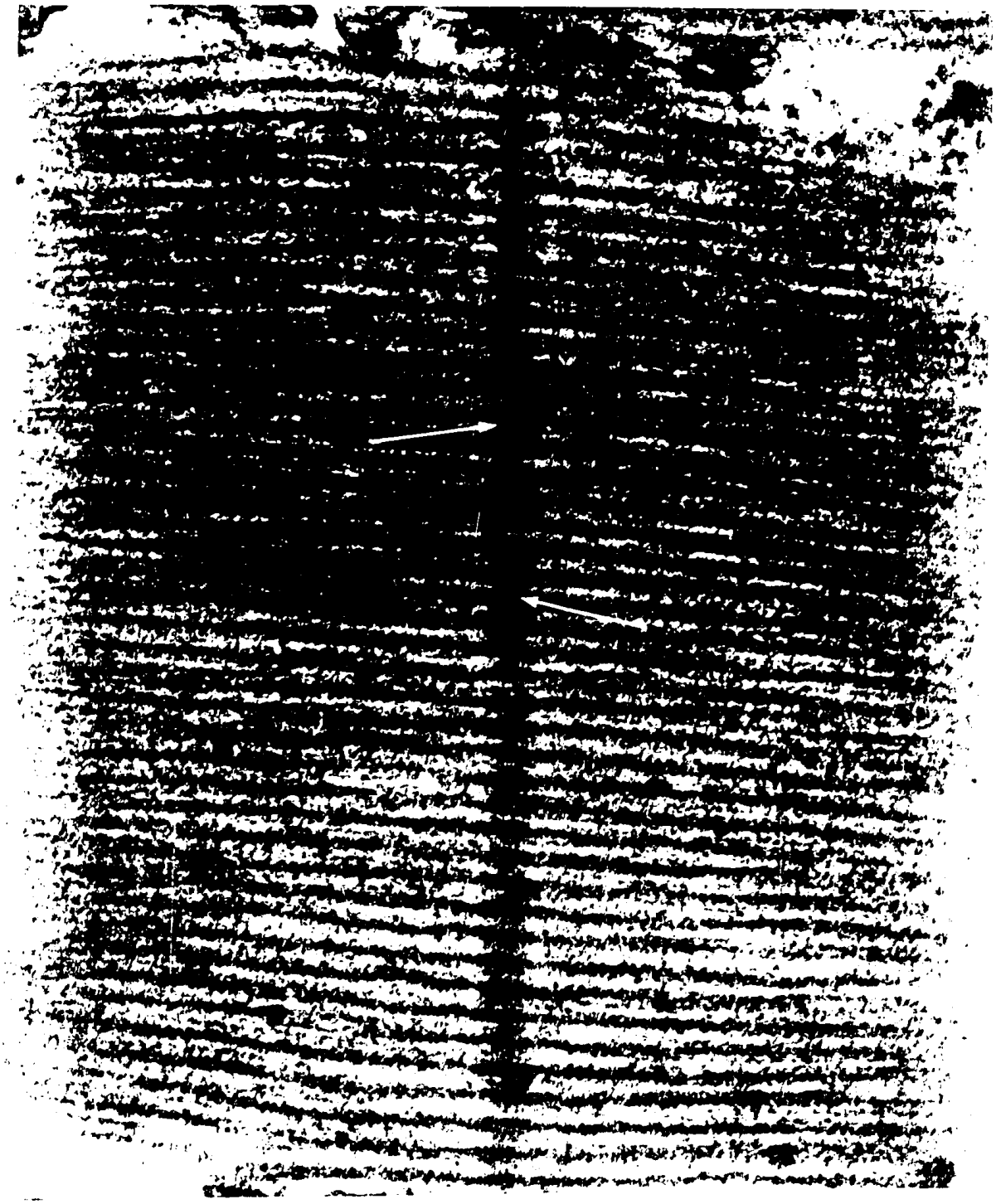

Z

Z
Z

Fig. 91 M line of red (Fig. 91) and white (Fig. 92) muscle
Fig. 92 fibers (superficial ventral flexor muscle). Note the presence of more dense component in M line of red muscle fiber (Fig. 91). The arrows show thin lines within M line.

ML., M line.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 87,600X.



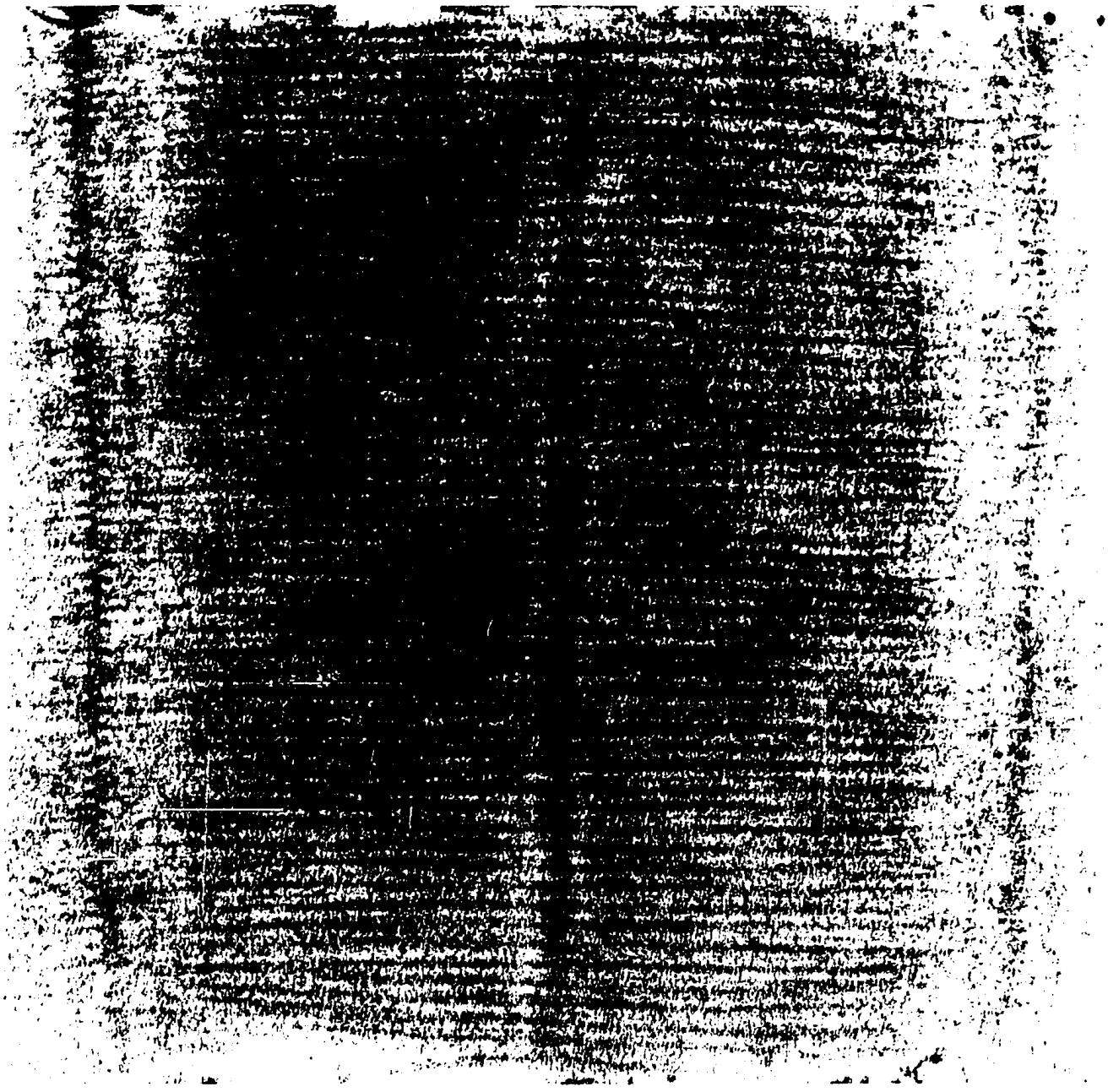


Fig. 93 (upper)
Fig. 94 (lower)

Transverse sections of red and white muscle fibers respectively (deep dorsal flexor muscle). Sections passed through M line, including parts of A band. Circles show six bridges of M line. Arrowheads show A band regions.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 87,600X.

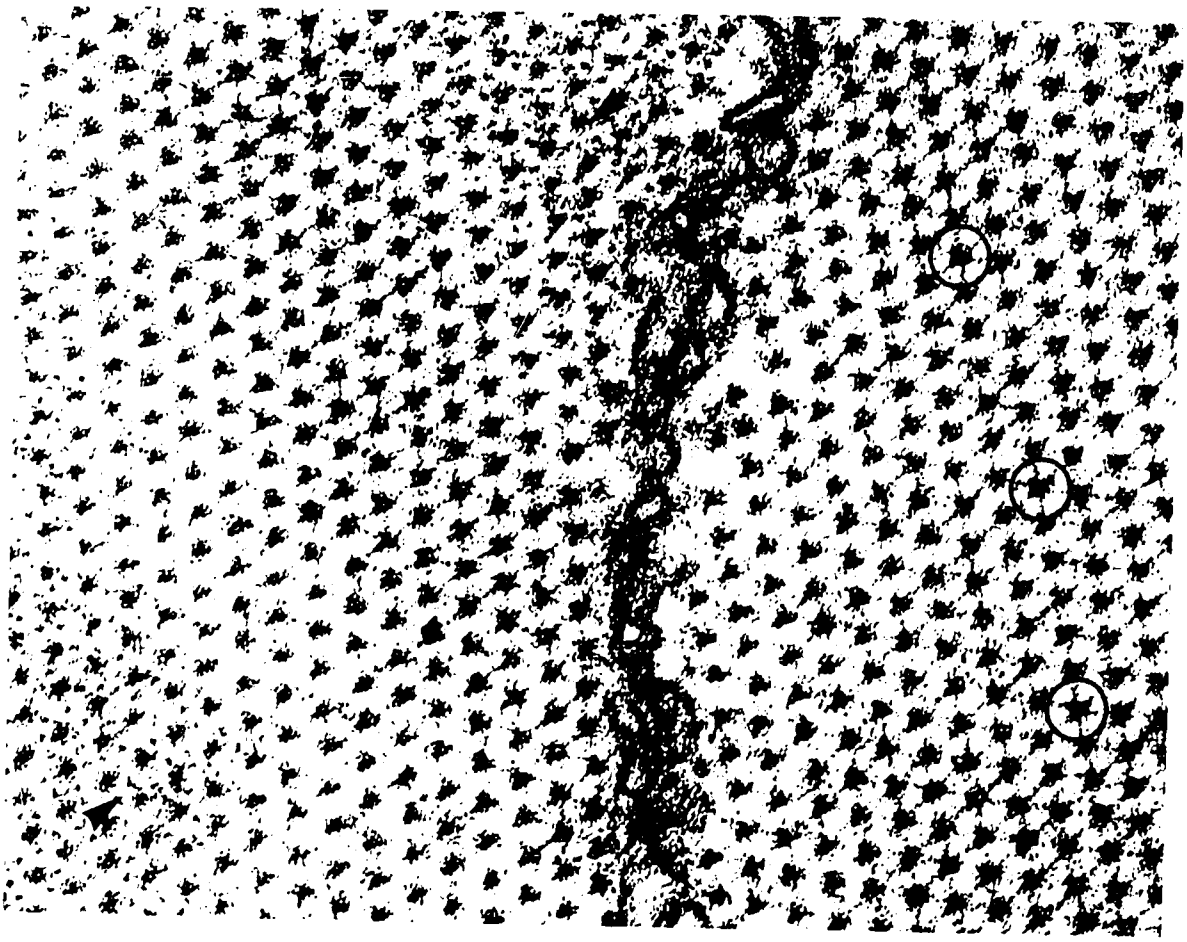


Fig. 95 A fibroblast is situated close to the sarcolemma of red muscle fiber (deep ventral flexor muscle). Note collagen fibrils outside the sarcolemma are in close association with the fibroblast.

C., collagen fibrils; Fb., fibroblast;
S., sarcolemma.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.


C
IFB
S

Fig. 96 Peripheral nuclei of red muscle fibers (deep dorsal flexor muscle). Note a part of a capillary at the upper left corner.

C., collagen fibrils; CP., capillary; N., nucleus; S., sarcolemma.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.

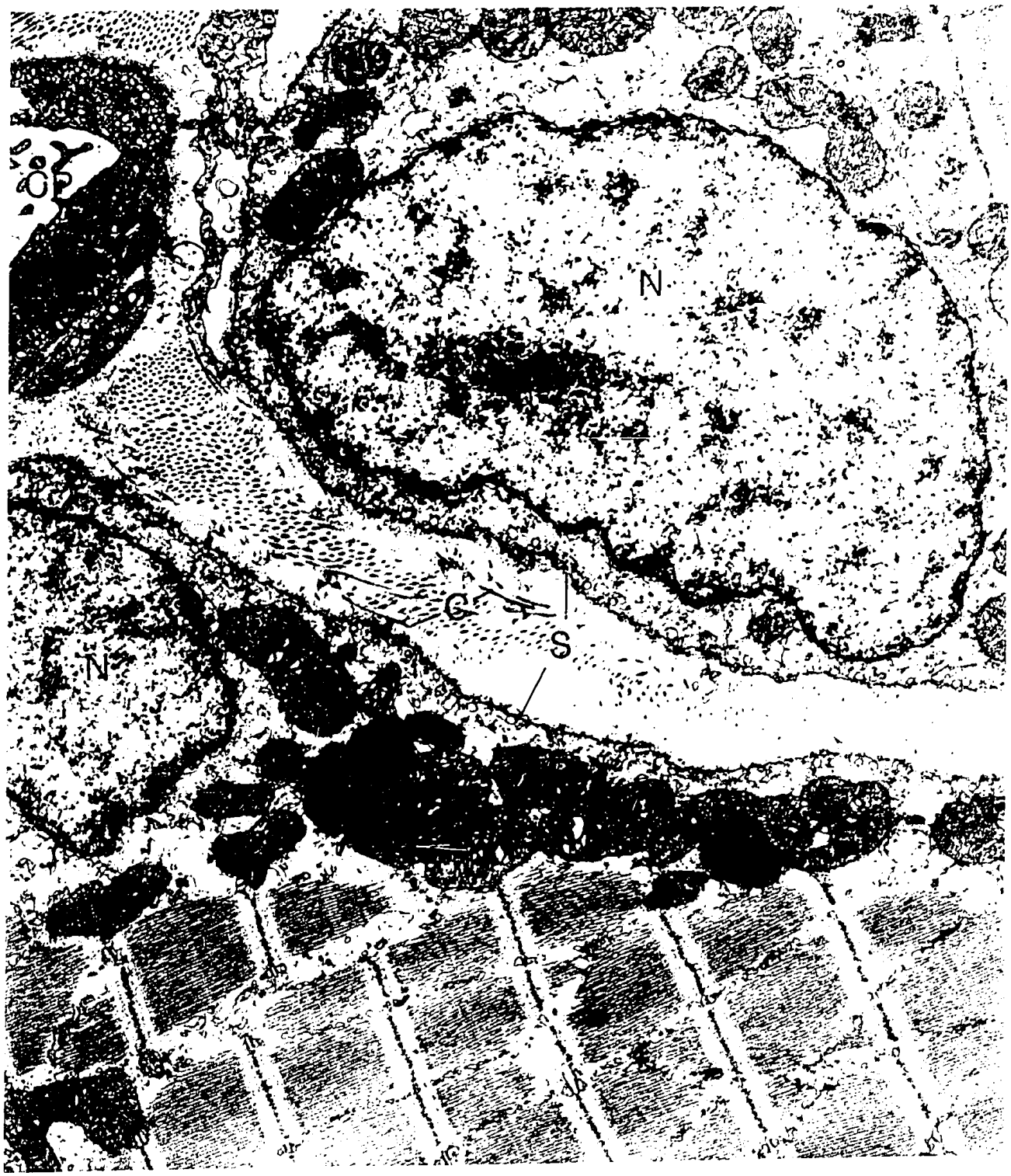

N
N
G
S

Fig. 97 (upper)
Fig. 98 (lower)

The arrows in the figures show pinocytosis vesicles of red muscle fibers (superficial flexor muscles).

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate.

51,600X; 87,600X.

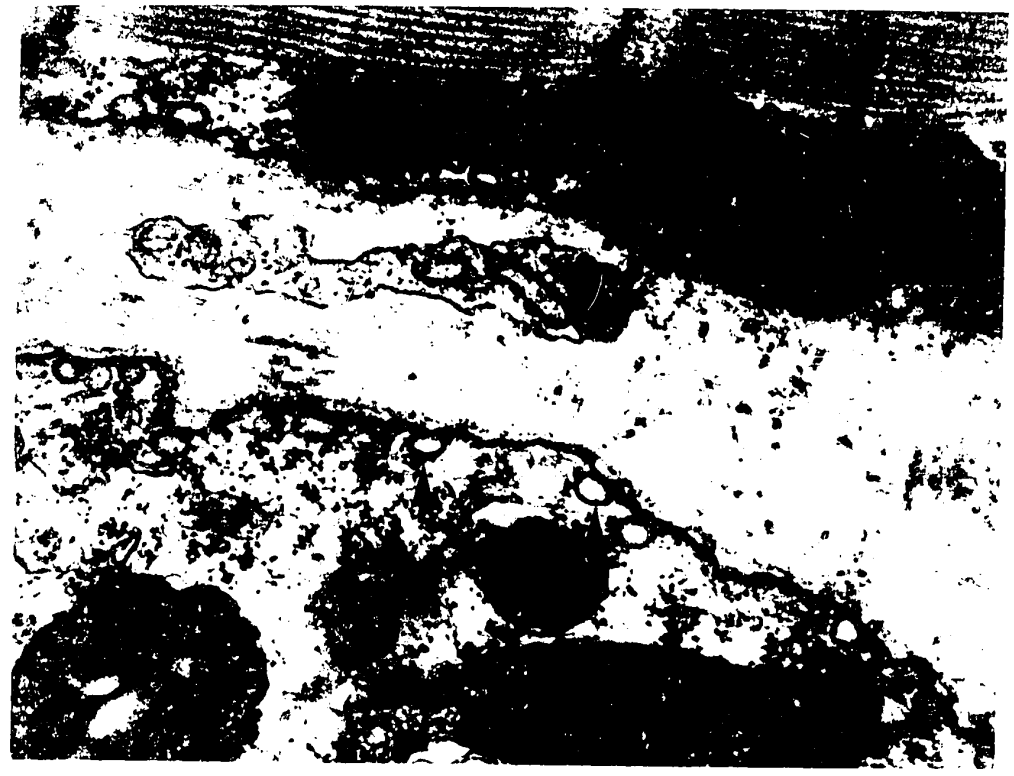


Fig. 99 A myosatellite cell situated close to the sarcolemma of red muscle fiber (hypochordal longitudinal muscle). The arrows show that the myosatellite cell is situated under the protein-polysaccharide coating of the muscle fiber.

L., lipid droplet; MC., myosatellite cell; S., sarcolemma.

Glutaraldehyde-osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 68,800X.


L
MC
S

Fig. 100 White muscle fibers of a four-week old embryo are shown in a transverse section. Note well-organized myofibrils and peripherally situated nucleus of central white muscle fiber.

N., nucleus.

Glutaraldehyde-paraformaldehyde, Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate.

15,600X.

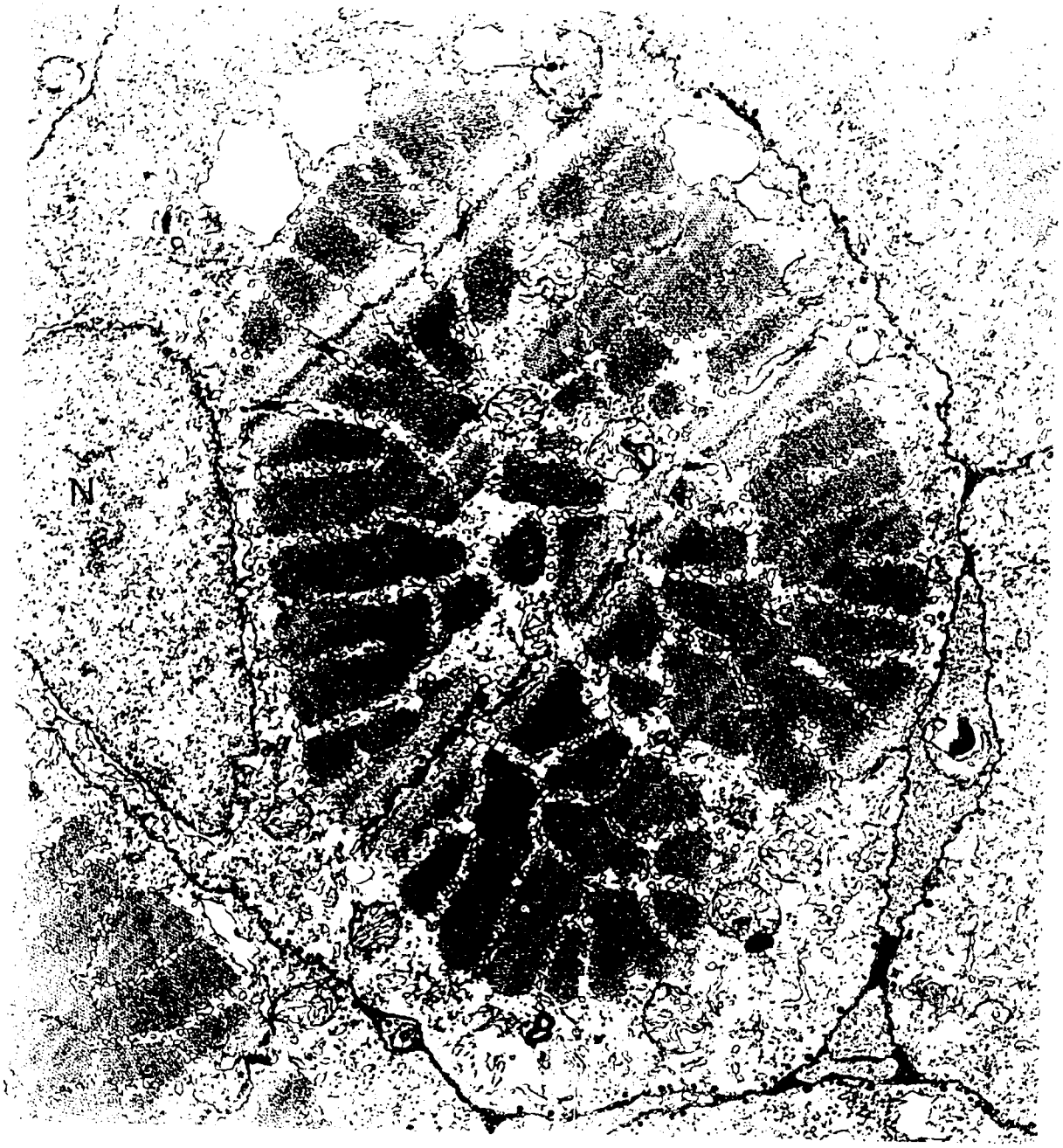

N

Fig. 101 Four-week old embryo. A number of undifferentiated cells and a number of cells containing myofilaments are shown. The arrows show the myofilaments.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.

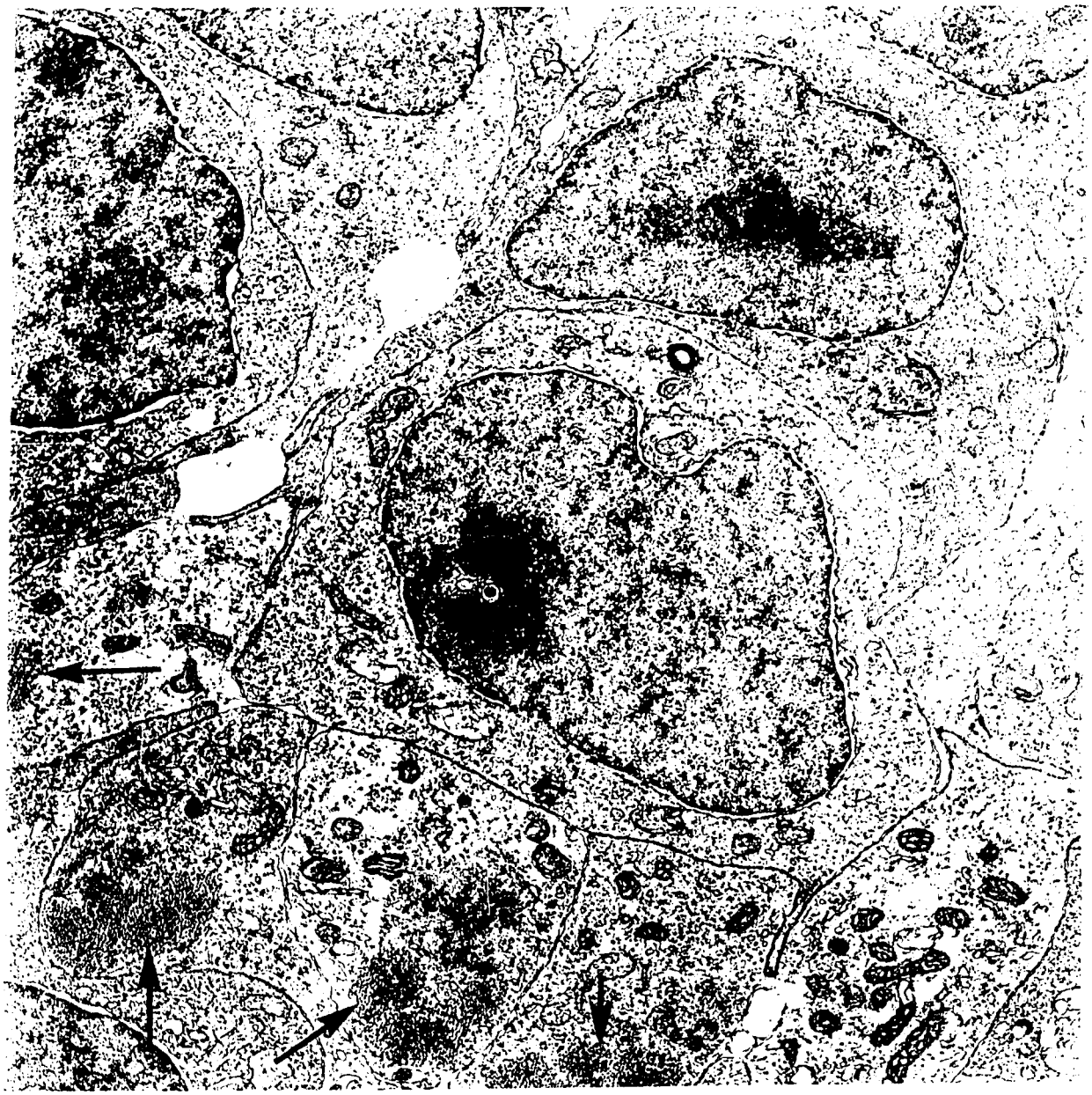
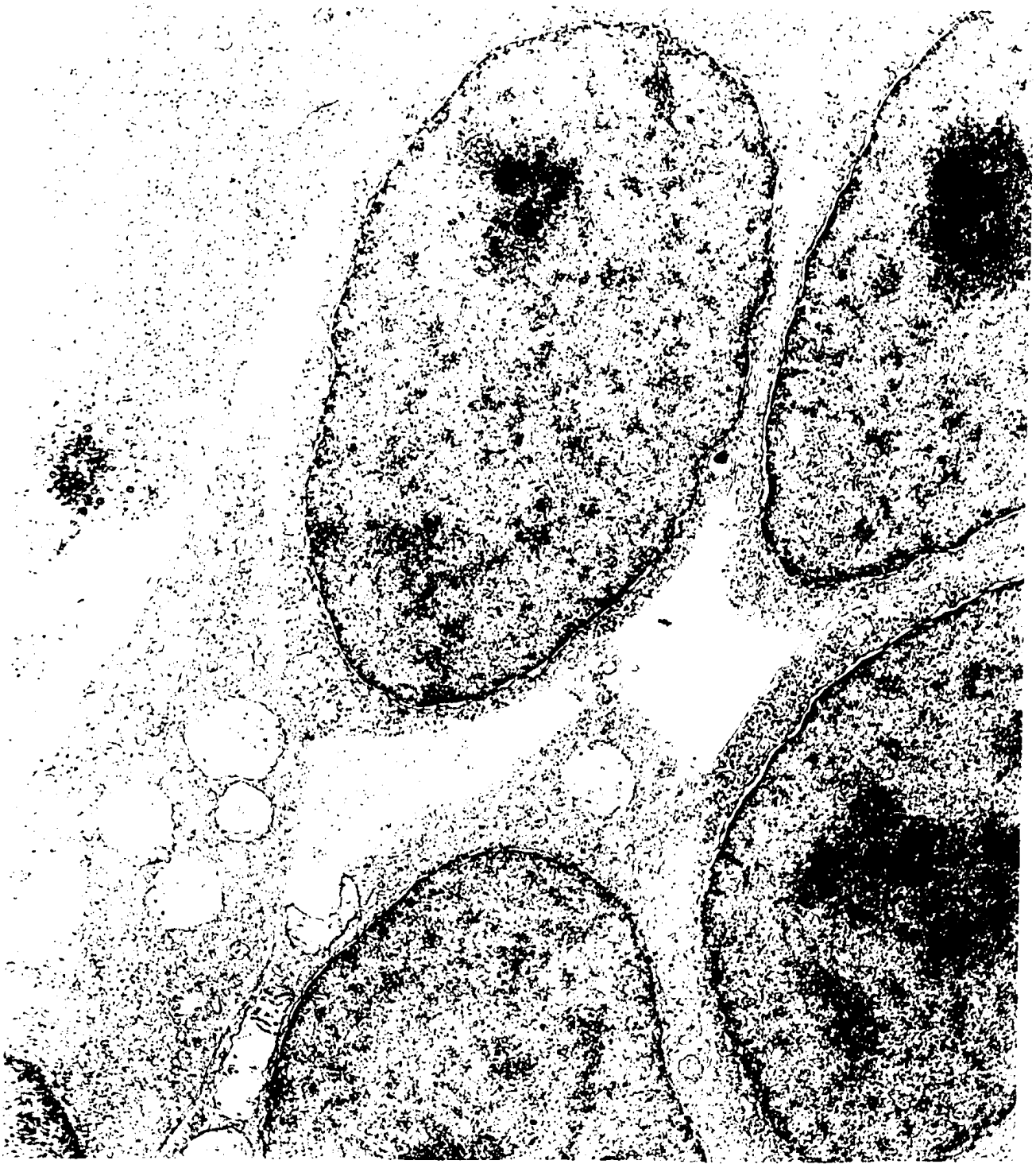


Fig. 102 Four-week old embryo. A number of undifferentiated cells with abundant free ribosomes and scanty rough-surfaced endoplasmic reticulum in cytoplasm is shown.

Fig. 103 Four-week old embryo. A number of undifferentiated cells with abundant rough-surfaced endoplasmic reticulum and scanty free ribosomes in the cytoplasm is shown.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 26,400X.



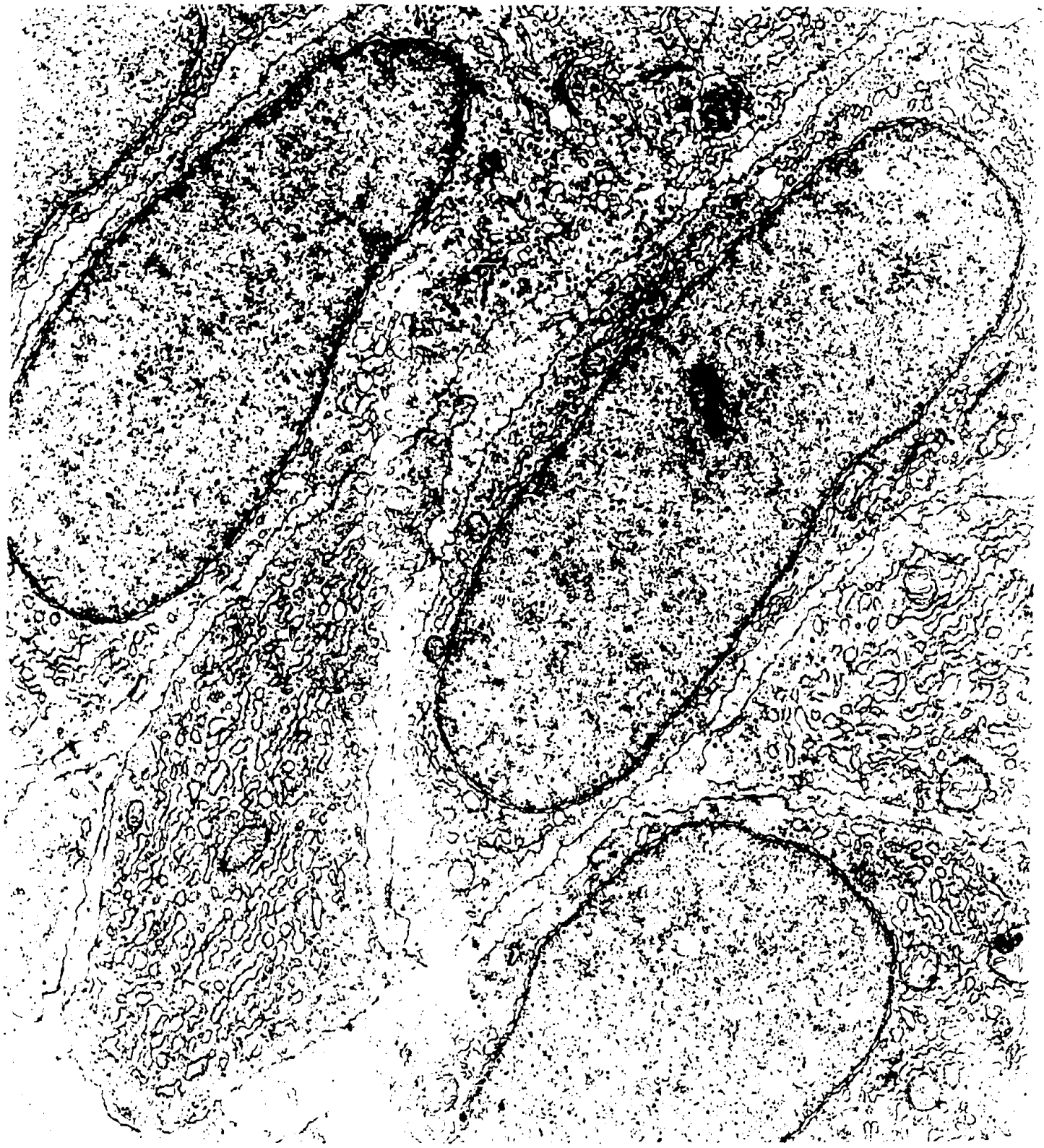


Fig. 104 Four-week old embryo. A number of cells with abundant rough-surfaced endoplasmic reticulum in the cytoplasm is in association with collagen fibrils, while the cells with abundant free ribosomes and myofilaments are organized in a group beneath them. The arrows show myofilaments.

C., collagen fibrils; N., nucleus.

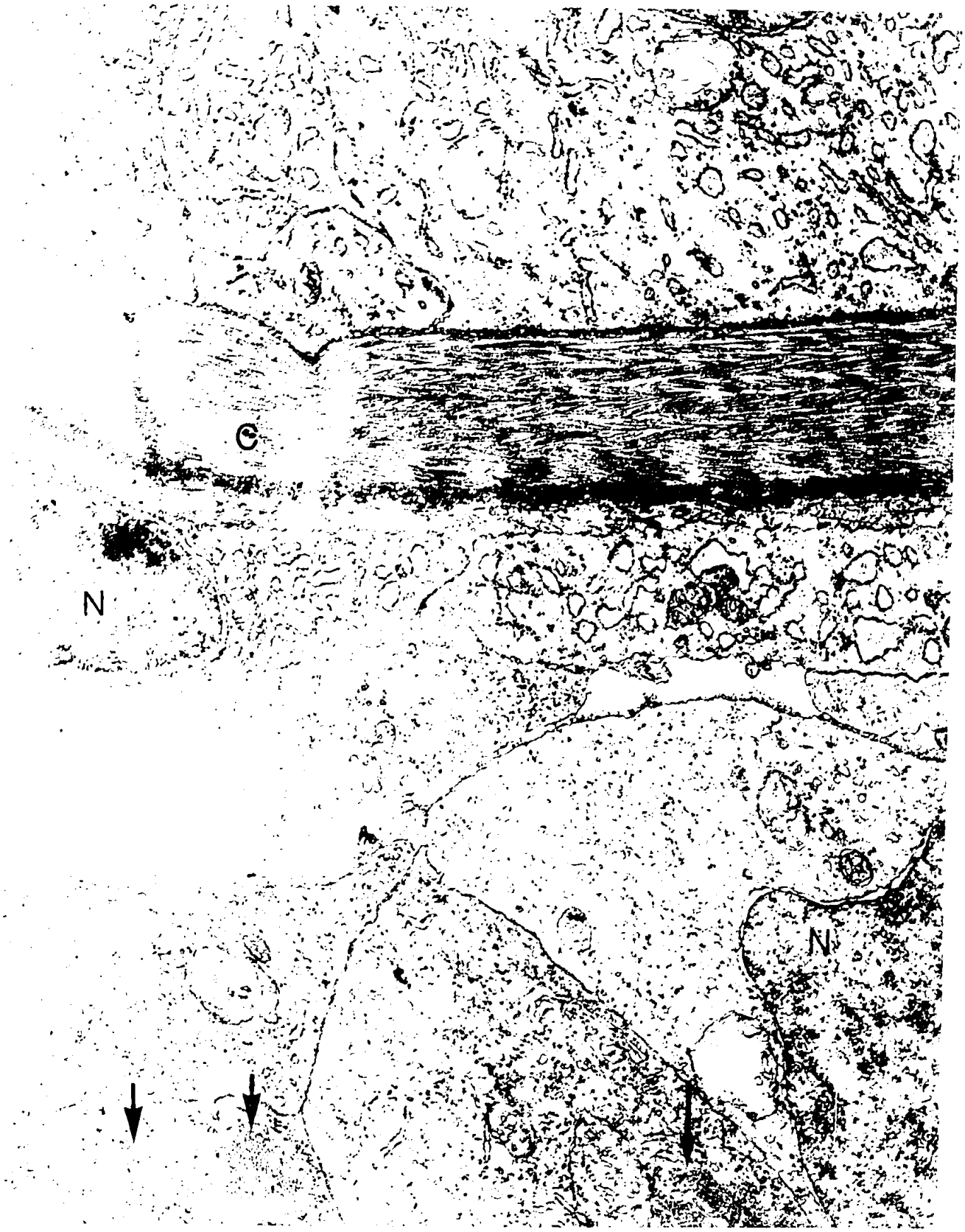

C
N
N

Fig. 105 A group of undifferentiated cells with their closely apposed plasma membranes. The arrows show the region of contact between two cells where plasma membranes are not clearly discernible.

L., lipid droplet; M., mitochondria.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.

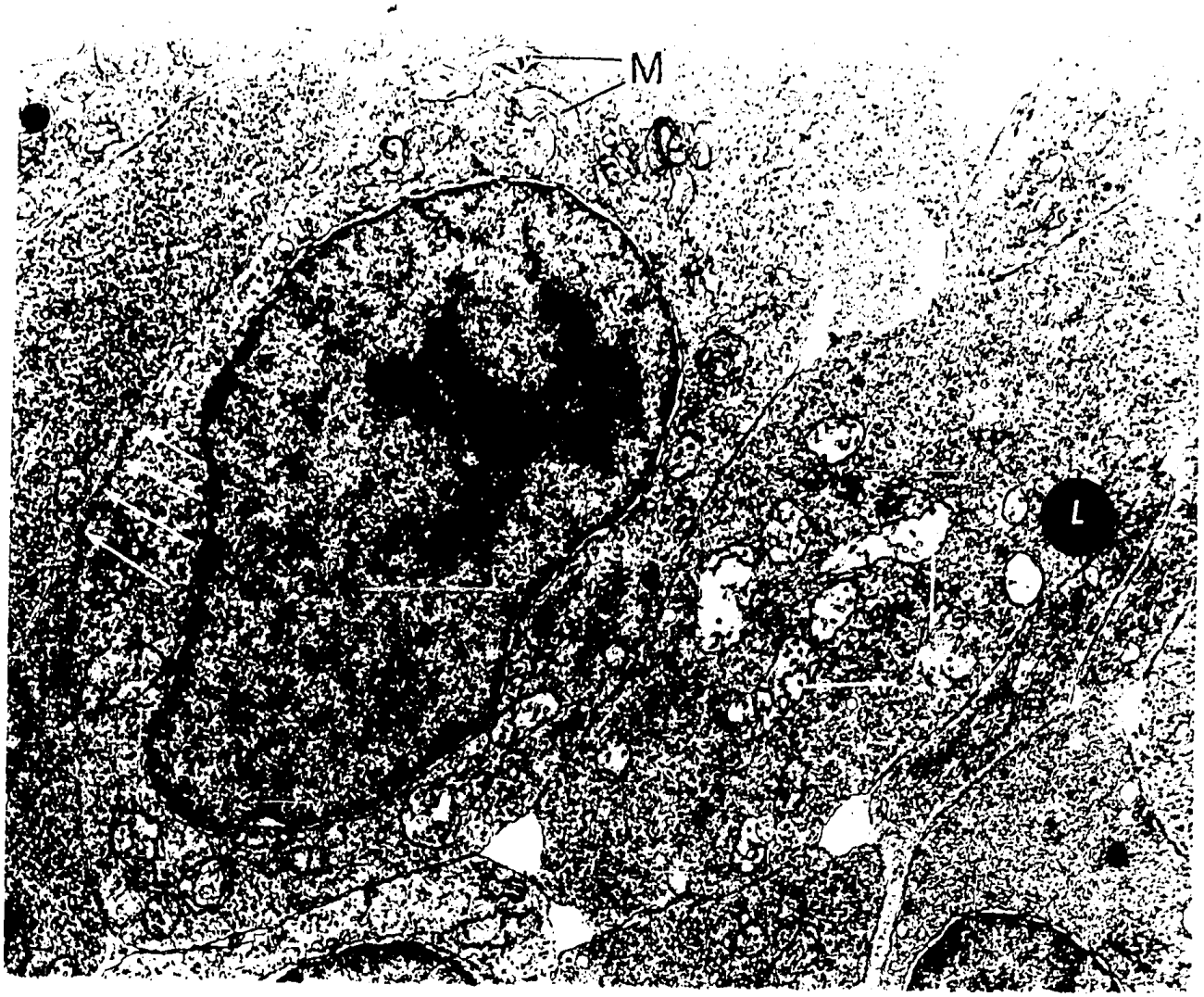

M
L

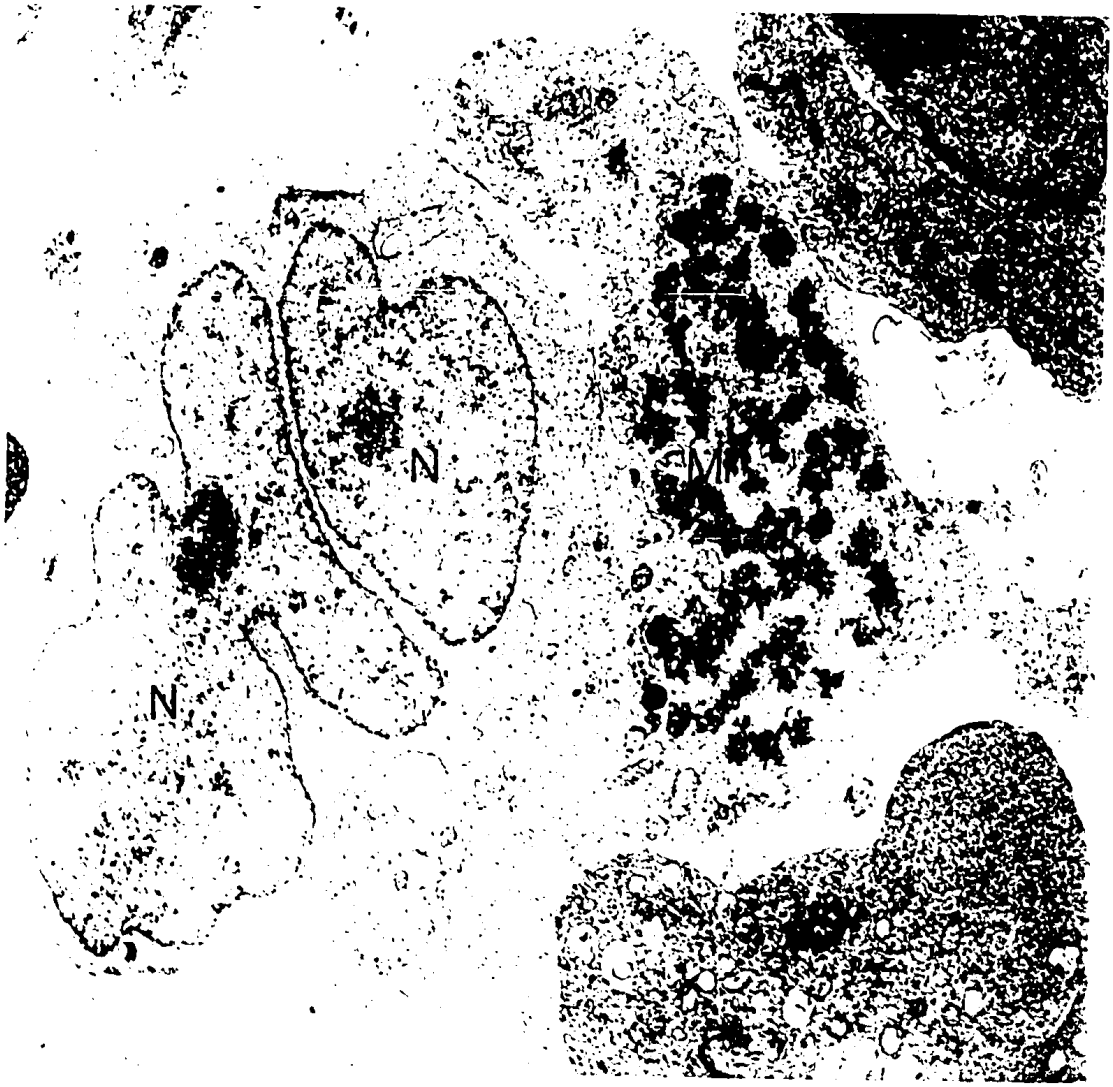
Fig. 106 Low power electronmicrograph showing an undifferentiated cell containing two nuclei and another with a mitotic figure.

Fig. 107 This is a higher magnification of a part of the above figure showing more clearly the mitotic figure of an undifferentiated cell.

MI., mitotic figure; N., nucleus.

Glutaraldehyde-paraformaldehyde, Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate.

7,200X; 35,200X.


N
N
M
C

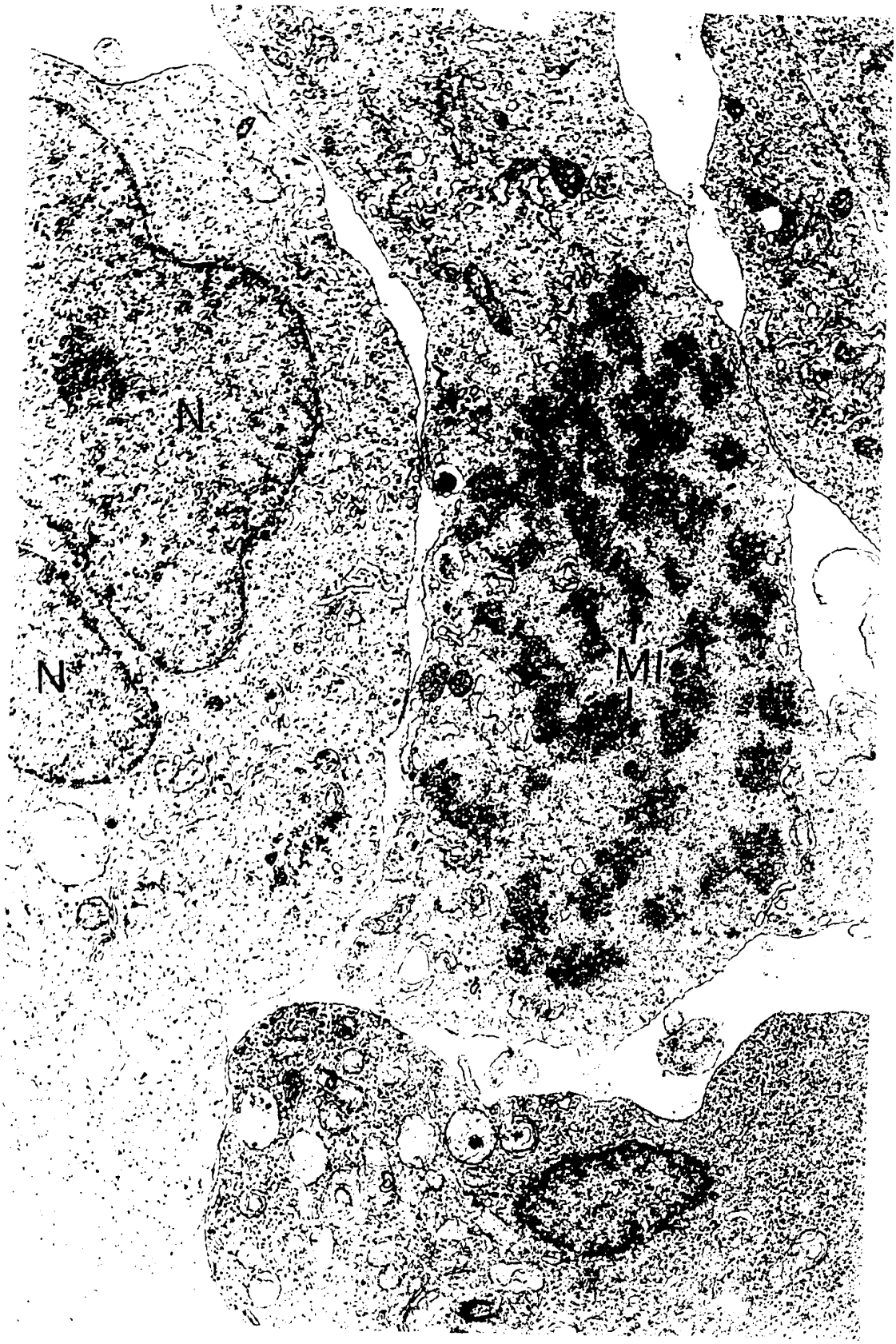

N
N
MI

Fig. 108 A myotube is embraced anteriorly by two pseudopodia of an undifferentiated cell. Note a pseudopodium from an undifferentiated cell lying in an interspace between two myotubes, separating them from each other.

PS., pseudopodium.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 26,400X.

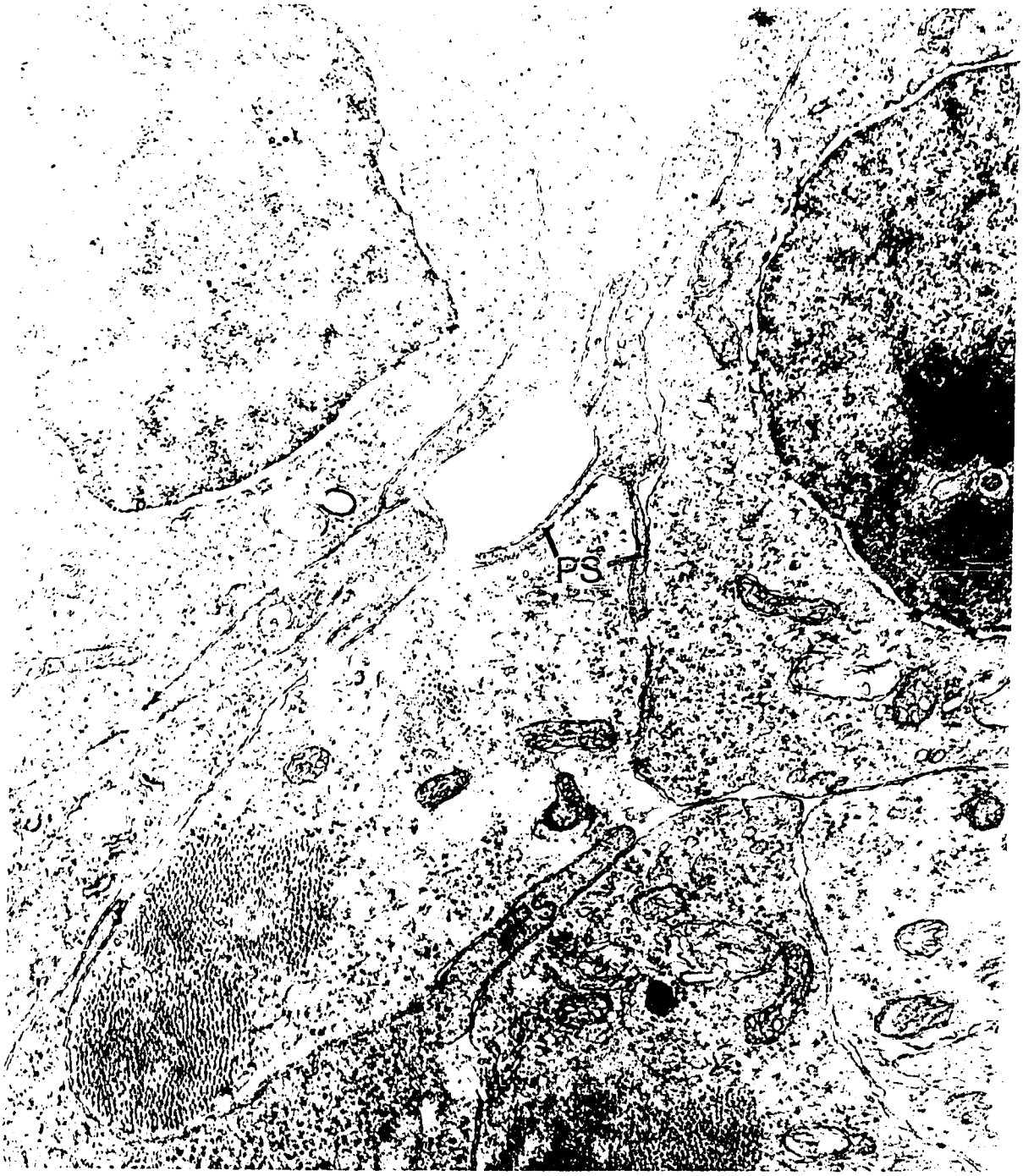

PS

Fig. 109 The arrows show the region of contact between two cells where plasma membranes are not clearly discernible.

PS., pseudopodium.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 51,600X.

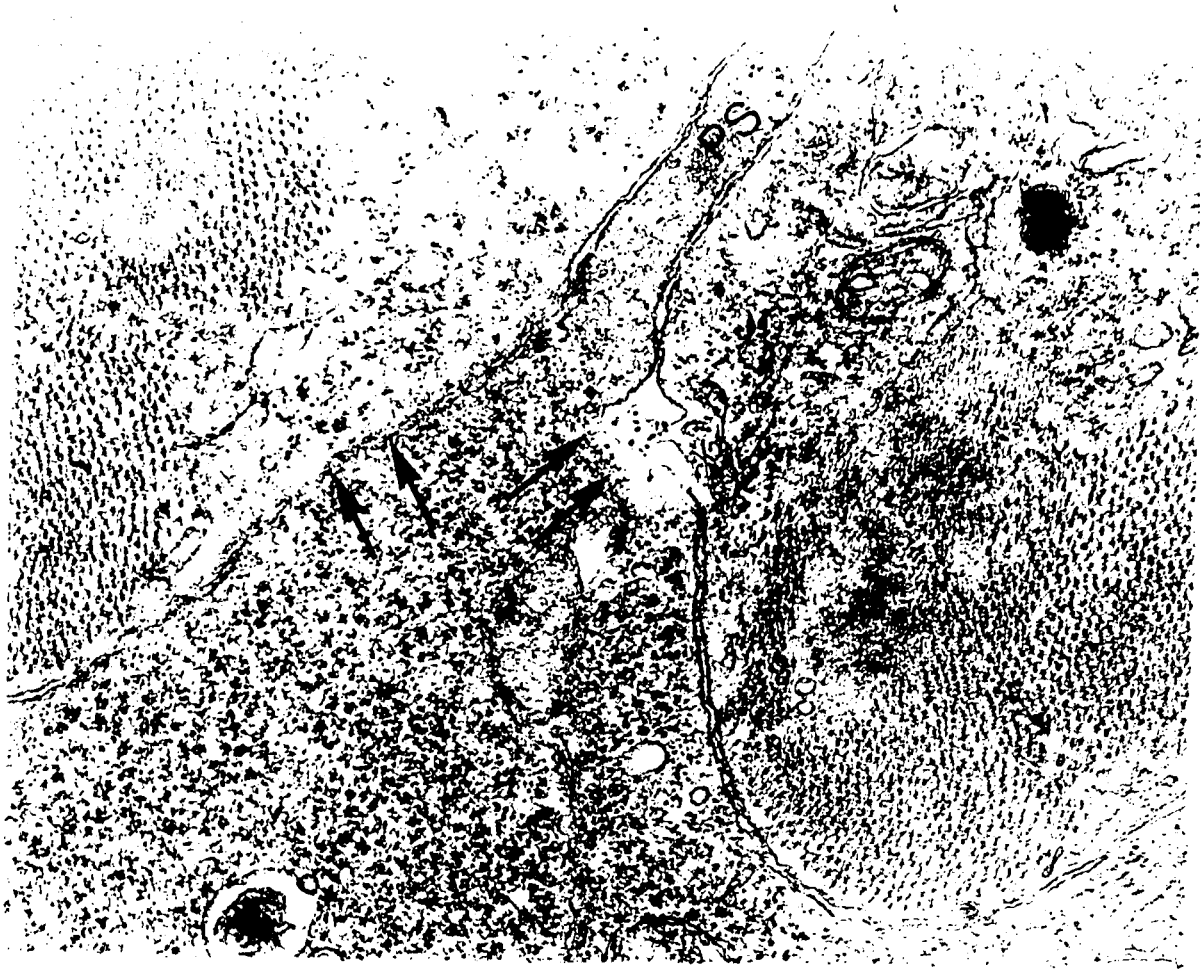


Fig. 110 A pseudopodium of an undifferentiated cell is occupying a depression on the wall of another undifferentiated cell. The arrow shows the region of contact between cells where plasma members are not clearly discernible. Note that a pseudopodium is lying in an interspace between two undifferentiated cells.

PS., pseudopodium; N., nucleus.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 51,600X.

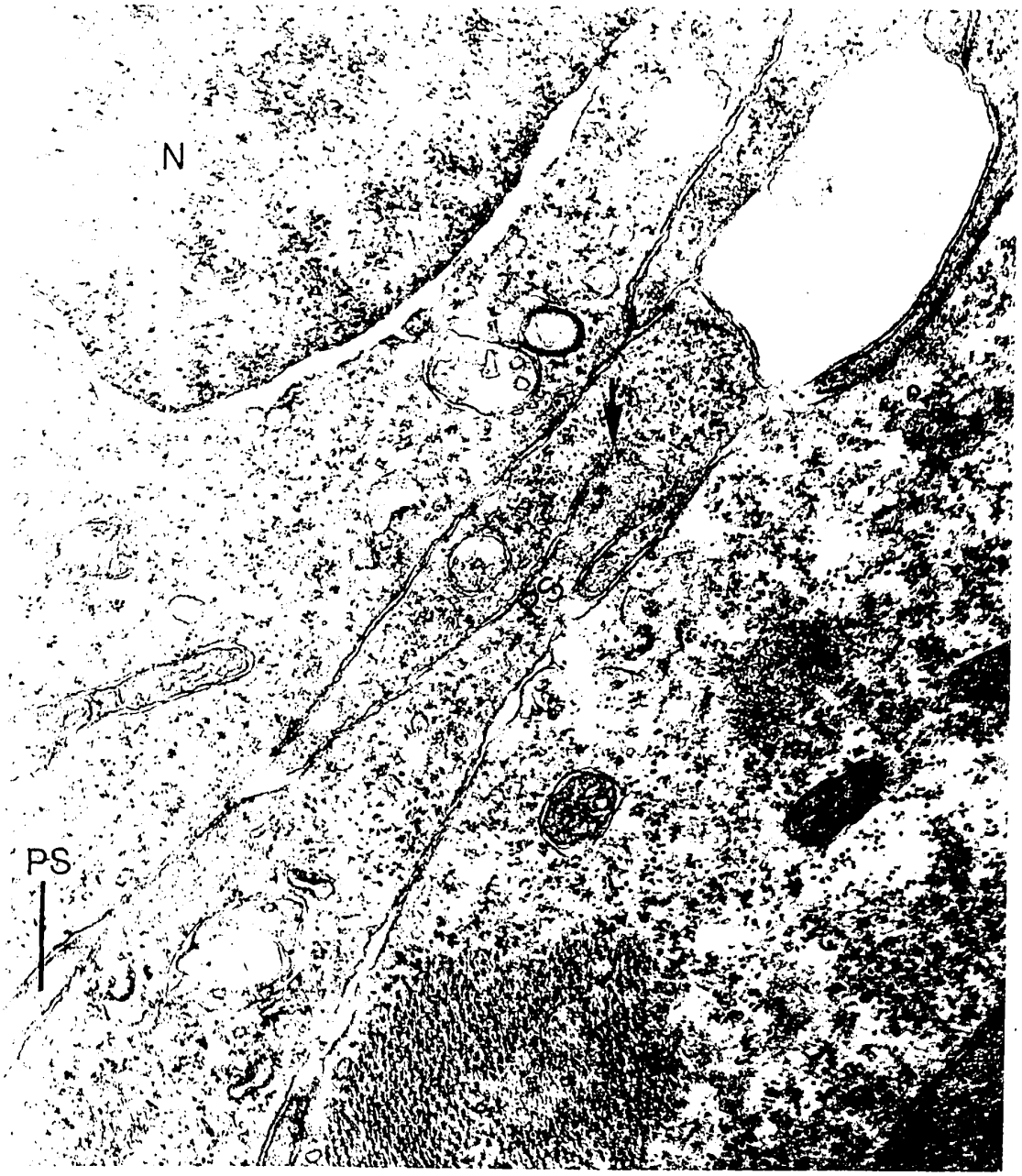

N
PS

Fig. 111 Sac fry. Myotubes with well-differentiated myofilaments. The arrows show discontinuities of apposed plasma membranes.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.

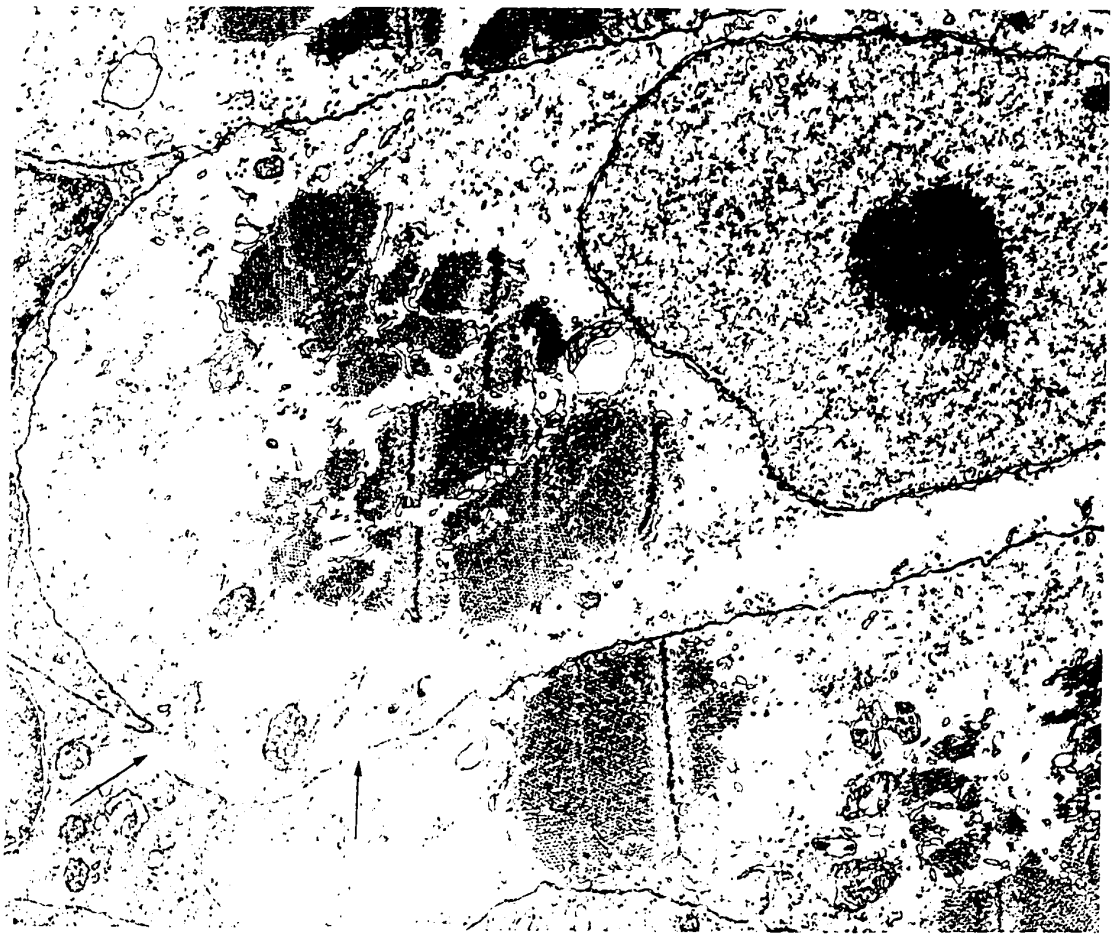


Fig. 112 Sac fry. Muscle cells with traces of organization of band patterns. Note undifferentiated cells (A, B, and C) in association with them.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 15,600X.

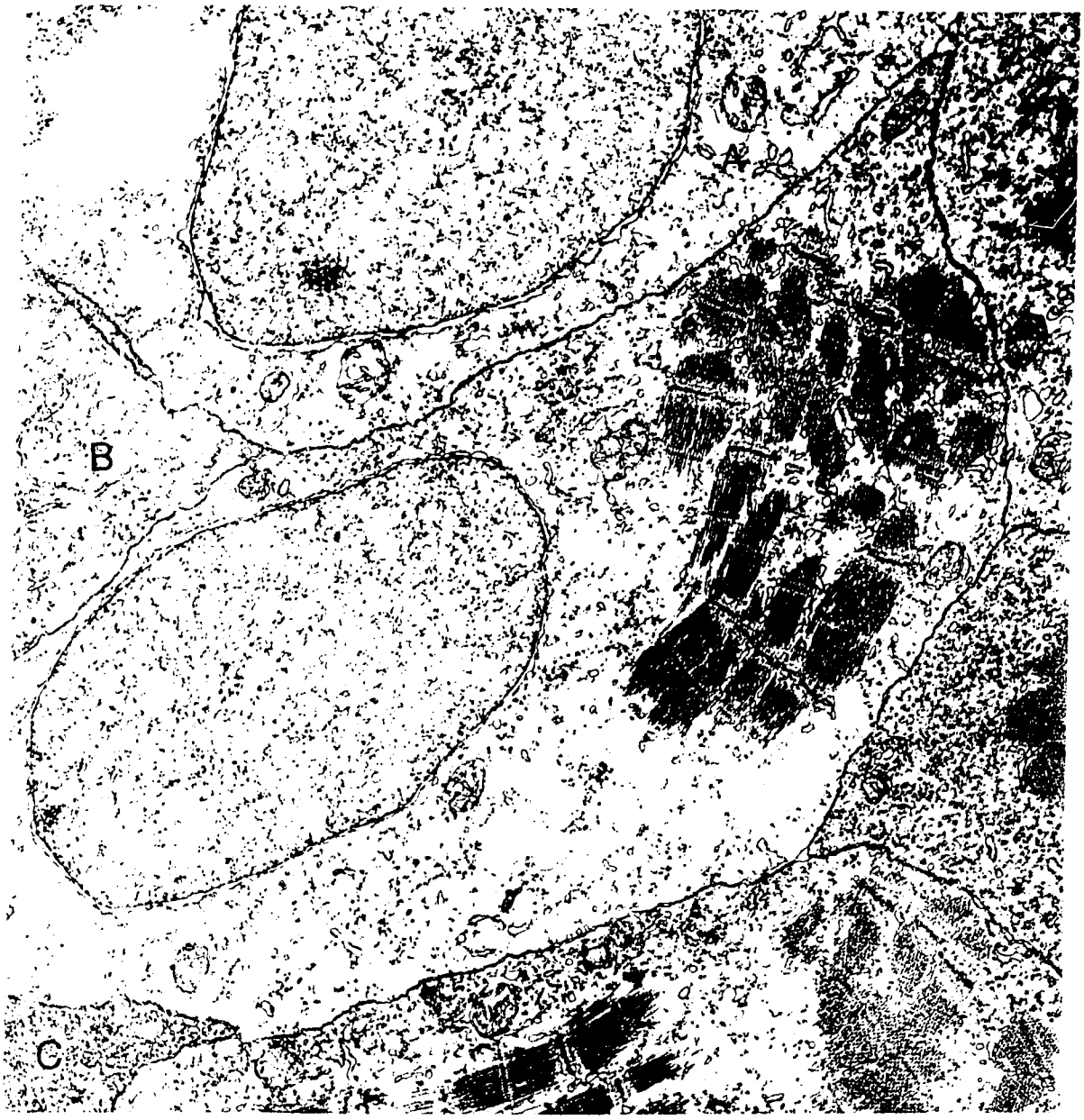
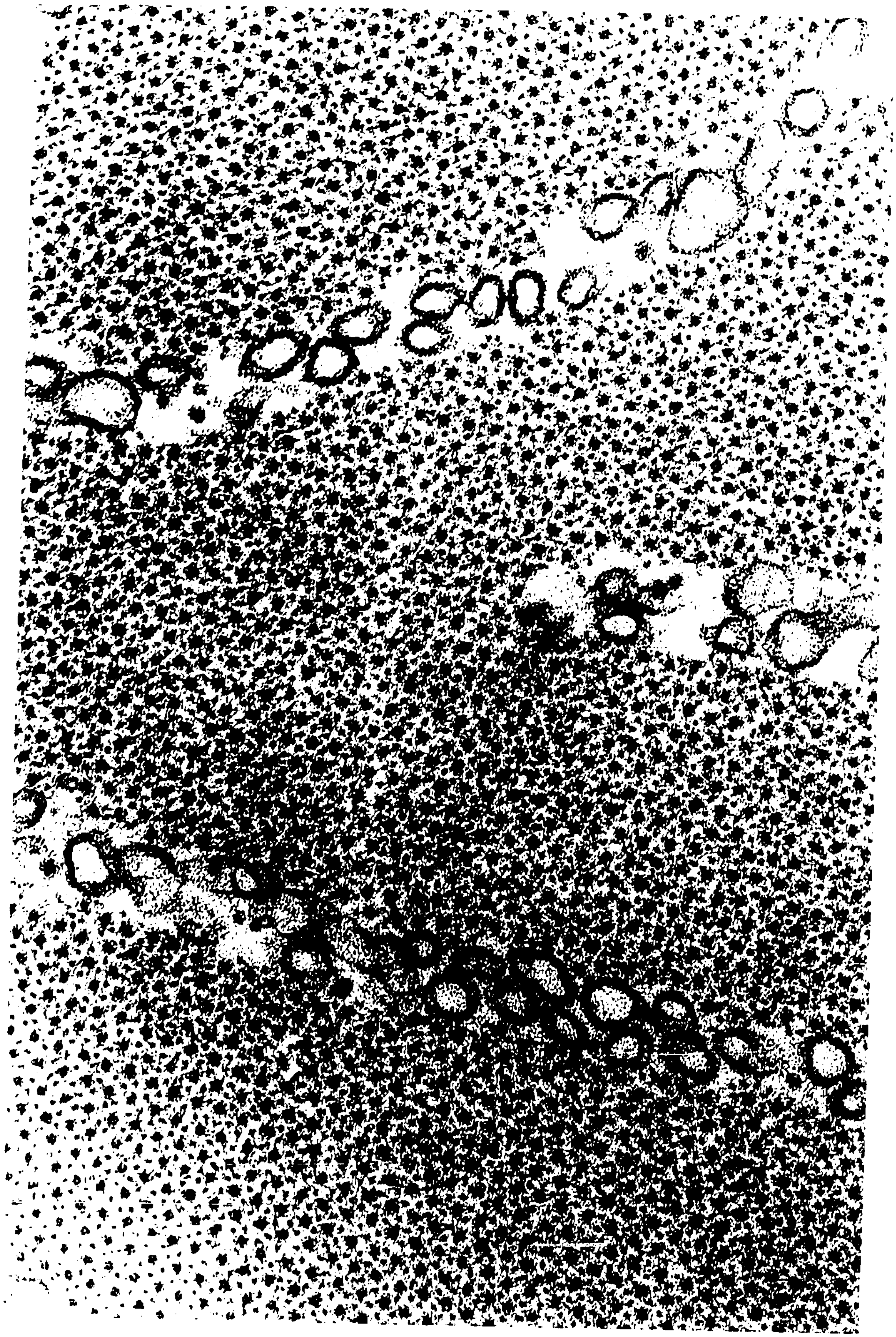

A
B
a
C

Fig. 113 Actin and myosin filaments of white muscle fibers
Fig. 114 of embryo and sac fry respectively. Note hexagonal arrangement of myofilaments.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Uranyl acetate, and Lead citrate. 146,000X.



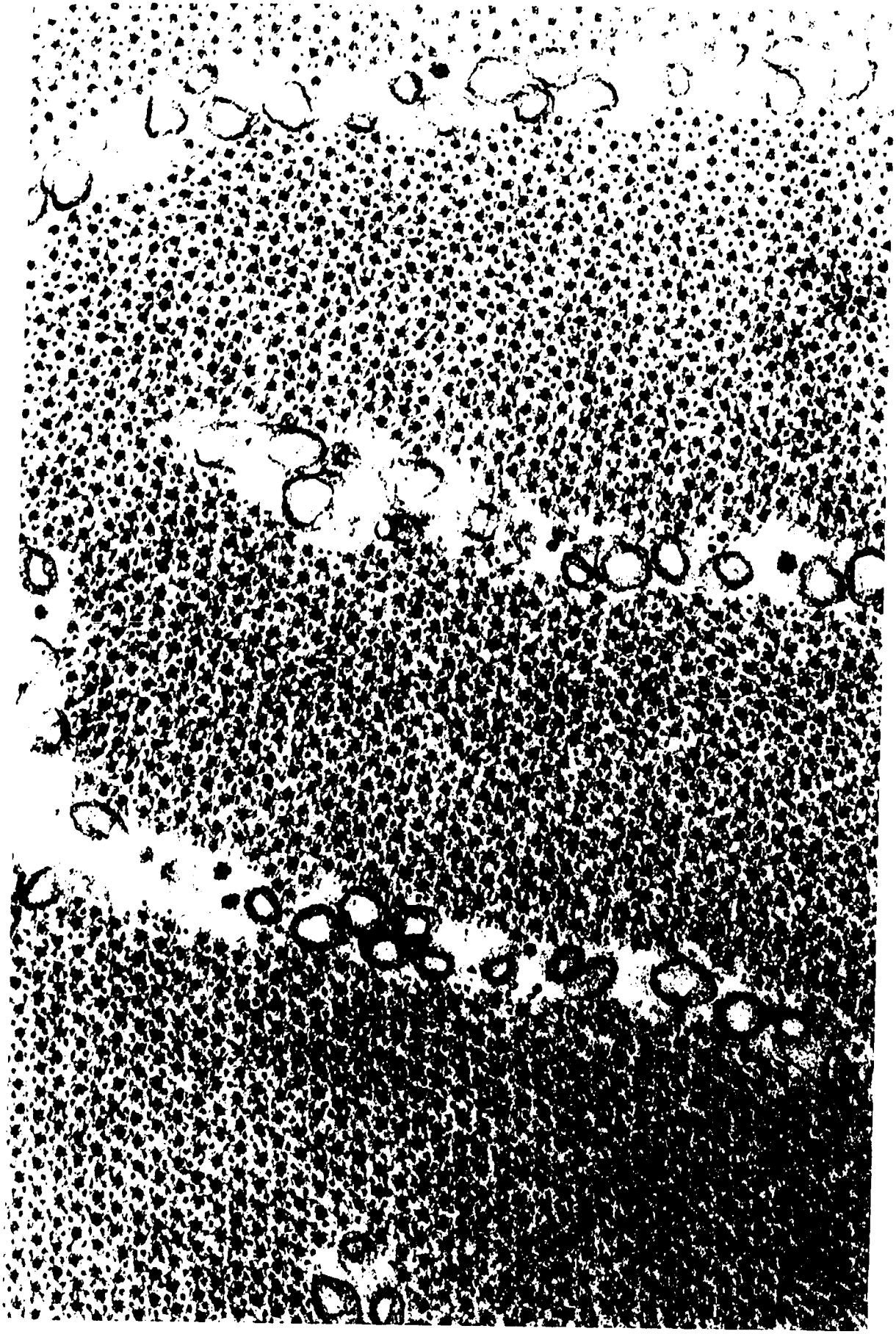


Fig. 115 Early free swimming specimen. Well-differentiated red muscle fiber. Note large number of mitochondria containing numerous cristae, lipid droplets containing electron-translucent central zones, and most of the myofibrils confluent with one another.

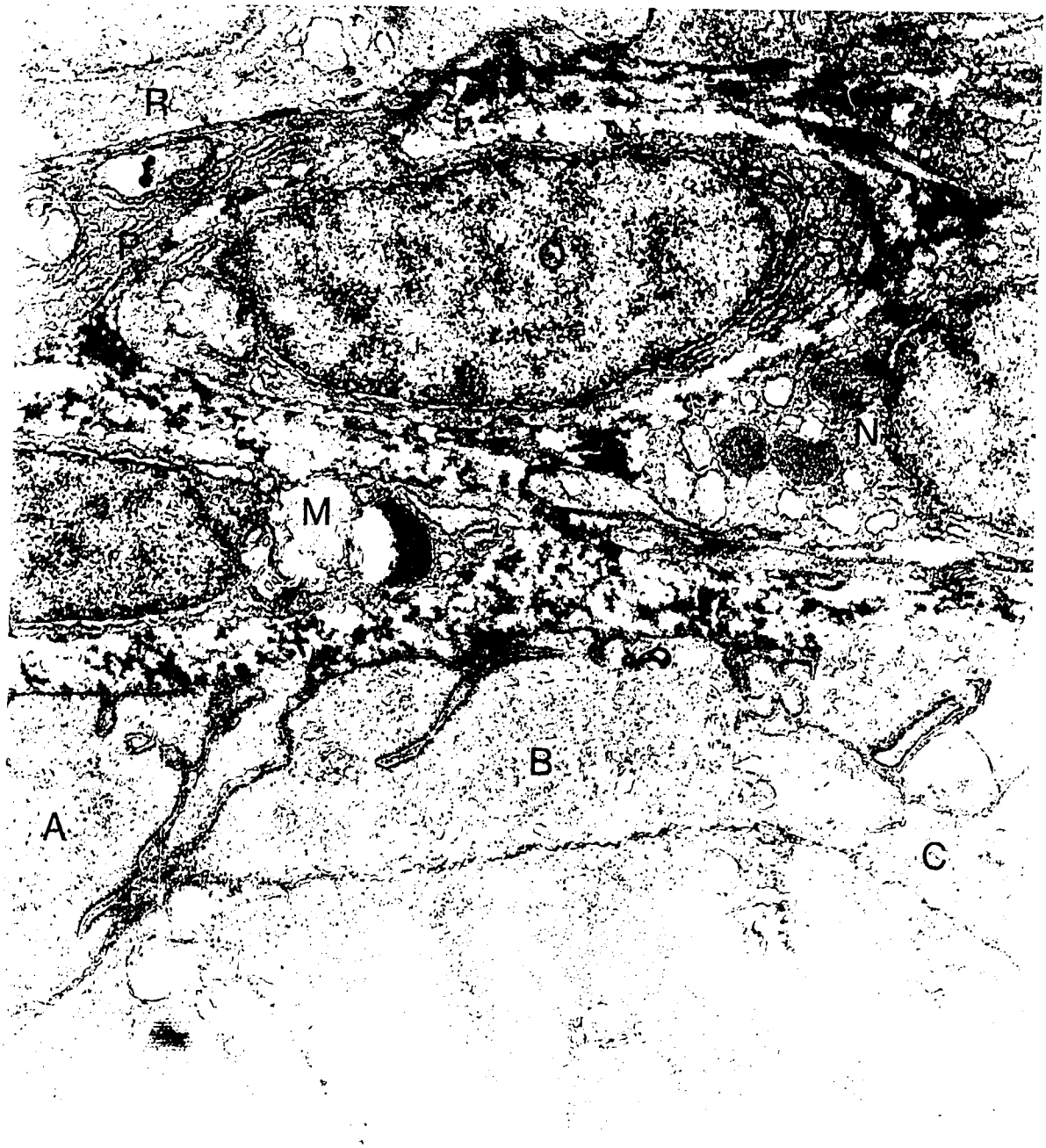
L., lipid droplet; M., mitochondria.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 21,000X.


L
L

Fig. 116 Early free swimming specimen. A part of differentiated red fiber in association with undifferentiated cells (A, B, and C). Some fibroblasts (M, N, O, P, Q, and R) are seen outside the muscle cells zone.

Glutaraldehyde in salmonid physiological solution - Osmium tetroxide, Araldite, Uranyl acetate, and Lead citrate. 26,000X.


R
M
N
A
B
C