DEVELOPMENT OF THE LATERAL MUSCULATURE IN THE TELEOST, *BRACHYDANIO RERIO*: A FINE STRUCTURAL STUDY

By

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We have carefully read the dissertation entitled Development of the lateral musculature in the teleost, Brachydanio rerio: A fine structural study. **submitted by** Robert Earle Waterman. **in partial fulfillment of the requirements of the degree of** Doctor of Philosophy **and recommend its acceptance. In support of this recommendation we present the following joint statement of evaluation to be filed with the dissertation.**

This dissertation fully meets our expectations and the University requirements for the degree of Doctor of Philosophy. It is a scholarly contribution to the field of developmental anatomy.

Mr. Waterman has accomplished an extensive and penetrating survey of the scientific literature relating to structure, function and development of myotomal muscle. He has provided in his investigations a significant and fundamental addition to that body of knowledge, particularly in regard to myotomal development and fine structure in fish. In the course of his study he has been able to demonstrate important similarities and differences between fish body musculature development and that of more widely studied vertebrates. The dissertation has also posed several problems and avenues for future investigation. Among these are the development of the sarcoplasmic reticulum and T-system in slow and fast muscle cells; the origin of collagen within the developing myotome; the relationship of emerging embryonic behavior to the process of myofilament formation and the acquisition of neuromuscular junctions; and the significance of intercellular communication via specialized myo-muscular junctions during early phases of myotomal development. It is clearly written and arranged, and is unusually well illustrated.

We enthusiastically recommend its acceptance.

DISSERTATION READING COMMITTEE: Douglas E. Kelly

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DEVELOPMENT OF THE LATERAL MUSCULATURE IN THE TELEOST, *BRACHYDANIO RERIO*: A FINE STRUCTURAL STUDY

INTRODUCTION

A wide range of variation in the morphology, biochemistry and function of skeletal muscle is known to exist within the animal kingdom. The composition of muscles may vary not only from species to species, but also from muscle to muscle, and within the same muscle of an individual animal.

Several comparative classifications of striated muscle fibers into two or three fiber types have been established, based on structural or physiological criteria. Among these are: red and white fibers (numerous authors); "Sarkoplasmareiche" and "Sarkoplasmaarme" fibers (Knoll, 1891); dark and light, opaque and clear fibers (Denny-Brown, 1929); "schmale" and "dicke" or "a- and b-fibers" (Wohlfart, 1937); fibers with "Felderstruktur" and fibers with "Fibrillenstruktur" (Krüger, 1950); narrow and broad fibers (Boddeke *et al.*, 1959); red, white and intermediate fibers (Ogata and Mori, 1964; Gauthier and Padykula, 1966); A, B and C fibers (Stein and Padykula, 1962), tonic and phasic fibers (Sommerkamp, 1928; Wachholder and V. Ledebur, 1930); slow and fast fibers, small-nerve slow-muscle fibre system and large-nerve twitch-muscle fibre system (Kuffler and Vaughan Williams, 1953a,b); and slow and fast motor systems (Barets, 1952, 1961).

The fact that these fiber types may be intimately intermingled within the same muscle often hinders physiological examination of their properties. In this regard, the conspicuous trunk muscle of

fish received some attention. In the majority of species it has been shown to contain at least two main types of striated muscle fibers, and in many teleosts, these are separated into rather distinct portions of the lateral musculature. This thus provides a valuable system for selective study of the properties of distinctly different kinds of vertebrate muscle cells.

Several workers have indicated that in the development of the lateral musculature these main fiber types also exhibit structural differences at early stages. However, few embryological descriptions exist which equal in quality the studies of the adult upon which our current understanding of fish musculature rests. This investigation is an attempt to clarify the developmental history of teleost skeletal muscle fiber types by describing the fine structure of the myotome and associated tissue at several developmental stages.

HISTORICAL REVIEW OF THE PROBLEM

I. ADULT MORPHOLOGY AND PHYSIOLOGY

A. Gross Anatomy

The somatic, or skeletal muscle of fishes may be categorized under the following divisions: muscles of the head, of the fins (paired and unpaired), of the pelvic and pectoral girdles, and of the trunk. The large trunk muscles, which constitute the main muscular mass, were first described in detail by Cuvier (1828). Basing his observations primarily on the perch, Cuvier considered the lateral muscle of each side as a single unit homologous to the sarcospinalis muscle of higher forms. Subsequent work, however, has shown that each lateral muscle consists of not one, but a series of "homologous transverse muscles" (myomeres), which are derived embryologically from somites (Owen, 1866; Shann, 1914). For reviews, bibliography and descriptions of the lateral musculature of various species see Shann (1914), Greene and Greene (1913), le Danois (1958) and Kafuku (1950).

The lateral musculature (m. lateralis, m. parietalis [Nursall, 1956]) of most fish extends as a series of myomeres on either side of the body from its most anterior insertions on the occipital region of the cranium and superior border of the scapular girdle, to the base of the caudal fin. Anteriorly, it also extends ventrad to the isthmus of the throat, as the spinal hypobranchial muscle (le Danois, 1958). The right and left halves of the lateral musculature are separated by the axial body structures and the dorsal and ventral medial connective tissue septa.

In fishes above Cyclostomes (Hyman, 1957; Jollie, 1962), each lateral muscle mass is separated into a dorsal (epaxial, dorso-lateral) and a ventral (hypaxial, ventro-lateral) portion by a horizontal connective tissue septum (transverse septum, horizontal skeletogenous septum) (Hyman, 1957; Lagler et al., 1963). This septum extends laterally from the vertebral column to the skin in the region of the lateral line. Posterior to the anus, the epaxial and hypaxial portions are nearly symmetrical, but anteriorly the anatomy of the ventral musculature is greatly altered by the presence of the abdominal cavity.

The constituent elements of the lateral musculature, the myomeres, are separated from each other by transverse connective tissue septa, the myosepta or myocommata. These appear at the body surface as tendinous inscriptions (le Danois, 1958; Lagler et al., 1963). Based on the external appearance of the myosepta (particularly in the caudal region), Nursall (1956) has distinguished, for comparative purposes, three types of lateral musculature architecture: 1) amphioxine, 2) cyclostomine, and 3) piscine. Of these, only the latter is pertinent to the present discussion.

The piscine type myomere is found in the cartilagenous and the bony fishes. It is " ≤ "-shaped with sharp flexures. The apices are labeled in the following manner (le Danois, 1958): that at the horizontal septum pointing anteriorly (L); the next dorsally (D_1); and dorsal to D_1 is (D_2). Similarly, the first apex pointed posteriorly below L is (V_1), and the next one ventrally is (V_2). In some teleosts, L may not exist as a single apex, but may be divided into two parts; one above and one below the horizontal septum. Anteriorly,

it is the fibers below V_2 which contribute most to the abdominal musculature (Nursall, 1956; le Danois, 1958).

In all piscine forms, however, the rather simple outward appearance of the myosepta belies the internal shape of the myomeres. Internal folding of the myosepta may be quite complex, especially in the more highly evolved forms, producing a series of overlapping cones. These may vary in number and extent and yield a complicated cross-sectional appearance of the lateral musculature (Nursall, 1956; le Danois, 1958).

Furthermore, in the piscine chordates, and especially in the Osteicthyes, supernumerary supporting structures may form within the lateral musculature, for example, dorsal ribs in the horizontal septum, neural spines in the dorsal median septum, pleural ribs in the pericoelomic fasciae, haemal spines in the ventral median septum posterior to the anus, and intermuscular bones in the myosepta (Lagler et al., 1963). Deep longitudinal ligaments may also be formed in some species by the fusion of neighboring myosepta along the medial margins of the myomeres (Nursall, 1956).

Cuvier (1828) also noted the presence of some slender, longitudinal strips of muscle (muscles greles) found dorsally and ventrally on either side of the median septa between the large lateral muscles. These were termed "supracarinales" (dorsal) and "infracarinales" (ventral) by Owens (1866). Their development varies between species. Their distribution has been discussed by Takahashi (1917) and le Danois (1958). Because of their gross anatomical relationships, they have been considered to act as elevators, retractors, and protractors of the unpaired fins and pelvic girdle by Cuvier (1828), Goto (cited by

Takahashi, 1917), Greene and Greene (1913), Shann (1914) and others (see Lagler et al., 1963). However, this has been disputed on the basis of electrical and mechanical stimulation studies by Takahashi (1917), who considered them to be homologous to the muscle of the dorsal and anal fins "which have become abortive or modified in accordance with the nondevelopment of the fine rays along those portions of the median line where they exist." They are separated from the main lateral muscle masses by fasciae, but are divided into segments by modified transverse septa (Shann, 1914). Although presumably differentiated from the dorsal and ventral extremities of the embryonic lateral muscle masses (Greene and Greene, 1913, Shann, 1914; Takahashi, 1917), the supra- and infracarinales have been considered by some authors as "deep trunk muscles" (Lagler et al., 1963) and are usually not included under the term of "great lateral muscle."

Very important to the present investigation, however, is an additional longitudinal subdivision of the lateral musculature. In the majority of adult teleosts two portions can be distinguished easily: a lateral band of small, often darkly colored fibers, and a larger medial portion composed of generally pale fibers. The lateral ("red") fibers are separated from the subjacent ("white") muscle fibers by a thin connective tissue fascia (Stirling, 1885; Shann, 1914; le Danois, 1958; Barets, 1952, 1961) and have been considered by some workers as forming an anatomically distinct muscular unit (Barets, 1952; Greene and Greene, 1913; Greene, 1913a). The absence of this fascia in goldfish, however, has been reported by Dubowitz and Pearse (1960).

The thin, dark lateral portion is better developed caudally,

thinning anteriorly, and in some forms it does not reach the pectoral girdle or occiput (le Danois, 1958; Shann, 1914; Kishinouye, 1923). In cross-section, it may appear as a wedge at the lateral margin of the horizontal septum; the base of the wedge facing laterally. Like the deeper fibers, this superficial muscle layer is divided by the myosepta into myomeric divisions, and by the horizontal septum into epaxial and hypaxial portions. The latter generally encompass the lateral nerve running in the lateral margin of the horizontal septum.

All muscle fibers of the lateral musculature extend the length of the myomere to insert on the myosepta. Their orientation with respect to the body axis varies, however. The fibers of the superficial portion run parallel, whereas the majority of the deeper fibers are oriented obliquely to the body axis. The exact orientation of the deep fibers varies in relation to their position within the myomere (Shann, 1914; Maurer, 1913).

"Red" and "white" fibers have been described in the Cyclostomes, Holostei, Branchiopterygi, Dipnoi, Chondricthyes and Teleostei (Bone, 1966). The present study will be concerned with the Teleostei, in which the two portions are more highly differentiated. A superficial portion of red fibers is well developed in the Clupes, Salmonides, Cyprinides, Atherines, and Mugilides (le Danois, 1958). It is more or less marked in the Acanthopteres, and has undergone a remarkable evolution in the Scombroides (maximally in the Tunny and Bonita [Kishinouye, 1923; Kafuku, 1950]) which sets these species apart from all other teleosts.

Several names have been applied to this superficial muscle. Because

of its striking development and color in some forms, it is commonly referred to as "dark meat," "mud-stread" (Lagler et al., 1963), or red muscle (fibers) of the lateral line (Gegenbaur, 1898; Chevrel, 1913). The Japanese term "Chiai," is also often used, although according to Kishinouye (1923), this term properly refers only to the deepest portion of the highly developed red muscle of the Scombroides; the term, "tiai" is given to the superficial dark fibers (Umemura, 1951a). Vogt (Agassiz and Vogt, 1845), who first introduced this red muscle into the scientific literature (see also figures in Agassiz, 1842), termed it "muscle cutene," or "m. superficialis trunci." Knaur (1910) and Maurer (1913) used the term "m. rectus lateralis," and Greene (1913a) proposed the term "m. lateralis superficialis" as opposed to "m. lateralis profundus," based on a study of the king salmon. The terms "superficial" and "deep" portions of the lateral musculature will be used throughout this study.

Although the separation of the superficial from the deep portion of the lateral musculature may be made quite easily in some forms on the basis of gross appearance and a difference in color, the gross anatomical division, as pointed out by Greene (1913a), is not as distinct as the histological. A clear distinction between them can presently be based on a combination of morphological, biochemical, and physiological criteria.

B. Microscopic Characterization.

The major histological differences between the superficial and the deep fibers of the lateral musculature (fiber diameter, myofibrillar appearance, fibril to sarcoplasm ratio, number of nuclei, fat

content, etc.) were established around the middle of the 19th century, and have been amplified and extended up to the present time. The work dealing with fish muscle histology prior to 1912 has been extensively reviewed by Prenant (1911), and more recently, although less extensively, by Barets (1961).

In what is recognized by most investigators as one of the earliest accounts of a histological difference between the superficial and deep portions of the lateral musculature, Leydig (1852) reported a relative abundance of nuclei in the red muscle, and an abundance of finely granular sarcoplasm in the superficial fibers. Two years later Stannius (1854) described the superficial muscle as a vascular, red, fatty meat which presents the appearance of tissue in the process of conversion into muscle. This was first disputed by Humphry (1872), who described his observations on the superficial muscle in a footnote to a long comparative anatomical discussion:

> "In the Fish...the lateral furrows are commonly occupied by muscular fibers which bear the transverse septa, but which are more closely connected with the skin, and peel off with it more easily than the rest of the lateral muscle. These fibers are more vascular than ordinary muscular fibers; and in a piece which I examined from a Dace they contained more oil than the other muscles." Further, "with the exception of the excess oil, they presented the usual microscopical characters of striped muscle." (p. 294)

Humphry's views were later supported by Shann (1914).

Although he did not use teleost material, Ranvier stimulated much of the subsequent inquiry regarding the structural and functional differences of striated muscle fibers. He noted a difference in fiber size between the smaller superficial (0.06 - 0.09 mm) and larger deep (0.15 - 0.18 mm) fibers in the "rays and torpedo" and described a

difference in the appearance of the striations; the transverse bands are more distinct in the deep fibers and the longitudinal striation more distinct in the superficial fibers (Ranvier, 1873). Perhaps most importantly, Ranvier also first established a contractility difference between the red (slow) and white (fast) muscles (Ranvier, 1874a).

Ranvier's histological observations on the rays were confirmed by Lavocat and Arloing (1875) and extended to several teleosts as well. These authors described fat cells in the interstitial tissue between the superficial fibers, and the compact arrangement of the fibrils within them. They were the first to note that the border line between the two main fiber types is not always sharp, and that there is often a mixture and a "gradual and imperceptible" gradation between the superficial and deep portions of the lateral musculature.

In a description of the gross anatomy and histology of the lateral musculature of the whiting, herring, mackeral, haddock, plaice and flounder, Stirling (1885) confirmed the smaller size and indistinct transverse bands in teased fibers of the superficial muscle. He noted "fat cells" between the deep as well as the superficial fibers, and showed in the mackeral that the superficial and deep fibers are separated by a connective tissue fascia.

A difference in cross-sectional appearance of the myofibrils of superficial and deep fibers was described and illustrated by Kölliker (1888); the deep fibers contain peripheral ribbon-shaped and central cylindrical fibrils; the superficial fibers have irregular ribbon-shaped fibrils. Kölliker also confirmed Leydig's observation of the large amount of sarcoplasm beneath the sarcolemma of the superficial fibers

and showed, in addition, that the fibers of the deep portion ("gewohnliche Rumpfmuskeln") also have a small peripheral cytoplasmic envelope. He pointed out that Van Gehuchten (1888) failed to show this in the same species (goldfish, Cyprinus carpio) and questioned the source of several of Van Gehuchten's illustrations.

Several workers described the relative amount of sarcoplasm in the muscle fibers of various fish (Ranvier, 1874b; Rollet, 1888 - dorsal fin musculature of Hippocampus; Retzius, 1890 - Myxine glutinosa) and variation in the amount of sarcoplasm was stressed by Knoll (1889, 1891) as being a more basic difference between muscle fibers than the cross-sectional fibrillar appearance. From a study of 35 species he concluded that the superficial fibers generally have a large peripheral band of sarcoplasm containing the nuclei, and are, therefore, "protoplasmareich," while the deep fibers contain only a small rim of cytoplasm and are "protoplasmaarm." He pointed out that, although the superficial muscle fibers are dark or red as a rule, in some cases where no red muscle can be distinguished grossly, microscopic examination reveals the presence of a region of thin, granular, "röthlichgelbe" fibers under the skin (Knoll, 1889). He also confirmed the observation of Lavocat and Arloing (1875) that there may be a definite transition zone in some species at the junction of the superficial and deep fibers.

Unlike Knoll, Lansimaki (1910) stressed the differences in cross-sectional appearance of the myofibrils. Based on a histological survey of various muscles within numerous teleosts and Petromyzon, he established three fiber types within fish muscle. The essential distinctions for these groups were: 1) all the fibrils in the cell are "bandförmig,"

2) all fibrils are cylindrical; and 3) both ribbon-shaped and cylindrical fibrils occur within the same fiber. Although previous workers had reported differences in fibril appearance between red and white muscle fibers (see Kölliker, 1888; Veratti, 1902), and he himself had noticed differences in the composition of various muscles, he did not specifically discuss the lateral musculature in terms of his fiber types.

An important series of articles concerning the structure and physiology of the lateral musculature of the King salmon was published by Greene (1912, 1913a,b) and Greene and Greene (1913). They noted that the superficial fibers were smaller and more uniform in size than the deep, and emphasized that the superficial fibers contain more intracellular fat during all stages in their life cycle (especially as the spawning migration begins).

Lansimaki's formulation of three fiber types within fish muscle was attacked by Bühn (1940) who noted after an examination of the musculature of seven teleost species, that fibers corresponding to Lansimaki's "types" are found in all muscles without apparent systematization, and further, that all intermediate degrees of fibrillar appearance can be observed. Bühn postulated that all variations in adult fibers may be traced to their origin from two embryological fiber types. A "primary" type, distinguished (in cross-section) by ribbon-shaped fibrils at the periphery and cylindrical fibrils located centrally, predominates at hatching time, but does not develop much beyond it. A "secondary" fiber type, with large areas of sarcoplasm between the sarcolemma and the fibrils develops subsequently. The cross-sectional appearance of mature primary fibers was presumed to arise as a consequence of the splitting

off of new cylindrical fibrils from the central edges of the ribbon-shaped fibrils, while that of the secondary fibers arises through the enlargement of the fibrils in the center of the cell. According to this scheme, only in older fibers do the fibrils within the secondary fibers approach the sarcolemma. In Bühn's view, the superficial portion of the lateral muscle is composed of secondary, and the deep portion of primary fibers.

Kirsche (1948), in describing the trunk musculature of several forms (primarily the guppy), confirmed the smaller size, greater sarcoplasmic content, and centrally located, radially arranged fibrils of the superficial fibers, but he proposed that the superficial muscle is a sensory organ similar to the muscle spindle of higher vertebrates.

Maser (1949) described a region ("horizontale Schicht") of small "sarcoplasmareiche" fibers dorsal and ventral to the horizontal septum in some fishes (e.g., Holocentrum sumara), and noted that they are separated from the superficial fibers by a distinct gap, as had been mentioned by previous workers (see Knoll, 1891; Rauther, 1945). Maser considered them to be a secondary differentiation of the deep portion of the lateral musculature bordering on the horizontal septum, rather than a medial extension of the superficial portion.

In the first of a series of reports on teleostean lateral musculature, Barets (1952) stressed the inadequacy of the histological criteria used until that time in attempting to establish meaningful differences in the nature of the fibers of the superficial and deep portions. He showed in the cat-fish, Ameiurus nebulosus (the lateral musculature of which is presumably representative of the majority of teleosts) that

certain histological characteristics are not sharply different, but vary in both the superficial and deep portions. Barets noted that no single criterion (e.g., fiber size, relative amount of sarcoplasm, form and arrangement of fibrils, vascularization) will allow an absolute distinction between the fibers of these portions in every species. He stressed, however, that a gradient of these characteristics does exist in both portions, and most importantly, that they are of opposite topographical orientation, allowing a rather distinct separation into the two major regions. These studies were later extended to include 24 species (Barets, 1961) and, although species differences were observed, the general conclusion regarding the inadequacy of single histological criteria was confirmed.

In a similar extensive investigation of 21 fresh-water teleosts, Boddeke et al. (1959) concluded that in each form, two types of muscle fiber can be distinguished on the basis of fiber diameter; "narrow" (25-45 μ) and "broad" (45-75 μ). A difference in sarcomere length was reported between the broad (deep) (1.82 μ) and narrow (superficial) (2.06 μ) fibers in all the forms they studied except the Eel and Thunderfish. They confirmed the fact that the "lateral line strip" (superficial portion) is generally composed of narrow fibers, but is not always sharply defined. Moreover, some narrow fibers are present at the lateral border of the deep portion. This latter condition was referred to as a "mosaic structure," and in some forms (e.g., salmon, trout, carp) the entire lateral musculature was considered as "mosaic." They confirmed the observation of Knoll (1891) that the narrow fibers are not always red, particularly if they do not occur in a well defined and

highly vascularized lateral line strip. Similarly, although the broad fibers are usually white, they may also be pinkish, as in the carp and salmon, which may be due to pigments other than myoglobin (Goodwin, 1954; Baalsrud, 1956). Thus, a specific correlation between fiber size (and presumably function) and color is not always possible.

Recently George and Bakdawala (1964) reported the presence of three general fiber types in the Cyprinid (Labeo rohita): small "red" (25-45 μ), larger "white" (ca. 135 μ) and "intermediate" (45-85 μ) and noted that the mitochondria at the periphery of the small superficial fibers are larger than those at the center of the cell.

The highly differentiated lateral musculature of the Scombroides (Mackerels, Seerfishes, Tunnis, Bonitos) has also been extensively described and compared with the lateral musculature of other teleost species. Kishinouye (1923) described two distinct portions of the red muscle in these forms: a deep "Chiai," and a more superficial and lighter portion. He reported that the "Chiai" is composed of very uniformly small fibers with faint striations, and he attributed their deep red color to the very rich blood supply. Ogata (1925) noted the greater lipid content of the "Chiai," and confirmed the small size of its fibers (20-50 μ) which was approximately 1/2 that of the white (deep) fibers. The comparative study of the distribution of red muscle in fish by Kafuku (1950) included several Scombroides, and demonstrated that in the skipjack (Katsuwonus pelamis) the deep red muscle formed the largest proportion of the trunk muscle of any species studied. Rayner and Keenan (1967) reported that in this same species, the anterior muscle fibers of the superficial portion are similar to those of the "Chiai"

muscle, while the posterior fibers are similar to the shite (deep) portion. They suggested that this might, in part, indicate a phylogenetic relationship between the superficial fibers and the deep red "Chiai" muscle.

In summary, it is generally recognized that the lateral musculature of the vast majority of teleosts contains two main histologically distinct types of fibers. The distribution of these fibers within the lateral musculature varies, but they are most often separated into a superficial portion composed of small, darkly colored fibers with irregular myofibrils and a relatively large amount of cytoplasm, and a larger deep portion composed primarily of large, pale fibers with little sarcoplasm and containing radially oriented ribbon-shaped fibrils peripherally and polygonal ones centrally. The superficial portion is usually more highly vascularized and contains more fat than the deep portion. The superficial portion may form a thin layer covering the lateral surface of the lateral musculature, or may be restricted to a muscular wedge at the lateral end of the horizontal septum. A region of fibers with a unique histological appearance ("intermediate fibers") are seen between the superficial and deep portions in some species, and in others, smaller fibers may be found throughout the lateral musculature ("mosaic structure").

C. Biochemistry.

Biochemical and metabolic differences have been reported between the superficial and deep portions of the lateral musculature in several species. Fat and glycogen content, and oxidative enzyme activity have been most extensively studied.

1. Fat. As noted in the previous section, a greater fat content of the superficial muscle has been indicated in several of the early histological investigations, but relatively few inquiries have been directed specifically toward the fat content of muscle. Most of these deal with the role of fat in migratory species (e.g., salmon).

Miescher-Rüsch (1880) in his extensive study of biochemical changes during the life cycle of the Rhine salmon, noted the presence of fat droplets within the lateral muscle, and particularly within the superficial fibers. He felt that this indicated a fatty degeneration.

Stirling (1885) also described rows of "refractive granules" in the sarcoplasm of the superficial fibers in several teleosts, and assumed they were of a fatty nature because they were blackened by osmium and soluble in ether. He agreed with Meischer-Rüsch that the fibers of the red muscle "present all the appearance of a muscle in a state of fatty infiltration or fatty degeneration," but he stated that these fatty granules may also have "some relation to the constructive metabolism of the fiber itself."

Boyd et al. (1898) also reported more fat in the "thin" than in the "thick" muscle of salmon, but they discarded the concept of fatty degeneration. They demonstrated that the extra- and intracellular accumulations of fat seen during feeding stages represent normal storage products, which are subsequently used up. When present, the intracellular fat droplets are prominent under the sarcolemma, and are distributed in rows separating the myofibrils; "tending to obscure the transverse striation."

Greene (1912, 1913a,b, 1926) confirmed the greater number of

intracellular fat droplets in the superficial fibers of the King salmon as opposed to the primarily intercellular fat deposition on the deep portion of the lateral musculature. Some intracellular fat was even present in the superficial fibers at death after spawning. In tracing the amount of muscular fat present at various times in the life cycle, he concluded that the stored fat was used as the "prominent and immediate source of energy during the spawning migration (Greene, 1913b).

Frazer et al. (1961) reported a higher fat content in the superficial portion of a number of teleosts, although there was significant species differences. A high intra- and extracellular fat content of the red muscle was also reported by George (1962) in the Mackeral (Rastrelliger knanagurla) and in the Cyprinid (Labeo rohita) (George and Bakdawala, 1964). Almost no fat was detected in the deep muscle, and only a small amount in the "intermediate" fibers of Labeo.

Recently, in electron micrographs of the superficial portion of the lateral musculature of the lingcod, Buttkus (1963) described vacuoles in the cells as "negative images of fat droplets dissolved during preparation." Although similar vacuoles were not seen in a micrograph of the deep fibers, he did not discuss relative fat content specifically (see also Buttkus and Tomlinson, 1966).

In brief, the superficial portion of the lateral musculature appears to contain more intra- and extracellular fat than the deep portion. In migratory forms, such as the salmon, there may also be a dramatic accumulation of fat within the superficial muscle during the feeding phase of the life cycle, which is subsequently depleted during the spawning migration. Metabolism of this stored fat presumably

supplies much of the energy required during this journey. In addition, in forms which contain "intermediate" fibers, these fibers contain "intermediate" amounts of fat relative to the superficial and deep fibers.

2. Glycogen. Studies of muscle glycogen in various teleosts are more numerous than those concerned with fat content and oxidative enzyme activity and have yielded more conflicting results. Early studies (e.g., Greene, 1921; Fontaine and Hatey, 1953) indicated that glycogen content in fish muscle (mixed red and white samples) were quite low (e.g., 0.015 - 0.16%; Greene, 1921). Later studies, however, revealed that muscle glycogen content may be greatly influenced by nutrition (Black et al., 1960; Hochachka and Sinclair, 1962), age, life cycle, sex, species, and particularly by exercise prior to sampling (Tomlinson and Geiger, 1962; Bone, 1966). Recent work summarized by Black et al. (1961) has produced values for muscle glycogen content of the lateral muscle of rested trout which are comparable with similar values reported for some mammals (e.g., 0.185 - 0.25%).

Black et al. (1960) showed in Rainbow and Kamloops trout that after vigorous exercise, muscle glycogen is apparently degraded to lactic acid, while after moderate exercise (i.e., sustained swimming), although the blood lactate level might be raised slightly, there is no decrease in muscle glycogen. These studies have been extended to show that the metabolism of glycogen may be extremely rapid (50% depletion in the first 2 minutes of vigorous activity) with rapid increases in muscle lactate and pyruvate. Furthermore, it has been demonstrated that muscle glycogen is restored slowly during recovery. It can be concluded, therefore, that "glycogen metabolism provides most, if not all energy

for severe muscular action" (Black et al., 1962).

George and Bakdawala (1964) demonstrated histochemically that the superficial fibers of Labeo rohita contain more glycogen than the deep and that the intermediate fibers contain intermediate amounts. It was suggested that the higher concentration of glycogen in the superficial (red) fibers is due to the preferential use of lipid in these fibers, and similarly, that the low glycogen concentration in the deep (white) fibers results from its preferential use there. It was also noted that the smaller of the deep fibers contain more demonstrable glycogen than the larger deep fibers.

Recently, Wittenberger and Diaciuc (1965) compared oxygen consumption and glycogen, pyruvate and lactic acid content of the red and white muscle of carp following direct electrical stimulation; a technique by which the uncertainties brought about by presampling struggle behavior are reduced. They found that with "moderate effort" the glycogen content of the white (deep) muscle decreases and the pyruvate content increases, while in the red (superficial) muscle, the glycogen and pyruvate content do not change, but the lactate content and oxygen consumption increase. With "exhausting effort," the situation for the deep muscle becomes similar to that after moderate effort. The superficial muscle, however, shows a slight decrease in glycogen content and oxygen consumption, but an enormous increase in lactate content. The fact that the "lactate:pyruvate ratio showed opposite variation in the two muscles and the oxygen consumption increased significantly in the red muscle only" was interpreted as indicating removal of metabolites from the deep muscle (liver lactate concentrations increased only

slightly). These workers concluded that the red muscle is a "burning place of catabolites produced in the white muscle," but did mention that the excess in lactic acid and in substrate for oxidation could come from other sources such as lipids and blood glucose.

Comparisons of glycogen content in the superficial and deep portions of the lateral musculature have also been made electron microscopically in the black mollie (Franzini-Armstrong and Porter, 1964a) and lingcod (Buttkus and Tomlinson, 1966). In both forms the superficial fibers were reported to be richer in glycogen than the deep fibers, and in the lingcod, this was confirmed by chemical analysis: the superficial muscle contained from 1-3 times the amount of glycogen in the deep portion (Buttkus and Tomlinson, 1966).

In summary, fibers of the superficial portion of the lateral musculature have been shown to contain more glycogen than the deep (and "intermediate") fibers, although the sensitivity of glycogen metabolism to various factors (particularly exercise) must be considered in interpreting available data. The apparent preferential use of glycogen by the deep fibers during strenuous exercise may indicate that glycogen supplies most of the energy for severe muscular action.

3. Oxidative Enzymes. Umemura demonstrated in the goldfish (Umemura, 1951a) and the silver carp (Umemura, 1951b,c) that the succinic dehydrogenase (SDH) activity of the superficial fibers is approximately 8 times that of the deep (white) fibers, and that the cytochrome oxidase and succinic oxidation in the superficial (red) fibers surpasses that of the liver and kidney. He also showed in the silver carp that the malic dehydrogenase (MDH) activity of the superficial muscle is greater than

that of the deep. Fukuda (1958) also found SDH activity to be much higher in the "dark" meat than in the "white" of 24 forms studied, and noticed that the SDH activity was higher in pelagic than in nonpelagic and fresh-water forms. He postulated that this may be correlated with the fish's mode of life.

Ogata and Mori (1964) studied the activity of numerous oxidative enzymes (including SDH) in the lateral musculature of teleosts, and in each case distinguished 3 fiber types on the basis of oxidative enzyme activity and fiber diameter: 1) small fibers of the superficial muscle, with high oxidative enzyme activity; 2) larger fibers of the deep muscle, with lower enzyme activities; and 3) an intermediate group of fibers in the transitional zone between the superficial and deep portions which are variable in size and enzyme activity. These authors also demonstrated the presence of three types of muscle fibers by these means in other vertebrates (Ogata, 1958a,b,c; Ogata and Mori, 1962), but they noted that in fish "the relations between the diameter of the muscle fibers and enzymatic activity were more irregular than those in mammals."

The presence of more than two fiber types based on size and enzyme activity was also demonstrated by Dubowitz and Pearse (1960) in the goldfish (Carassius auratus). The uniformly small (20-25 μ) red (superficial) fibers reacted strongly for all oxidative enzymes tested (SDH, lactic dehydrogenase [LDH], DPN-diaphorase), and also for phosphorylase. The deep muscle was mosaic, and contained 3 groups of fibers: 1) small (10-20 μ) fibers which reacted strongly for both oxidative enzymes and phosphorylase, but which contained fewer mitochondria than the superficial fibers, and which were generally located subjacent to the super-

ficial muscle; 2) intermediate (25-40 μ) fibers which reacted strongly for oxidative enzymes but less strongly for phosphorylase; and 3) large (50-75 μ) fibers with weak, or no, reaction for either oxidative enzymes or phosphylase. Dubowitz and Pearse assumed that "phosphorylase activity reflects the ability of the muscle fiber to utilize and synthesize glycogen," and, therefore, fibers with high phosphorylase activity presumably depend on intrinsic glycogen for energy. If this is so, they reasoned, an inverse relationship between phosphorylase and oxidative enzymes might be expected, and this was indeed found by these workers in the muscles of higher vertebrates. Thus they suggested that the direct correlation in goldfish muscle fibers may indicate a real difference between the metabolism of muscle from warm and cold blooded animals.

George (1962) confirmed the high content of SDH in the red muscle of the Mackeral and demonstrated its location in the mitochondria. He also noted that the lipase activity of the red fibers was about 4 times greater than that of the white fibers, and concluded that the red muscle "is well adapted for an aerobic metabolism to use fat as the chief fuel for muscular activity and the white for an anaerobic metabolism to use mainly glycogen." In an extension of these studies to several cyprinids, George and Bakdawala (1964) found that SDH and lipase activity and the capacity for "in vitro" oxidation of the fatty acids sodium butyrate and sodium malate was greatest in the superficial, lowest in the deep, and intermediate in the "intermediate" fibers. In addition, it was demonstrated that the superficial fibers readily oxidize both butyrate and malate, while the deep fibers oxidize only malate.

Bilinski (1963) and Jonas and Bilinski (1964) studied the oxidation rate of C^{14}-labeled fatty acids *in vitro* in strips of red and white muscle from rainbow trout (*Salmo gairdneri*) and Sockeye salmon. By measuring the amount of $C^{14}O_2$ produced, they concluded that in both species, both muscle types contain an enzyme system which will oxidize fatty acids and acetate, but that the superficial muscle is considerably more active in this respect than the deep. Fatty acid oxidation was very low in the deep fibers and "it remains to be established whether the low activity found is of importance '*in vivo*' " (Bilinski, 1963).

In brief, the superficial fibers in a number of species exhibit greater lipase, and oxidative enzyme activities than the deep (and "intermediate") fibers. They also are capable of greater oxidation of certain fatty acids "*in vitro*." These findings are consistent with the proposal that the superficial fibers utilize the aerobic metabolism of fat as their chief energy source (George, 1962). The fact that they may also contain significant amounts of phosphorylase may be correlated with their increased glycogen content (see Section C,2).

4. *Other Biochemical Differences*. Of other biochemical differences reported, that of myoglobin content is one of the most important. Hamoir (1953) stressed that, although there has been much mention of myoglobin in fish muscle, little clear evidence of this has been presented (see also Prosser and Brown, 1961; Lemberg, 1949 [cited by Hamoir, 1953]). He electrophoretically separated and identified myoglobin from the superficial portion of the lateral musculature of the carp (*Cyprinus carpio*), but did not compare the superficial portion with the deep portion.

Boddeke et al. (1959) demonstrated that "myohemoglobin" is not present in the "broad" (deep) but only in the superficial ("narrow") fibers in 21 teleost species, and that this accounts for the red color of the superficial fibers when they are located in a well-defined lateral line strip.

Differences in vitamin content have been reported by Mori et al. (1956). They determined microbiologically the amounts of certain vitamins in muscles of 7 teleosts, in 4 of which the red and white muscle was studied separately. The red muscle was found to be rich in thiamine, riboflavin, and vitamin B_{12}, but contained somewhat less niacin than the deep muscle (see also Braekkan, 1956). A similar situation was established in the Mackeral (Scomber scombus) and halibut (Hippoglossus hippoglossus) by Braekkan (1956). This study also revealed that the red muscle was rich in pantothenic acid.

For other differences (e.g., cytochrome c, minerals) see the discussions of Hamoir (1953) and Umemura (1951a).

D. Electrophysiology and Pharmacology.

Although little is known regarding the electrophysiological and pharmacological properties of the lateral musculature of teleosts in general, distinct differences have been demonstrated between the superficial and deep muscle fibers of the tench (Tinca tinca) and cat-fish (Ameiurus nebulosus) by Barets and collaborators (Barets, 1952, 1955, 1961; Barets and LeTouze, 1956; Barets et al., 1956; Barets and and Pecot-Dechavassine, 1959). In both species the superficial portion exhibited the greater susceptibility to the depolarizing substances, potassium chloride (KCl), acetylcholine (Ach), and butyrylcholine

(Buch), and presented a different contractile response when comparative strips were immersed in appropriate concentrations of these substances. It responded with a slow contraction which varied in amplitude in proportion to the magnitude of the concentration used. This contraction was maintained for long periods until the muscle was washed with saline. The deep muscle gave a short contraction and returned to the relaxed state in less than 4 minutes. The resting membrane potential of the superficial fibers was lower (ca. 55 mv) than that of the deep fibers (ca. 90 mv).

The two portions of the lateral musculature also differed in their contractile response to both direct stimulation and stimulation of the motor nerve. The superficial muscle exhibited no visible response following a single stimulation (irregardless of intensity), but a slow, sustained contraction was observed with repeated shocks; the amplitude of the contraction increased proportionally with the frequency of stimulation. The deep muscle, on the other hand, responded "coup-pour-coup" with a twitch response for each stimulus. No propogated action potential was recorded from the superficial fibers. In deep muscle fibers a local potential followed by a "spike" was recorded in the cat-fish, but in the tench, only dispersed, local potentials or "abortive" spike potentials, but no typical spikes with overshoot, were recorded. This difference was attributed to a difference in the pattern of motor innervation of the deep muscle in the two species: those of the cat-fish are innervated only at the ends of the fiber near the myoseptum, while those of the tench receive multiple endings distributed along the length of each fiber (Barets, 1955, 1961; Barets et al., 1956; Bone, 1964).

An attempt was made to determine whether there is a difference in

the diameter of the motor axons supplying the superficial and the deep fibers as is the case in the fast and slow muscle fibers of the frog (Kuffler and Vaughan Williams, 1953a). Two classes of axons (based on diameter) were measured in the ventral roots of the teleosts and the number of small axons per root decreased posteriorly. Because this paralleled the decrease in sympathetic innervation, it was felt that only the larger fibers (8-14 μ) innervate the lateral musculature, and that there is no significant difference in the diameter of the axons innervating the two portions. It was concluded that in teleosts the superficial fibers constitute a "slow," and the deep fibers a "fast" motor system* whose susceptability to depolarizing agents, contractile responses, and resting membrane potentials are directly comparable with the well-studied "slow" and "fast" muscle fibers of the frog. But, certain characteristics are not observed in the frog; namely, the lack of a difference in the diameter of the motor axons, and variations in innervation patterns of the deep, "fast" fibers. Based on this latter observation, an evolutionary development of neuromuscular mechanisms within the teleosts was suggested from a low level resembling the crustaceans (tench) to a high one closely comparable to the Amphibia (cat-fish).

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* Because direct stimulation of a muscle fiber provoked the same contractile response as stimulation of the motor nerve, Barets defines a "motor system" as a group of motor units of the same type.

E. Fine Structure.

Few fine structural studies of the lateral musculature of young or adult teleosts are found in the literature (Buttkus, 1963; Buttkus and Tomlinson, 1966; Slautterback, 1966; Franzini-Armstrong and Porter, 1964a,b; Franzini-Armstrong, 1964; Kilarski, 1965, 1966, 1967).

The most extensive and complete descriptive report is that of Franzini-Armstrong and Porter (1964a) who examined the trunk myotomes of the black mollie (Mollienesia sp.) in specimens about 1/3 adult size. They found that the majority of the fibers are "white" (deep), and that there is a well-developed strip of "red" (superficial) fibers along the lateral line. Although primarily concerned with a description of the sarcoplasmic reticulum (SR), they confirmed many of the light microscopical data at greater resolution. The larger, deep fibers (6-12 μ) have a marginal band of sarcoplasm containing only occasional mitochondria and some glycogen. The superficial fibers are smaller (4-8 μ) with broad marginal zones of sarcoplasm containing large numbers of mitochondria and glycogen particles. Both the superficial and deep fibers contain branching, ribbon-shaped myofibrils. Cylindrical or polygonal fibrils are also present near the center of some of the larger fibers. The sarcomeres of the myofibrils of both fiber types are 1.4 - 1.6 μ long and have dense M-bands.

The thick filaments are hexagonally arranged and each thick filament is surrounded by six thin filaments in the A-band region. In the M-band, "cross-bridges" connecting the thick filaments contribute to the density of this region. The thick filaments often appear as hollow

tubules, particularly in the M-band. Their diameter was shown to vary with the fixation procedure; decreasing from 150 Å following glutaraldehyde fixation to 130 Å after osmium tetroxide. In the I-band the thin filaments are more irregularly arranged, but assume a parallel alignment as they approach the Z-band. Glycogen granules may be seen between the thin filaments in the I-band.

No significant differences in the sarcoplasmic reticulum (SR) of the superficial and deep fibers were reported, and a common description of the SR in the lateral musculature was given (see also Franzini-Armstrong and Porter, 1964b; Franzini-Armstrong, 1964). The longitudinal elements occupy nearly the full interfibrillar space, so that "one sarcoplasmic sheet and its content of sarcoplasmic reticulum profiles is shared by two adjacent fibrils." The longitudinal cisternae are dilated over the I-band forming "terminal sacs" on either side of the T-tubule located over the Z-band, and are also confluent over the H-band region. No continuity was observed between the SR and the cell membrane, but areas of continuity between the SR and the nuclear envelope were described.

Funnel-shaped areas of continuity were observed between the T-tubules and the plasmalemma in glutaraldehyde fixed specimens. The T-tubules measure 20-40 mμ when fixed in glutaraldehyde fixation, and 15-30 mμ after osmium tetroxide. Dense material was seen at regions where the T-tubules and terminal sacs are adjacent, and the SR membrane facing the tubule was occasionally scalloped. "Finger-like projections" containing glycogen granules extend from the surfaces of the muscle fibers and protrude into the T-systems of neighboring fibers. A simi-

lar appearance of the sarcoplasmic reticulum in fibers of the superficial and deep portions of the lateral musculature in several other freshwater and marine teleosts has been described by Kilarski (1965, 1966, 1967), although cell processes within the T-tubules were not reported.

The few specific ultrastructural differences between superficial and deep fibers which have been reported relate to mitochondrial and sarcomere morphology. More numerous mitochondria with well developed cristae have been reported in the superficial fibers by Kilarski (1967). Buttkus (1963) stated that in the lingcod, mitochondria in the superficial fibers display the "characteristic cristae type found in mammals," while those of the deep fibers "seem to possess a more tubular structure." A small difference in sarcomere length has been reported for six species by Kilarski (1965, 1966, 1967), the sarcomeres of the superficial fibers being 0.3 - 0.5 μ longer than the deep fibers. The H-band appears comparatively more distinct in the deep fibers (Buttkus, 1963; Buttkus and Tomlinson, 1966; Kilarski, 1967), and a much shorter I-band in the deep fibers of the lingcod was described by Buttkus (1963).

F. Function.

The presence of two main fiber types in the lateral musculature of numerous fish raises the question of a possible difference in their function.

As mentioned previously, Kirsche (1948) proposed that the superficial muscle is a sensory organ similar to the muscle spindle of higher vertebrates, but this idea was later discredited by the work of Barets (1952, 1955, 1961) who demonstrated that the nerve endings described

by Kirsche are actually small motor nerves.

Braekkan (1956) proposed that because of its high fat content, and because "the anatomical situation of the red muscle prevents it from taking part in the main muscular work," it is likely that the main purpose of the red muscle is not mechanical work, but that it functions as a metabolic organ like the liver. This concept has been supported by the work of Wittenberger and Diaciuc (1965) and Mori et al. (1956) but has been attacked by many investigators (Buttkus, 1963; George, 1962; see discussion below).

Other workers have suggested that the two main fiber types may perform separate functional roles related to their different contractile properties. One such proposal, stemming from Ranvier's original suggestion that pale muscles are "muscles d'action," and the red are "equilibrateurs" (Ranvier, 1874a), is that the superficial muscle may perform a postural role. It is directly derived by analogy of the superficial fibers of the lateral musculature to the slow muscle fibers of the frog, which were postulated to serve in maintaining posture in this form (Kuffler and Vaughan Williams, 1953b). This proposal has been accepted by Barets (1952) and others for fish muscle, but as pointed out by Ritchie (1928) and Andersen et al. (1963), since fish live in a dense medium and are nearly neutrally bouyant, the maintenance of posture is not a major problem for aquatic forms. A variation of this view is that of Chevrel (1913) who admittedly without experimental evidence postulated that contraction of the superficial muscle in fish is more or less like that of mammalian red muscle, and hypothesized that it may sustain the tail in a curved position as the fish turns.

Another school of thought holds that both the superficial and the deep portions of the lateral musculature are important in propulsion, but that they may produce different swimming behavior. It has generally been presumed that the superficial fibers are active during slow, sustained swimming and the deep fibers are active during rapid swimming as first proposed by Lavocat and Arloing (1875). Variations of this idea are supported by George (1962) who added that the deep muscle may also be involved in "maintaining body balance."

The opposite view, that the deep muscle is used in "continuous" (i.e., slow-sustained) and the superficial portion during "vigorous" swimming, was concluded by Gerebtzoff (1956) and by Buttkus (1963) who stated that the superficial muscle is "designed for rapid acceleration."

The only direct experimental evidence concerning the role of the two main fiber types in swimming behavior in teleosts comes from extracellular recordings by means of concentric electrodes from the "Chiai," the deep, and the superficial portions of the lateral musculature of the highly developed tuna (Katsuwonus pelamis) by Rayner and Kennan (1967). In specimens anesthetized with sodium pentobarbitol or propiopromazine hydrochloride, the "chiai" muscle showed electrical activity in "almost all cases in which any swimming movements could be seen," and the size and frequency of the recorded spikes, and the duration of activity bursts, were clearly correlated with the amplitude, frequency, and form of the observed swimming behavior. Some electrical activity was also recorded from the superficial muscle during "low frequency swimming movements," but none from the deep (white) muscle. The deep muscle showed increasing activity at "high tail beat frequencies" in

tranquillized fish, but no activity was recorded from the deep portion under pentobarbitol, even after strong tactile stimulation. It was concluded that basal swimming in this species is normally accomplished entirely by the "chiai" and/or superficial muscle.

Similar data were obtained by Bone (1966) from electrodes implanted in the myotomal muscles of "spinal" dogfish. He demonstrated that only the red (slow) fibers are active during slow, sustained swimming, whereas the white (fast) fibers are active during vigorous movements. Based on these results and a biochemical analysis of muscle glycogen and fat before and after exercise, Bone concluded that the "slow and fast muscle fibers in the dogfish myotome represent two separate motor systems, which operate independently and utilize different metabolites." He suggested that this also applies to all fish having red and white fibers in their lateral musculature. His conclusion was supported by Andersen et al. (1963) who hypothesized that, in the hagfish, the powerful and numerous fast fibers, by virtue of their peripheral location and rich blood supply, are used when "maximal and rapid propulsive movements are required," and that the slow fibers "may be sufficiently powerful to carry out the slower movements of ordinary life." Furthermore, although not directly discussed by the authors (see Bone, 1966), support for this idea may also be drawn from the data of other studies (Bainbridge, 1960, 1962; Boddeke et al., 1959), and particularly from the conclusion of Barets (1961) that, in teleosts, the superficial and deep portions of the lateral musculature form a fast and a slow motor system.

II. EMBRYOLOGY

A. General Terminology of Somite Development.

During the 19th century knowledge accumulated concerning the origin of the somites, and the tissues derived from them (reviewed by Williams, 1910; Boyd, 1960). In the earliest period of descriptive embryological research, it was thought that the primitive mesodermal segments corresponded directly to the vertebrae and became such by ossification (Kaestner, 1890). For this reason, they were called "Urwirbel" or "protovertebrae."

One of the first consistent theories concerning somite differentiation was published by Remak (1855) who conceived of the newly formed "Urwirbel" (in the chick) as a hollow, cuboidal mass of cells. Remak felt that the medial-ventral edge gives rise to: 1) two leaf-like processes which grow around the notochord to unite with the contralateral processes thus forming the perichordal sheath ("Wirbelkörpersäule"); and 2) a cellular mass which grows into the cavity forming a core ("Urwirbelkerne"). According to this concept, the core soon fuses with all the neighboring walls except the dorsal, yielding a two-component structure with an epithelial upper wall ("Rükentafel" or "Muskelplatte") and a medial mesenchymal mass ("Urwirbelkernmasse").

Once Remak had shown that the "Urwirbel" contains the anlagen of certain body musculature as well as the vertebrae, the term became inappropriate. However, it was used without question until Goette proposed the word "segment" in 1875 (cited in Williams, 1910) and is still sommonly used in a nonliteral sense today. "Mesoblastic somite," or "somite" began to be used as substitutes for "Urwirbel" and "proto-

vertebre" during the latter decades of the 19th century, and Williams (1910) adopted the term "somite" as referring to "one of the blocks of mesoderm formed by the segmentation of the vertebral or somitic plate," and "segment" as including a pair of somites with their corresponding nephrotomes. Knowledge that the primitive mesodermal segments give rise to several structures also led to attempts to determine which portions of the somite produce each tissue. The origin of the vertebrae, intervertebral discs, and associated structures was taken up by Gegenbaur, His, Götte, Froriep, Von Ebner and others, and this aspect has been reviewed by Williams (1910). Other studies dealt with the formation of voluntary muscle.

Remak's conception of the three-layered nature of the "Urwirbel" was modified for the chick by Schenk (1868) who felt that each "Urwirbel" has only a central and a peripheral portion. The central part was presumed to give rise to the vertebrae, cartilage, muscle and other structures around the notochord; the peripheral part, composed of long cells oriented radially to the center, was discussed in much less detail.

Rabl (1888) stated that the dorsal wall of the "Urwirbel" ("Muskelplatte" of Remak) actually becomes a two-layered structure, the "Hautmuskelplatte" (dermomyotome). Only the inner of these layers was thought to form muscle, and was, therefore, termed the "Muskellamelle" (myotome). The outer layer, assumed to give rise to the dermal connective tissue, was named the "Cutislamelle" (dermatome).

Hatschek (1888) proposed calling Remak's "Urwirbelkernmasse" the "Sklerablatt" (sclerotome).

B. Somite Differentiation in Teleosts.

Although several early accounts of teleost embryology contain

some discussion of somite and muscle differentiation (Vogt, 1842; Lereboullet, 1854, 1861), most are primarily concerned with problems of fertilization, oöplasmic segregation, cleavage, and the formation of germ layers. They are based primarily on observations of oiving eggs, and have been admirably discussed by Oppenheimer (1936, 1947). Initial studies regarding the formation and internal differentiation of the somites specifically were hampered by a lack of sufficient well-staged material, insufficient knowledge of the species used, inadequate histological technique, and by attempts to transfer the terminology and conceptual framework based primarily on the chick directly to teleost development. Particularly troublesome were: 1) uncertainties which arose regarding the presence or absence of various laminae or layers within the newly-formed somites; 2) attempts to account for the fate of these various layers, and 3) a dispute concerning the presence or absence of a cavity in somites of teleost embryos.

Oellacher (1872) briefly reported that in trout, unlike the chick, the "Urwirbel" is a solid, oval cell mass with a single peripheral layer of longer cells and a nucleus of rounded, polygonal cells, but this was denied by Hoffman (1883). Ehrlich (1875), a student of Schenk, reported that the peripheral part of the "Urwirbel" in trout embryos (Salmo fario) gives rise not only to the subcutaneous connective tissue, but to the myosepta of the trunk musculature as well. He noted, however, that the cells of the peripheral part apparently change shape prior to their differentiation into connective tissue elements, forming a layer of flattened cells with oval nuclei on the external surface of the "Urwirbel" (Figs. 1a,b). The "light regions" observed by him beneath this flattened

cell layer presumably became a part of the "Urwirbelkerne." It is interesting that, although Ehrlich's Figure 2 (Fig. 1b) is one of the most accurate 19th century representations of myotomes in older teleost embryos, he apparently did not recognize the "light regions" as a superficial layer of muscle cells.

In 1888, Ziegler applied Rabl's concepts to bony fishes and mentioned that in many teleosts (Salmo, Esox, Labrax) the outer layer of the myotome supplies a "Muskellamelle" and is, therefore, not exclusively a "Cutisblatte" in some vertebrates (Ziegler, 1888).

The compact nature of the newly formed somites in trout embryos as reported by Oellacher (1872) was confirmed by Henneguy (1888) who stressed that, although the peripheral cells are arranged in a regular layer, the somites never contain a cavity which is an extension of the coelom. Felix (1897) also confirmed Oellacher's description of the newly formed somites in the trout and salmon, and illustrated mitotic figures within them. He suggested that the presence of radially oriented, large nuclei in the lateral cells indicates the future formation of the "Cutisblatte."

In the United States, Wilson (1891) published a monograph on the embryology of the sea bass which included a brief section on the somites. He was apparently unconcerned with the questions of "Urwirbellamelle" and their fates, and merely recorded what he saw. He, too, described the somites as solid, rectangular structures composed of an inner mass of polygonal cells and an outer layer of cuboidal cells forming a smooth surface. These soon undergo gross changes in shape; elongating dorso-ventrally and assuming a characteristic "chevron" shape. Because

he was uncertain about the formation of the connective tissue (i.e., skeleton, "wandering cells"), he implied that all somitic cells elongate into muscle cells, extending the entire length of the somite and containing nuclei with conspicuous nucleoli. The beginning of elongation appeared to be indicated by the loss of the smooth surface in the region of the notochord on the medial side of the somite.

At later stages, Wilson observed that the muscle mass is divided at the level of the notochord into a dorsal and a ventral portion by a wedge of small cells with inconspicuous nuclei and nucleoli (Fig. 1c). He labelled these as "connective tissue (?) cells," since in larval stages several connective tissue cells were present in this region (Fig. 1d). Wilson's work, although extensive, was not immediately incorporated into the European literature.

The first specific study of the internal differentiation of the somite in teleost embryos was published by Kaestner (1892). It was based primarily on the trout, although Syngnathous, Gobius, and Hippocampus were also examined. Kaestner, too, described the "Urwirbel" as solid structures with a one-layered peripheral epithelium ("Rinde") which is sharply demarcated from a central group of irregularly oriented cells ("Kern") (Fig. 1e) and, like Henneguy (1888) and Wilson (1891), he showed that the shape of the "Urwirbel" is affected by the general change in shape of the embryo; becoming elongated dorso-ventrally and decreasing in lateral dimension.

His description of the latter development of the "Urwirbel" differed from those of previous investigators, however, and was based in part on accurate observation, and in part on the conclusions of his former study

concerning the "Rükentafel" ("Muskelplatte") of Remak in chick and duck embryos (Kaestner, 1890).

Kaestner proposed that the "Urwirbel" gradually becomes a two-component structure, with a one-layered lateral lamella composed of cylindrical cells, and a multilayered medial lamella formed by the "Kern" and the medial portion of the "Rinde." Like Wilson (1891), Kaestner reported that the process of muscle fiber formation begins near the notochord in the medial wall of the medial lamella (Fig. 1e), and spreads laterally, dorsally and ventrally, gradually involving the cells of the "Kern" and the medial lamella.

At this point, according to Kaestner, the sclerotome forms the ventral end of the medial lamella and begins to grow dorsally between the muscle fibers and the notochord, leaving a myotome composed of a medial layer of muscle cells ("primary lateral muscle"), and a lateral lamella which is still undifferentiated (Fig. 1f).

Kaestner's description of the myotome at a still older stage (Fig. 1g) is essentially identical to that of Ehrlich (Fig. 1b). However, Kaestner was the first to interpret the "light regions" of Ehrlich as a superficial layer of "young" muscle cells. Both the young muscle cells and the squamous epithelium covering them were thought to be derived by mitosis from the cells of the lateral lamella, the medial daughter cells forming the muscle cells, and the lateral ones becoming the squamous epithelium.

This configuration of the myotome was maintained for some time, modified only shortly before hatching by the horizontal septum which divided the large medial muscle mass into dorsal and ventral divisions

(Fig. 1h).

From 1892 to 1913, a series of papers dealing with the comparative anatomy and embryology of numerous species, including the teleosts; Chondrostoma nasus, Esox lucius, Salmo fario and Tinca fluviatus was published by Maurer (1892a,b, 1894, 1906, 1913). His views of teleost development differed sharply from those of Kaestner and most previous workers, and were greatly influenced by his studies of Cyclostomes, Acipenser, and Siredon (Maurer, 1892b). Although many of his embryological descriptions were later refuted, they were widely disseminated and influenced much subsequent work.

Unlike Kaestner and others who had shown that the newly formed somites are solid structures, Maurer essentially adopted the concepts of Rabl, and described the newly formed "Urwirbel" as consisting of a medial lamella ("Muskelplatte") separated by a conspicuous cavity ("Urwirbelhöhle") from the lateral lamella ("Cutisblatte") (Fig. 1i). He maintained that there is a basic plan of "Urwirbel" differentiation in all vertebrates: 1) a major portion of the medial lamella giving rise to striated muscle ("Muskelblatte") and a minor portion (ventral border) to the sclerotome; and 2) the lateral lamella ("Cutisblatte" or "Cornium") giving rise to the dermis and not to muscle (Maurer, 1906).

Maurer confirmed that myogenesis begins medially near the notochord, and like Kaestner, also observed two regions within the lateral muscle mass of recently hatched trout larvae: 1) a deeper portion, many fibers thick, which forms the majority of the trunk muscle; and 2) a superficial layer of presumably young fibers, each of which contains a large "Fibrillengruppe" medial to the nucleus (Maurer, 1894). The

deep fibers were thought to arise from the "Muskelplatte." However, the origin of the superficial muscle layer remained uncertain. Because he conceived of the newly formed "Urwirbel" as a hollow structure, Maurer was forced to disagree with Kaestner's proposed origin of these cells by mitosis from the lateral lamella. He suggested initially that the superficial muscle layer may arise by migration of cells dorsally and ventrally between the medial lamella and the "Cutisblatte" (Maurer, 1892a) but later changed his opinion, and considered them to originate by "pinching off" from the cytoplasm at the lateral border of the medial lamella (Maurer, 1894, 1906) (Fig. 1j).

Maurer was not certain about the fate of the medial and lateral muscle layers. He apparently did not associate them with the superficial (red) and deep (white) portions of the adult lateral musculature in teleosts which he himself had described (Maurer, 1892a). In fact, he did not refer to either Ryder or Vialleton who had earlier suggested an association of the embryonic muscle layers and the red and white muscle of the adult (see discussion below).

The compact nature of the somites in trout was again confirmed by Swaen and Brachet (1900, 1902) who attributed both the changes in shape undergone by the somite as a whole, and the act of segmentation itself, primarily to changes in shape and orientation of the peripheral cells. Their description of the fate of the somite ("Sclέromyotome") differed somewhat from that of previous investigators. They felt that some time after a somite has formed a small cavity appears near its ventral extremity. The cells in this area lose their epithelial nature and form mesenchyme, thus separating the original somite into a superior

portion (myotome) and a ventral portion (sclerotome).

Swaen and Brachet confirmed that muscle cells first form medially near the notochord, and one of their illustrations (Fig. 70, Pl. VII) shows two distinct cellular regions within each myotome of an older trout embryo: 1) a lateral layer one or two cuboidal cells thick, and 2) a deeper region with many nuclei arranged in rows separated by rather indistinct cell outlines.

Ryder (1882) proposed that the outer plates of dark colored muscle in the cod "appear to be derived by delamination from the same somites from which the dorsolateral and ventrolateral plates have differentiated," but Vialleton (1902) apparently unaware of this work, claimed to be the first to suggest the direct relationship between the two muscle layers observed in fish larvae and the red and white muscle layers of the adult. He demonstrated that the lateral muscle of the hatching trout is limited externally by a thin sheet composed of tall cylindrical cells with their nuclei lateral to the fibrils. Vialleton favored Kaestner's view that this layer arises from the external lamina of the "Urwirbel," and called it the "lame de Kaestner." He disagreed, however, with Kaestner's interpretation. According to Vialleton, as development of the muscle progresses, the "lame de Kaestner" does appear as if formed by a row of muscle fibers covered on the outside by an epithelium, but this is an illusion produced in the cells of this layer by the flattened nuclei and cytoplasm laterally, and the myofibrils medially. His opinion is clouded, however, when he admits that a layer of nucleated protoplasm may occasionally be observed outside the "lame de Kaestner." He reported that new muscle bundles appear in this protoplasm and in

this way cause an increase in width of the "lame de Kaestner." New fibers were thought to appear in a similar manner in the medial muscle mass, particularly near the "lame de Kaestner."

Shortly before hatching, the lateral muscle cells could easily be identified by the greater development of their contractile bundles in contrast to the small peripheral fibrils in the muscle cells immediately subjacent to them. The differences in the cross-sectional appearance of the fibrils of the lateral and medial muscle cells in just-hatched larvae are not the same differences as in older stages, however, and Vialleton showed in alevins that the myofibrils of the lateral muscle cells are not uniform bundles, but are arranged in "twisted bands" or "small islets." He also pointed out that the lateral muscle layer in older stages forms a wedge which penetrates between the dorsal and ventral portions of the main muscle mass near the lateral line.

In 1911 Sunier published an account of somite differentiation in teleosts based on the Muraenides (Moray eels). He discussed the differentiation of the somites as units and demonstrated gross changes in shape similar to those described previously for trout. He emphasized that there is never any contact between the ends of adjacent myotomes.

Sunier confirmed that the newly-formed somites are solid, but he felt that at the beginning of somite development, there is no distinction between a lateral epithelial layer and irregular inner cells as described by Kaestner (1892). Sunier believed that all previous descriptions were inaccurate because the investigators could not discern cell borders, which he claimed to be able to do. The essential distinction in Sunier's work as compared to prior authors was that the process of myogenesis

begins medially with the formation of cells ("en forme de plaque") which extend the length of the somite, and become multinucleate, presumably by amitosis. The remaining cells differentiate in two ways: 1) a single intermediate layer of cells elongates to form muscle cells each with a single centrally-located nucleus; and 2) the lateral-most cells flatten to form an external sheet ("feuillet externe") which apparently does not increase in mass and becomes very thin due to the growth of the myotome (Fig. 1k).

Unfortunately, Sunier did not suspect the fundamental importance underlying his observations, for he stated that there is no difference between the medial and lateral muscle cells, and he did not include references to either Ryder or Vialleton.

Sunier described small masses of cells at the dorsal and ventral somite borders, and interpreted these as growth zones which give rise to the connective tissue and sclerotome. Moreover, since the cells of the "feuillet externe" could not be observed at later stages, he proposed that this thin sheet ultimately becomes discontinuous and also forms a part of the connective tissue.

After Sunier, there was an almost total lack of studies dealing with somite development and differentiation in the teleosts, although Fischelson (1966) has recently reported the presence of a "myocoelom" in somites of the Gattung, Tilapia (Cichlidae), and Mahon and Hoar (1956) have included a brief mention of muscle development in the Chum Salmon (Oncorhynchus keta). Several papers primarily concerned with adult morphology, however, do contain references to the development of the superficial and deep muscle fibers. Most of these are directed toward

explaining the presumed "primitive" appearance of the superficial fibers (Chevrel, 1913), and are variations of the old statement of Stannius (1854) that the dark superficial layer appears to be in the process of becoming muscle.

One such attempt to account for the more "embryonic character" of the superficial muscle fibers was made by Kirsche (1948) who noted that the difference between the average diameters of the superficial and deep fibers in the guppy (Lebistes) increases during development. Because he could detect no significant differences between the myoblasts in the myotomes at earlier stages, he concluded that the myofibrils form rapidly in the deep fibers and, more slowly, in the superficial fibers. It will be recalled that Bühn (1940) had expressed the opposite opinion, namely, that the superficial fibers are "secondary" fibers, which appear later and become more like the deeper "primary" fibers as they mature. Barets (1961) has pointed out that although Bühn's concept is contrary to many embryological studies, and although morphological differences between the superficial and deep portions of the adult lateral musculature strongly suggest underlying developmental differences, all specific interpretation of the data in this regard seems premature.

Regarding the deep fibers, Greene (1913b) accounted for the great diversity in diameter (40-250 μ) of the deep muscle cells in young salmon by assuming that the "fibers are undergoing longitudinal cleavage which is very unequal," and Maser (1949), assuming that the smaller fibers of the deep muscle (which generally occur toward the lateral surface) are young fibers, suggested that perhaps new fibers are always produced laterally. One might have hoped that the increased resolution of the

electron microscope would add significant information to these problems. However, to date, the only fine structural studies of teleost muscle development are those of van Breémen (1952) and Yamamoto (1965).

Van Breémen studied embryos of the guppy (Lebistes reticulatus) which, according to the earliest electron microscopical techniques, were fixed in formalin, embedded in paraffin and rubber cement, and sectioned with a razor blade. The electron micrographs are, therefore, primarily of historical interest, and his conclusions must be regarded with caution.

Yamamoto examined primarily fibrillogenesis within the developing trunk musculature of the Medaka (Oryzius latipes). The location of the myoblasts illustrated within the myotome was not explained, and no distinction was made between superficial and deep portions. His results are discussed below in relation to the observations of this investigation.

The preceding review of the literature clearly indicates that the lateral musculature of many teleosts affords a system for the selective examination of distinctly different types of adult vertebrate skeletal muscle fibers, and for the study of their embryological differentiation. It also points out, however, the confusion which exists concerning the internal differentiation of the teleost somite, and the lack of recent histological and ultrastructural data with which to evaluate proposed theories regarding the development of the two main adult fiber types. This investigation, therefore, was undertaken to: 1) explore structural differences between the fibers of the superficial and deep portions of the lateral musculature in an adult teleost; and 2) study the development of these differences by examining normal somite differentiation with special emphasis on the myotome.

MATERIALS AND METHODS

I. EXPERIMENTAL ANIMALS

The experimental animal chosen for this investigation was the zebrafish (Brachydanio rerio [Hamilton-Buchanan]). This small aquarium fish is a tropical species native to India (Ceylon, Bengal, Madras). It is a member of the family Cyprinidae, and like other members of this family (carps, minnows, goldfish), has been frequently used in light microscopical studies of teleost development (Creaser, 1934). Although these researches provide a basis for future investigation of zebrafish embryology, they contain very little histological, and no ultrastructural information; particularly concerning older embryonic and larval stages.

Advantages of the zebrafish as an experimental animal are: 1) the almost year-round availability of eggs; 2) the small size and relative transparency of the embryo and chorion (egg membrane); 3) the rapid rate of development; and 4) the fact that as a Cyprinid, the superficial and deep portions of the lateral musculature are well developed and distinctly separated.

II. MAINTENANCE OF FISH

Adult zebrafish, obtained from local pet shops, were maintained in glass aquaria in schools of 12-16 animals. The ratio of males to females was approximately 2:1 and no attempt was made to separate the sexes. The water temperature of the tanks was maintained at 75-80°F with a thermostatically controlled heater, and aeration was used.

The bottom of the aquarium was covered either with a single layer of glass marbles or with a layer of fine sand and gravel.

The fish were fed both dry commercial and/or frozen brine shrimp

(Artemia). Best production of eggs was obtained when dry food was fed in the morning followed by brine shrimp in the afternoon.

III. PROCEDURES

A. Adult: Tissue Preparation.

For electron microscopy the trunks of small zebrafish (1.5 cm) were dissected and placed immediately into ice-cold chrome-osmium fixative (Dalton, 1955) containing a final osmium concentration of 2.5% for 1-2 hours, followed by postfixation in ice-cold 10% formalin for 1 hour. Dehydration at room temperature and embedding in Epon was according to the procedure recommended by Luft (1961).

For light microscopy of paraffin embedded material, adult zebrafish (3-4 cm long) were fixed in 10% ("neutralized") formaldehyde, and double embedded in nitrocellulose and picolyte paraffin. Formalin was injected into abdominal cavity with a syringe, the ventral body wall was then opened, and the fish immersed in fresh fixative. Fixation time was 7-13 days. Blocks were sectioned at 8-10 μ after being soaked overnight in a 1:1 mixture of 60% alcohol and glycerol. Paraffin sections were stained with hematoxylin and eosin.

For histochemistry, the midbody regions of adult zebrafish (3.5-4 cm long) were dissected from decapitated animals and frozen immediately on dry ice. They were then mounted in a cryostat, and sections cut at a setting of 12-14 μ at minus 15-20°C. The sections were placed on albuminized slides and allowed to air dry for 5-10 minutes after which they were subjected to the following incubation procedures:

1. Succinic Dehydrogenase (SDH). The procedure for the locali-

zation of SDH activity was essentially that of Ogata and Mori (1964). Unfixed sections were placed into a solution containing 0.2 M Na_2Succinate.$6H_2O$ (5 ml), 0.1% nitro-blue tetrazolium (NBT) (10 ml), and 0.2 M phosphate buffer (5 ml). The reaction was accomplished in Coplin jars at 33-36°C for 30 minutes to 2 hours. The sections were then fixed in 10% formalin (15-20 min) and mounted in glycerine jelly.

2. Sudan Black B. Following fixation in Baker's formaldehyde (Baker, 1944) for 1 hour at room temperature, sections were stained for "total lipid" with 0.7% Sudan Black B in propylene glycol (Pearse, 1960). Counterstaining was omitted. Staining time was 5-20 minutes at room temperature. After washing, the slides were mounted in glycerine jelly.

3. Oil Red O. Both unfixed and sections fixed in Baker's formaldehyde for one hour were stained for "neutral lipids" with Oil Red O in iso-propanol (Pearse, 1960). Incubation was from 10-25 minutes both at room temperature and at 33-36°C. Counterstaining with Meyer's hemalum was done in some cases. The slides were mounted in glycerine jelly.

B. Embryonic: Manipulation of Living Specimens.

Eggs were collected from the bottom of the tank by means of a dip-tube, and kept in finger bowls at room temperature until needed. They were observed with a binocular dissecting microscope, or were placed in a standard depression slide, covered with a coverslip, and observed at higher magnification.

The developmental stage of the embryos was determined under the dissecting scope, and selected specimens were pipetted into a small

Petri dish. Only clear (transparent) embryos of normal appearance were selected. Late embryonic stages were anesthetized with a small amount of MS222 (Tricaine methanesulfonate, Sandoz Pharmaceuticals) for observation. The stages chosen were based on somite number, and according to the stages established by Hisaoka and Battle (1958).

The chorion was rapidly removed from embryos by means of a pair of watchmaker's forceps, and the embryos were pipetted immediately into the fixative. Embryos distorted or obviously damaged during these procedures were discarded. Free swimming larvae were pipetted directly into the fixative.

C. Embryonic Tissue Preparation.

A number of fixative systems were tried during the early stages of this study, but adequate preservation of embryonic material proved elusive. Best results were obtained after primary fixation with a 1/2 dilution of Karnovsky's aldehyde mixture (Karnovsky, 1965) followed by postfixation with osmium tetroxide, and variations of this procedure were used for each stage examined.

In general, embryos were fixed in diluted Karnovsky's fluid at room temperature or on ice for from 30 minutes to 7.5 hours (most often 1 hour). They were then washed at room temperature or on ice in several changes of buffer (approximately adjusted for molarity). Both phosphate and dichromate buffers were used. Total time of washing ranged from 10 minutes to 21 hours, but was 10-30 minutes in most cases. Postfixation was in ice-cold 2.5% OsO_4 in either phosphate or dichromate buffer for 30 minutes to 1 hour. Dehydration was in a graded series of ethanol, beginning with 50%. Total dehydration time was approximately 1 hour and

was accomplished at room temperature. The embedding procedure was essentially that of Luft (1961). Following 2 changes of propylene oxide, the tissue was placed in a 50% mixture of propylene oxide and Epon 812 (with accelerator) for 3-6 hours, then into an equal volume of Epon for "overnight." The embryos were then placed into a 35°C oven for 1-2 hours, at which time they were oriented under a dissecting microscope and returned to the 45°C and 60°C ovens for approximately 24 hours each. Following polymerization, the specimens were mounted on aluminum slugs with epoxy glue.

The small size and transparency of the embryos permitted rather precise orientation, allowing sections to be cut from the same group of somites at each stage examined. The midbody somites, numbers 17-20, were selected because: 1) they are easily identifiable in all stages studied by virtue of their position just posterior to the anus and the tail extension of yolk, and 2) they are representative of the trunk musculature. Being posterior to the abdominal cavity, problems arising from the formation of the abdominal musculature were not encountered.

Developmental stages utilized were selected on the basis of observation of the midbody somites in living embryos in an attempt to provide: 1) a stage prior to muscle differentiation (20-somite), 2) a stage displaying the first appearance of myoblasts (25-somite), 3) an embryonic stage in which the lateral musculature is functioning (33-somite), and 4) a free swimming larval stage (3-4 days posthatching).

Sections (1-2 μ thick) were cut with glass knives on a Porter-Blum II ultramicrotome, mounted on glass slides, stained for approximately 30 seconds with methylene blue-azure II (Richardson et al., 1960), and

coverslipped with either Epon or "Permount" resin. The midbody somites were located by examination of the thick sections cut in this manner. Because the total number of somite pairs of the embryos was known prior to fixation, the number of the somite being sectioned at any time was determined by counting the number of somites sectioned in a cranial direction from the unsegmented mesoderm. In larvae, the midbody somites were located by their position immediately behind the anus.

Once the number and orientation of the somites to be sectioned had been determined, thin sections showing gold to silver interference colors were cut with the Porter-Blum II microtome using a DuPont diamond knife. Thin sections were placed on carbon-coated grids and stained with half-saturated aqueous uranyl acetate followed by alkaline lead citrate (Reynolds, 1963), each for 20-30 minutes at room temperature. Some grids were singly stained.

Sections were examined with an RCA-2A electron microscope operated at 40 kv.

Thin sections cut at various levels were examined from a minimum of 2 specimens at each developmental stage studied.

OBSERVATIONS

I. ADULT STAGES

Cross-sections through the midbody region of adult zebrafish (Fig. 2) reveal the typical appearance of fish trunk muscle. The lateral musculature is divided by the vertebral column and dorsal and ventral median septa into symmetrically right and left halves, each of which in turn is divided into a dorsal (epaxial) and a ventral (hypaxial) portion by a horizontal septum extending from the vertebral bodies to the skin on either side.

When viewed laterally, the myomeres have the typical piscine " ≤ " configuration. They are separated by myosepta which are relatively straight in their deep course and pass medially and anteriorly from the skin to the median connective tissue and axial body structures. Supra- and infracarinales, and portions of the unpaired fin musculature are seen between the median septum and the dorsal and ventral tips of the lateral muscle masses.

In sections of fresh tissue, the majority of the fibers appear pale or white, but a well-developed, wedge-shaped strip of light reddish-brown fibers is seen at the lateral end of the horizontal septum. In histological preparations (Fig. 3a), this superficial portion of the lateral musculature is seen to be composed of rather small, highly vascularized, fibers and is separated from the deep portion by a thin fascia. This fascia becomes thicker in more mature specimens (Fig. 3b). The superficial muscle, like the deep, is divided by the horizontal septum and myosepta. It does not form a continuous layer along the

entire lateral surface of the myomere, but thins rapidly dorsal and ventral to the horizontal septum. Only a few small, flattened cells are occasionally seen beneath the skin near the dorsal and ventral ends of the myomere, and it is unclear whether these belong to the superficial portion. The lateral nerve is seen within the horizontal septum near the apex of the wedge of superficial fibers.

Superficial fibers in the mature adult vary in diameter from 6 μ to 35 μ. The smaller of these are characteristically found along the fascia at the medial margin of the muscle; the larger ones are found laterally. The sarcoplasm of the superficial fibers is distributed peripherally as well as among the myofibrils (Fig. 3b).

Fibers of the deep portion are generally much larger than those of the superficial wedge. A well-developed region of "intermediate" fibers is seen laterally within the deep muscle near the horizontal septum. These fibers are generally intermediate in size between the superficial and deep, and are separated from the superficial fibers by the fascia. The smallest intermediate fibers are found laterally along the fascia, and they merge with the remaining deep fibers medially. The sarcoplasm forms a thick rim and is more thinly distributed among the fibrils than in the superficial fibers. The intermediate fibers are more vascularized than the remainder of the deep muscle (Fig. 3a,b).

The majority of the deep fibers display a very narrow rim of sarcoplasm, and are uniformly filled with myofibrils (Figs. 3,5).

In young adult zebrafish the intermediate and deep muscle fibers are approximately equal in size (Fig. 3a). In the mature adult (Figs. 3b,c), however, the average size of the deep fibers is significantly

greater than that of the intermediate fibers.

The three fiber regions may also be distinguished by their lipid content and succinic dehydrogenase (SDH) activity. Oil Red O and Sudan Black B staining (Fig. 3b) reveal a higher lipid content in the superficial fibers, almost no intracellular fat in the majority of the deep fibers, and an intermediate amount in the intermediate fibers. Similarly, intense SDH activity is seen within the superficial fibers (Fig. 3c), almost none within the deep fibers, and an intermediate activity in the intermediate fibers.

The pattern of SDH activity is paralleled by the mitochondrial distribution within the fibers of the three regions. In the superficial fibers numerous mitochondria surround the myofibrils and are dispersed among them. The intermediate fibers have a rim of densely packed mitochondria, with a few scattered between the central myofibrils (Fig. 4), and the majority of the deep fibers have only a few mitochondria around the periphery of the cell or in small accumulations at one side of the fiber (Fig. 5). There may also be a few widely scattered throughout the fibrils.

Glycogen particles can be observed in all fibers in electron micrographs, but the superficial and intermediate fibers appear to contain more than do the deep fibers. The glycogen particles are distributed among the fibrils and within the peripheral sarcoplasm. Large accumulations of glycogen particles can be observed in the peripheral sarcoplasm of some superficial fibers, but they are not obvious within deep fibers (Figs. 6a,b).

Myofibrils of all fiber types exhibit distinct Z-, I-, A-, H-,

and M-bands (Fig. 6), and measurements of sarcomere length in routine electron micrographs revealed no significant differences between superficial and deep fibers. The range of values for the distance between Z-bands in both fiber types was 1.75 - 2.0 μ. Myofibrils with no observable I-bands, presumably due to maximal contraction, were excluded. The range of values for measurements of the A-band width in these same preparations was 1.4 - 1.7 μ in both the superficial and deep fibers.

No differences in the sarcoplasmic reticulum were observed between the superficial and deep muscle fibers although preservation of membranous structures in adult fish following osmium-formaldehyde fixation was generally not as satisfactory as in embryonic and larval specimens after Karnovsky aldehyde fixation. In both cell types numerous longitudinal cisternae are present in the cytoplasm between adjacent fibers as described in the black mollie by Franzini-Armstrong and Porter (1964a).

T-tubules are present over each Z-band in all cell types. No continuity was observed between the T-tubules and the cell membranes or the nuclear envelope.

Presumed neuromuscular junctions were occasionally observed in the lateral musculature. No fine structural differences were noted between those of the superficial, intermediate, or deep muscle fibers (Figs. 6c-e).

No complex folding of the muscle cell membrane or cytoplasmic specialization was observed in the region of nerve-muscle contact. The axonal profiles generally lie in shallow depressions of the muscle cell surface. They contain small mitochondria and numerous vesicles. A uniform space of 510-530 Å separates the nerve and muscle cell membranes, and contains a line of increased density approximately midway between

the two cells. The surface of the axon opposite the muscle cell is covered by a Schwann cell.

II. DEVELOPMENTAL STAGES

A. 20-Somite Embryos.

The embryos are approximately 24 hours postfertilization, and correspond to stage 20 of Hisaoka and Battle (1858).* The tail has begun to elevate from the yolk, and a short extension of yolk extends posteriorly from the main yolk mass. At this stage, somites No. 17 and 18 are located lateral to the posterior tip of this extension. Twitching of muscle cells can be detected at high magnification within the anterior somites of embryos having only 17 somites; by the 20-somite stage muscular movements are visible under the dissecting microscope. All embryos selected were twitching prior to fixation, but no movements were detected within the midbody somites.

In horizontal sections (Fig. 7a) the unsegmented portion of the somitic mesoderm is seen as a cellular column, 4-6 cells wide, extending posteriorly from the newly forming somites on either side of the notochord and neural keel. Cells of the unsegmented mesoderm are polygonal in outline and are rather loosely aggregated. The cells at the medial and lateral surfaces appear to be more closely apposed, but are not elongated. Each cell contains a single nucleus, which may be irregular in outline and have rather deep indentations (Fig. 8a). The cytoplasm contains numerous mitochondria, smooth membranous cisternae and vesicles of the Golgi apparatus, many small profiles of rough endoplasmic reticulum, groups of "free" ribosomes, and much glycogen (Fig. 8a). In

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* The stages established by these authors will be used subsequently.

addition, lamellated membranous structures occur frequently throughout the cytoplasm. These have a complicated fine structure, and are often associated with glycogen granules and mitochondria (Fig. 8b). They are less frequent at later stages and have not been observed following primary osmium fixation. They are tentatively considered to be fixation artifacts. Very few specialized intercellular attachments are seen, with the exception of small "embryonic" junctions (see p. 59). Although some extracellular material is occasionally observed, no distinct external lamina (Fawcett, 1966) surrounds the unsegmented mesoderm.

The newly formed midbody somites appear as relatively compact cellular units separated by distinct intersomitic furrows (Figs. 7a,b). Their lateral surfaces become rounded and smooth, with the exception of blunt cellular protrusions which are most numerous near the lateral ends of the intersomitic furrows and at the dorsal and ventral ends of the somite. Soon after their formation, the somites begin to assume the chevron shape characteristic of embryonic and early larval stages (Fig. 7b). As development proceeds, the cells of the somite become more closely apposed, with a concomitant decrease in intercellular spaces.

The majority of the cells retain their polygonal shape and appearance, but the peripheral cells are more closely apposed in all planes of section, thus forming an epithelial somitic cortex (Fig. 9). Narrow cytoplasmic extensions are seen to originate from many cells and insert between adjacent cells (Fig. 10). There is no indication of a myocoel.

Intercellular junctions observed between the somitic cells at this stage may perhaps be classified best as "embryonic" or "focal intermediate"

junctions (see Kelly, 1967; Kelly and Luft, 1966). Very small embryonic junctions are more numerous, and occur between adjacent cells regardless of their location within the somite. They are characterized by a short region of parallel alignment of adjacent prominent cell membranes separated by approximately 100-120 Å. Some dense intercellular material is seen, and some fibrillar components may appear on the cytoplasmic side, but these embryonic junctions lack the highly ordered structure of mature desmosomes (Fig. 9, inset).

"Focal intermediate junctions" consist of a longer region of uniform separation (about 150-160 Å) between opposed, and again rather prominent, cell membranes. However, there appears to be no increased cytoplasmic density at the junction. Like the smaller junctions, these also occur between both peripheral and central cells (Fig. 10).

Portions of centrioles and flagella are frequently observed in thin sections at this stage. In longitudinal section (Fig. 11) a flagellum is seen to project from one of two centrioles at its base into a membrane bounded sleeve. In cross-section (Fig. 10, inset) the flagella contain 9 peripheral structures which are occasionally seen to be tubular. Although there is some material at the center of the flagellum, a central doublet was not observed. No more than one flagellum was observed per cell in thin sections, and there did not appear to be any definite polarity of these structures regarding either the location of the centriole pair within the cells, or the orientation of the flagellar shafts within the somite as a whole. A collection of smooth surfaced membranous cisternae and microtubules is associated with the centriole pair. Microtubules also are found coursing throughout the cytoplasm.

Mitotic figures are frequently seen within the unsegmented mesoderm and the newly-formed somites, and focal intermediate junctions are commonly present between a dividing cell and adjacent cells (Fig. 12).

Although elongated cells with lightly staining nuclei (presumptive myoblasts) are sometimes found near the medial surfaces of somite No. 17 at this stage (Fig. 7b), no distinct muscle cells are present in the midbody somites. The most medial cells of the youngest midbody somite (No. 20) show no signs of elongation or of myofilament formation (Fig. 13). The nuclei of these cells, however, may appear less dense than those of the cells lateral to them.

Some flocculant extracellular material is characteristically seen at this stage between the medial somitic cells and the spinal cord (Fig. 13), but not at the lateral surface of the somite. In the intersomitic furrows, very little extracellular material can be found, and almost no specialized cell contacts are observed between cells of adjacent somites (Fig. 10).

B. 25-Somite Embryos.

These embryos are approximately 26 hours postfertilization, and are between stages 20 and 21. The development of the tail has progressed, and the tail extension of yolk is becoming more sharply delineated. Strong, but slow, contractions occur at irregular intervals within the anterior somites. Because of the gross movements produced, it is difficult to determine whether contractions are also occurring within the midbody somites.

The general appearance of the somites in histological preparations is similar to that of the 20-somite stage with the important exception

that one or two layers of elongated cells have formed on the medial surface of each midbody somite (Fig. 14). The more lateral cells retain the polygonal shape and fine structural appearance of previous stages, including flagella and microtubules (Fig. 15).

The more medial of the elongated cells are myoblasts* containing groups of thick and thin myofilaments of uncertain length (Fig. 16). Each myoblast contains a single elongated nucleus with one or more prominent nucleoli and dispersed chromatin. The appearance of the myoblast nuclei is in sharp contrast to that of the dense nuclei of the majority of the more lateral cells. No indication of cell or nuclear division has been seen in any of the myoblasts, although mitotic figures were observed in cells lateral to them. The mitochondria and other organelles appear similar to those of earlier stages. Dense membrane-bounded structures are often observed in the myoblasts. They are of unknown origin, but appear similar to structures which have generally been included into the lysosome family of organelles. A decrease in over-all cytoplasmic density was evident in many of the

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* Much confusion exists in the literature regarding the terminology applied to cells in various stages of myogenesis. The terminology used in this paper is based on the fine structural appearance and is as follows: "presumptive myoblast" a mononucleate, elongated cell which contains no myofilaments but which will presumably develop into a "myoblast" - an elongated cell containing myofilaments, but no large myofibrils; "young muscle cell" - a mono- or multinucleate cell containing well-developed myofibrils; and "muscle cells" - cells whose appearance approaches that of the muscle cells of the adult.

myoblasts in comparison with that of other somite cells.

The myofilament bundles are generally, but not always, aligned parallel to the long axis of the cell, and are often associated with areas of increased free ribosome content. They are located both near the cell surface and toward the center of the cell. They are composed of aligned thick and thin filaments and contain occasional presumptive Z-bands but no distinct sarcomeres (Fig. 16).

In sections, some myoblasts appear to extend the entire length of the somite, while others do not. Whether the latter condition reflects a true variation in the degree of elongation of the myoblasts, or merely arises because the plane of section is slightly oblique to the long axes of some cells could not be determined. Small embryonic, but no focal intermediate junctions are occasionally observed between the myoblasts and presumptive myoblasts.

Centrioles were observed within myoblasts and presumptive myoblasts, and in a single case, a flagellum was seen to originate from a myoblast. Microtubules are frequent occupants of the myoblasts and presumptive myoblasts, and are generally aligned parallel to the long axis of the cell. They occur near the cell membrane in many cases (Fig. 17).

Some extracellular material was observed along the medial surfaces of the somites but a lamellated basal lamina like that surrounding the spinal cord and notochord was not seen (Figs. 14a,b). As in the 20-somite embryo, little extracellular material is present on the lateral surfaces of the somites and in the intersomitic furrows.

C. 33-Somite Embryos

These embryos are approximately 40-45 hours postfertilization and between stages 22 and 23. Retinal pigmentation is well developed and small melanophores are scattered over the head and body. A continuous fin-fold circumscribes the posterior end of the tail. Circulation has been established within the major vessels, but no segmental circulation was observed within the midbody region. Strong contractions of the lateral musculature occur at frequent intervals, twisting the embryo about within the chorion.

The cross-sectional appearance of the midbody somites is illustrated in Figure 18a. Because of their pronounced chevron shape, the dorsal and ventral portions of the same somite, and the middle portion of the somite immediately posterior to it are seen. The somites are now separated by developing myosepta, and have elongated dorsoventrally and narrowed mediolaterally. They measure approximately 150-160 μ from dorsal to ventral tip and are about 30 μ wide dorsally and 50 μ ventrally.

At this stage, three distinct cellular regions can be observed within the compact somitic mass (Figs. 18a,b): 1) darkly staining "external cells" on the lateral surface of the somite beneath the epidermis; 2) a single lateral layer of lightly staining young "superficial muscle cells" extending from the dorsal to the ventral pole of each somite, and 3) several layers of young "deep muscle cells" medially. The general fine structural and topographical relationships of these three cellular regions are illustrated in Figure 19, which represents an area similar to that included within the rectangle of Figure 18a.

The external cells are extremely flattened, and are characterized by their densely staining nuclei and cytoplasm (Fig. 20). They contain mitochondria, glycogen and rough ER cisternae, but no myofibrils or clusters of filaments and ribosomes characteristic of myoblasts. In both cross- and horizontal sections the thin cytoplasmic processes of these cells are seen in close association with several subjacent superficial muscle cells, to which they may be attached by small embryonic junctions. Similar attachments occur between adjacent external cells, and overlapping of external cell processes is frequent (Fig. 19). The external cells form a continuous layer of flattened cells covering the entire lateral surface of the superficial muscle cells. They separate the myotome from the overlying epidermis.

Portions of cell processes containing either pigment granules or numerous vacuoles (0.1 - 0.4 μ) are also often present beneath the epidermis and presumably belong to melanophores or mesenchymal cells. They are always separated from the superficial muscle cells by processes of external cells (Fig. 19), and are not to be confused with them.

Because the middle portion of the somite present in a given cross-section is closely comparable to the middle portion of the somite immediately anterior to it, the superficial muscle cells may be seen in Figure 18a to form a continuous cellular layer along the lateral aspect of the myotome. Each superficial muscle cell extends the entire anterior-posterior dimension of the somite (about 65 μ) and is characterized by the presence of a single, elongated, centrally located nucleus with one or two prominent nucleoli, and especially by a large myofibrillar bundle along its medial margin (Figs. 18a,19,21).

The majority of the superficial muscle cells are roughly cuboidal or flattened in cross-section. They are approximately 7 μ wide in the region of the nucleus, and narrow slightly near their ends in the region of the myosepta. Small cell processes are often seen at the corners of the cells (Figs. 19,21).

Their cytoplasm generally appears less dense than that of the subjacent deep muscle cells, but contains the same organelles. In addition to the characteristic myofibrillar bundle medially, smaller myofibrils and groups of myofilaments may also be found throughout the cytoplasm (Fig. 21).

At the midnotochordal level, several cells extend the width of the myotome forming a cellular wedge, the base of which faces laterally (Figs. 18a,22). These flattened cells are considered to be superficial muscle cells because of their continuity with that layer dorsally and ventrally, the over-all density of the cytoplasm, and the presence of a single group of myofibrils medial to the nucleus. An external cell profile, from which processes extend medially between the superficial cells, is characteristically seen at the lateral ends of these cells. No indication of the horizontal septum which later occupies this region is visible at this stage.

In contrast to the superficial muscle cells, the deep muscle cells are characterized in cross-section by a number of rather small myofibrils distributed around the periphery of each cell, and a central core of cytoplasm containing the mitochondria, nuclei, ribosomal clusters and other organelles (Fig. 23). The mitochondria appear swollen and their matrix is less dense than that in the superficial muscle cells.

Free ribosomes and glycogen particles are present. Although the average cross-sectional area of the superficial and deep muscle cells is approximately equal at this stage, the dimensions of the deep muscle cell population vary much more widely. Smaller cells are characteristically located just medial to the superficial muscle cell layer (Figs. 19,23, 24) but do not form a continuous layer.

The deep muscle cells, like the superficial ones, extend the entire length of the somite. Since some of them are now oriented obliquely to the superficial muscle cells, however, their entire length is often not included in a single horizontal section.

In contrast to the mononucleate condition of the superficial muscle cells, deep muscle cells may have up to three nuclei distributed along the length of the cell and may properly be called "myotubes" (Konigsberg, 1965) (Fig. 25a).

Microtubules are seen in both the superficial and deep muscle cells. They are oriented more or less parallel to the long axis of the cell and are frequently located near myofibrils (Fig. 25b). These structures are comparable in appearance to the microtubules of earlier stages, with components of the spindle apparatus of dividing cells, and with the tubules of the flagella.

Centrioles (Figs. 22,26) and flagella (Fig. 26) were occasionally observed in deep but never in superficial muscle cells.

In favorably oriented sections, individual myofibrils within both superficial and deep muscle cells are seen to extend the entire length of the somite. The number of sarcomeres in such instances is 31-33 with regions of insertion at either end which cannot be described as complete sarcomeres.

Myofibrils of both cell types exhibit Z-, I-, A-, H-, and M-bands and frequently branch (Figs. 24,25,27). The sarcomere lengths of myofibrils in both superficial and deep muscle cells measure about 1.8 - 1.9 μ in routine electron micrographs. In sections including the entire width of the myotome it was noted that the sarcomere lengths generally increased from those of the fibrils in the most medial deep muscle cell to those of the myofibrillar bundle in the superficial muscle cells.

In myofibrils of both superficial and deep muscle cells, an hexagonal arrangement of thick filaments exists in the H- and A-band regions, and the thin filaments are also occasionally hexagonally arranged around each thick filament within the A-band (Fig. 19, insert). This hexagonal packing is generally seen near the center of the larger fibrils and is less regular toward their periphery. It also seems to be less regular in the myofibrils of the superficial cells. The thick filaments appear tubular in both H- and A-band regions, and bridges in the M-band are present between adjacent thick filaments.

Cisternae of the sarcoplasmic reticulum (SR) are seen in close association with most of the organized myofibrils (Fig. 27). In both the superficial and deep muscle cells the longitudinal elements of the SR are found parallel to the filaments over the I- and A-band regions. There is an enlarged terminal sac on either side of the Z-band, and a region of transverse confluence of the cisternae over the H-band. Elements of the SR are located between adjacent fibrils and between the fibrils and cell membrane. The transverse tubule of the "T-system" occurs at the level of the Z-band and forms the third, and middle

element of the triad with the terminal sacs on either side. Definite confluence of the T-tubules with the cell membrane was not observed. No differences in development and arrangement of the SR were observed between superficial and deep muscle cells.

The space between somites measures about 0.5 - 1 μ and contains fibrillar material with no apparent organization (Fig. 28). A periodicity is evident along some of these fibrils (Fig. 29a), and they presumably represent collagen.

Although the cells of each somite seem to form a relatively independent unit, numerous small cytoplasmic processes are seen within the developing myoseptal region. The short processes project from both superficial and deep muscle cells, but are often more evident between the ends of the superficial muscle cells. In many instances their point of origin corresponds with the terminal insertions of a cell's myofibrillar bundles, and they may interdigitate with like processes from a neighboring superficial muscle cell across the myoseptal region. Some of these may form specialized attachments with similar processes from muscle cells of adjacent somites (Fig. 29b). These attachments are similar to the focal intermediate junctions of earlier stages, and to those found most frequently at this stage near the ends of adjacent muscle cells of the same somite (Figs. 29b,30a).

Similar cell processes and attachments have not been observed between external cells (Fig. 28) which are closely associated with the superficial muscle cells. Neither external cells nor fibroblastic cells at the medial ends of the myosepta appear to penetrate the myoseptal region, but the possibility that they occasionally do so cannot be

excluded since not all profiles of cellular processes in this region can be directly traced to muscle cells.

At this stage a well-developed basal or external lamina is present beneath the epidermis (Fig. 24 and others), around the neural tube (Fig. 28) and between the cells of the notochord and its fibrous sheath (Fig. 22), but not around either individual muscle cells, or the myotomal unit. A layer of dense material exists at points along the medial surface of the myotome, particularly adjacent to the spinal cord (Fig. 25a) and extending into the myoseptal region. No such layer is present between the external cells and superficial muscle cells, nor immediately adjacent to the lateral surface of the external cells (Figs. 19,28).

Neuronal profiles are first seen within the lateral musculature at this stage and are characteristically located just medial to the anterior ends of the superficial muscle cells very close to the developing myoseptum (Fig. 30b). They may be identified by their low cytoplasmic density, by numerous vesicular profiles, and by their similarity to neuronal processes at later stages.

D. Larval Stage.

The free-swimming zebrafish larvae utilized were approximately 5-6 mm long and had the adult number of somites (34) (Fig. 31). They were fixed approximately 7 days postfertilization. Hatching generally occurs on the third or fourth day. Larvae of this stage are not constantly active, but show bursts of vigorous contractions of the body musculature followed normally by relatively long periods of quiet. Circulation is well established in the segmental vessels of the midbody region.

The somites remain chevron-shaped, but the myosepta are now beginning to course anteriorly and medially as in the adult (Fig. 31c). This diagonal course is less pronounced at the midnotochordal level in the region of the horizontal septum (Fig. 31b, arrow). Seen in cross-section (Fig. 31a), the somites have approximately doubled in dimension by comparison with those of the 33-somite embryo, and now measure about 250 μ dorsoventrally, 60 μμ across dorsally and 70-80 μ across ventrally. As in the 33-somite embryo, each myotome is narrowed at the midnotochordal level, but a well-developed horizontal septum is now present which divides the lateral musculature into dorsal and ventral portions. The lateral nerve lies within the lateral margin of this septum.

In histological preparations two distinct regions are readily apparent within each lateral muscle mass; a single layer of superficial muscle cells, and several layers of deep muscle cells. The external cells are not easily distinguished in such preparations, although electron micrographs reveal their presence (Fig. 33 and others). Melanophores and highly vacuolated cells are seen between the muscle cells and the epidermal basement membrane (Figs. 31a,c).

No capillaries were observed within the lateral musculature.

An area similar to that within the rectangle of Figure 31a is seen in Figure 32 at higher magnification.

Flattened external cells are present (Fig. 33). They have the same general appearance as in the 33-somite embryo, but no overlapping of external cell processes is seen, and gaps of varying extent occur between adjacent external cells (Figs. 34a,35,37a). The membranes of superficial muscle cells are exposed to the epidermal basement membrane

in regions between adjacent external cells, and patches of electron-dense material are often seen near the muscle cell surfaces. In some cases, external cell processes are observed between superficial muscle cells and vacuolated cells (Fig. 39a), but gaps occur here, too. Small embryonic junctions are occasionally observed between the superficial muscle cells and the external cell processes.

The superficial muscle cells form a single layer of small flattened cells covering the entire lateral surface of the lateral musculature from the dorsal to the ventral pole, interrupted longitudinally by the myosepta, and within each myomere, by the horizontal septum. They are generally more flattened than in the 33-somite embryo, and now measure only 1.5 - 4.5 μ in width at their centers. The superficial muscle cell layer curves medially at the horizontal septum to encompass the lateral nerve, but in contrast to the previous stage, no widened cells extend across the myotome (Fig. 32).

Myofibrils of the superficial muscle cells are both polygonal and ribbon-shaped in cross-section. At this stage they tend to be more polygonal and irregular than those of the deep muscle cells, and are situated in the medial part of the cell. Some mitochondria are found among the myofibrils and along the medial cell margin, but no definite alignment with respect to the fibrils has been observed. In most sections, the mitochondria appear darker than those in the deep muscle cells. This is primarily due to the greater density of the mitochondrial matrix.

Nuclei of the superficial muscle cells are located either lateral to the compact myofibrils or at one edge of the cell. They were never

observed medial to the myofibrils (Fig. 35). No more than one nuclear profile was observed within a cell but a nuclear profile was often located near the myoseptal end, and not always at the center as in the 33-somite embryo.

Accumulations of smooth surfaced cisternae and Golgi membranes (Fig. 37a) occur more often in the superficial, than in the deep muscle cells.

Single centrioles were occasionally found in superficial muscle cells, but no centriole pairs or flagella were observed.

The largest deep muscle cells measure about 15 by 30 μ, and most of them are much larger in cross-sectional area than the superficial muscle cells. The cross-sectional area varies in size, but in the previous stage, the smaller cells occur subjacent to the superficial muscle cells.

Some of these small cells contain well-developed myofibrils, while others do not. A few are characterized by a dense and darkly staining nucleus and cytoplasm and have been termed "dark cells" (Figs. 32,37). Their nature is uncertain, but they have been identified tentatively as muscle cells because, in one case, filaments were observed in them which showed some evidence of alignment (Fig. 37b).

With the exception of some of the smaller cells, the deep muscle cells are almost completely filled with ribbon-shaped and polygonal fibrils (Fig. 36). The cytoplasm and its contents form a thin peripheral rim, and in some cases also, a small cytoplasmic core in the center of the cell. In cross-section, the fibrils often branch and appear "V"- or "Y"-shaped. There is a tendency for the ribbon-shaped myofibrils to

be radially arranged around the periphery in some cells, but a clear separation or orientation of the polygonal and ribbon-shaped myofibrils is not present in all cells.

Mitochondria are found either at the periphery or scattered between the myofibrils, but are not aligned with respect to the sarcomeres.

As in the previous stage, the deep muscle cells can be seen extending the entire length of the somite (about 100-110 μ). No more than a single nucleus was observed in any deep muscle cell in cross- or horizontal section, but because of the increased obliquity of their alignment with respect to the superficial muscle cells, a deep muscle cell is almost never seen in its entirety in a horizontal section, and the number of nuclei per cell remains uncertain.

No centrioles or flagella were seen in any deep muscle cells.

As in the previous stage, the appearance of the sarcomeres in both the superficial and deep muscle cells is similar. Distinct Z-, I-, A-, H-, and M-bands are visible (Fig. 40a). An hexagonal arrangement of the thick and thin filaments is now more frequently present in the myofibrils of both the superficial and deep muscle cells. The hexagonal arrangement of thin filaments around each thick filament is, in general, still less rigid in the superficial than the deep muscle cells. The cross-bridges between thick filaments in the M-band are more readily demonstrable than in the 33-somite embryo.

The T-tubules of the triad are located over the Z-band region in both cell types, but there is an important difference between deep and superficial cells in the number and distribution of the longitudinal cisternae of the sarcoplasmic reticulum. In cross-sections through the

A-band (Fig. 35), the superficial muscle cells are characterized by the presence of round or oval cisternal profiles, which are of more uniform size and shape than cisternae in the corresponding regions of the deep muscle cells. Moreover, they are more often arranged as double sets between the fibrils in contrast to single rows characteristic of the deep cells. The same configuration of the SR is also indicated in longitudinal sections cut nearly parallel to the filaments. In the superficial cells (Fig. 39a), several sets of longitudinal cisternae are seen between each myofibril, whereas in the deep muscle cells (Fig. 40a), a single row of cisternae is generally observed. Each myofibril of the superficial cells, therefore, may have its own sheath of SR, while in the deep muscle cells, adjacent fibrils may share a common set of longitudinal cisternae.

The tubules extending from each terminal cisternum anastomose in the H-band region in both muscle cell types (Figs. 38-41), resulting in the presence of cross-sections of cisternae at this location in longitudinal section. This anastomosis is more evident in the superficial cells.

Nerve processes are observed within, or near, the myosepta (Figs. 32, 39b,c, 40a, 41). When seen between adjacent muscle cells, they are free of surrounding supportive cells. In all sections nerve processes associated with the superficial muscle cells were characteristically and solely located medial to the ends of the cells (Figs. 32,40). Neuronal processes in relation to deep muscle cells were observed both near the myosepta and along their length. However, whether the deep muscle cells receive multiple endings or endings from more

than a single neuron was not determined.

Because of the lack of postsynaptic specializations in the neuromuscular junctions of teleosts (see Table I, p. 78), absolute identification of motor end plates is difficult. In some instances, however, nerve and muscle cell membranes are parallel and separated by a uniform space of 320-420 Å. This space contains an electron-dense material and a single line of increased density approximately midway between the cell membranes. These regions may or may not be present at all areas of adjacent nerve and muscle cell membranes (Fig. 39c). When a region of uniform membrane separation containing dense material does occur, the muscle cell membrane in this region appears more prominent than the adjacent cell membrane. At some regions of uniform membrane separation (Fig. 41), an accumulation of primarily spherical vesicles is seen, and these areas may represent neuromuscular junctions.

The myosepta are well-developed collagenous structures extending from the epidermal basement membrane to the median septa and the connective tissue enveloping the axial structures.

Short protrusions with specialized attachment sites such as were found in 33-somite embryos were not observed in the larval stage, but long cellular processes are present within the myosepta (Figs. 39b,41). These may be either portions of fibroblasts or of cells associated with neuronal processes.

As the myofibrils approach the end of the cell adjacent to the myoseptum, filaments are seen to extend from the terminal Z-band and approach the sarcolemma as described in the Medaka by Yamamoto (1965) (Figs. 40a,41). They vary in length, and may form bundles of both

thick and thin filaments, or thin filaments alone. There is a region of increased density at the cell membrane, but the exact mode of junction between filaments and cell membrane has not been precisely ascertained.

No specialized cell attachments were observed between the muscle cells, and no connective tissue fascia is present between the superficial and the deep muscle cells. Small regions of increased separation between adjacent muscle cells are frequent (Fig. 36). They contain a dense material and are generally observable in cross-sections at the point of junction between several muscle cells. However, no continuous layer of density surrounding individual muscle cells has been seen, nor were connective tissue elements present between adjacent muscle cells. The myotome is surrounded on the medial, dorsal and ventral surfaces by a layer of electron-dense material (the external lamina). A rather indistinct region of density is also present adjacent to the cell membranes of the muscle cells in the myoseptal region. This density probably also represents an external lamina because a distinct external lamina is observed in sections cut normal to the cell surfaces in the myosepta (Fig. 40b). Regions of dense material are present between the lateral surfaces of superficial muscle cells and external cell processes (Fig. 34). At other points, the superficial muscle cells and external cells remain closely apposed (Fig. 33). Small patches of very dense material are seen between the external cells and the dermal collagen, and between the superficial muscle cells and the collagen in the gaps between adjacent external cells.

DISCUSSION

Observations on the zebrafish confirm for this species much of the previous descriptive data from other teleosts. Some of the conflicting observations, particularly regarding ultrastructural details, will be more fully discussed. Topics are arranged under the general headings: 1) Adult Lateral Musculature, 2) Developmental Stages, and 3) Comparison of Developmental and Adult Stages.

I. ADULT LATERAL MUSCULATURE

The electrophysiological properties of the superficial and deep portions of the lateral musculature are not known, but these muscles appear histologically and histochemically similar to those of the tench (also a cyprinid) which have been shown to constitute a slow and fast motor system (Barets, 1961). The physiological properties of the intermediate fibers have not been examined in any species.

Table I demonstrates the close agreement of descriptions of the fine structure of the neuromuscular junctions in several teleosts. No major differences have been reported between junctions in fast and slow muscles of other species, and the fact that no differences were observed between the neuromuscular junctions in the lateral musculature of the zebrafish is consistent with the possibility that the superficial and deep portions represent different motor systems.

The ultrastructure of the adult lateral musculature is similar to that of the trunk muscle of the black mollie as described by Franzini-Armstrong and Porter (1964a) except that these authors did not discuss a region of intermediate fibers. An abundant sarcoplasmic reticulum is present in all muscle fibers, and no differences in the organization

TABLE I

A COMPARISON OF THE FINE STRUCTURE OF NEUROMUSCULAR JUNCTIONS IN THE ZEBRAFISH LATERAL MUSCULATURE WITH THOSE PREVIOUSLY DESCRIBED IN OTHER TELEOST MUSCLES

	Fish	Muscle	Character	Description of Junction	Reference
1.	Zebrafish (Brachydanio)	Lateral: Superficial fibers Deep fibers Intermediate fibers	 Red Pale ?	All: oval endings. Fit into shallow depressions. No subjunctional folds. Cleft ca. 510-530 Å.	This investigation.
2.	Killifish (Fundulus)	Extrinsic Eye	Slow	No (or very little) folding of muscle cell membrane. Cleft ca. 400-500 Å.	Reger, 1959,1961.
3.	Seahorse (Hippocampus)	Dorsal Fin	Red(twitch)	Axon ending rounded and fits into smooth depression. No folds. Cleft ca. 600 Å.	Bergman, 1964a.
4.	Seahorse (Hippocampus)	Dorsal Fin	Red (twitch)	May be associated with nuclei. Cleft ca. 750 Å. (As above)	Bergman, 1967.
5.	Snakefish (Ophiocephalus)	Levator pectoralis Flexor pectoralis	Red Pale	Both: no folds. Resembles junctions of fundulus extrinsic eye muscles.	Nakajima, 1962.
6.	Goldfish (Carassius)	Levator pectoralis Levator pinnae abdominus	Red Pale	Both: oval or spherical endings. Fit into smooth depressions. No folds, similar to frog and snake slow muscles.	Nishihara, 1966.

TABLE II

LOCATION OF THE T-TUBULE IN VARIOUS TELEOST SKELETAL MUSCLES

Position of T-tubule	Fish	Muscle	Reference
Z-band	Seahorse (Hippocampus)	Dorsal fin	Bergman, 1964a,b;1967
Z-band	Killifish (Fundulus)	Branchial	Philpott & Goldstein, 1967
Z-band	Black mollie (Mollienesia)	Lateral (superficial & deep)	Franzini-Armstrong & Porter, 1964a
Z-band	Black mollie (Mollienesia)	Lateral (deep?)	Franzini-Armstrong & Porter, 1964b; Franzini-Armstrong, 1964; Gorden et al., 1967; Slautterback, 1966; Peachey, 1966
Z-band	Lingcod	Lateral (superficial & deep)	Buttkus, 1963; Buttkus & Tomlinson, 1966
Z-band	Goldfish (Carassius)	Fin (m. levator pinnae lat. abdom. & m. levator pinnae pectoralis)	Nishihara, 1966
Z-band	Snakefish	Fin (m. levator pinnae pectoralis & m. flexor pinnae pectoralis)	Nakajima, 1962
Z-band	Sculpin (Clinocottus)	Fin (pectoral)	Peachey, 1966
Z-band	Guppy (Lebistes)	Lateral	Franzini-Armstrong & Porter, 1964b
Z-band	Lesser sand-eel (Ammodytes)	Lateral (superficial & deep)	Kilarski, 1965, 1966, 1967
Z-band	Perch (Perca)	Lateral (superficial & deep)	Kilarski, 1965, 1966, 1967
Z-band	Pike (Esox)	Lateral (superficial & deep)	Kilarski, 1965, 1966, 1967

TABLE II - CONTINUED

Position of T-tubule	Fish	Muscle	Reference
Z-band	Tench (Tinca)	Lateral(superficial & deep)	Kilarski, 1965, 1966 1967
Z-band	Pond-loach (Misgurnus)	Lateral(superficial & deep)	Kilarski, 1965, 1966 1967
Z-band	Flounder (Pleuronectes)	Lateral(superficial & deep)	Kilarski, 1965, 1966 1967
Near A-I junction	Killifish (Fundulus)	Extrinsic eye	Reger, 1961
Z-band	Species same as Nos. 11-15	Extrinsic eye	Kilarski, 1965, 1966, 1967
Z-band	Toadfish (Opsanus)	Swim bladder	Fawcett & Revel, 1961
Z-band	Burbot (Lota)	Swim bladder	Kilarski, 1964

of the sarcoplasmic reticulum between the superficial and deep fibers have been observed in either species. The location of the T-tubules over the Z-band in all fibers of the lateral musculature agrees with the situation described in most other teleost muscles (Table II, p. 79) (see also Smith, 1964).

The absence of a marked variation in sarcomere length between the superficial and deep fibers in the zebrafish is contrary to the reports by Boddeke et al. (1959) and by Kilarski (see p. 28) of a significant difference in sarcomere length between superficial and deep fibers in a number of teleosts, both closely and distantly related to the zebrafish. Measurements of both sarcomere and A-band length in the zebrafish varied from 1.75 - 2.0 μ (a range of approximately 0.25 μ). Differences between superficial and deep fibers were no greater than those between adjacent fibers in the superficial or the deep portions, or between similar cells in different preparations. Differences were attributed primarily to variations in the state of contraction, but may also be due in part to differences in orientation of the cells with respect to the plane of section. Even if more precise measurements in the future reveal significant differences in sarcomere length between the superficial and deep muscle fibers, they will be far less than those between "slow" (long sarcomeres) and "fast" (short sarcomeres) fibers in Arthropods (Jasper and Pezard, 1934; Smit, 1958), used as standards for comparison by Boddeke et al. (1959).

Some of the ultrastructural differences reported by Buttkus (1963) and Buttkus and Tomlinson (1966) (see p. 28) between superficial and deep muscle fibers of the lingcod were not confirmed for the zebrafish.

Myofibrils of both fiber types exhibit distinct H- and M-bands at all stages from the 33-somite embryo to the adult, and variations observed in the width of the I-band can be attributed to differences in the degree of contraction of the myofibrils at the instant of fixation.

II. DEVELOPMENTAL STAGES

A. Ultrastructure.

1. Mitochondria. A general increase in mitochondrial size and the number of cristae was noted during myogenesis in the zebrafish. Similar observations have been made in chicks (Allen and Pepe,1965; Dessouky and Hibbs, 1965) and rabbits and mice (Allbrook, 1962). The difference in appearance of the mitochondrial cristae of the superficial and deep fibers of the lingcod claimed by Buttkus (1963) was not confirmed in the zebrafish.

Przybylski and Blumberg (1966) noted an increase in mitochondrial matrix density concomitant with filament formation in chick myoblasts (following glutaraldehyde fixation). This was not seen in myoblasts of the 25-somite zebrafish embryo (Fig. 14). In the 33-somite embryo and larval stages, however, increased mitochondrial density was observed in the superficial, but not in the deep muscle cells (Fig. 21). The significance of this increase is not known, but since there was no gradient of mitochondrial density across the myotome, it apparently represents a true distinction between the mitochondria of the two cell types.

2. Glycogen. The amount of glycogen within myoblasts at various stages of myogenesis has been discussed by several investigators. In Medaka (Yamamoto, 1965) and in rat myoblasts (Bergman, 1962), glycogen

in the form of numerous particles appears relatively late. According to Hay (1963), large glycogen particles appear only rarely in developing muscle cells of Amblystoma. Others have reported that glycogen particles (150-450 Å) become a common cytoplasmic component approximately at the time of myofilament formation in chick myoblasts (Allen and Pepe, 1965; Dessouky and Hibbs, 1965; Firket, 1967), and increase in amount as development progresses. In the zebrafish, abundant small glycogen particles (150-250 Å) are present at all stages, and larger glycogen particles are seen in the adult (based on a comparison of uranyl acetate and alkaline-lead staining) (Revel, 1964; Revel et al., 1960).

3. Microtubules. Microtubules occur in cells of the unsegmented mesoderm and newly-formed somites, and in myoblasts of the 25- and 33-somite embryos, but have not been seen in muscle cells of larvae fixed by the same technique. They have also been reported in chick myoblasts following primary glutaraldehyde fixation, but a difference of opinion exists regarding their possible relationship to the myofilaments.

Przybylski and Blumberg (1966) described tubular structures (ca. 150 Å diameter) aligned parallel to the long axis of myoblasts in chick branchial somites. On the basis of: 1) presumed continuity of these tubules with the thick (100 Å) myofilaments; 2) the tubular cross-sectional appearance of the thick myofilaments; and 3) the close approximation of the tubules with developing myofibrils, the microtubules were implicated in the "formation of the thick filaments of the A-band." A similar relationship between the thick (primary) filaments and microtubules (ca. 200 A diameter) in developing dipteran flight muscle has also been proposed by Auber (1962, 1964, 1965a,b) and extended to developing rat

muscle (Auber and Couteaux, 1964). Firket (1967) and Fischman (1967) also described microtubules oriented primarily parallel to the long axis of the cell in chick myoblasts, but they observed no structural connection with myofibrils or filaments, and denied that tubules transform into filaments.

This opinion is supported by the findings in the zebrafish. The relative paucity of microtubules in proportion to the myofilaments, and the lack of any demonstrable continuity between microtubules and filaments in the vast majority of cases, make the suggestion of a developmental connection between these structures extremely doubtful. Short sections of presumed microtubules can be seen in close proximity to small groups of myofilaments in some myoblasts (Fig. 27a), but the absolute identity of these images as microtubules, and their possible continuity with filaments is difficult to ascertain. In such cases, stereo-electron microscopy might yield valuable information.

Fischman (1967) has suggested that if microtubules are associated with cytoplasmic streaming, this might play a role in initial myofilament alignment. Alignment of microtubules parallel to the long axis of the cell may also indicate that they play a role in cell elongation. This alignment may be entirely passive, however. Whether or not microtubules play a role in myogenesis, and what that role might be, are unresolved questions.

Adult muscle fibers may contain microtubules (Sandborn et al., 1967), but their absence from adult zebrafish material is probably due to primary osmium fixation.

4. Flagella. Flagella (cilia) have previously been described

in dermatomal, mesenchymal and myotomal cells of branchial somites in chick embryos (Przybylski and Blumberg, 1966). They were frequently seen in myotomal cells prior to elongation and the appearance of myofilaments, but their presence at later stages was not mentioned by these authors. In the zebrafish, flagella were observed in cells prior to the appearance of myofilaments, in a myoblast of a 25-somite embryo and in deep muscle cells at the 33-somite stage (Fig. 42). With the single exception of two flagella within a deep muscle cell (Fig. 26c), no more than one recognizable flagellum was observed per cell. The frequency with which portions of flagella are seen in thin sections of early stages suggests that all mesodermal cells may be flagellated. Since cilia and flagella generally project into cavities or the external environment and are, therefore, indicators of the polarity of the cells which line surfaces and cavities, the apparent lack of polarity or orientation of the flagella within newly formed somites of the zebrafish may be a consequence of the same fundamental mechanism which prevents cavity formation within the somite. In newly formed somites of the chick similar flagella appear to project into the "sclerocoel" from the apical ends of the cells lining this somitic cavity (Trelstad et al., 1967). In contrast to the somites, flagella of the developing ependymal and endodermal cells of the zebrafish project into the cavities of the neural tube and the posterior portion of the intestine.

5. Multinucleation. The bulk of recent investigation utilizing primarily tissue culture techniques and employing radioactive tracers, hybridization, etc., has indicated that the formation of multinucleate vertebrate skeletal muscle fibers occurs principally, if not entirely,

by cell fusion (Holtzer et al., 1957; Firket, 1958; Stockdale and Holtzer, 1961; Cooper and konigsberg, 1961; Bassleer, 1962, 1963; Strehler et al., 1963; Konigsberg, 1963; Yaffe and Feldman, 1965; reviewed by Konigsberg, 1965). Many electron microscopical investigations of vertebrate muscle development also claim to support the concept of cell fusion (Bergman, 1962; Allbrook, 1962; Hay, 1963; Price et al., 1964; Firket, 1967; Fischman, 1967). This conclusion was based on the lack of observed mitotic figures within multinucleate myoblasts, and on the occurrence of regions of cell membrane discontinuity and apparent cytoplasmic confluence between adjacent cells. In some cases, rows of vesicles presumed to indicate former cell membranes were observed across regions of discontinuity. Both of these were seen in zebrafish embryos (especially following primary osmium fixation). It must be stressed, however, that the absence of mitotic figures does not of itself rule out the possibility that mitosis, or some other mode of nuclear replication, exists, and the static features of membrane discontinuities and vesicles do not per se allow conclusions regarding cell fusion. There is also much evidence suggesting that such membrane discontinuities are fixation artifacts (e.g., see Rosenbluth, 1963; Franzini-Armstrong and Porter, 1964a).

6. Myofilament Formation and Fibrillogenesis. Based on studies combining electron microscopical with biochemical and biophysical techniques (Hanson and Huxley, 1957; Hanson and Lowy, 1963; Huxley, 1963), most investigators agree that the thick and thin myofilaments seen in electron micrographs of developing muscle cells correspond to the proteins myosin and actin, but controversy exists regarding the sequence of their formation during myogenesis. The precedence of thick filaments

(Firket, 1967) and of thin filaments (Allen and Pepe, 1965; Obinata et al., 1966) have both been reported. Most investigators, however, have described simultaneous appearance of both thick and thin myofilaments (Ferris, 1959; Hay, 1963; Price et al., 1964; Dessouky and Hibbs, 1965; Przybylski and Blumberg, 1966). Thick and thin filaments also appear simultaneously in the Medaka (Yamamoto, 1965) and in the zebrafish (Fig. 16). Some of the disagreements in the literature may be due to differences in technique (e.g., PTA staining), in the relative concentrations of thick and thin filaments present in the cytoplasm (Fischman, 1967), in the time of sampling, and to species differences.

The importance of sampling time is illustrated by the rapid development of the myotome in the zebrafish (Fig. 42). Myofilament formations begin in the medial most cells of the midbody somites between the 20- and 25-somite stages (a period of approximately 1.5 hrs.), and by the 33-somite stage (14-17 hrs. later), the myotome is composed of two muscle cell types each containing well-formed myofibrils.

Neither the manner in which randomly oriented myofilaments or filament bundles become aggregated into myofibrils, nor the sequence of appearance of the characteristic sarcomere bands (particularly of the Z-band) have been similarly described by all authors. Sarcomeres of early myofibrils have been described as being either longer or shorter than in the adult (Hay, 1963; Price et al., 1964), as being essentially the same as the adult (Bergman, 1962; Heuson-Stiennon, 1965; Firket, 1967; Fischman, 1967), or as exhibiting an increase in the width of A- or I-bands and the gradual emergence of the H- and M-bands (Allen and Pepe, 1965; Yamamoto, 1965). The sarcomere lengths

in young muscle cells of the 33-somite zebrafish embryo (1.8 - 1.9 μ) were approximately equal to those of the adult (1.75 - 2.0 μ). An increase in sarcomere length similar to that in insect muscle (Aronson, 1961; Shafiq, 1963; Auber, 1965c) has not been reported in vertebrate muscle, and Fischman (1967) suggested that this indicates a difference in growth mechanisms between dipteran and vertebrate muscle.

Because of the limited number of stages examined and the rapid rate of development, the early phases of fibrillogenesis in the zebrafish were not completely revealed. The myoblasts of the 25-somite embryo contained randomly oriented filaments and bundles of organized thick and thin myofilaments in which no sarcomeres were evident. This may have been due to a combination of short fibrils and an oblique plane of section, or may be truly representative of the appearance of the first filament bundles. In the next stage examined (33-somite), well-developed myofibrils were seen containing all the striations of adult sarcomeres. Knowledge of these "end points" will be helpful to future studies of intermediate stages in fibrilogenesis.

Firket (1967) reported that in chick tissue culture preparations, no myofilaments were observed in uninucleate cells, but other workers have reported the presence of filaments (Bergman, 1962; Allbrook, 1962; Price et al., 1964; Fischman, 1967), and immature myofibrils with recognizable sarcomeres (Przybylski and Blumberg, 1966), in uninucleate myoblasts from chick and other species. All these investigators agree that most of filament and myofibril development occurs in multinucleate cells (chick, mouse, rat, rabbit). This was also the case for the deep muscle cells of the zebrafish. The uninucleate deep myoblasts

of the 25-somite embryos contained bundles of filaments, and the multinucleate deep muscle cells contained well-developed myofibrils at the 33-somite stage. In contrast, the uninucleate superficial muscle cells of the 33-somite embryo contained large myofibrillar bundles, and are a clear example of advanced myofibril formation in uninucleate vertebrate muscle cells.

7. Connective Tissue Relationships. Separation of the somite, and portions of it, from the connective tissue compartment by an external lamina is apparently concomitant with somite differentiation, since no layer of fibrillar material surrounds the column of unsegmented mesoderm. The myotomal external lamina forms later than the basal or external laminae of the epidermis, spinal cord and notochord. This is in close agreement with the observations of Low (1966a,b) in the chick, and in general agreement with Allbrook (1962) and Price et al. (1964) in regenerating muscle cells, and with Przybylski and Blumberg (1965) for chick somites. There also appears to be a definite sequence of its formation which roughly parallels that of myoblast formation. External lamina material appears first at the medial surface of the somite (Figs. 13,14), is seen at the ends of the somites in the developing myoseptal region of 33-somite embryos (Figs. 28-30), but is not evident along the lateral surfaces of the superficial muscle cells until the larval stage (Fig. 34).

Production of an external lamina is perhaps an important step in the establishment of a particular environment conducive to cellular differentiation, but it may not be essential. A cellular layer might also be an effective barrier, and the fact that the lateral surface of the myotome appears to be closely covered by a layer of external cells

throughout most of prehatching development, whereas much of the medial surface of the somite is more or less exposed to the connective tissue compartment at earlier stages, could be a specific factor in the differentiation of two muscle cell types. The close association between the single layer of external cells and the single layer of superficial muscle cells subjacent to it certainly suggests a possible discrete developmental relationship between them, but is not conclusive evidence for such a dynamic process.

Connective tissue elements invade the lateral musculature late in development. Several days after hatching, no fibroblasts, and only occasional areas of electron-dense material, were observed between adjacent muscle cells, and no connective tissue fascia was present between the superficial and deep muscle cells.

The myosepta also develop late in relation to the muscle cells. The intersomitic furrows in the 20- and 25-somite embryos are almost totally devoid of extracellular material (Figs. 10,16) while in the 33-somite embryo, some fibrillar material with definite periodicity is present between consecutive myotomes (Fig. 29a). The origin of this material (presumably collagen) is uncertain. Cell processes containing much rough endoplasmic reticulum are present within the well-developed myosepta in larva, but the apparent absence of fibroblastic cells within the myoseptal region, and the general reduction of mesenchymal cells at the 33-somite stage is noteworthy. A possible source of this material might be the muscle cells, although this study does not provide any positive evidence in support of this proposal.

The horizontal septum appears toward the end of embryonic develop-

ment (between the 33-somite stage and several days after hatching), as has been described in other forms (Emelianov, 1935; Fischelson, 1966). Emelianov noted that the horizontal septum in teleosts is recognizable by light microscopy as a very thin connective tissue layer at the level of the notochord approximately at the time of vertebral cartilage formation, and that it is not preceded by any separation between the epaxial and hypaxial portions of the myotome. This also seems to occur in the zebrafish, but details of its development are lacking. The presence of widened superficial muscle cells in the region of the future horizontal septum, and the external cell processes which extend between them at the 33-somite stage, raise the possibility that these cell types may be involved in its formation.

8. Motor Behavior. Certain ultrastructural observations may be correlated with the development of motor behavior, and raise important questions for future research. All previous descriptions of the development of contractility and swimming behavior in teleosts agree that, as in the zebrafish, the motions of the embryo, the larva, and the adult are distinctly different (White, 1915; Tracy, 1926; Coghill, 1933; Barron, 1941; Sawyer, 1944; Hooker, 1952; Harris and Whiting, 1954; Whiting,1955; Fischelson, 1966). It might be expected, therefore, that the structural basis for these movements are different, and not necessarily just an increase in the complexity of an initial morphology.

The movements of young teleost embryos are slow and irregular, producing a slight curvature of the trunk which increases in strength and rapidity. These "coil movements" were exhibited by the 33-somite embryos prior to fixation. The midbody somites are almost certainly

active, although the gross movements produced make absolute determination of this difficult. Because both the deep and superficial muscle cells at this stage contain myofibrils which extend the length of the somite and are equally associated with elements of the sarcoplasmic reticulum and T-tubules, it cannot be determined on this basis whether both or only one cell type is responsible for these movements. With the exceptions of Tracy (1926) and Barron (1941), all of the investigators mentioned above state that the initial contractions in teleosts are myogenic. The observation that the first neuronal processes seen in the myotome of the 33-somite embryo appear to have a close and specific relationship to the superficial muscle cells, raises the possibilities that the myogenic phase may begin earlier than was proposed on the basis of light microscopical examination, and that these cells may be primarily responsible for the first contractions, or at least are the first to come under neuronal control. Whether the superficial muscle cells are indeed innervated earlier than the deep muscle cells, and whether the neuromuscular contacts observed at this early stage are functional neuromuscular junctions remain to be explored.

Whiting (1955) has reported that in the trout (Salmo irideus), as in selachian embryos, early transmission of contraction between adjacent myotomes is also myogenic rather than neurogenic. In selachian embryos, the first contractions are apparently transmitted from myotome to myotome at regions of overlap between the ends of myoblasts across the developing myosepta (Balfour, 1878; Paton, 1911; Wintrebert, 1920; Te Winkel, 1948; Harris and Whiting, 1954; Whiting, 1955). Connection of adjacent myotomes across developing myoseptal regions through muscle

cell processes and specialized cell attachments in the 33-somite embryo (Fig. 29b) constitutes a previously undescribed relationship in teleosts, and may be analogous to the overlapping myoblasts of Chondrichthyes (although these have been described only at the light microscopic level). As in selachians (Te Winkel, 1948; Harris and Whiting, 1954), these connections appear to have only a brief existence, since they are not present in the 25-somite embryo, nor in larvae (Fig. 42). These cell attachments may serve as physical stabilization for consecutive myotomes during myoseptal development, or they may also serve as points of electrical intercommunication between myotomes. In view of the general lack of innervation of the myotome, focal intermediate junctions seen between adjacent myoblasts of the same somite at this stage may serve as sites of transmission between these cells. Electric coupling has been demonstrated in adult tissues between both electrically active and nonexcitable cells (Burnstock et al., 1963; Bennett et al., 1963, 1967a,c,d; Furshpan, 1964; Loewenstein and Kanno, 1964; Kuffler and Potter, 1964; Loewenstein et al., 1965; Loewenstein, 1966; Kuffler et al., 1966; Penn, 1966). Electron microscopical examination of these tissues has revealed the presence of specialized cell attachments (tight or "close" junctions, septate desmosomes), which presumably represent sites of low resistence (Karrer and Cox, 1960; Curtis, 1962; Dewey and Barr, 1962, 1964; Farquhar and Palade, 1963, 1965; Wiener et al., 1964; Berrill and Sheldon, 1964; Pappas and Bennett, 1966; Bennett et al., 1967b). Similar intercellular electric coupling has also been demonstrated in embryos of the squid (Loligo pealii) (Potter et al., 1966) and chick (Sheridan, 1966). In the chick, tight and "close" junctions have been described between the

electrically-coupled cells (Trelstad et al., 1966, 1967).

The term "focal intermediate junction" was applied to the larger attachment sites seen in zebrafish embryos because no dense line is evident between the two electron-dense cell membranes, and because the distance between the centers of these two dense lines is slightly greater (150-160 Å) than that in a tight junction. However, because a "unit membrane" structure was not resolved, and since the observed structure of attachment sites is in part a function of fixation procedures (Dewey and Barr, 1962; Trelstad et al., 1966, 1967), the similarity in appearance of the focal intermediate junction with "tight junctions" in other systems (see Loewenstein et al., 1967a) (Fig. 3, insert), and their apparently specific spatial and temporal relationships within the myotome of the developing zebrafish embryo warrents further ultrastructural and electrophysiological investigation of their structure and function.

The characteristic movement of larvae ("flutter response") is presumably due to a rapid alteration of right and left coil movements (Tracy, 1926), and is under neuronal control. In the zebrafish, neuronal processes and presumptive neuromuscular junctions are present in association with both superficial and deep muscle cells, and the possible relative contributions of these two cell types to the "flutter response" is not known.

The dissimilarity in organization between the sarcoplasmic reticulum of the superficial and deep muscle cells in the larval stage was not observed in the 33-somite embryo, nor in the adult (see also Franzini-Armstrong and Porter, 1964a), and may be related to differences in the

functioning of the two muscle cell populations. Recent work attempting to associate the amount and distribution of the sarcoplasmic reticulum with specific functional properties of diverse muscle cells has indicated that, in general, slow muscle fibers tend to contain reduced amounts of sarcoplasmic reticulum (reviewed by Porter, 1961; Peachy, 1961; Smith, 1964; Hess, 1967, and others), but the correlation is not sufficiently close to derive any conclusion in the present case.

B. Comparison with Previous Embryological Descriptions.

Observations on somite differentiation in the zebrafish compare most closely with the description of Sunier (1911), although certain important exceptions were noted. In agreement with Sunier, this study has shown that the newly formed somites are relatively compact cellular units, without a cavity, and with no indication of a separation into distinct medial and lateral layers. A peripheral layer of more closely apposed cells and an inner region in which the cells are more loosely aggregated can be distinguished (Fig. 7), although this was denied by Sunier. Sunier's proposed "equivalence" of all early somitic cells cannot be evaluated from structural data alone, but the cells of newly formed somites appear ultrastructurally similar (Fig. 9). An important exception to this generalization may be the fact that nuclei of the most medial cells in a newly formed somite appear to be less dense than those of most of the more lateral cells. In this characteristic, they are similar to myoblast nuclei, although the cells are not elongated.

Myogenesis was demonstrated to begin at the medial surface of the somite, but it was not established whether cell elongation occurs first in the region of the notochord as in other teleosts (Wilson, 1891;

Kaestner, 1892; Maurer, 1892, 1894; Sunier, 1911).

Interpretation of older descriptions "after the fact" must be undertaken with caution, but it is interesting to compare the appearance of the midbody somites of the 33-somite zebrafish embryo (Figs. 18a,b) with previous illustrations of a similar stage in different species (Fig. 1).

Of the nonmyoblastic cells associated with the compact myotome in the 33-somite embryo, the dispersed cells at the dorsal and ventral tips of the myotome, and those between the myotome and axial body structures medially are presumably of either sclerotomal or neural crest origin, but details of their formation were not investigated.

A second group of nonmyoblastic cells, the external cells, are not dispersed, but form an epithelium which is closely apposed to the lateral surface of the superficial muscle cells. It is suggested that the external cell layer is what was described as the flattened "peripheral part of the Urwirbel" by Ehrlich (1875), the "squamous epithelium" by Kaestner (1892), and the "feuillet externe" by Sunier (1911). The existence of such a layer in other fish was denied by Maurer (1894) and Vialleton (1902), but its presence in the zebrafish is now clearly established.

The external cells probably arise by flattening of the most lateral cells of the somite, as suggested by Sunier (1911). This conclusion is supported by three observations: 1) the compact somite mass, including the external cells, is about 4-6 cells wide at the level of the spinal cord in all developmental stages studied; 2) the external cells are always observed in close relationship with the superficial

muscle cells; and 3) in somites posterior to (and therefore younger than) the midbody somites in 33-somite embryos, the most lateral cells were seen to contain dense nuclei, and although flattened, they did not extend the length of the somite. This cell layer has been presumed by all investigators who recognized its presence to disappear eventually and become a part of the connective tissue. Part of the previous difficulty in observing this cell layer undoubtedly arose from an inability to resolve the external cell processes, which in older stages may be as little as 200 Å thick.

The continuous external cell layer with overlapping processes present in the 33-somite embryos does become discontinuous in larvae several days after hatching, but even at this stage, the cells maintain close contact with the superficial muscle cells, and are separated from them only at scattered points by dense material. Their position between the myotome and epidermis, and their apparent derivation from the somite, suggest that the external cells might be considered as forming the dermatome in this teleost. The fact that no cavity forms between the myotome and external cell layer is consistent with the general reduction of cavity formation during organogenesis in teleosts (Witshi, 1956). The possible role of external cells in the formation of the epidermal basement membrane, in the development of the adult dermal structures, and perhaps in the differentiation of the superficial muscle cells with which they maintain an intimate contact, remain to be explored.

III. COMPARISON OF DEVELOPMENTAL AND ADULT STAGES

The observations on the zebrafish strongly suggest that the young superficial and deep muscle cells first seen in 33-somite embryos are

two distinct muscle cell types, and that they may develop into the superficial and deep portions of the lateral musculature of the adult as reported in trout by Vialleton (1902). This opinion is based on their respective locations within the myotome and adult musculature, and more specifically, on structural differences which tend to become increasingly similar to the obvious differences of the adult fibers.

For example, the two main muscle cell types (superficial and deep) can be distinguished at every stage by the cross-sectional appearance of their myofibrils. The single large myofibrillar bundle along the medial margin of each superficial muscle cell in the 33-somite embryo (Fig. 19) apparently develops into the group of polygonal myofibrils in larvae (Fig. 33), and ultimately into the irregularly arranged fibrils of the adult superficial muscle fibers (Fig. 4). In addition, incorporation of mitochondria among the fibrils seen in larvae is retained and accentuated in the adult. The small, peripheral myofibrils characteristic of the deep muscle cells, on the other hand, apparently increase in size, eventually filling the cell and forcing the cytoplasm and organelles into a peripheral position, thus conforming to classical descriptions of vertebrate muscle development. The pattern of long, ribbon-shaped fibrils at the periphery and centrally located polygonal fibrils which characterizes adult deep muscle fibers is well established during the first few months of larval existence (Fig. 5).

The difference between the mean diameter of the superficial muscle cells and that of the majority of deep muscle cells was found to increase from essentially no difference in the 33-somite embryo to a very significant difference in the mature adult. Kirsche (1948) found essentially

the same increase in the guppy, but his conclusion that the "embryonic" appearance of the smaller superficial fibers in the adult arises from the fact that the myofibrils develop rapidly in the deep fibers and are retarded in the superficial fibers must be questioned. It is true that in size and greater sarcoplasm-to-fibril ratio the superficial fibers are more similar to myoblasts than are the deep fibers. They are apparently not "embryonic," however, in the sense that they later transform into deep muscle fibers. The observations of this investigation indicate that the two fiber types exhibit differences from the time of their formation and differentiate along separate pathways leading to dissimilar adult configurations. Therefore, they should be considered as distinct cell lines, each with its own characteristic rate of development (which may not necessarily be equated with increase in size).

The exact mode of superficial muscle cell formation remains an interesting problem, and hinges on the presence of smaller cells immediately subjacent to the superficial muscle cells in 33-somite and larval specimens. Unlike the superficial muscle cells, the small cells do not form a continuous layer, but they are characteristically found at the border between the superficial and deep muscle cell populations. Only very small myofibrils (or none at all) are discernible in these cells in 33-somite embryos (Fig. 23). Superficial muscle cells certainly differentiate later than the more medial of the deep muscle cells, but the existence of the smaller cells, and the fact that the average diameters of the superficial and deep muscle cell populations are approximately equal in the 33-somite embryo, do not allow a distinction between the following several general possibilities of their initial

formation.

There may be a gradual lateral progression of myoblast formation originating from the medial surface of the somite, which eventually includes the superficial muscle cells, and which is combined with an initial difference in the relative growth rates of the two cell types, such that the superficial cells increase in size more rapidly than the subjacent cells. The facts that the average width of the superficial muscle cells is less at the larval stage than it is in the 33-somite embryo, and that the superficial muscle cell population is significantly smaller than the deep muscle cell population at the larval stage, may argue against an initial increased growth rate of the superficial cells. However, these conditions may be attributed to other factors expressed subsequent to the initial appearance of the superficial muscle cells (e.g., differences in the number of nuclei per cell).

A second possibility is that of two independent centers of initial myoblast formation: one at the medial surface of the somite leading to the differentiation of the deep muscle cells; and another lateral in the somite, which is expressed between the 25- and 33-somite stages, and which leads to the formation of the superficial muscle cell layer.

Finally, it is possible that the small cells seen laterally at the 33-somite and larval stages and/or the dark cells seen in the larvae may differentiate into the intermediate fibers of the adult. If this were true, the intermediate fiber population would have an origin equally as early as the superficial muscle cells and the majority of the deep muscle cells, and would not be a later secondary differentiation of the deep portion of the lateral musculature in the sense of Maser

(1949). In regards to the differentiation of the intermediate fibers, it was noted that in the youngest specimen examined in which a distinct intermediate fiber population was seen (1.5 cm adult) (Fig. 3a), the average diameters of the intermediate and deep fibers are approximately equal, whereas in the mature adult (Figs. 3b,c) the majority of the deep fibers are significantly larger than the intermediate fibers.

It is apparent that confirmation of the proposal that the superficial and deep muscle cells develop into the superficial and deep portions of the lateral musculature must await the explanation of two outstanding observations: 1) the fact that in both the 33-somite embryo and larva several days after hatching, the superficial muscle cells form a continuous single cell layer along the entire lateral surface of the myotome, whereas in the youngest adult stage examined (1.5 cm), the superficial portion of the lateral musculature is a wedge-shaped muscle several layers thick located near the lateral margin of the horizontal septum; and 2) the fact that the intermediate fibers, which form a well-defined region in the deep portion of the adult lateral musculature, are not evident even in larvae several days after hatching. Both of these problems may be associated with the formation of the fascia between the superficial and intermediate fibers, and the vascularization of the lateral musculature. Neither of these has occurred by several days after hatching.

The development of differences in color and SDH activity was not analyzed, but variations in fat and glycogen content apparently arise relatively late, and were not seen in the larvae.

SUMMARY AND CONCLUSIONS

As in other cyprinids, the lateral musculature of the adult zebrafish consists of two main divisions: 1) a highly vascular superficial portion composed of dark, relatively small fibers with high fat content and SDH activity; and 2) a deep portion composed primarily of large, pale, poorly vascularized deep fibers showing little SDH activity and containing little fat. "Intermediate fibers" located at the lateral edge of the deep portion are generally larger than the superficial fibers, but smaller than the more medial deep fibers, and they contain more fat, exhibit greater SDH activity, and are more vascularized than true deep fibers. They are considered to be a specialized region of the deep portion of the lateral musculature. The superficial and intermediate fibers probably also contain a higher concentration of glycogen than the deep fibers, on the basis of electron microscopical evidence.

The superficial and deep portions probably correspond to slow and fast motor systems, as has been shown in other fish species. However, it is noteworthy that unlike many other vertebrate slow and fast systems, the fibers of both the superficial and deep portions contain sarcomeres of similar dimensions and fine structure, have T-tubules located over each Z-band, and contain abundant sarcoplasmic reticulum. Moreover, no fine structural differences have been noted between the neuromuscular junctions of the superficial, intermediate, and deep fibers.

This study provides a general outline of myotomal differentiation, and certain fine structural landmarks of myogenesis in the midbody somites:

1) No indication of cell elongation or myofilament formation is seen in the compact, newly formed somites of the 20-somite embryo.

2) A single layer of uninucleated myoblasts containing bundles of myofilaments has formed at the medial surface of the somites in the 25-somite embryo. The cells immediately lateral to them may be elongated (presumptive myoblasts), but the majority retain an undifferentiated appearance.

3) The myotome in the 33-somite embryo is composed of two distinct types of young muscle cells, superficial and deep. The uninucleate superficial cells, each characterized by a large medial myofibrillar bundle, form a continuous single cell layer along the lateral surface of the myotome. Several flattened superficial cells also extend across the width of the myotome at the midnotochordal level. The deep muscle cells, each containing several small myofibrils peripherally and a central core of cytoplasm with up to three nuclei, form the bulk of the myotome. Myofibrils of both superficial and deep muscle cells exhibit the striations and sarcomere length characteristic of the adult, and are associated with sarcoplasmic reticulum and T-tubules. The presence of certain additional small cells subjacent to the superficial muscle cells raises questions regarding the initial development of the two muscle cell types. These are discussed.

4) The superficial and deep muscle cell populations are still evident in larvae several days after hatching, although their distinguishing characteristics differ from those of the previous stage.

5) Microtubules and flagella were observed in somitic cells at all stages except the adult and larval. No transformation between

tubules and myofilaments was obvious. The apparent lack of orientation of flagella within newly formed somites may reflect the basic mechanism which prevents cavity formation in the teleost somite.

This study suggests that the embryonic and larval superficial and deep muscle cells differentiate into the superficial and deep portions of the adult lateral musculature. However, final confirmation of this view will require further study of larval stages in regard to: 1) the possible transition of the single layer of superficial muscle cells covering the entire lateral surface of the young larval myotome into the wedge-shaped superficial portion of the adult lateral musculature; and 2) the genesis of the intermediate fiber population, which is not present in the larva.

The connective tissue septa and external lamina of the myotome form relatively late in relation to the muscle cells.

A layer of flattened "external cells" covering the lateral surface of the myotome in the 33-somite embryo is considered to form the dermatome in this species, although its role in the formation of adult dermal structures was not investigated. A possible influence of these external cells in the differentiation of the superficial muscle cells is also suggested.

Some of the fine structural observations are discussed in relation to the development of motor behavior. The fact that the first neuronal processes seen within the myotome (33-somite stage) have a specific relation to the superficial muscle cells, suggests that the superficial cells may be innervated before the deep muscle cells, and that the "neurogenic" phase of behavior may begin at this early stage. A

distinct difference between the superficial and deep muscle cells with respect to arrangement of the sarcoplasmic reticulum was observed in larvae. This may be correlated with differences in the behavior of these two cell types at this stage.

Intermyotomal connections across developing myoseptal regions occur by the 33-somite stage. The muscle cell processes and specialized attachments involved appear to have a brief existence. Their possible roles in stabilization of the somites during myoseptal development and as points of electrical intercommunication are discussed.

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FIGURES

ABBREVIATIONS

A	Axon	FL	Flagellum
AO	Aorta	G	Golgi membranes
BL	Basal lamina	GL	Glycogen
BM	Epidermal basement membrane	HP	Hypaxial musculature
C	Centriole	HS	Horizontal septum
CAP	Capillary	I	Intermediate muscle fiber
CH	Chromosomes	IC	Infracarinales
CM	Cell membrane	ISF	Intersomitic furrow
COL	Collagen	L	Lateral nerve
CV	Caudal vein	M	Mitochondrion
D	Deep muscle fiber	MB	Myoblast
DC	Dark cell	MIF	Mitotic figure
DFF	Dorsal fin fold	MF	Myofilaments
DMC	Deep muscle cell	MFB	Myofibrillar bundle
DS	Dorsal median septum	MS	Myoseptum
E	Epidermis	MT	Microtubules
EC	External cell	MY	Myomere
EJ	Embryonic junction	MYF	Myofibril
EP	Epaxial musculature	N	Nucleus
ER	Rough endoplasmic reticulum	NC	Notochord
F	Fascia	NU	Nucleolus
FC	Fibroblastic cell	P	Melanophore
FIJ	Focal intermediate junction	PF	Polygonal-shaped myofibril

PM	Presumptive myoblast
R	Ribosomes
RF	Ribbon-shaped myofibril
S	Superficial muscle fiber
SC	Supracarinales
SH	Notochordal sheath
SK	Skin
SM	Smooth surfaced cisternae and vesicles
SMC	Superficial muscle cell
SOM	Somite
SPC	Spinal cord
SWC	Schwann cell
T	T-tubule
TS	Terminal sac
UM	Unsegmented mesoderm
V	Vertebral column
VC	Vacuolated cell
VL	Vesicle
VS	Ventral median septum
VFF	Ventral fin fold

Figure 1: Representations of figures adapted from the publications of previous investigators illustrating their views of somite ("Urwirbel") development in teleosts.

a-b. Two stages of "Urwirbel" development in trout according to Ehrlich (1875): a) The "Urwirbel" consists of a large central part (u_1) and a peripheral part (u_2) composed of a single cell layer (30-day trout, from Taf. XV, fig. 1); b) The cells of the peripheral part (u_2) at a later stage have flattened to form a squamous epithelium, which was thought to form connective tissue and the myosepta. The central part (u_1) is now composed of muscle fibers. The "light regions" (r) beneath the squamous epithelium were presumed to become a part of the central part (older trout, from Taf. XV, fig. 2).

c-d. Somite appearance in the sea bass according to Wilson (1891): c) Wilson implied that all somitic cells elongate into muscle cells (m) with the exception of some small cells with inconspicuous nuclei tentatively identified as "connective tissue (?) cells" (c.t.?) (from fig. 110); d) The somite is later composed of muscle cells with fibrils at their periphery and a central cytoplasmic core. A wedge of "connective tissue elements" (c.t.) is now present at the level of the notochord (N) (from fig. 136).

e-h. Four stages of "Urwirbel" differentiation in trout as viewed by Kaestner (1892): e) A newly formed "Urwirbel" with a peripheral epithelial layer ("Rinde") (p) and a central group of cells ("Kern") (k). Muscle development begins at the medial surface near the notochord (15-somite trout from fig. 28); f) The "Urwirbel" later becomes a two-component structure with a medial and a lateral lamella (ml) (ll) (31-somite trout, from fig. 29); g) The sclerotome (s) forms from the medio-ventral border of the "Urwirbel" and differentiates into the "Bildungsgewebe." Cells of the lateral lamella divide. The medial daughters form a layer of "young" muscle cells (y) at the lateral surface of the myotome. The lateral daughters form a squamous epithelium (sq) (7 mm trout, from fig. 30); h) The appearance of the "Urwirbel" shortly before hatching resembles that of the previous stage, with a large "medial muscle lamella" (ml) and a single "lateral muscle lamella" (ll) covered by a squamous epithelium (sq). The horizontal septum now divides the lateral musculature into epaxial and hypaxial divisions (14 mm trout, from fig. 31).

Continued on next page

Figure 1 (Continued):

i-j. "Urwirbel" development in salmon as conceived by Maurer (1894): i) The "Urwirbel" consists of a medial wall giving rise to a "Muskelblatte" (mb) and a sclerotome (s), and a lateral wall, the "Cutisblatte" (c). The two walls are separated by a cavity, the "Urwirbelhöhle" (uh) (11-day salmon, from fig. 26); j) Muscle development begins medially in the "Muskelblatte" involving the formation of grooves (g) and the establishment of cytoplasmic regions in which fibrils subsequently appear. Cells of the lateral muscle layer seen in slightly older embryos presumably formed by "pinching off" from the lateral border of the "Muskelblatte" (arrow) (22-day salmon, from fig. 27).

k. Horizontal section through a trunk somite of a moray eel at 7 days according to Sunier (1911). Myogenesis begins medially with the formation of multinucleate muscle cells "en forme de plaque" (pl). Remaining cells differentiate into a lateral layer of uninucleate muscle cells (lm), and the lateral-most cells flatten to form a "feuillet externe" (fe). No contact was observed between the ends of adjacent somites (Muraena species No. 1, from fig. 13b).

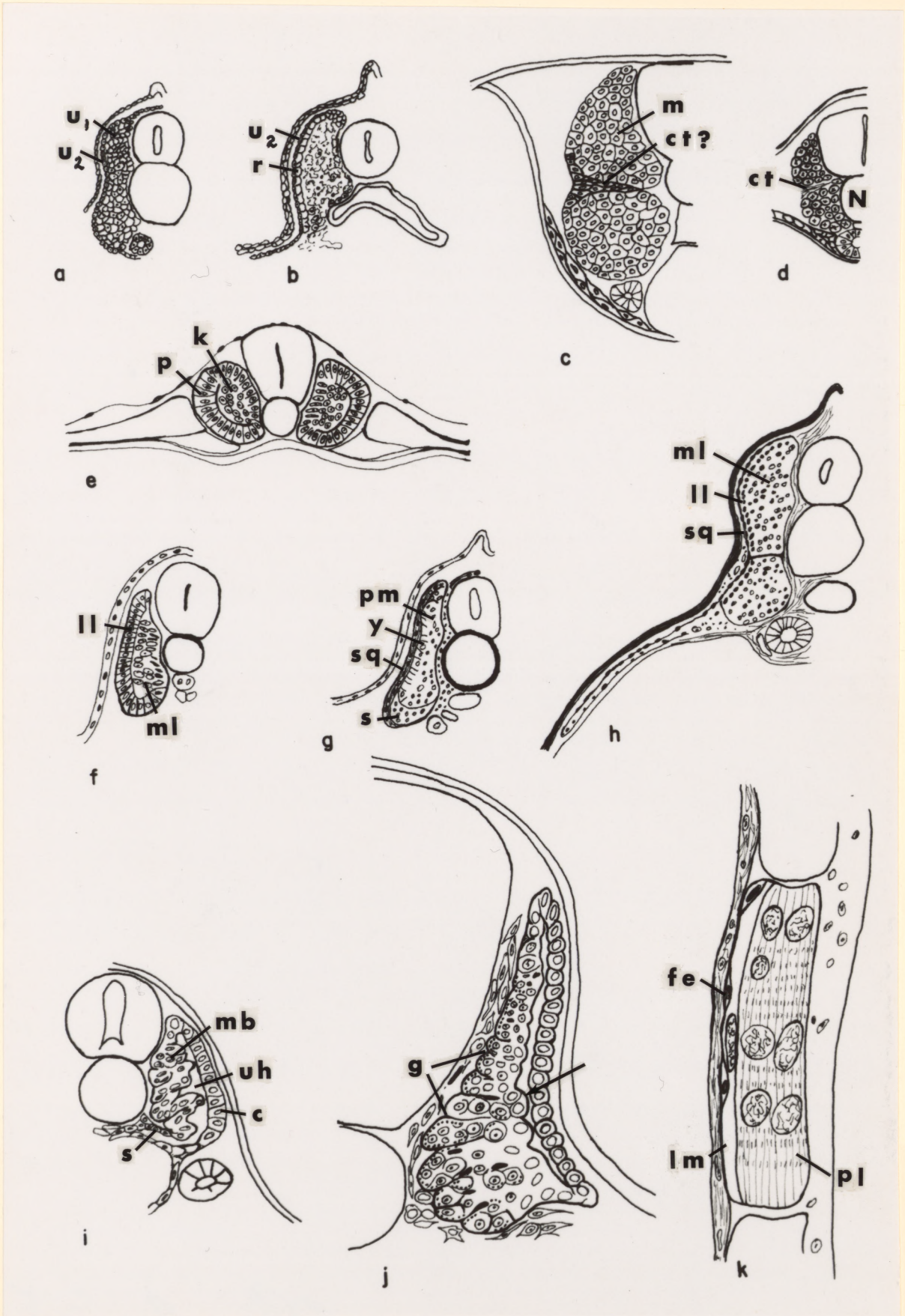
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Figure 2. Semidiagrammatic cross-section of an adult zebrafish illustrating the general anatomy of the midbody somites (Nos. 17-20). Right and left halves of the lateral musculature are separated by the vertebral column (V) and the dorsal and ventral median septa (DS) (VS). Each half is divided into an epaxial (EP) and hypaxial (HP) portion by the horizontal septum (HS), and is comprised of a wedge-shaped, superficial portion (stippled) and a larger, deep portion (unstippled). Portions of 5 myomeres (MY) separated by myosepta (MS) are seen on either side. Infracarinales (IC), supracarinales (SC), lateral nerve (L) and skin (SK) are also illustrated. Drawn by projection of a frozen section. Stained with Oil Red O and hemalum. Magnification approximately 66 X.

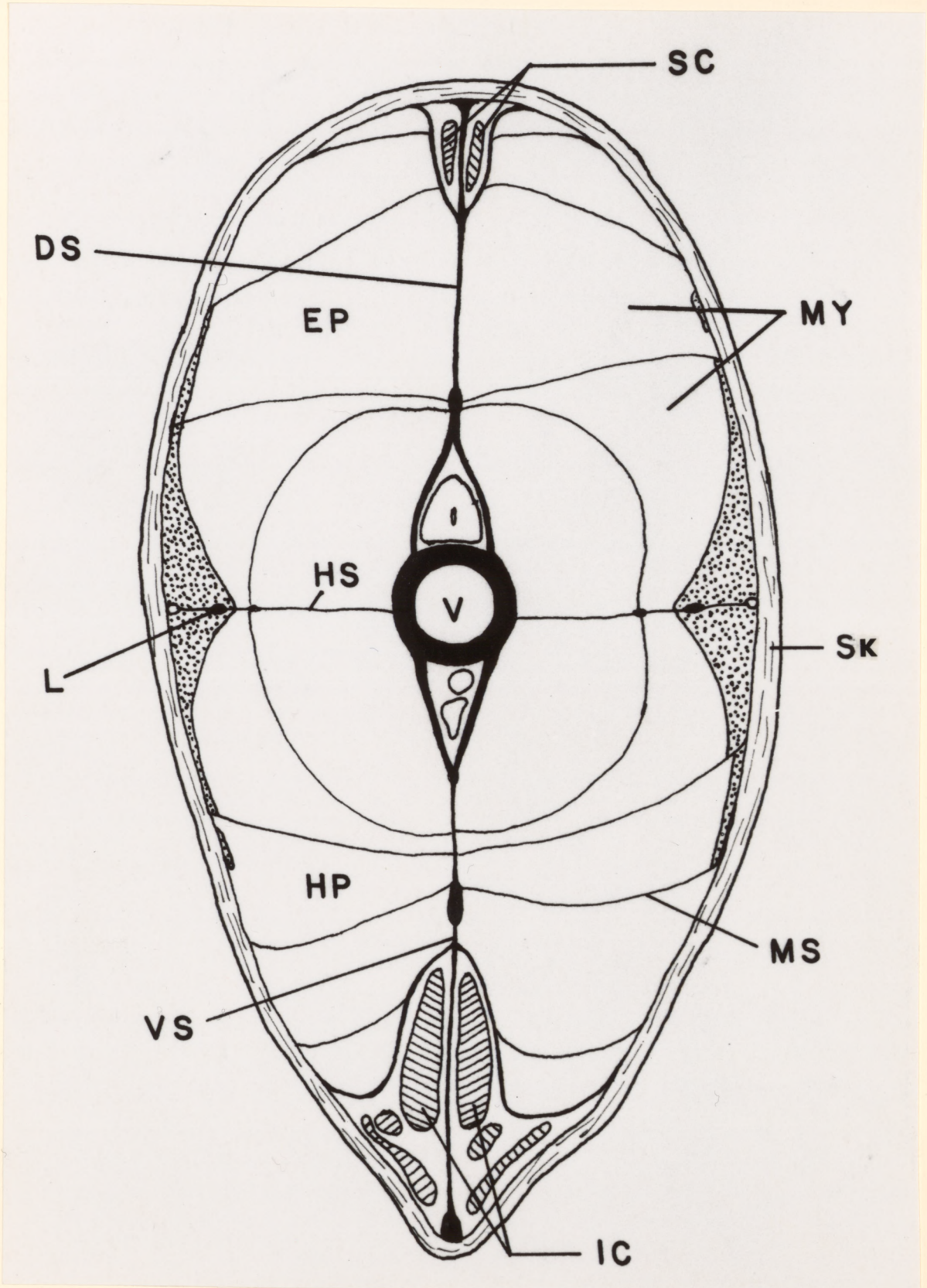
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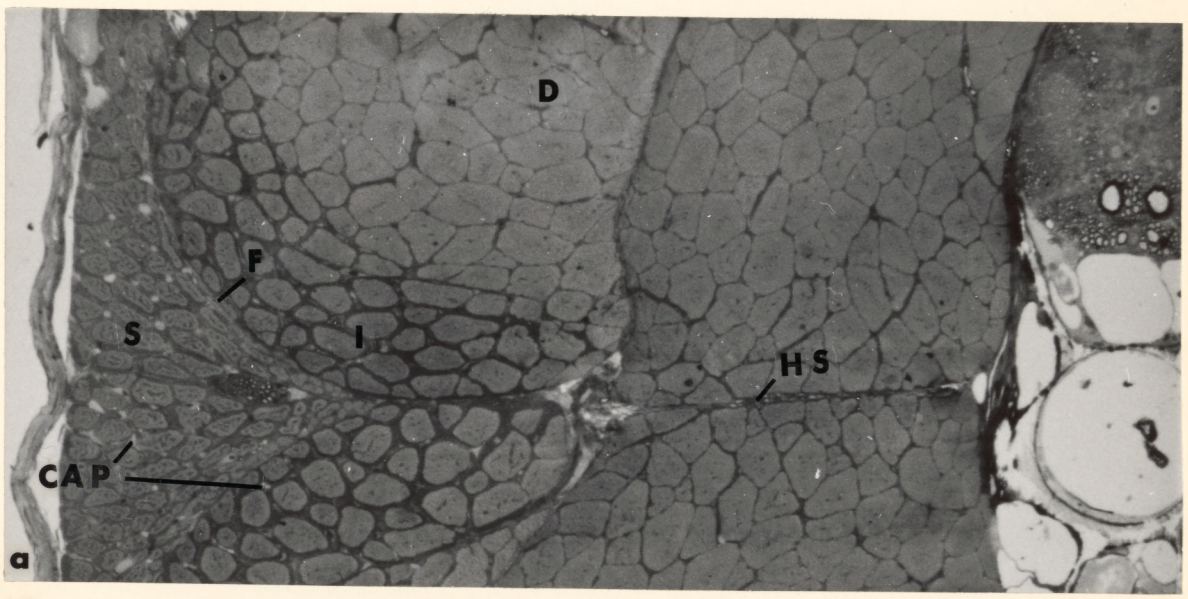
Figure 3. a) Thick (2μ) section through the lateral musculature of a young (1.5 cm) adult zebrafish showing the general histological appearance of the superficial (S), intermediate (I), and deep (D) muscle fibers near the horizontal septum (HS). Capillaries (CAP) are prevalent among the superficial and intermediate fibers, but almost totally absent between the deep fibers. A thin fascia (F) separates the superficial portion from the deep portion of the lateral musculature. Osmium-formaldehyde fixation. Stained with methylene blue-azure II. Magnification approximately 175 X.

b) Frozen section from a mature (4 cm) adult zebrafish stained with Sudan Black B to demonstrate the relative amounts of intracellular lipid (black) in the superficial (S), intermediate (I) and deep (D) muscle fibers. The lateral nerve (L) stains intensely. Magnification approximately 85 X.

c) Frozen section nearly adjacent to that shown in Figure 3b, illustrating the relative amounts of SDH activity (black) within the superficial (S), intermediate (I), and deep (D) muscle fibers. Magnification approximately 85 X.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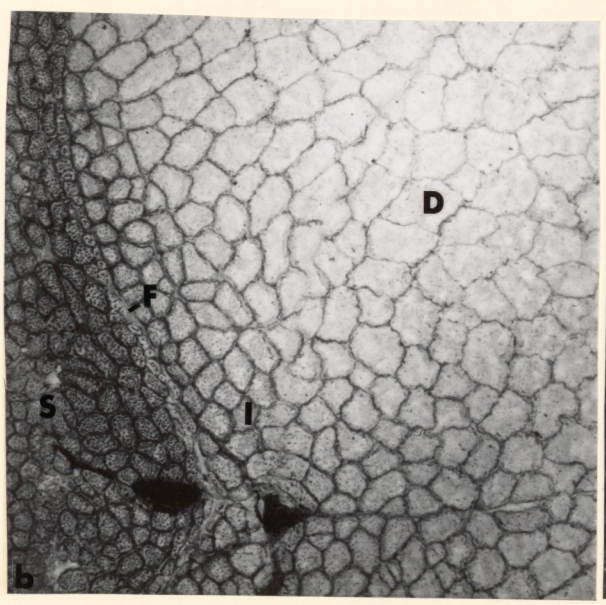
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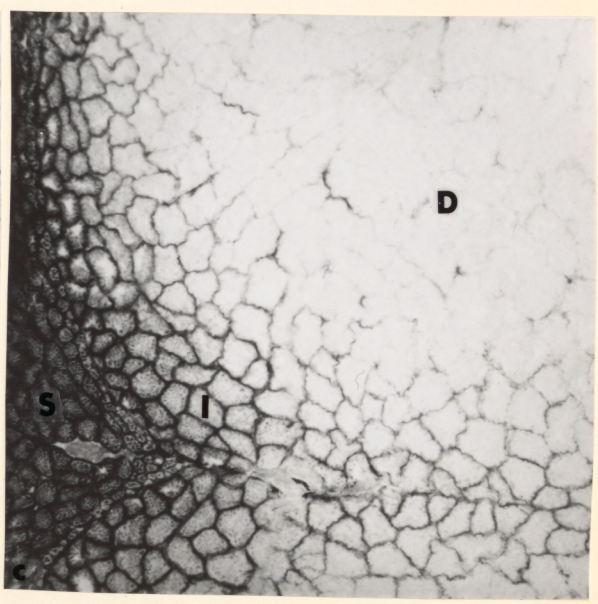
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Figure 4. Electron micrograph showing a portion of the lateral musculature in the midbody region of a young (1.5 cm) adult zebrafish. Very small superficial fibers (S) with irregularly arranged myofibrils (MYF) surrounding groups of mitochondria (M) are seen near the medial end of the lateral nerve (L). They are separated from larger, intermediate fibers (I) of the deep portion of the lateral musculature by a thin connective tissue layer (F). Numerous mitochondria lie within the peripheral cytoplasm of the intermediate fibers. Axons (A), nuclei (N), capillary (CAP). Osmium-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 7790 X.

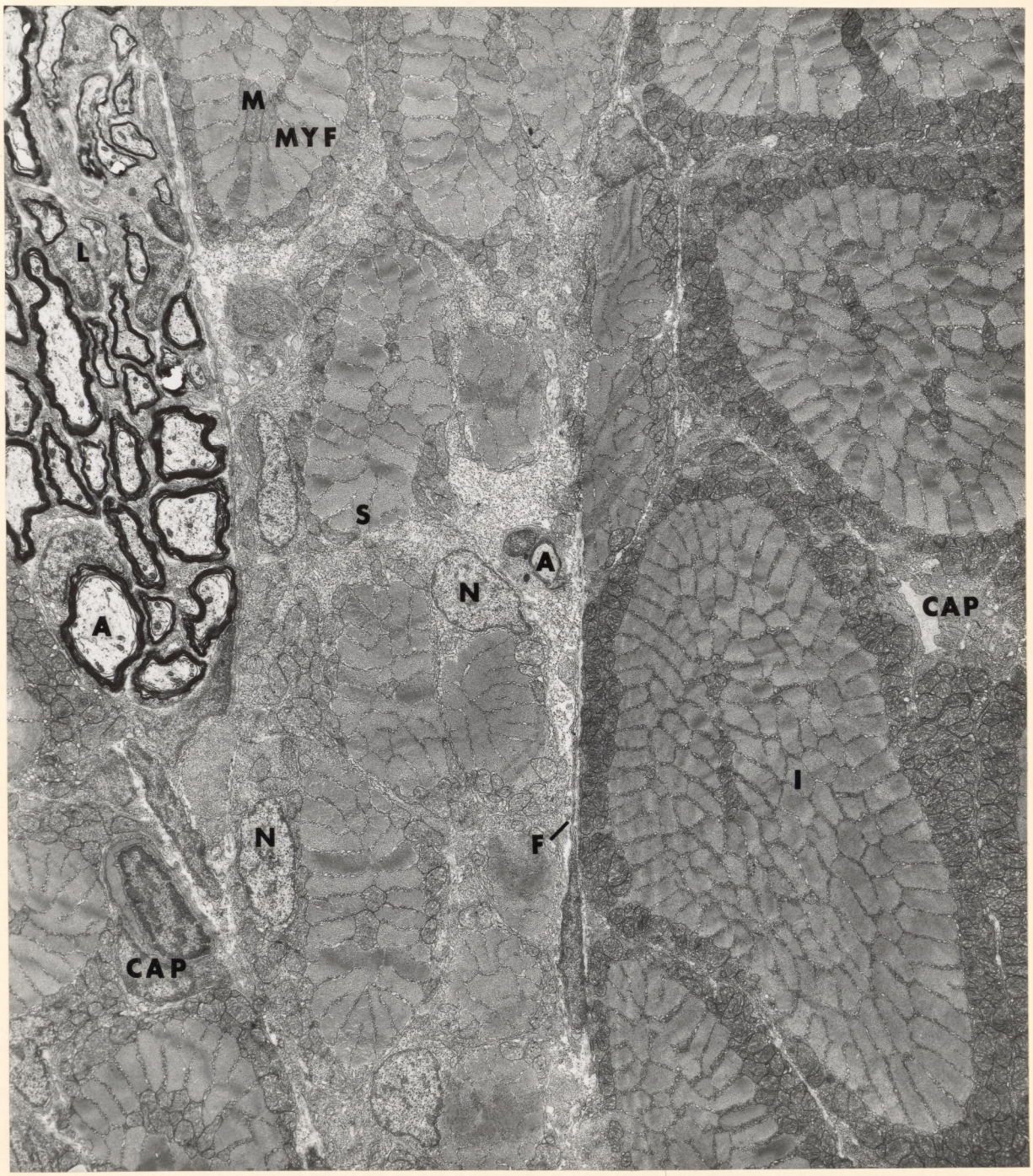
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Figure 5. Portions of several deep muscle fibers from a young (1.5 cm) adult zebrafish as seen in oblique cross-section. They contain radially arranged, ribbon-shaped myofibrils (RF) at the periphery, and polygonal-shaped fibrils (PF) near the center. Mitochondria (M) are seen in this peripheral rim of cytoplasm and among the fibrils. Osmium-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 4090 X.

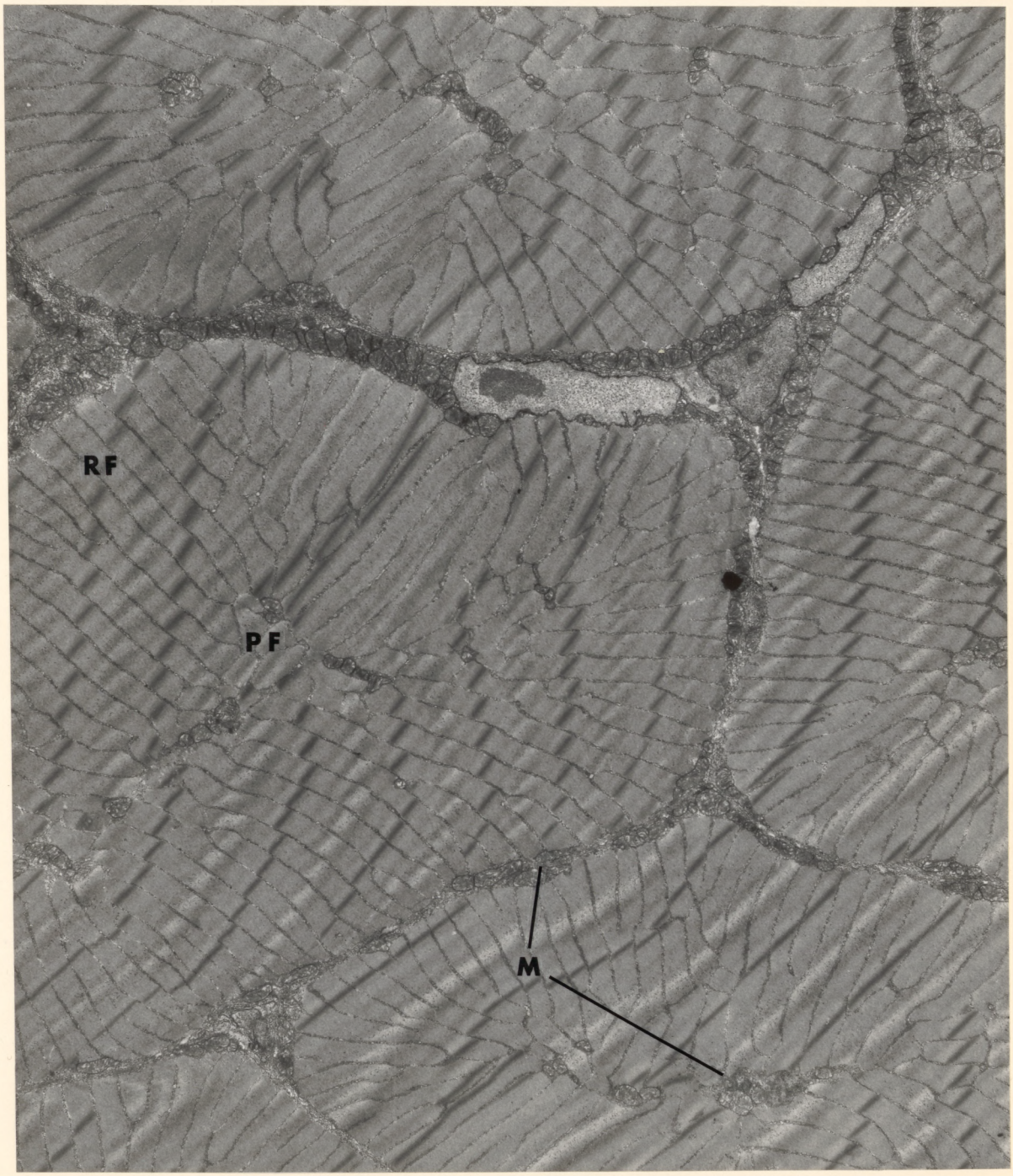
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Figure 6. a) Horizontal section showing a portion of a superficial muscle fiber from a young (1.5 cm) adult zebrafish. An accumulation of glycogen particles (GL) are seen in the peripheral cytoplasm and between the myofibrils. The myofibrils exhibit distinct Z-, I-, A-, H- and M-bands. T-tubules of the triads are located over the Z-bands (arrows). Osmium-formaldehyde fixation. Stained with lead. Magnification approximately 8550 X.

b) Portion of a deep muscle cell in the same section as Figure 6a illustrating the relative amount of glycogen (GL) in the deep fibers. Few mitochondria are evident. Triads are found at the Z-band level (arrows). Osmium-formaldehyde fixation. Stained with lead. Magnification approximately 8550 X.

c-e) Presumed neuromuscular junctions are present in relation to the three main fiber types of the lateral musculature of young (1.5 cm) adult zebrafish. No significant fine structural differences between them have been noted. The nerve endings contain vesicles (VL) and small mitochondria (M). They are separated from the muscle cell membrane by a uniform distance of 510-530 Å (arrow) and are covered by Schwann cell processes (SWC) over the opposite surface. c) Junction on a superficial fiber, d) Junction on an intermediate fiber, e) Junction on a deep fiber. Osmium-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 194,000 X.

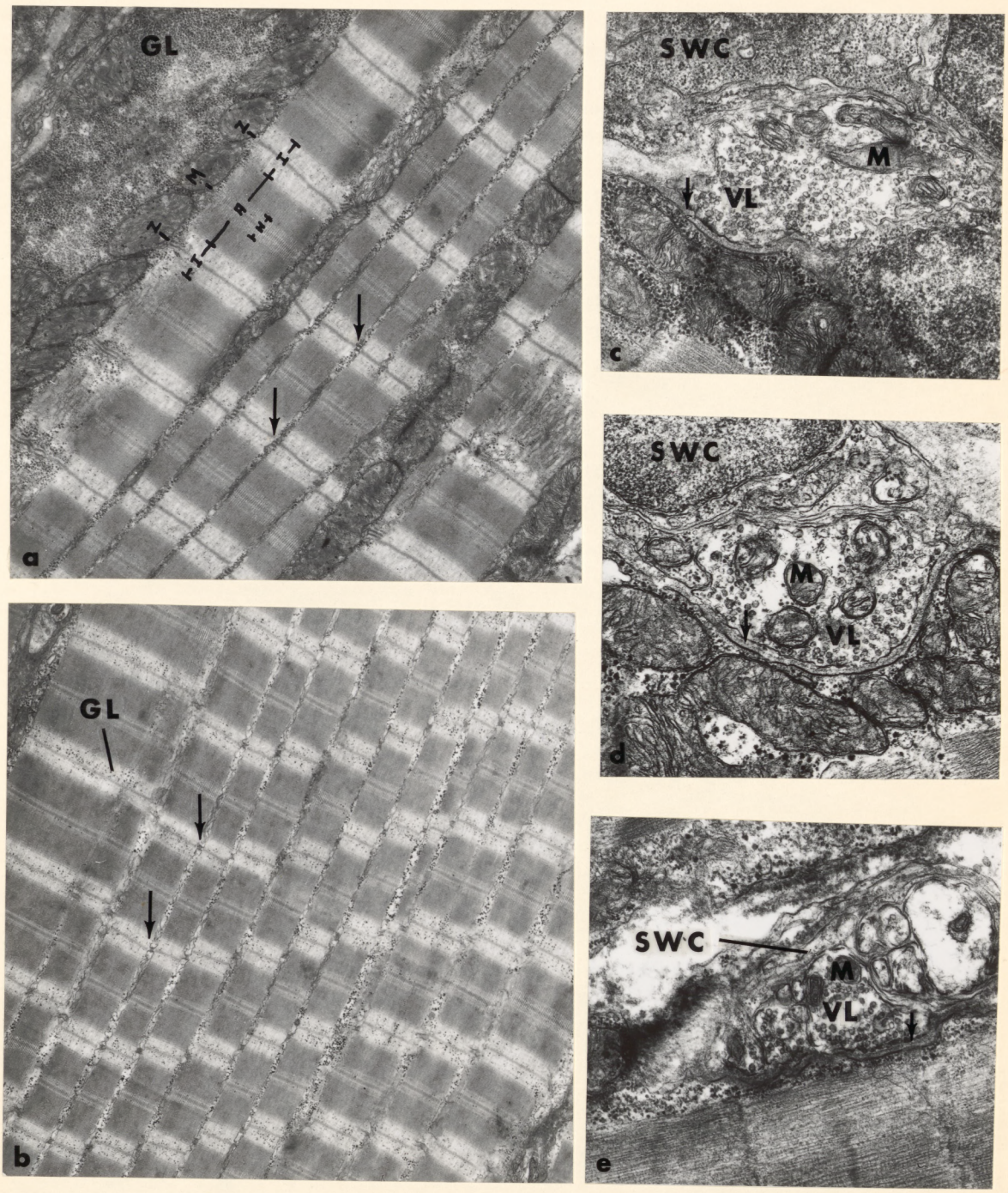
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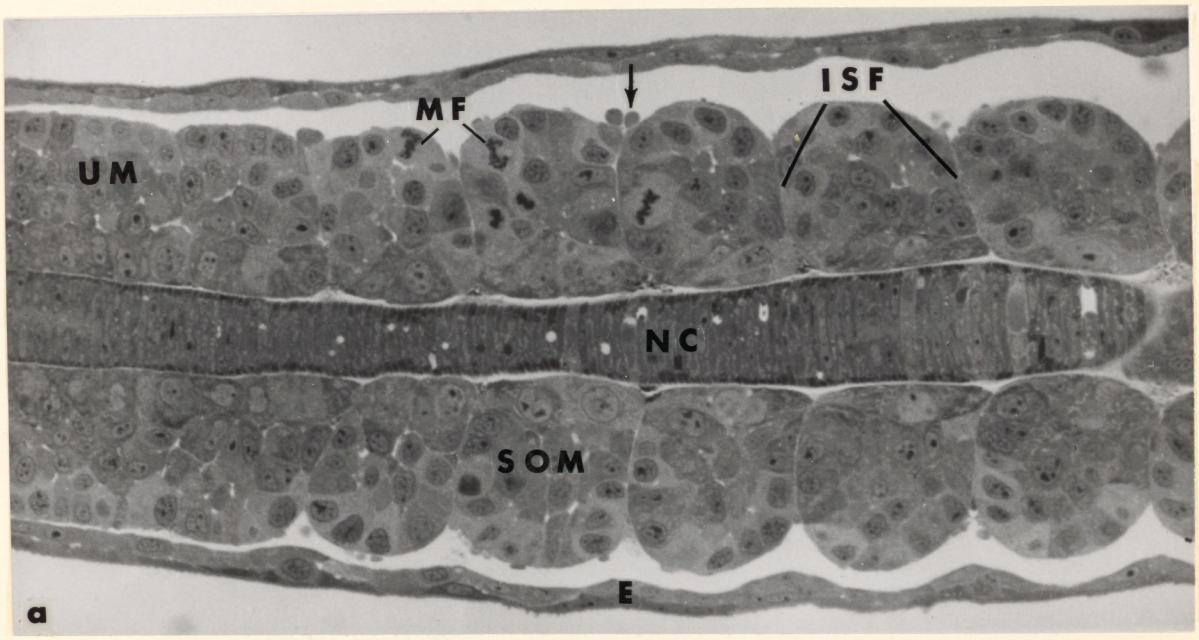
Figure 7. a) Horizontal 1 μ section from a 20-somite embryo illustrating the general appearance of the unsegmented somitic mesoderm (UM) and newly formed midbody somites (SOM) at the level of the notochord (NC). The somites are compact cellular units separated by intersomitis furrows (ISF). Small protrusions (arrow) often occur near the lateral margin of these furrows. The peripheral cells of each somite are more closely apposed than the centrally located cells. No distinct cavity is present within the somites. Mitotic figures (MF) are frequent. (E) Epidermis. Glutaraldehyde-formaldehyde fixation. Stained with methylene blue-azure II. Magnification approximately 466 X.

b) Oblique parasagittal 1 μ section through the midbody somites of a 20-somite embryo. The plane of section passes through the lateral cells of somite No. 20 posteriorly (right) and approaches the medial surface of No. 15 anteriorly. Elongated presumptive myoblasts (PM) are seen medially in the more anterior somites, but no muscle cells are observed. Numerous small protrusions (arrows) occur at the lateral ends of the intersomitic furrows and over the dorsal and ventral surfaces of the somites. A portion of the neural keel (K) is seen dorsally. Rectangle indicates an area similar to that shown in Figure 9. Glutaraldehyde-formaldehyde fixation. Stained with methylene blue-azure II. Magnification approximately 466 X.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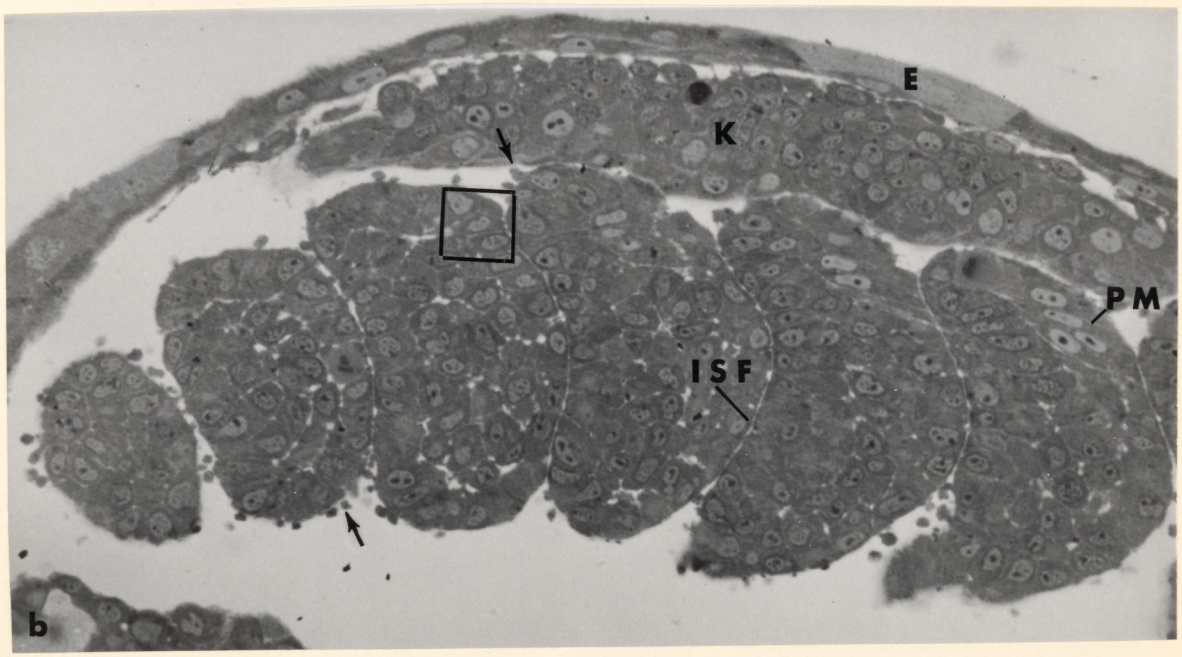
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ISF
b

Figure 8. a) A horizontal section showing cells of the unsegmented mesoderm just posterior to the midbody somites in a 20-somite embryo. Each polygonal cell contains a single, irregularly shaped nucleus (N), numerous mitochondria (M), Golgi membranes (G), small profiles of rough endoplasmic reticulum (ER), and numerous glycogen particles (GL) (accounting for much of the cytoplasmic density in this lead-stained preparation). Lamellated, membranous structures are frequently observed at this stage (arrows). Glutaraldehyde-formaldehyde fixation. Stained with lead. Magnification approximately 8360 X.

b) A portion of a cell from somite No. 18 of a 20-somite embryo. Several typical lamellated membranous structures are shown. These structures may often appear near a mitochondrion (M) or confluent with them (arrow). They are tentatively identified as a fixation artifact. A centriole (C) is also shown. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 17,400 X.

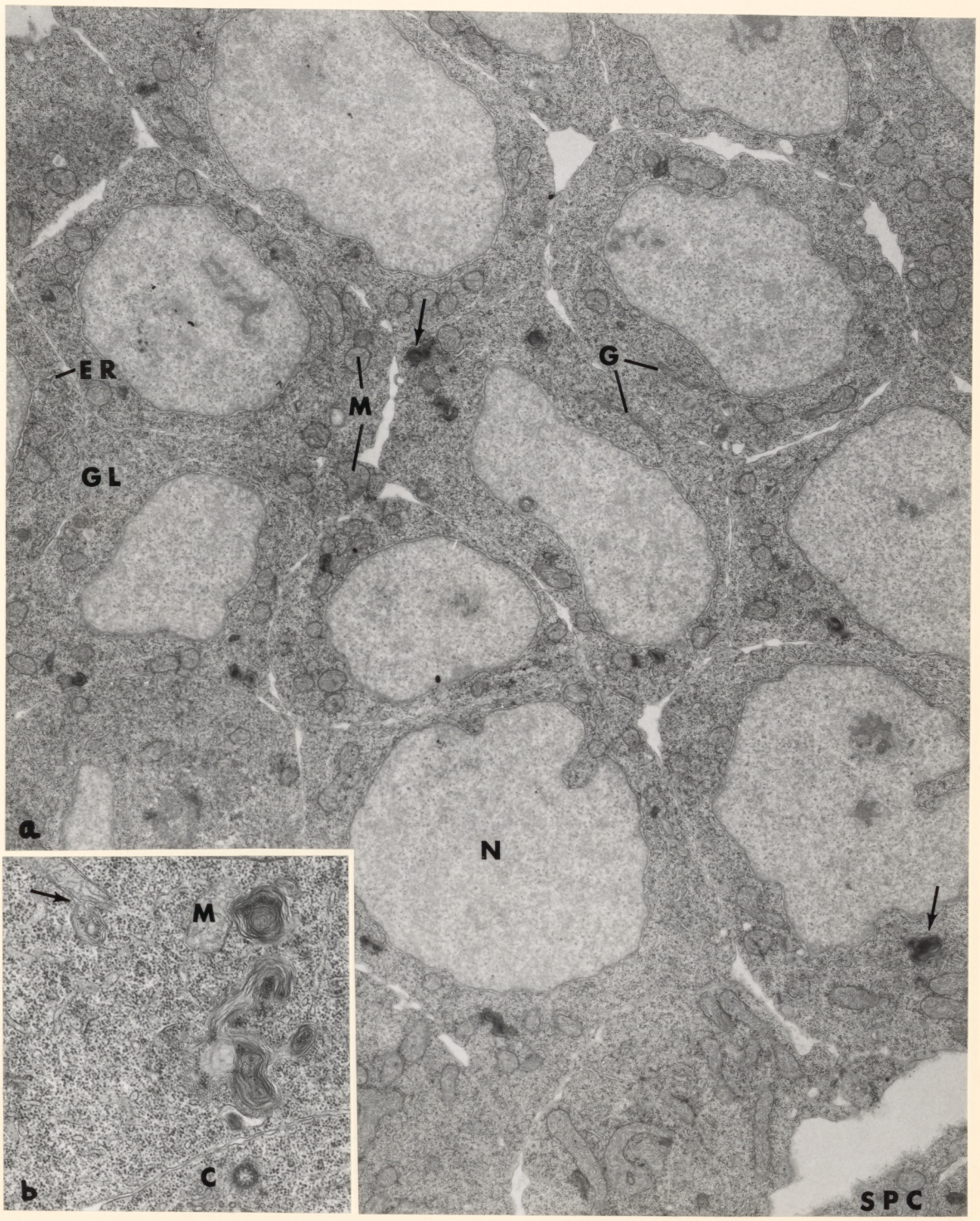

ER
M
G
GL
a
N
SPC
M
C
b

Figure 9. A portion of somite No. 18 from a 20-somite embryo similar to that indicated by the rectangle in Figure 7b. The peripheral cells (arrows) are more closely apposed than the centrally located cells (lower left). All cells retain the general polygonal shape and fine structure of the unsegmented mesodermal cells. Intercellular spaces (*) are plentiful, but no distinct cavity is present. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 7220 X.

Insert: Embryonic junction (EJ). Magnification approximately 19,000 X.

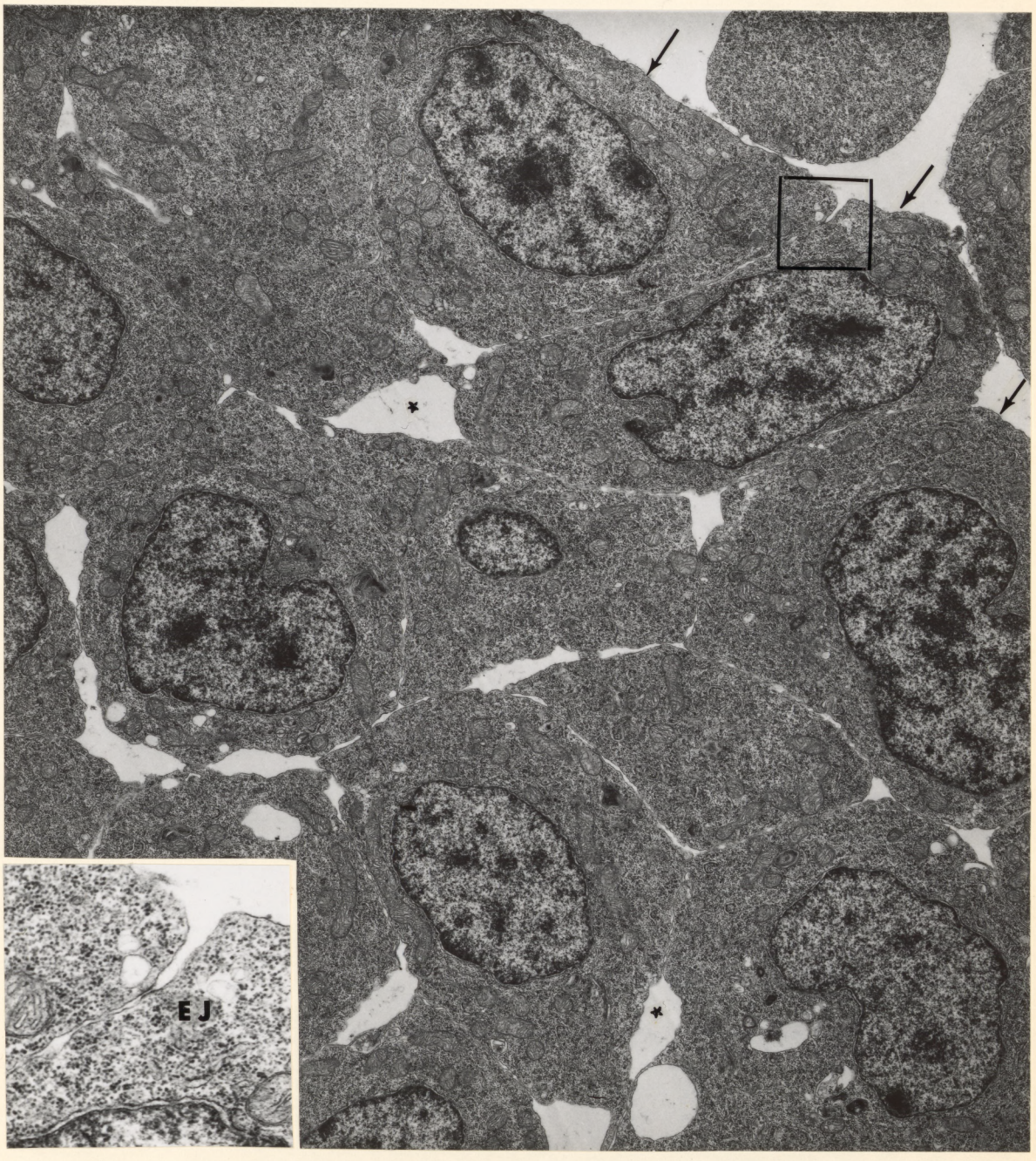

EJ

Figure 10. Portions of somites No. 19 (left) and 18 (right) from a 20-somite embryo, and the intersomitic furrow between them. Cytoplasmic processes (arrows) frequently extend from the cells and insert between adjacent cells. Focal intermediate junctions (FIJ) are shown. Some flocculant material is present within the intersomitic furrow, but few direct cell contacts are observed between adjacent somites. Flagella are commonly seen (see insert). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 8170 X.

Insert: Focal intermediate junction and flagellum seen at higher magnification. Nine peripheral elements are present within the flagellum, but a center doublet is not evident. Magnification approximately 54,500 X.

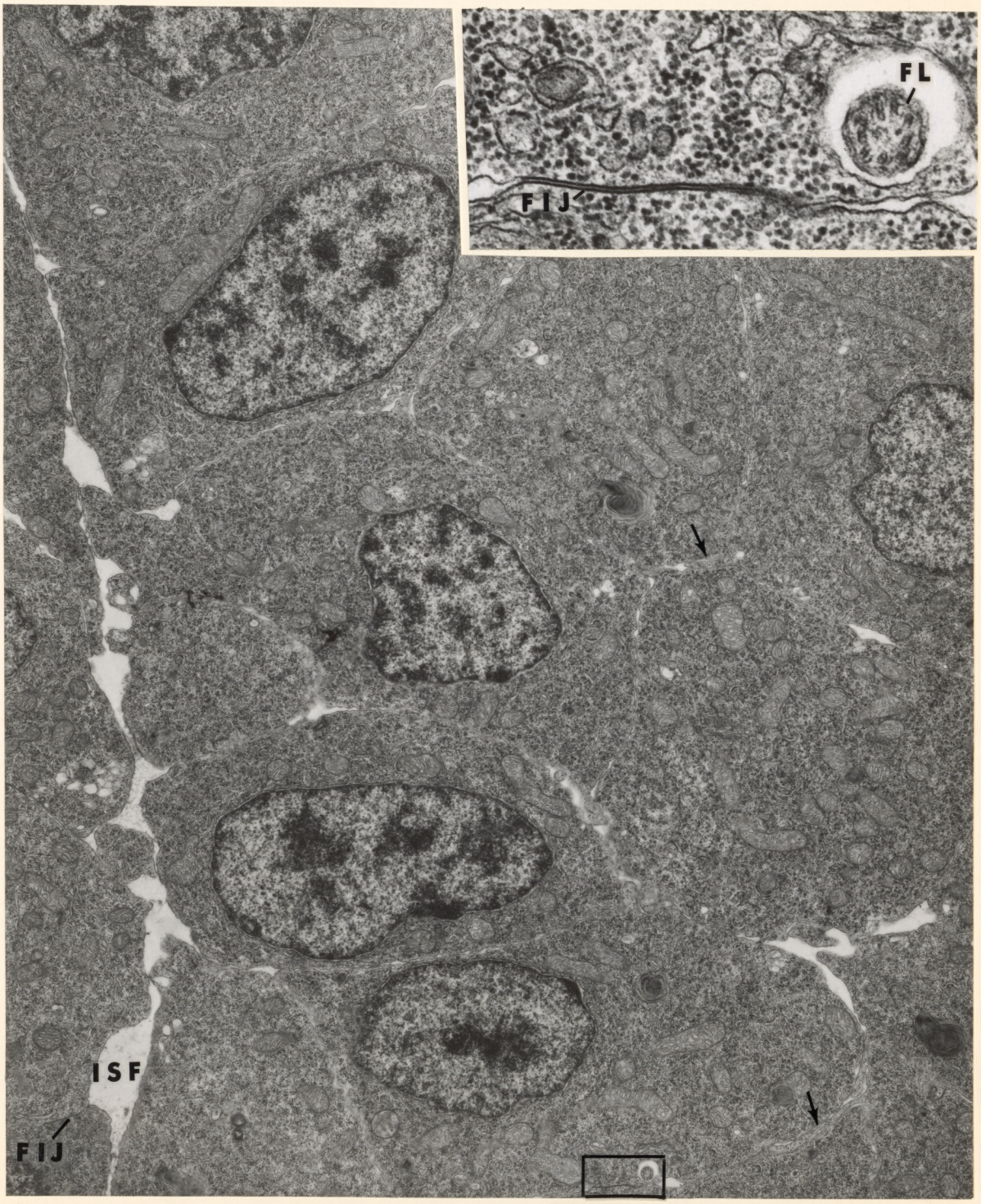

FL
FIJ
ISF
FIJ

Figure 11. A flagellum (FL) in somite No. 18 of a 20-somite embryo is seen projecting into a membranous sleeve (arrows) which is not continuous with the cell membrane in this plane of section. A centriole (C), smooth surfaced membranous cisternae and vesicles (SM), and microtubules (MT) associated with the flagellum are located at its base. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 14,800 X.

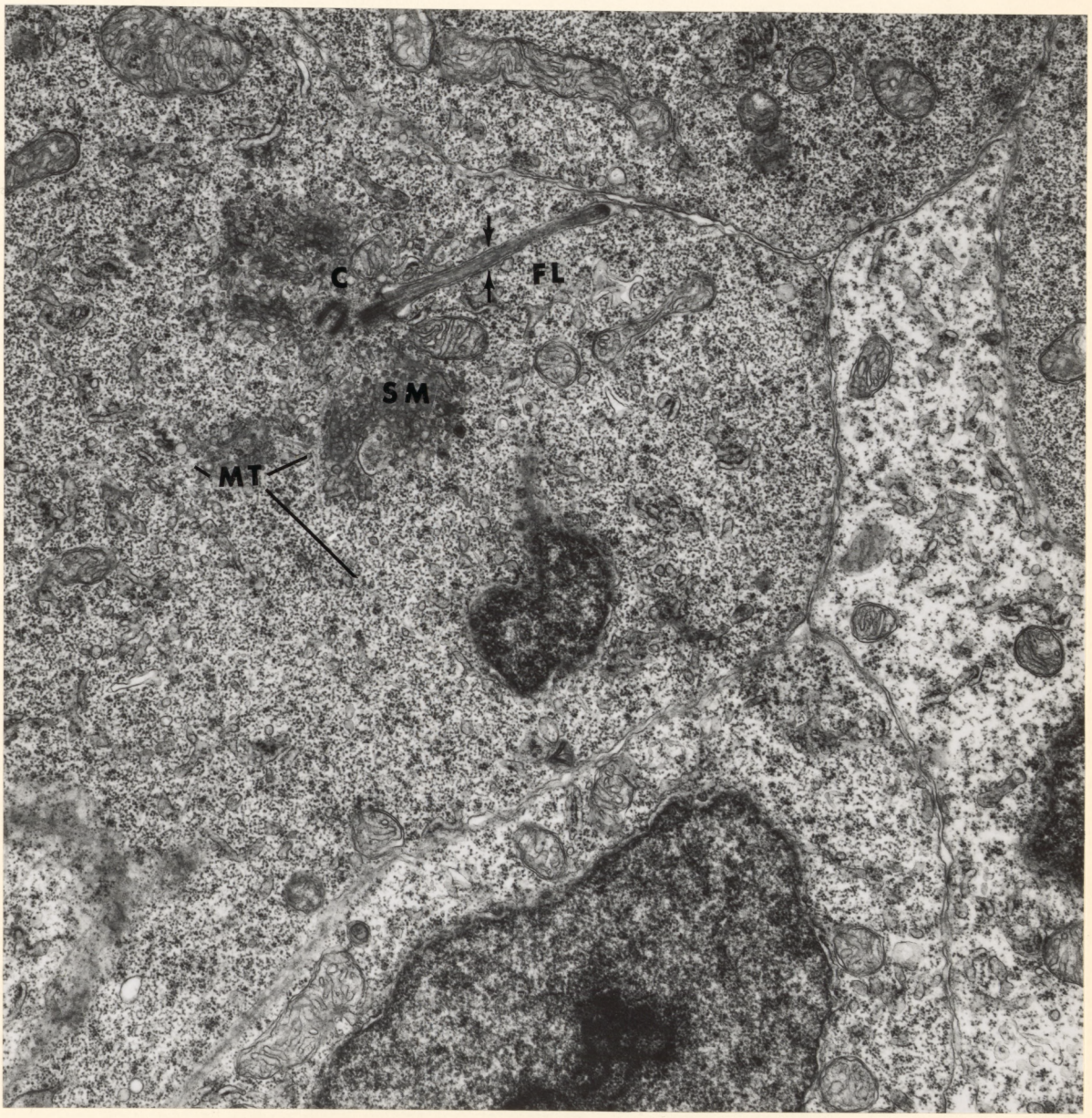

C
FL
SM
MT

Figure 12. An electron micrograph of a mitotic cell in somite No. 19 of a 20-somite embryo. Focal intermediate junctions (FIJ) are seen between the dividing cell and adjacent cells near the intersomitic furrow (ISF). Microtubules (MT) of the spindle, and a centriole (C) in cross-section are shown in the insert. Chromosomes (CH). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 17,300 X.

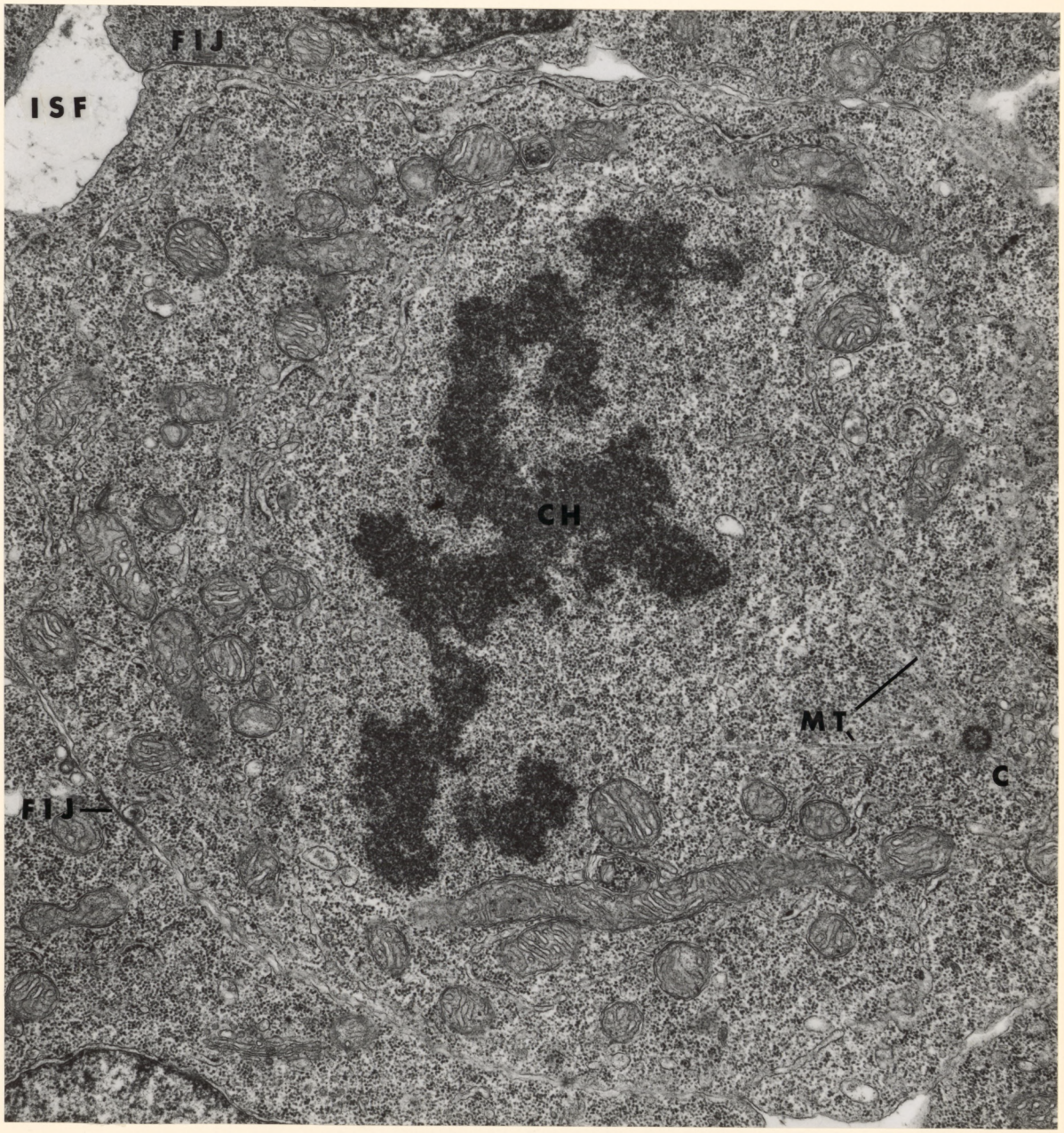

FIJ
ISF
CH
MT
C
FIJ

Figure 13. Cells at the medial surface of somite No. 20 of a 20-somite embryo are shown in horizontal section. No sign of cell elongation or myofilament formation is observable, although their nuclei (N) are somewhat less dense than those of more lateral cells. Some flocculant material (arrows) is characteristically seen along the spinal cord (SPC) and the medial somite surface. Similar material is also present in the intercellular spaces, but is less conspicuous. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 11,200 X.

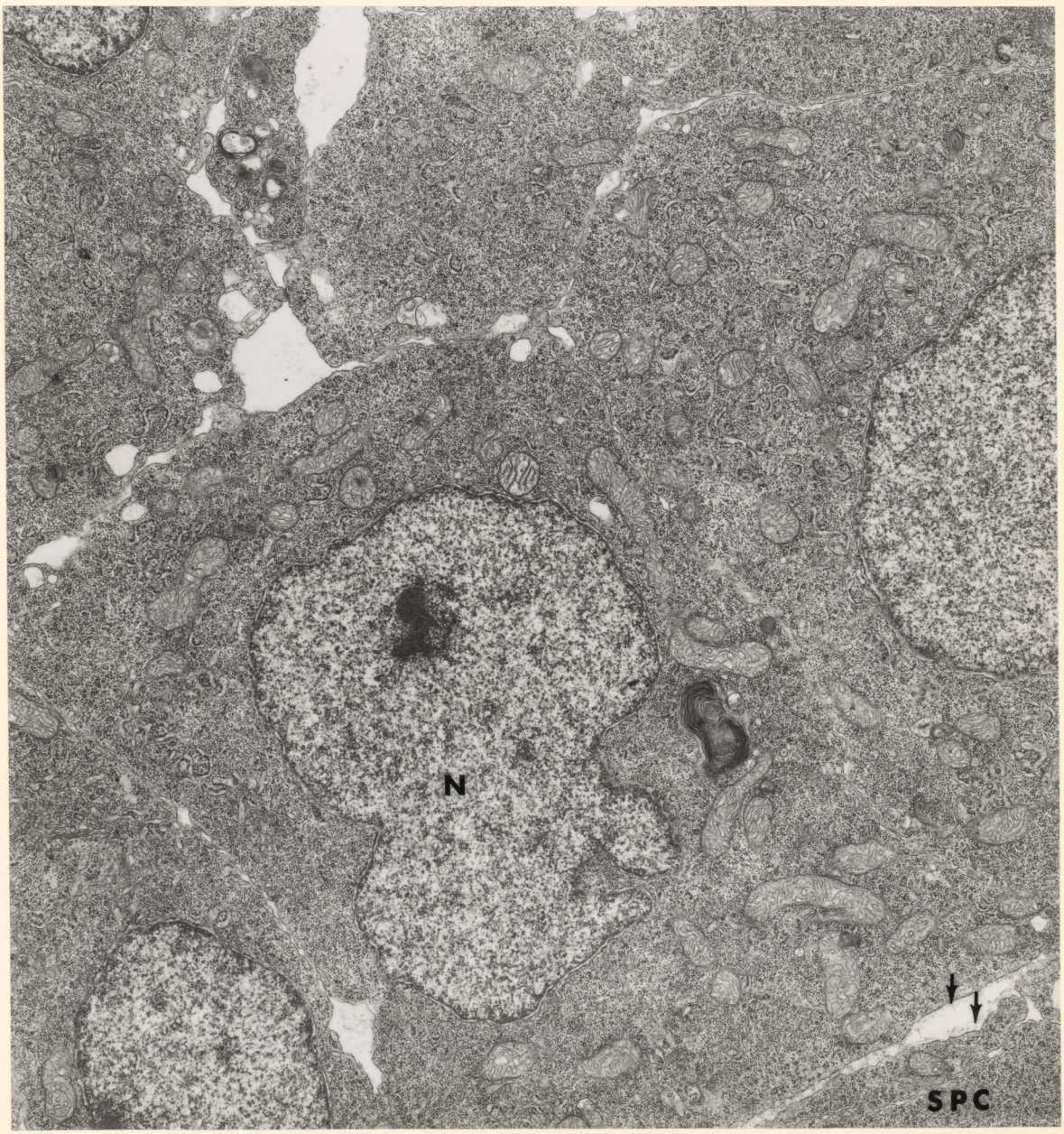

N
SPC

Figure 14. a) Oblique cross-section of somite No. 18 from a 25-somite embryo. A layer of myoblasts (MB) is seen at the medial surface of the somite. Each myoblast contains organized bundles of thick and thin myofilaments (MF) and clusters of ribosomes (R). Myoblast nuclei (N) are less dense than those of more lateral cells. A basla lamina (BL) is present between the cells of the notochord (NC) and its fibrous sheath (SH). Some flocculant or fibrillar material is also present at the medial surface of the somite (arrow). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 8740 X.

b) The region between the spinal cord (SPC) and myoblasts (MB) at the medial surface of somite No. 18 from a 25-somite embryo is shown in cross-section. A basal lamina (BL) surrounds the spinal cord, and some flocculant material (arrow) is found along the medial surface of the somite. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 28,800 X.

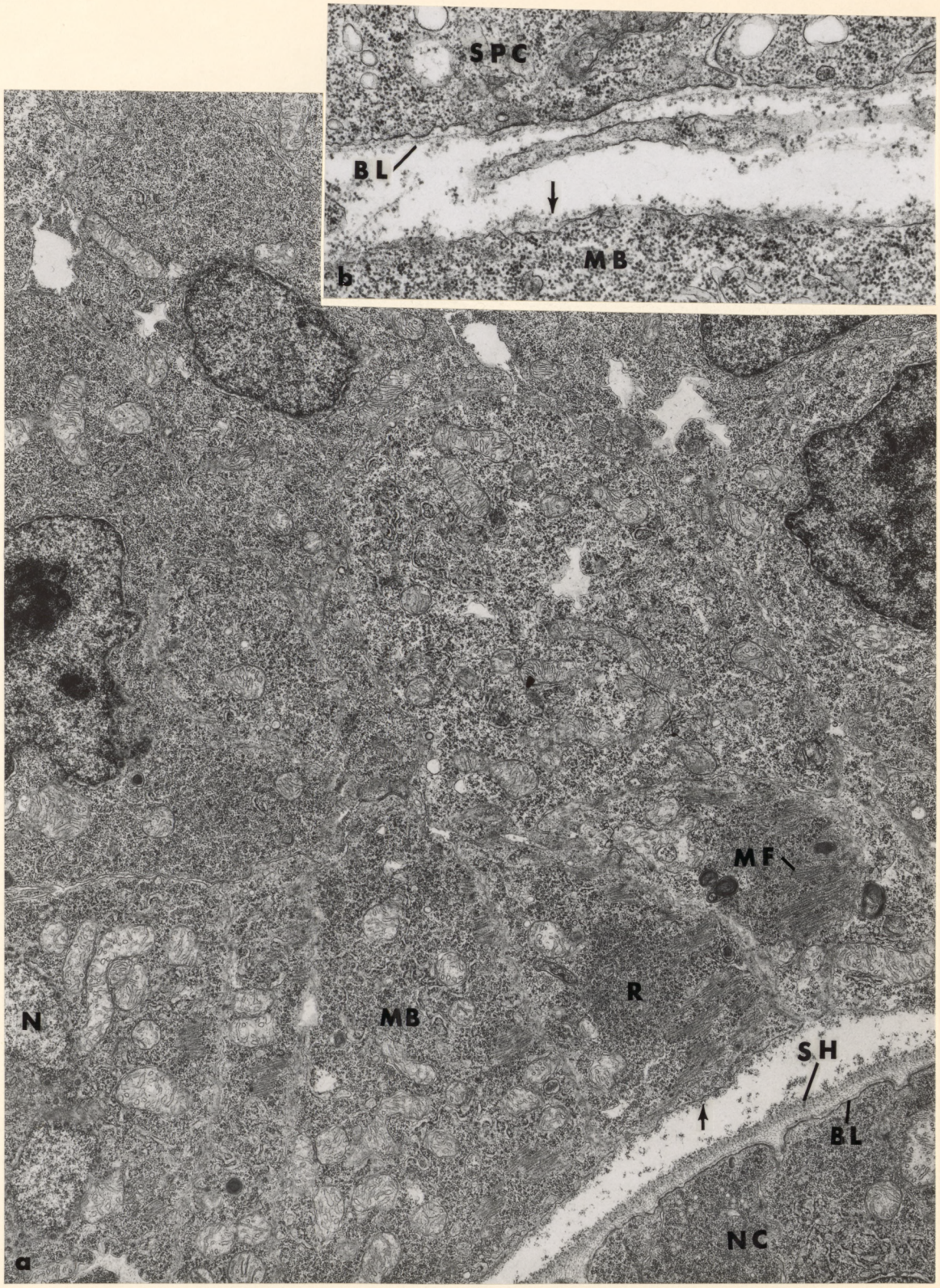

SPC
BL
MB
b
MF
R
N
MB
SH
BL
NC
a

Figure 15. Cells lateral to the myoblasts and presumptive myoblasts in 25-somite embryos retain the shape and fine structural appearance of previous stages as illustrated in this portion of somite 22. A flagellum (FL) is seen in longitudinal section extending into a membranous sleeve which is continuous with the cell membrane (CM). Also seen are microtubules (MT) and an embryonic junction (EJ). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 24,300 X.


MT
EJ
FL
CM
MT

Figure 16. Slightly oblique parasagittal section through the layer of myoblasts (MB) at the medial surface of somite No. 20 from a 25-somite embryo. Each myoblast contains bundles of myofilaments (MF), ribosomal clusters (R), and a single nucleus (N) with dispersed chromatin and up to 3 distinct nucleoli (NU). Lysosome-like structures (arrows) are also frequently observed in such myoblasts. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 8170 X.

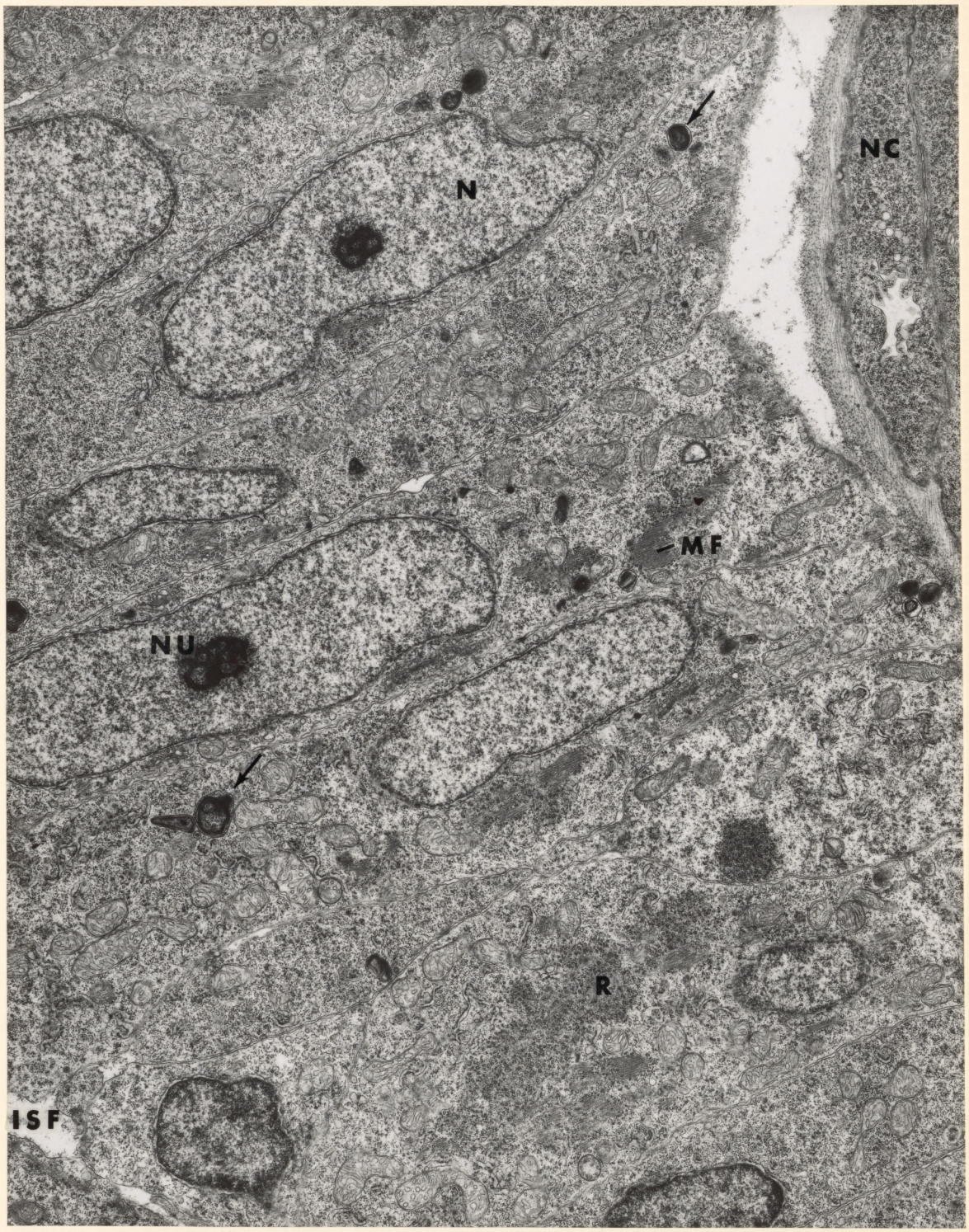

N
NC
MF
NU
R
ISF

Figure 17. a-b) Portions of 2 myoblasts in somite No. 20 from a 25-somite embryo. Microtubules (MT) are shown oriented primarily parallel to the long axis of the cell. No definite continuity of microtubules and myofilaments (MF) has been observed. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately: a) 39,000 X, b) 33,600 X.

c) Portions of several presumptive myoblasts containing microtubules (MT) in somite No. 19 from a 25-somite embryo. A mitotic nucleus (MIF) is also illustrated. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 17,700 X.

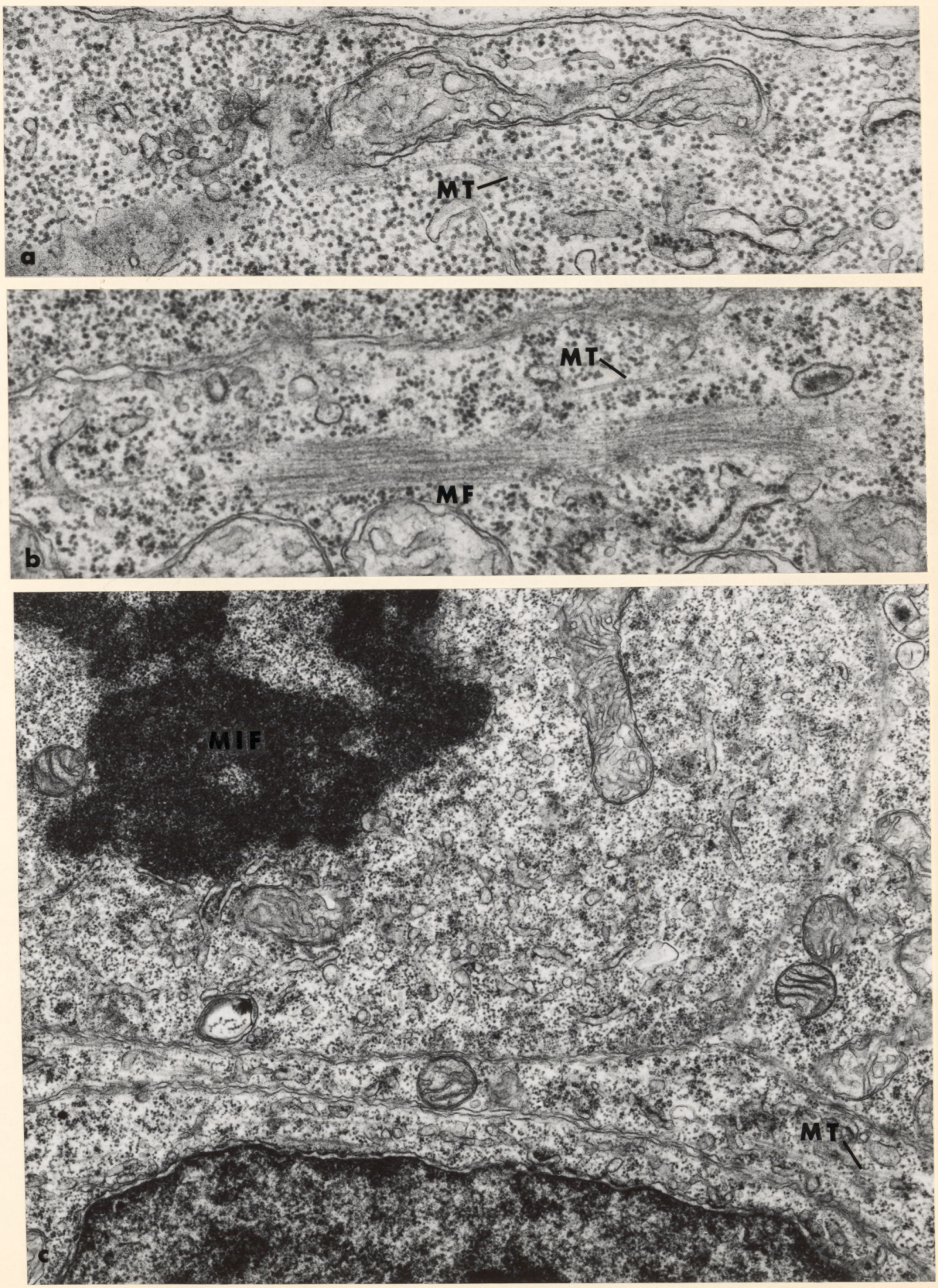
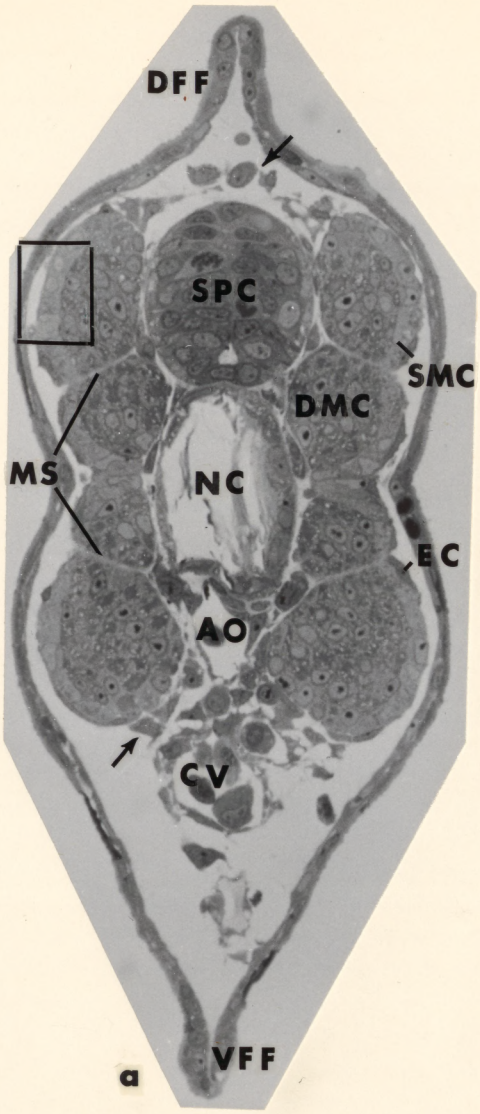

MT
a
MT
MF
b
MIF
MT
c

Figure 18. a) A 1 μ section of a 33-somite embryo showing the mid-portion of somite No. 19 and the dorsal and ventral portions of somite No. 18. Three cellular regions may be distinguished within the somite: 1) external cells (EC); 2) superficial muscle cells (SMC); and 3) deep muscle cells (DMC). Dispersed cells (arrows) around the compact somitic mass are presumably of either sclerotomal or neural crest origin. The aorta (AO), caudal vein (CV), and the dorsal and ventral fin-folds (DFF) (VFF) are also seen. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 448 X.

b) Horizontal 1 μ section from a 33-somite embryo at the level of the spinal cord illustrating the same three cellular regions of the modbody somites shown in Figure 18a (somite No. 21 at right). Darkly staining external cells (EC) cover the lateral surface of the myotome. The myotome is composed of two muscle cell types: 1) a layer of superficial muscle cells (SMC), each with a single centrally located nucleus (N), and a large myofibrillar bundle (MFB) along the medial cell margin; and 2) several layers of deep muscle cells (DMC), each of which has numerous small, peripheral myofibrils (MYF) and may contain up to 3 nuclei. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 448 X.


DFF
SPC
SMC
DMC
MS
NC
EC
AO
CV
VFF
a

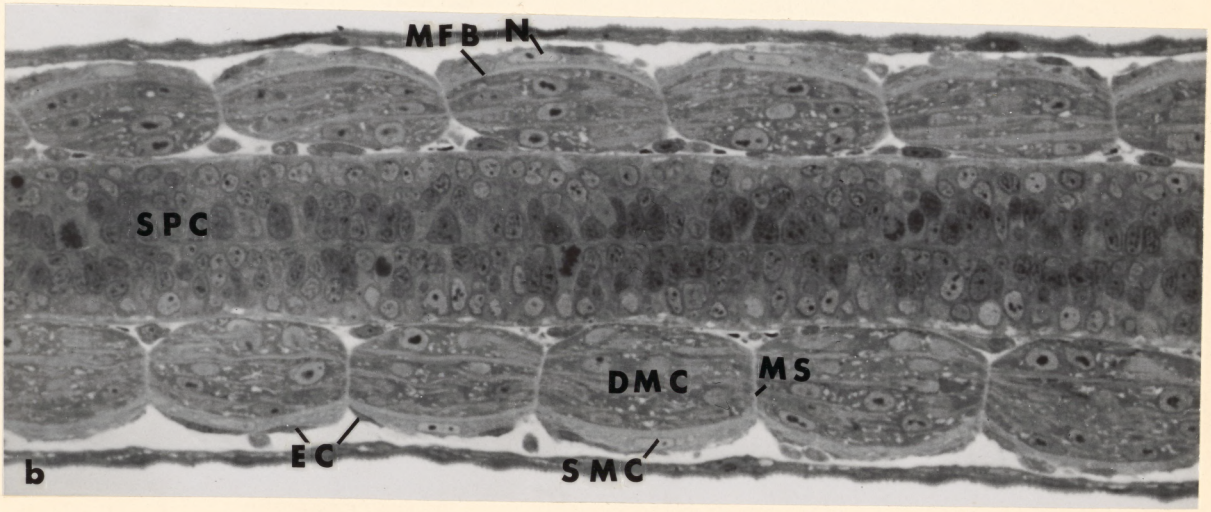

MFB
N
SPC
DMC
MS
EC
SMC
b

Figure 19. An electron micrograph representing an area similar to that indicated by the rectangle in Figure 18a, illustrating the relationships of deep muscle cells (DMC), superficial muscle cells (SMC), and processes of several external cells (EC). Deep muscle cells contain a central cytoplasmic core and several small myofibrils (MYF) located peripherally. Superficial muscle cells contain a large myofibrillar bundle (MFB) along the medial cell margin and a single, centrally located nucleus (N). Mitochondria of the superficial muscle cells usually appear more dense than those of the deep muscle cells. Smaller cells occur subjacent to the superficial muscle cells. External cell processes cover the lateral surface of the myotome, separating it from the epidermis (E) and vacuolated cells (VC) in the subepidermal space. Overlapping of external cell processes is frequent. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 9880 X.

Insert: Hexagonal arrangement of thick and thin myofilaments is indicated by arrow. Magnification approximately 57,700 X.

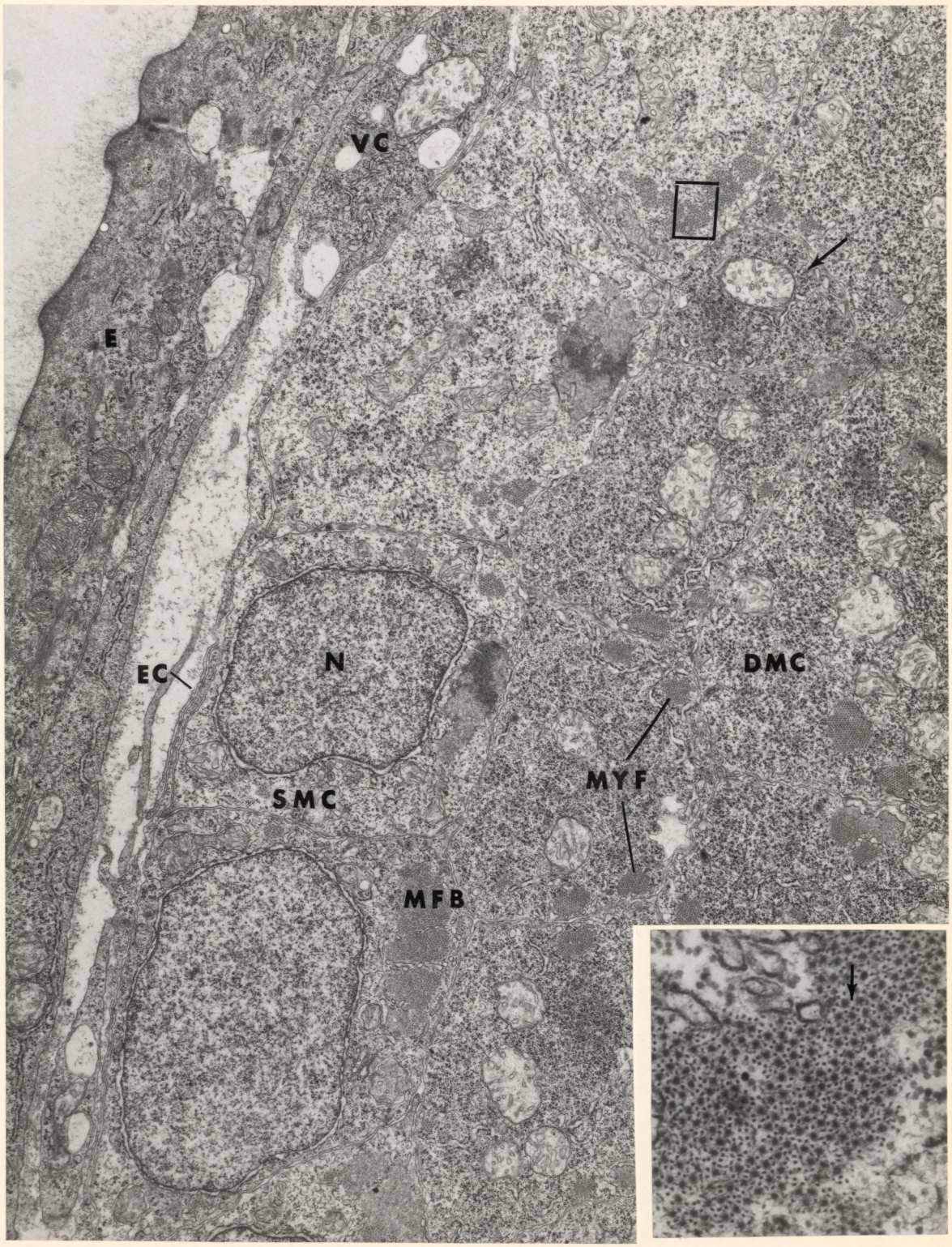

VC
E
EC
N
DMC
MYF
SMC
MFB

Figure 20. Cross-section of the dorsal extremity of somite No. 18 from a 33-somite embryo. A portion of an external cell (EC) is visible. It contains a dense nucleus (N) and cisternae of the rough endoplasmic reticulum (ER) in an extremely thin cell process (arrow). Each external cell is in close contact with several superficial muscle cells (SMC). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 7700 X.

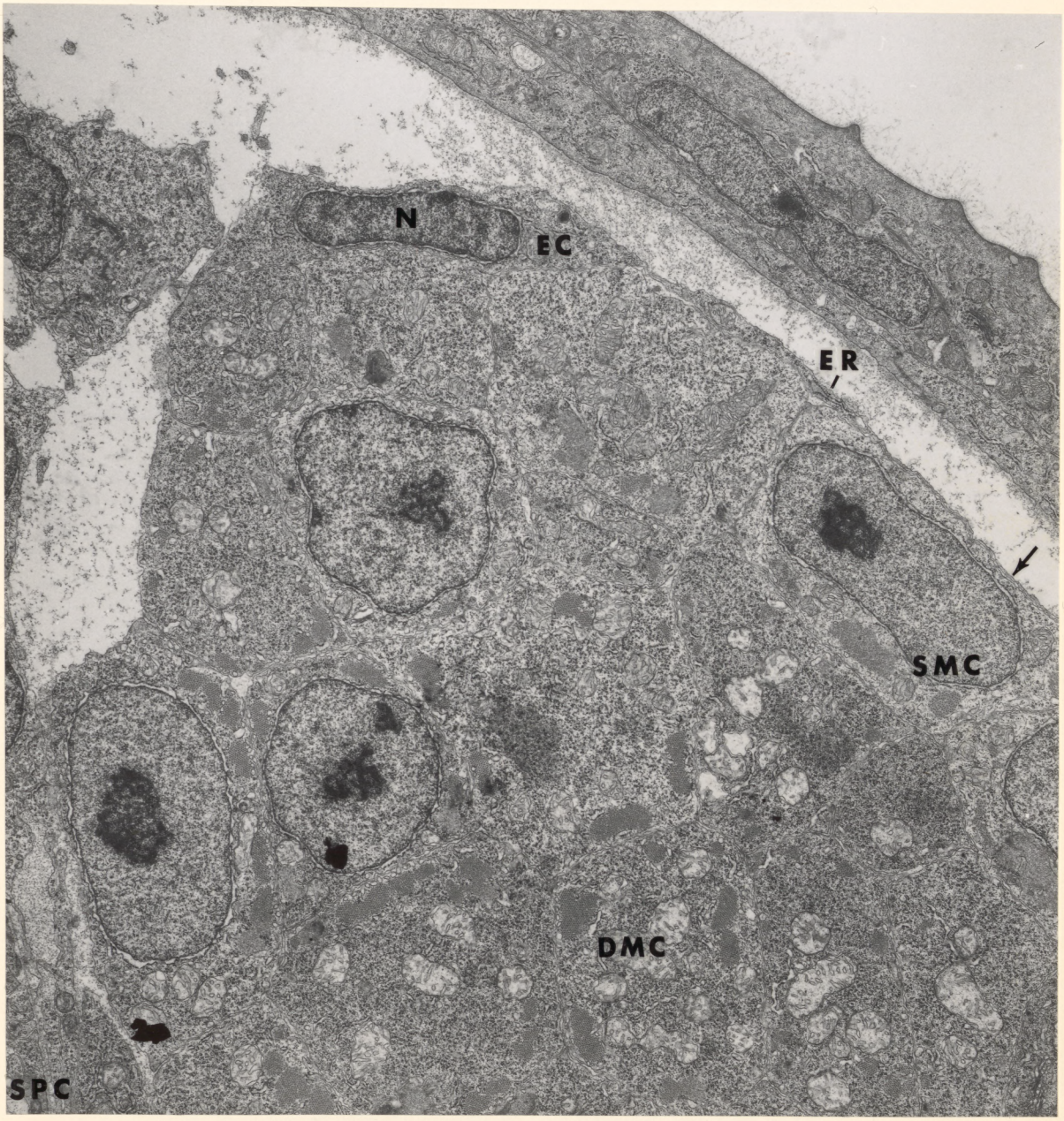

N
EC
ER
SMC
DMC
SPC

Figure 21. Portions of two superficial muscle cells in somite No. 18 from a 33-somite embryo. Each is characterized by a single large myofibrillar bundle (MFB) medially, and a single nucleus (N) centrally. Smooth surfaced membranes (SM), some rough endoplasmic reticulum (ER), and a few small myofibrils (MYF) are also present. Cisternae of the sarcoplasmic reticulum (SR) are associated with the myofibrillar bundles, which show an hexagonal arrangement of both thick and thin myofilaments. Overlapping external cell processes (EC) cover the lateral surface of the superficial muscle cells. Mitochondrial matrix density appears slightly more dense in the superficial than in the deep muscle cells. Small cell processes (arrows) are characteristic of both young superficial and deep muscle cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 18,700 X.

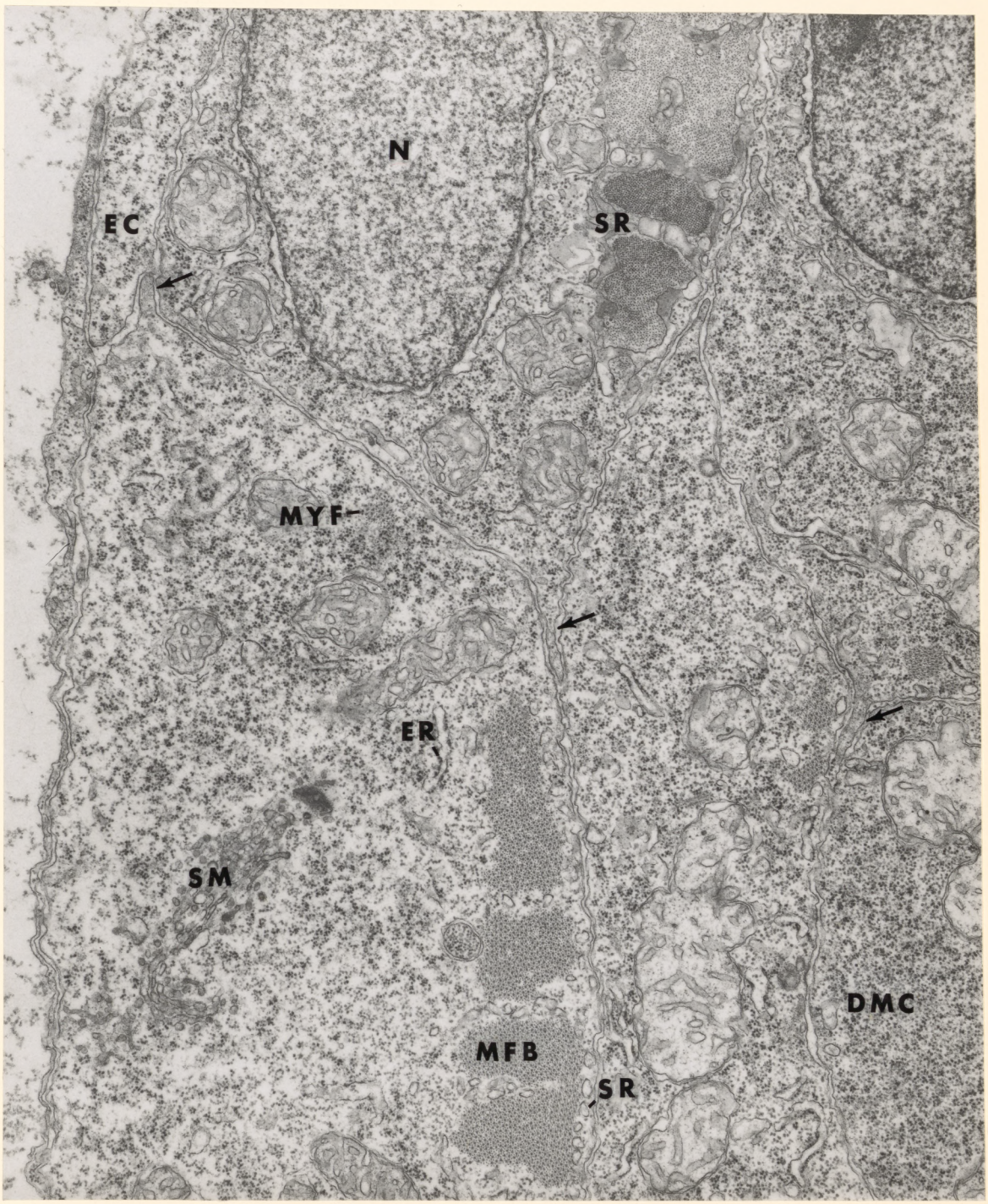

N
EC
SR
MYF
ER
SM
DMC
MFB
SR

Figure 22. Flattened superficial muscle cells (SMC) in the 33-somite embryo (somite No. 19) extend the entire width of the myotome at the midnotochordal level. Short external cell processes (arrows) extend medially between the lateral margins of these cells. No horizontal septum is yet present. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 7310 X.

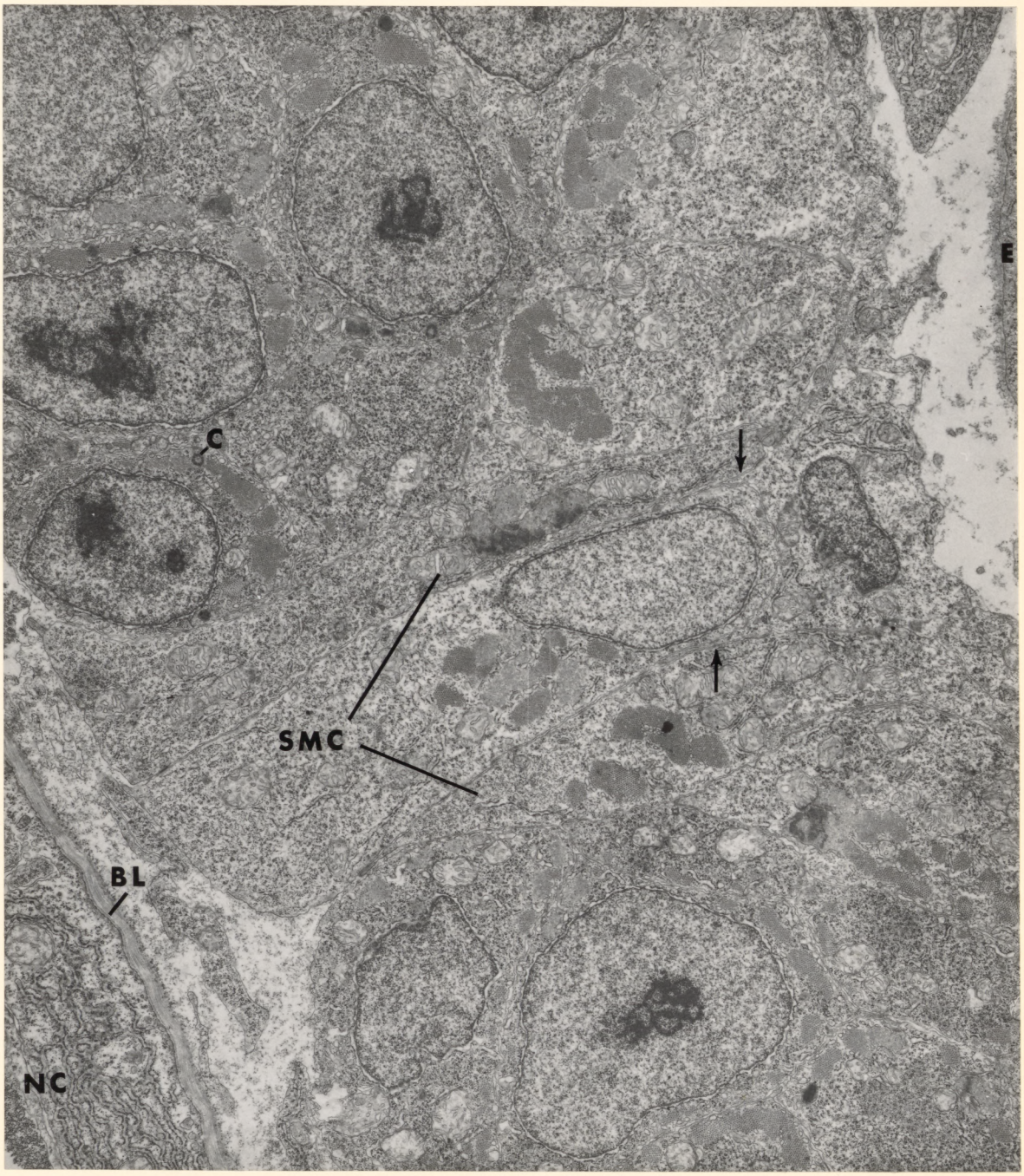

E
C
SMC
BL
NC

Figure 23. Cross-section of somite No. 18 from a 33-somite embryo illustrating the appearance of the myotome at the level of the spinal cord. Each deep muscle cell contains a central core of cytoplasm with nuclei and other organelles and a number of small peripheral myofibrils (MYF). The average cross-sectional area of the majority of the deep muscle cells is approximately equal to that of the superficial muscle cells (SMC). Smaller cells (arrows) are most frequently seen subjacent to the superficial muscle cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 7790 X.

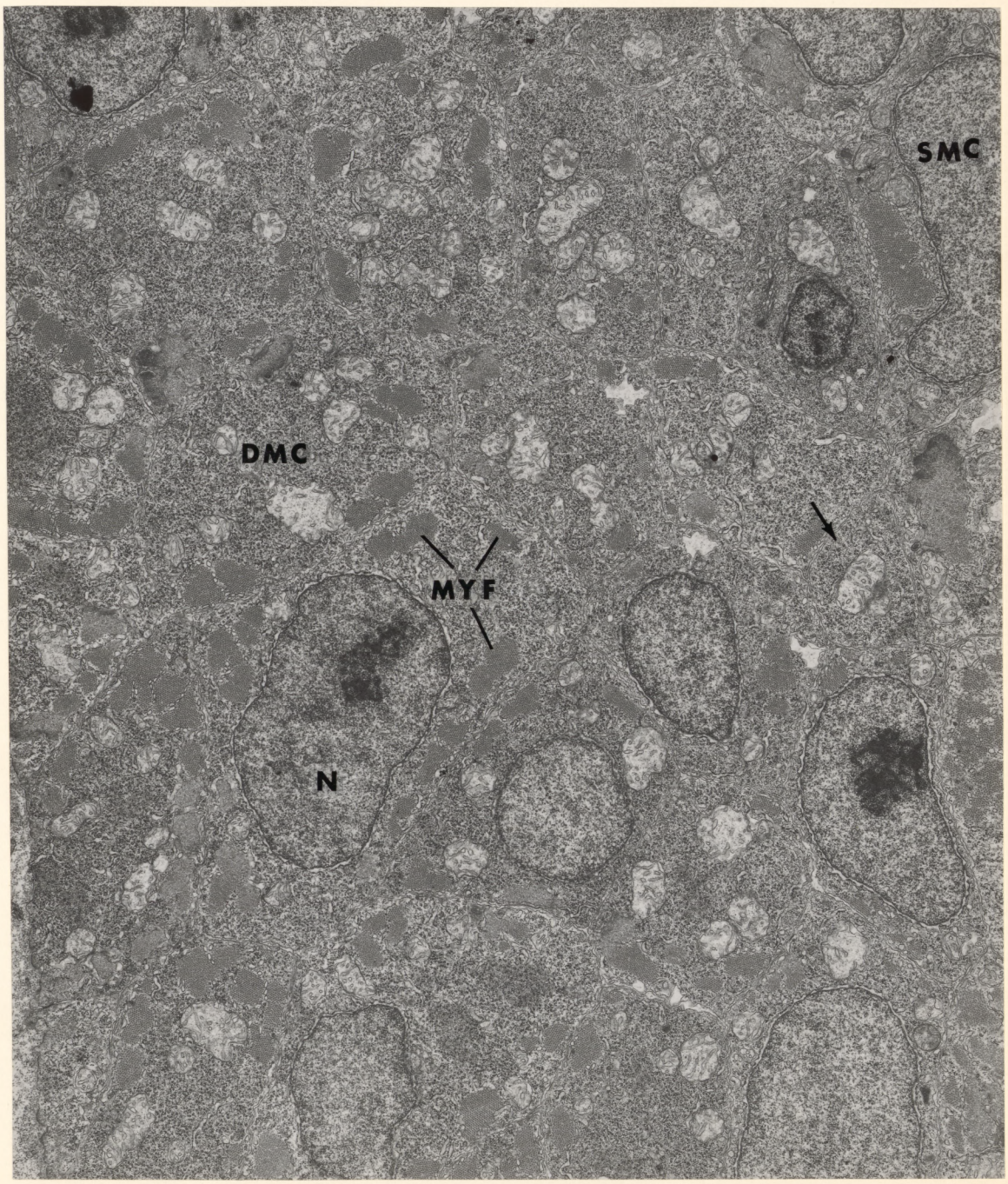

SMC
DMC
MYF
N

Figure 24. Horizontal section at the level of the spinal cord (SPC) showing the midportion of somite No. 19 from a 33-somite embryo. Myofibrils of all muscle cells exhibit distinct Z-, I-, A-, H-, and M-bands, and are associated with elements of the sarcoplasmic reticulum (SR). T-tubules occur at the level of each Z-band in well-developed myofibrils. The region between the double arrows is thought to represent obliquely sectioned cell membranes, and presumably does not represent cell fusion. A smaller cell (arrow) is visible subjacent to a superficial muscle cell (SMC). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 7040 X.

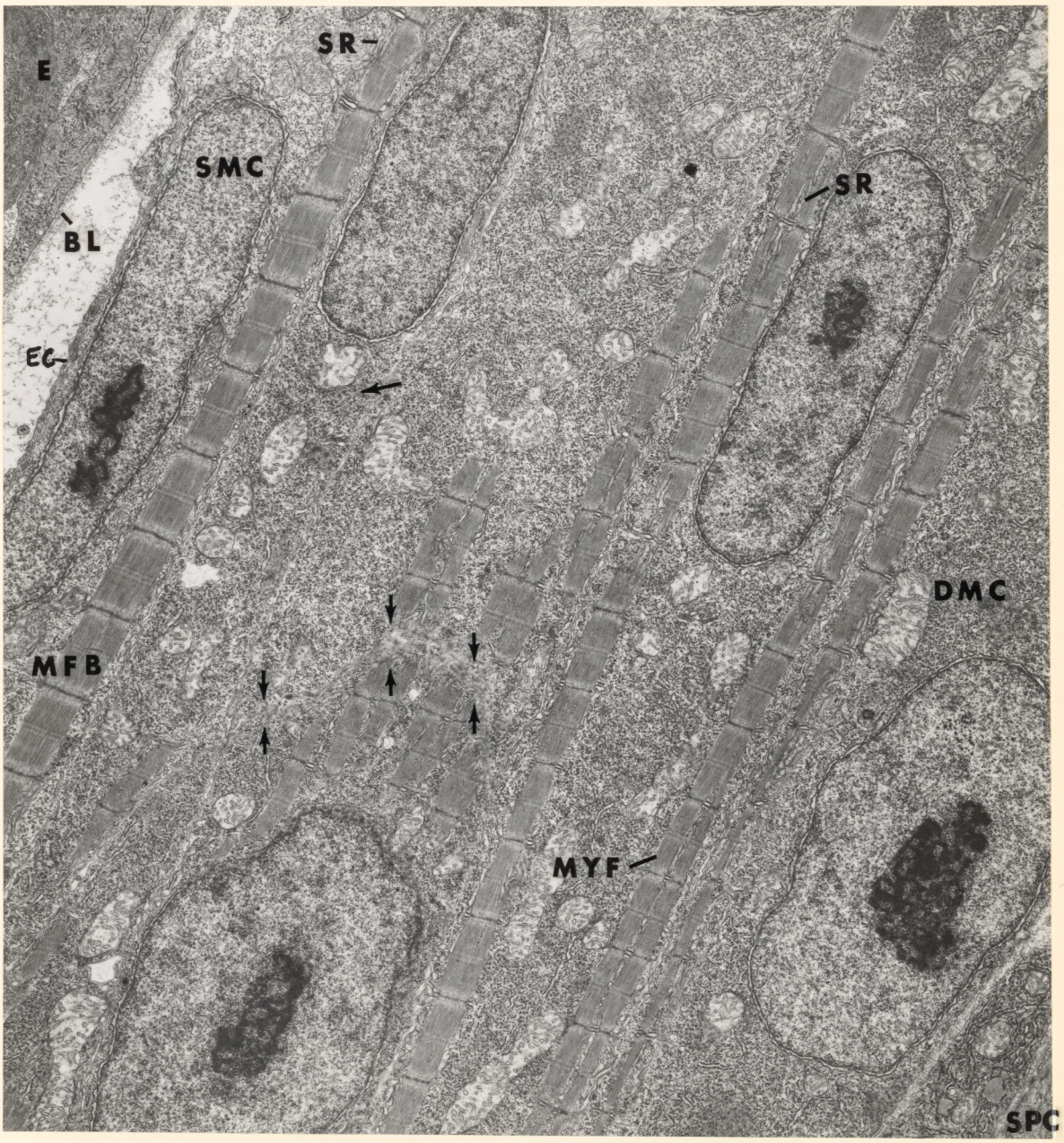
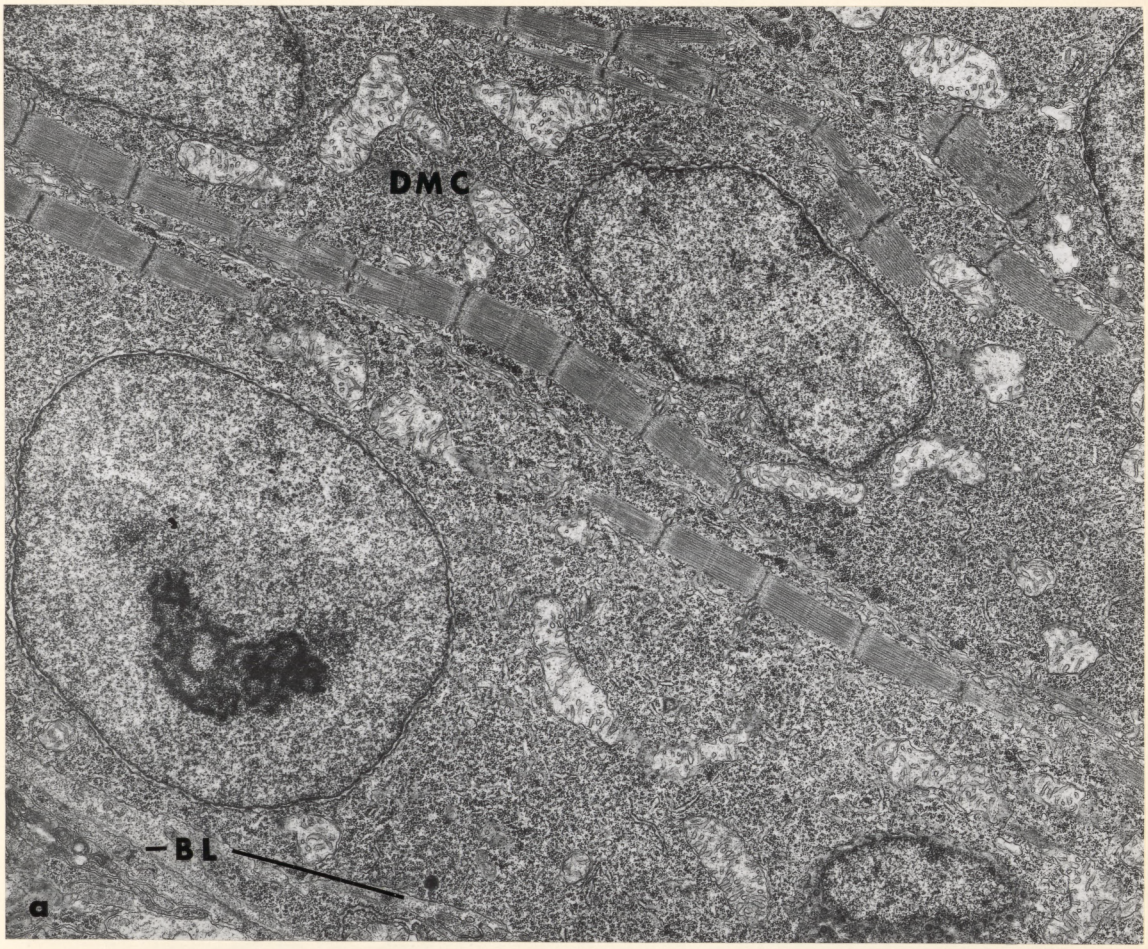

E
SR
SMC
SR
BL
EC
DMC
MFB
MYF
SPC

Figure 25. a) Horizontal section showing portions of two multinucleate deep muscle cells near the medial side of the myotome in a 33-somite embryo. The nuclei in the center cell represent 2 of 3 nuclei within this cell. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 8360 X.

b) Portion of a microtubule (MT) in a deep muscle cell of a 33-somite embryo. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 32,600 X.


DMC
BL
a

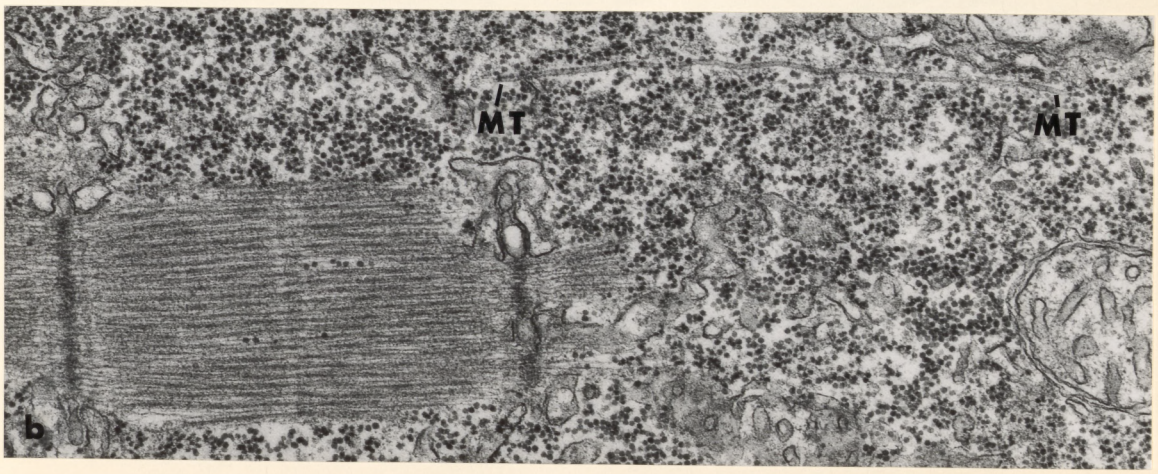

MT
MT
b

Figure 26. a-d) Horizontal sections of several deep muscle cells from a 33-somite embryo. They contain flagella (FL) and centrioles (C). The shafts of the flagella may be aligned either perpendicular or parallel to the long axis of the cell. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately: a-c) 16,000 X, d) 11,100 X.

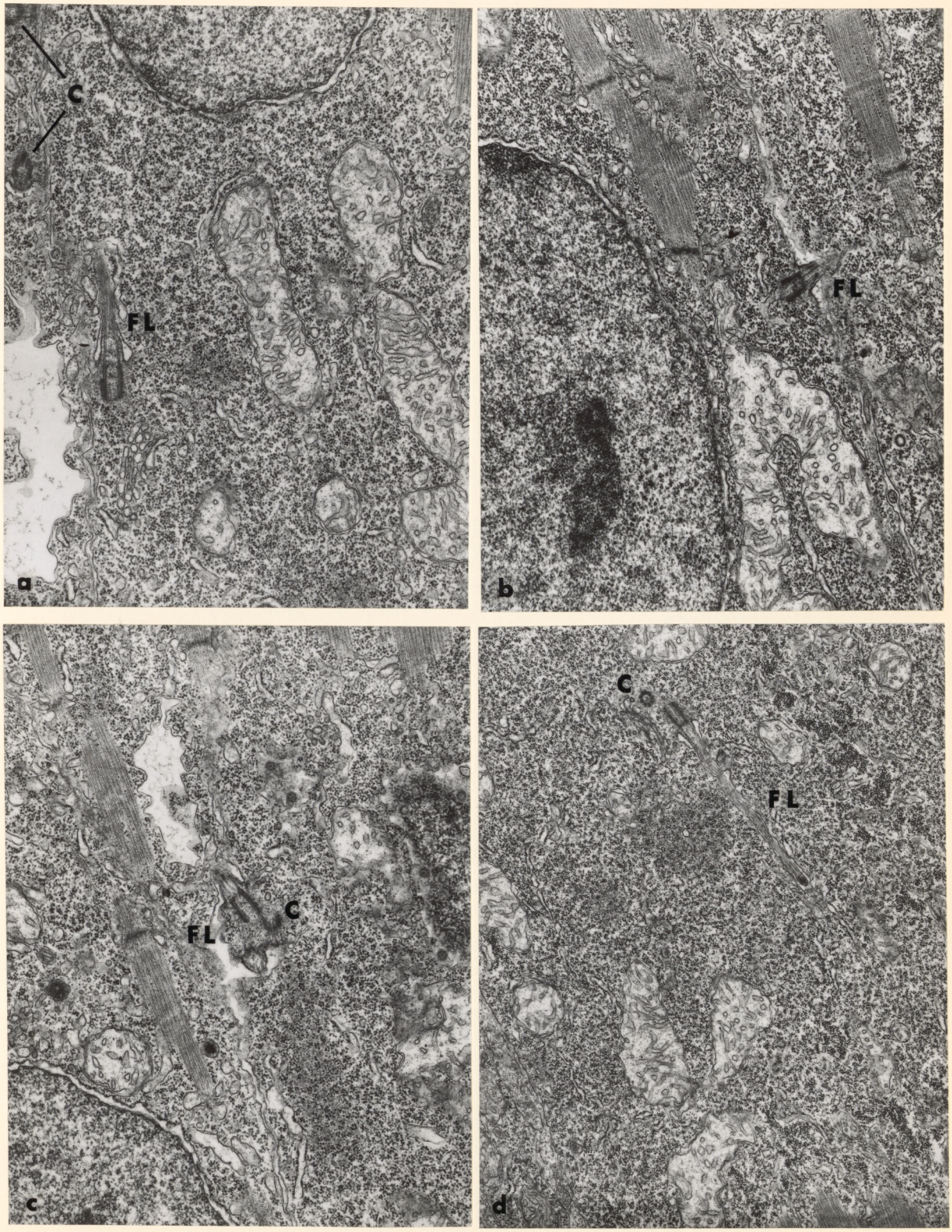
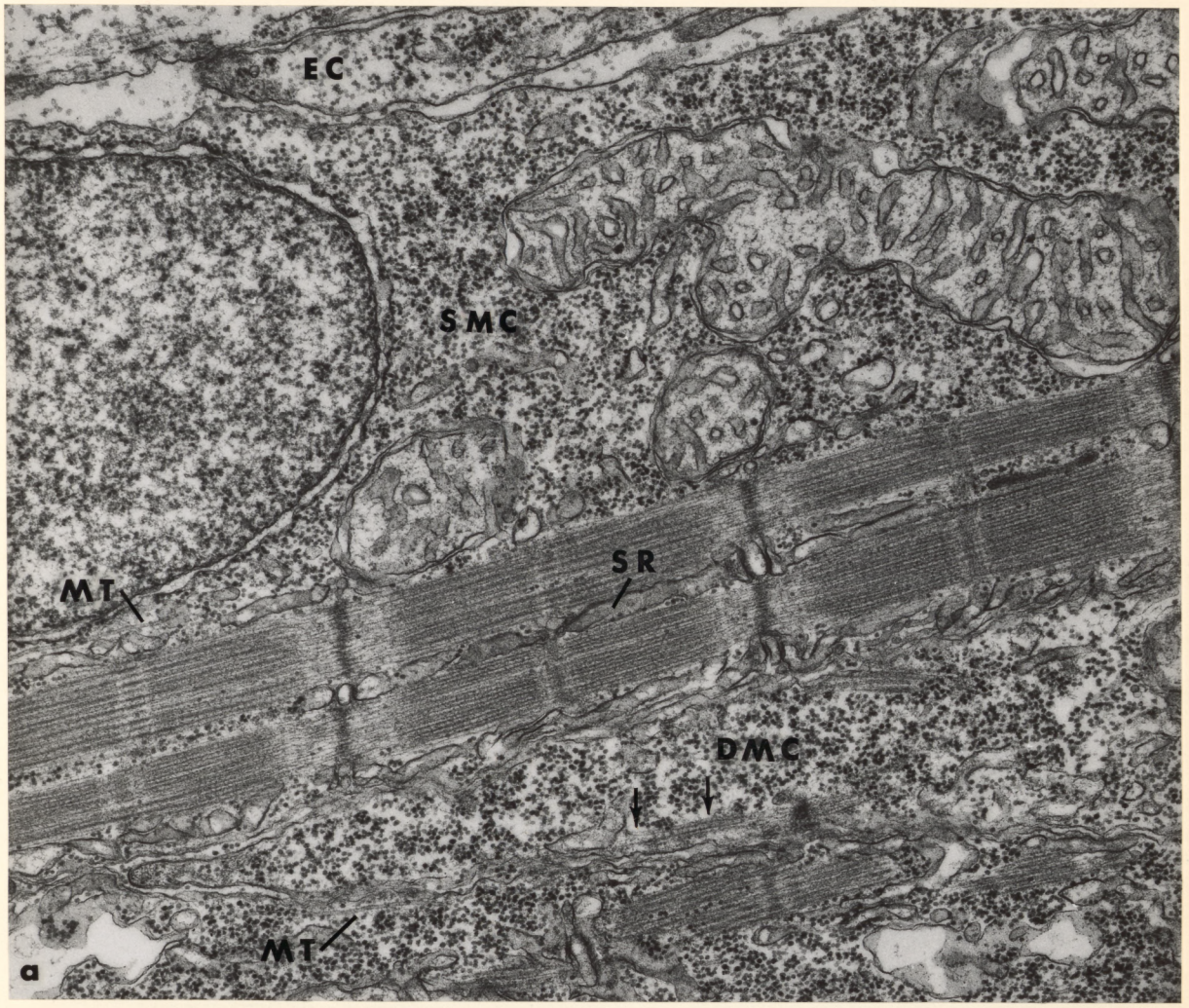

C
FL
a
FL
b
C
FL
c
C
FL
d

Figure 27. Comparison of myofibrils from superficial (a) and deep (b) muscle cells. Both micrographs are of horizontal sections from a 33-somite embryo. Myofibrils of both cell types have sarcomeres measuring 1.8 - 1.9 μ. No significant difference in the development of the sarcoplasmic reticulum (SR) or T-tubules of the two cell types is evident. Microtubules (MT) are present in both cell types.

a) Portions of a superficial muscle cell and two deep muscle cells are visible. An image which might represent a microtubule is seen in close proximity to myofilaments (arrows) in one of the deep muscle cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 29,400 X.

b) A portion of a myofibril in a deep muscle cell. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 29,400 X.


EC
SMC
SR
MT
DMC
MT
a


SR
T
b

Figure 28. Hotizontal section illustrating the developing myoseptal region (MS) between two midbody somites of a 33-somite embryo. Some of the cell processes within this region can be traced to muscle cells (arrows). Fibroblastic cells (FC) are commonly present at the medial margin of the developing myosepta, but processes of fibroblastic cells or of external cells (EC) are never seen entering the myoseptal region. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 9040 X.

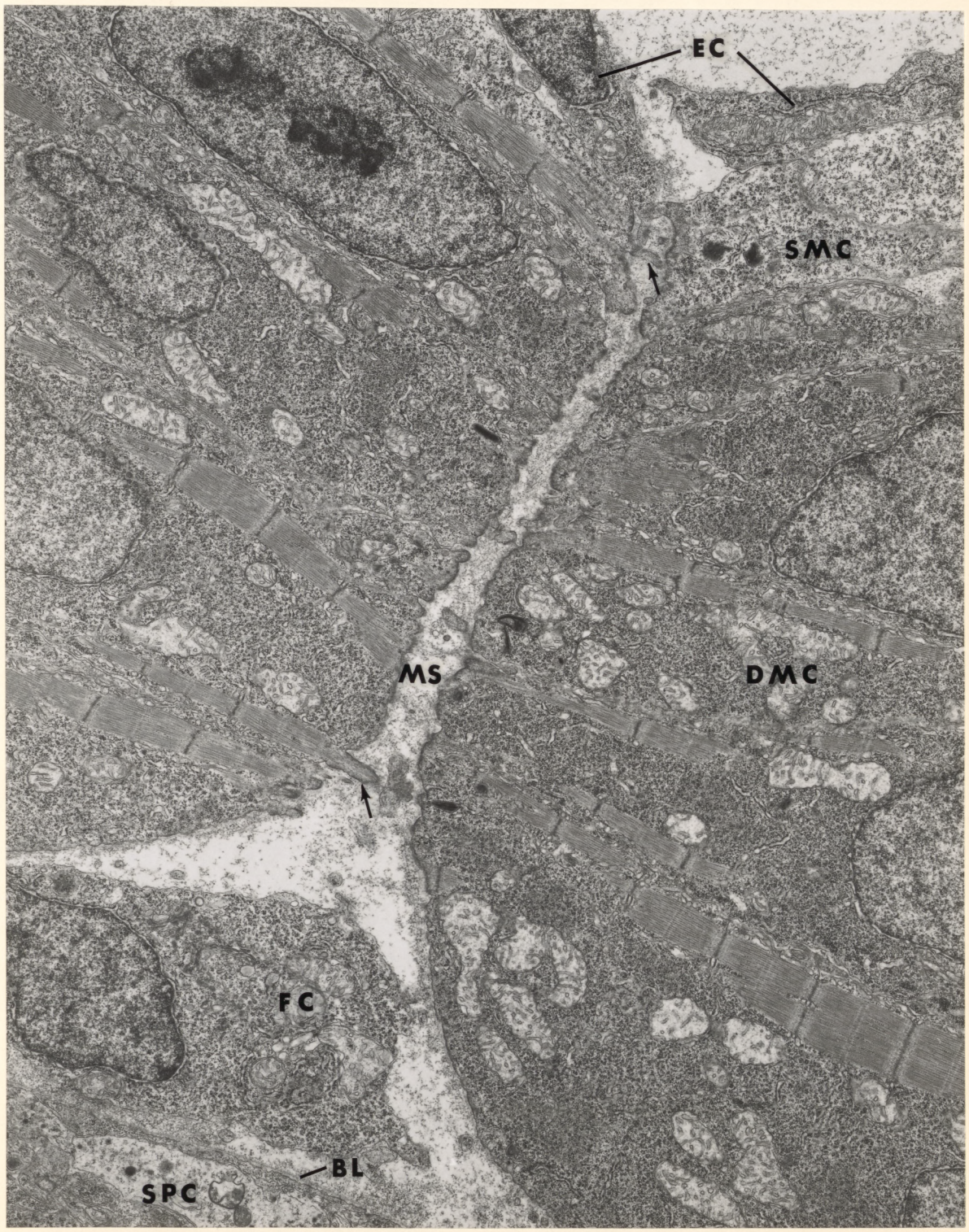

EC
SMC
MS
DMC
FC
BL
SPC

Figure 29. Developing myoseptal regions of 33-somite embryos shown in horizontal section.

a) A portion of the medial end of a developing myoseptal region containing some periodic collagen fibrils (COL) . A small cell process from a deep muscle cell extends into this region and is surrounded by external lamina material (BL). A focal intermediate junction (FIJ) is present between adjacent deep muscle cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 25,900 X.


MS
BL
COL
DMC
FIJ
FC
a

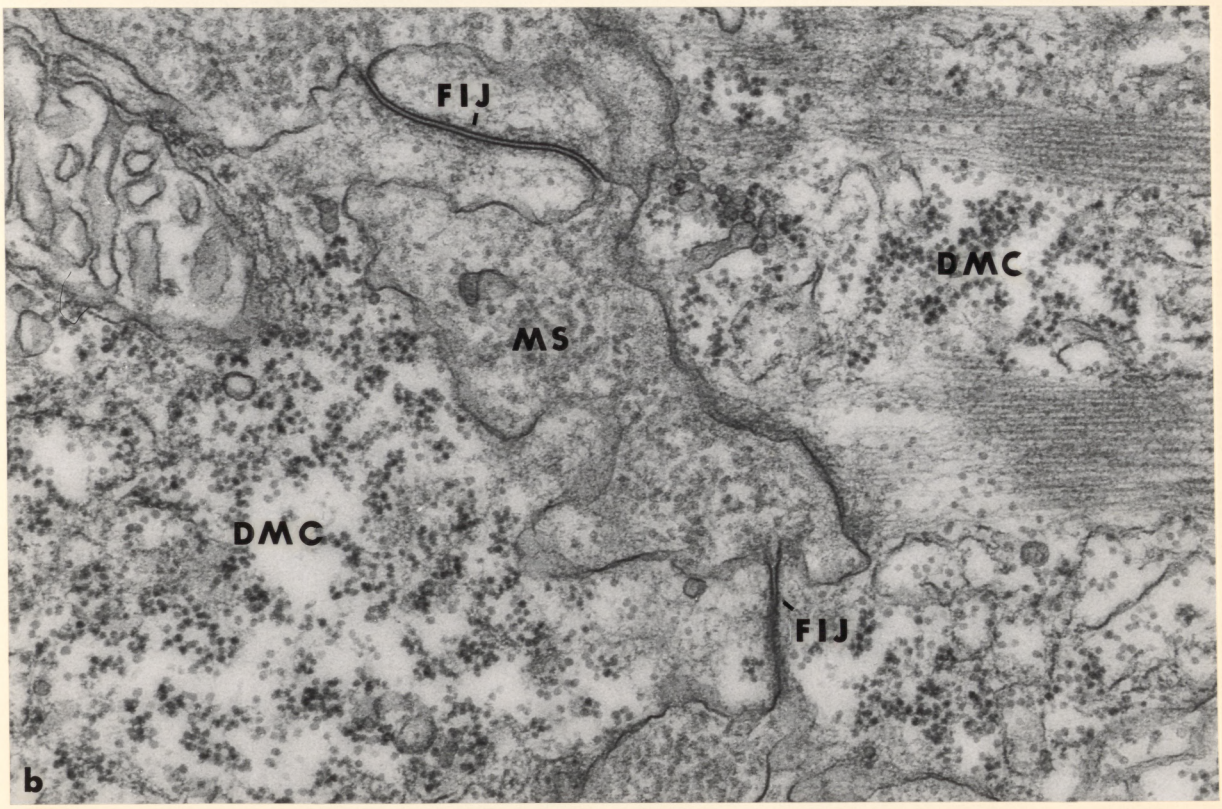
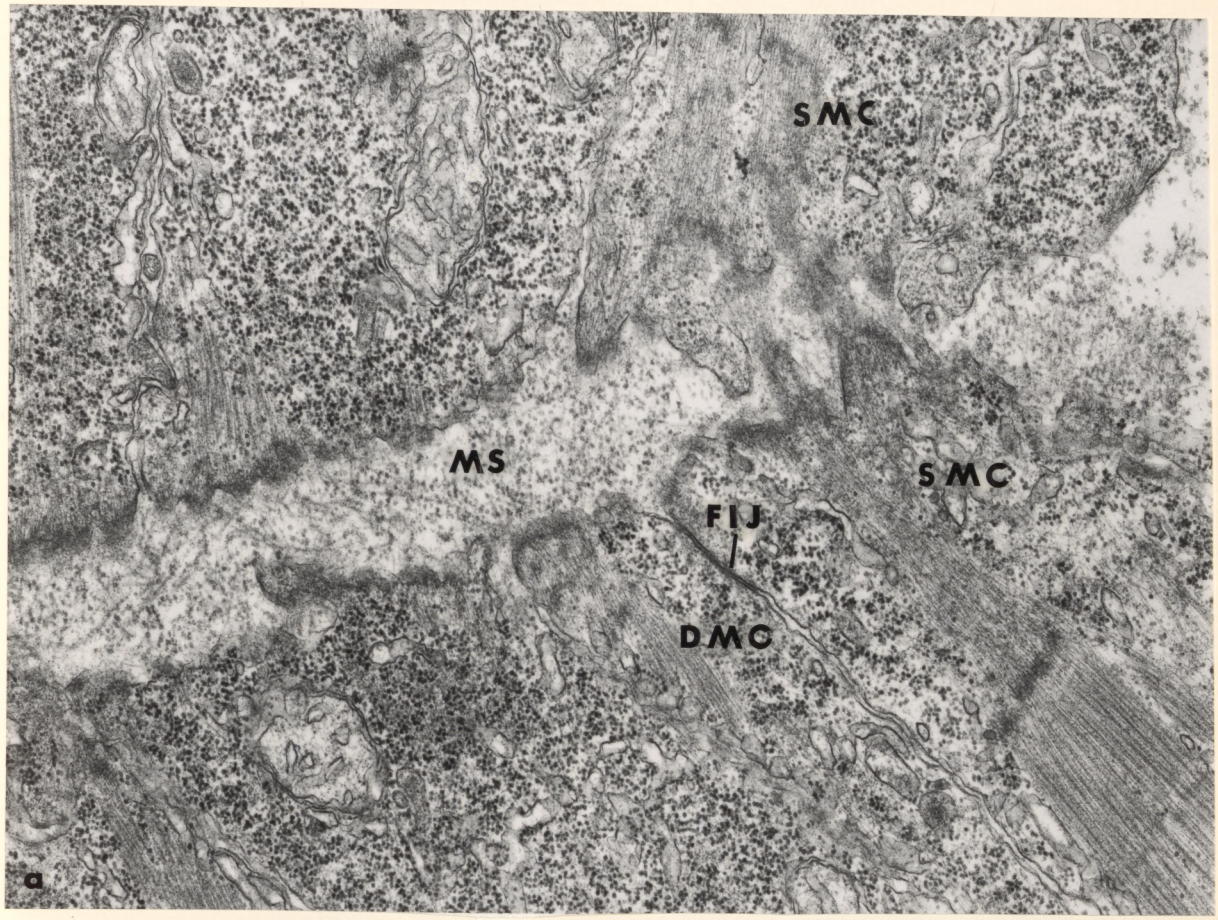

FIJ
DMC
MS
DMC
FIJ
b

Figure 30. a) Lateral end of a developing myoseptal region in a 33-somite embryo. Tongues of superficial muscle cells (SMC) extend into this region, and a focal intermediate junction (FIJ) is seen between a superficial and a deep muscle cell (DMC). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 25,900 X.

b) Neuronal processes (A) seen in the midbody somites of a 33-somite embryo are located medial to the anterior ends of superficial muscle cells near the developing myoseptum (MS). These nerve processes contain numerous (synaptic?) vesicles (VL), small mitochondria (M), and have cytoplasm of low density. They are not surrounded by Schwann cells. Osmium-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 25,300 X.


SMC
MS
SMC
FIJ
DMC
a

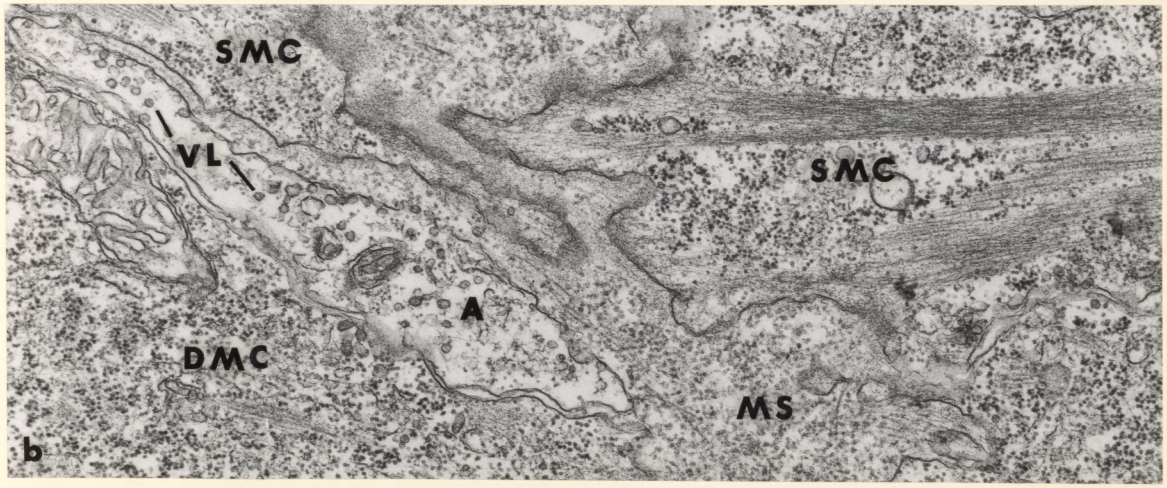

SMC
VL
SMC
A
DMC
MS
b

Figure 31. Thick 1 μ sections of zebrafish larvae 3-4 days post-hatching. Glutaraldehyde-formaldehyde fixation. Stained with methylene blue-azure II.

a) Cross-section of somites Nos. 18 and 19. The myotome is composed of a single layer of superficial muscle cells (SMC) and several layers of deep muscle cells (DMC). The deep muscle cells are significantly larger than the superficial muscle cells at this stage. External cells are difficult to discern in histological preparations. Melanophores (P) and numerous vacuolated cells (VC) are seen beneath the epidermis (E). Well developed myosepta (MS) separate adjacent somites, and a horizontal septum (HS) no divides each myotome into dorsal and ventral portions. Magnification approximately 430 X.

b) Horizontal section of an entire larva. The section plane passes through the spinal cord (SPC) posteriorly and the notochord (NC) anteriorly. Dorsal and ventral to the horizontal septum, the myosepta pass anteriorly and medially from the skin to the axial structures, while at the level of the horizontal septum, they are nearly perpendicular to the body axis (arrow). Magnification approximately 36 X.

c) Horizontal section of the midbody somites at the level of the spinal cord. Superficial muscle cells (SMC) are oriented parallel to the body axis, whereas the deep muscle cells (DMC) course obliquely to it. Magnification approximately 48 X.

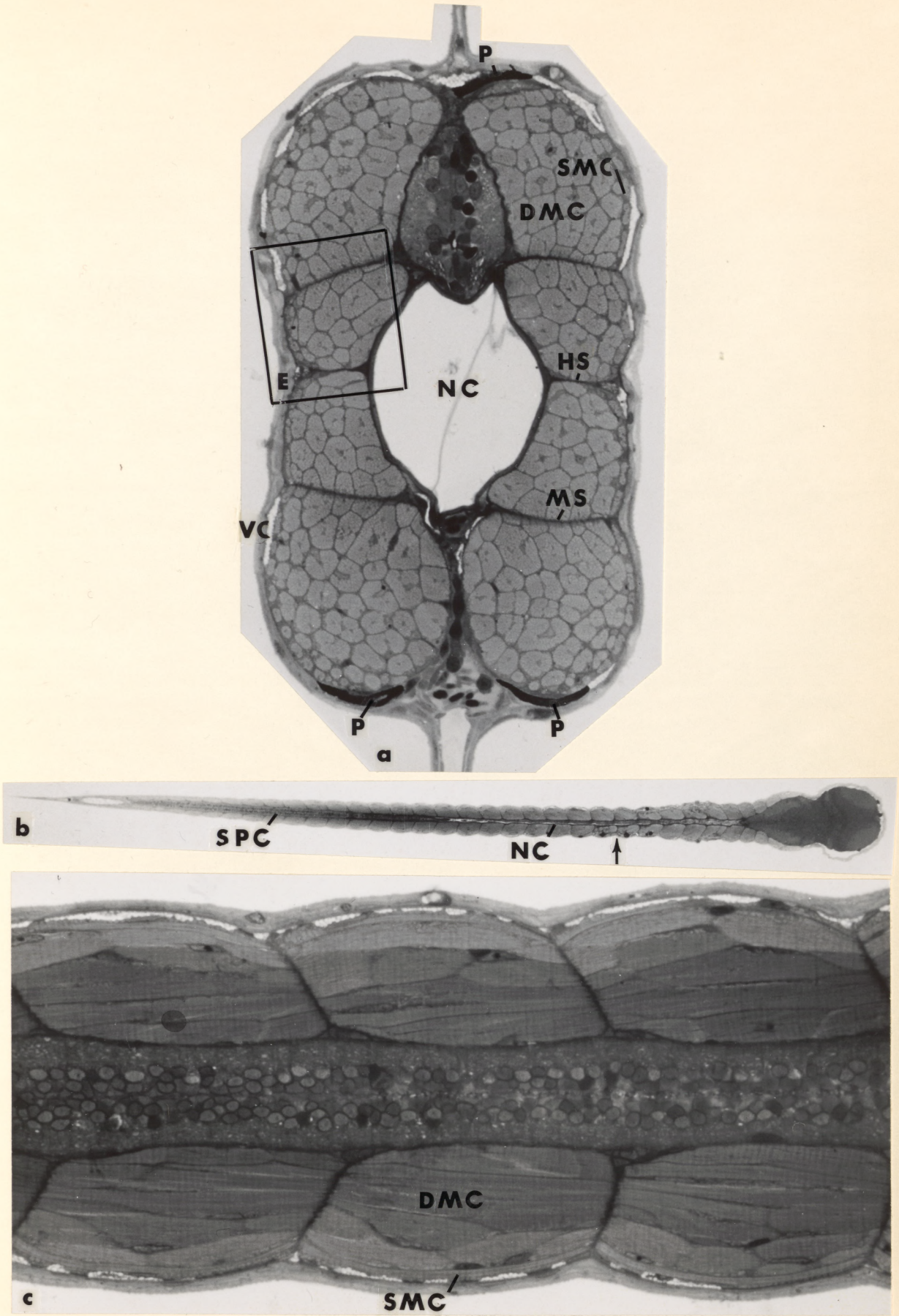

P
SMC
DMC
E
NC
HS
MS
VC
P
P
a
b
SPC
NC
DMC
SMC
c

Figure 32. Electron micrograph of an area similar to that enclosed by the rectangle in Figure 31a, illustrating the general organization of the myotome. Neuronal profiles (A) are present throughout the lateral musculature. Dark cells (DC) with dense nuclei and cytoplasm, and small cells (arrows) are occasionally located subjacent to the superficial muscle cells. The myosepta (MS) and horizontal septum (HS) are well developed. No capillaries are visible in the lateral musculature at this stage. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 3580 X.

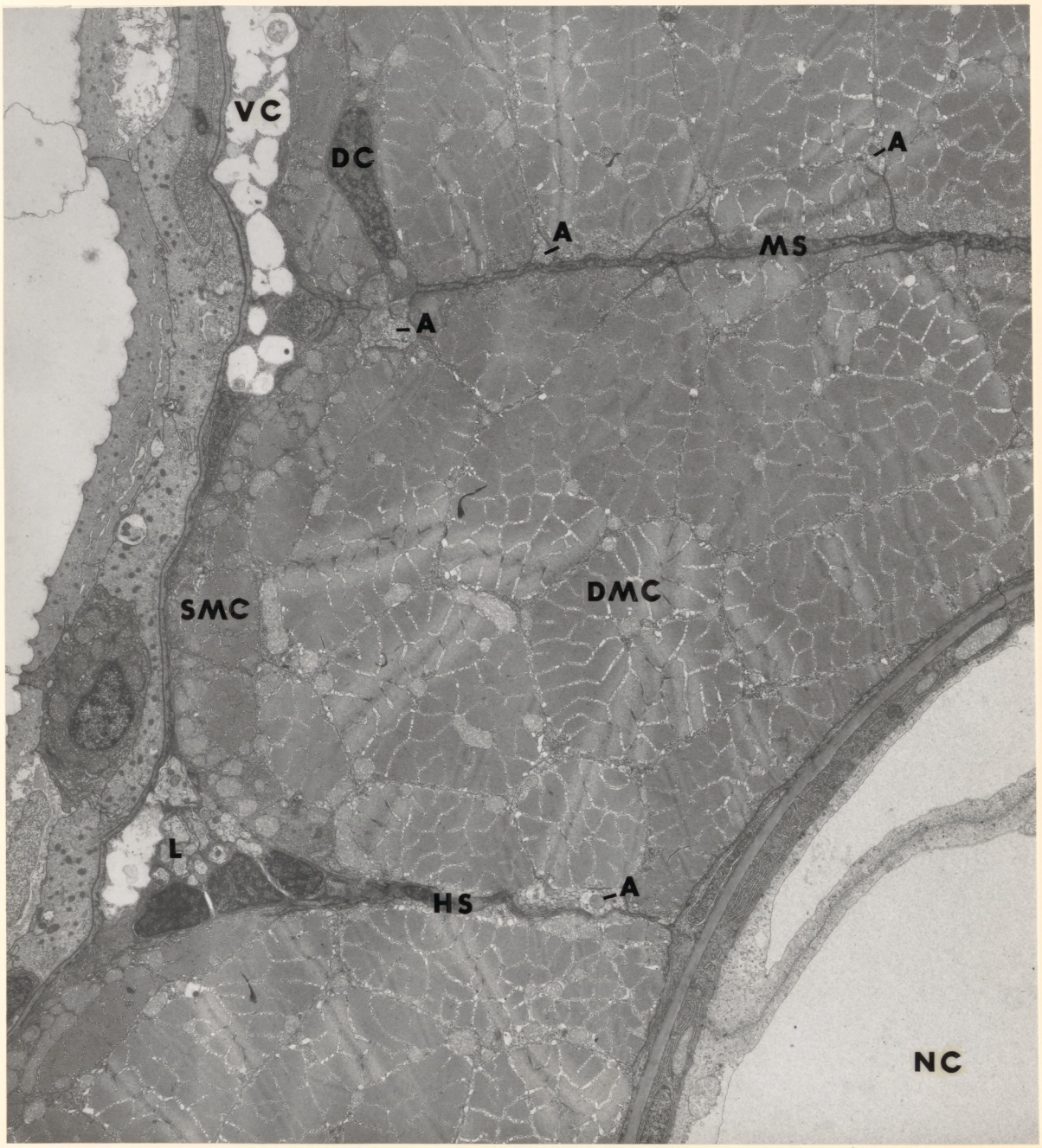

VC
DC
A
A
MS
A
A
SMC
DMC
L
HS
A
NC

Figure 33. Cross-section of the ventral portion of somite No. 17 from a larva. Flattened external cells (EC) are still present between the superficial muscle cells (SMC) and the epidermal basement membrane. The latter is composed of the basal lamina of the epidermis (BL) and several layers of dermal collagen (COL). The mitochondrial matrix density is greater in the superficial than in the deep muscle cells, and the myofibrils (MYF) of the superficial muscle cells appear smaller and more irregular in cross-section than the ribbon-shaped myofibrils (RF) in the deep muscle cells. Glutaraldehyde-formaldehyde fixation. Staining with uranyl acetate and lead. Magnification approximately 11,200 X.

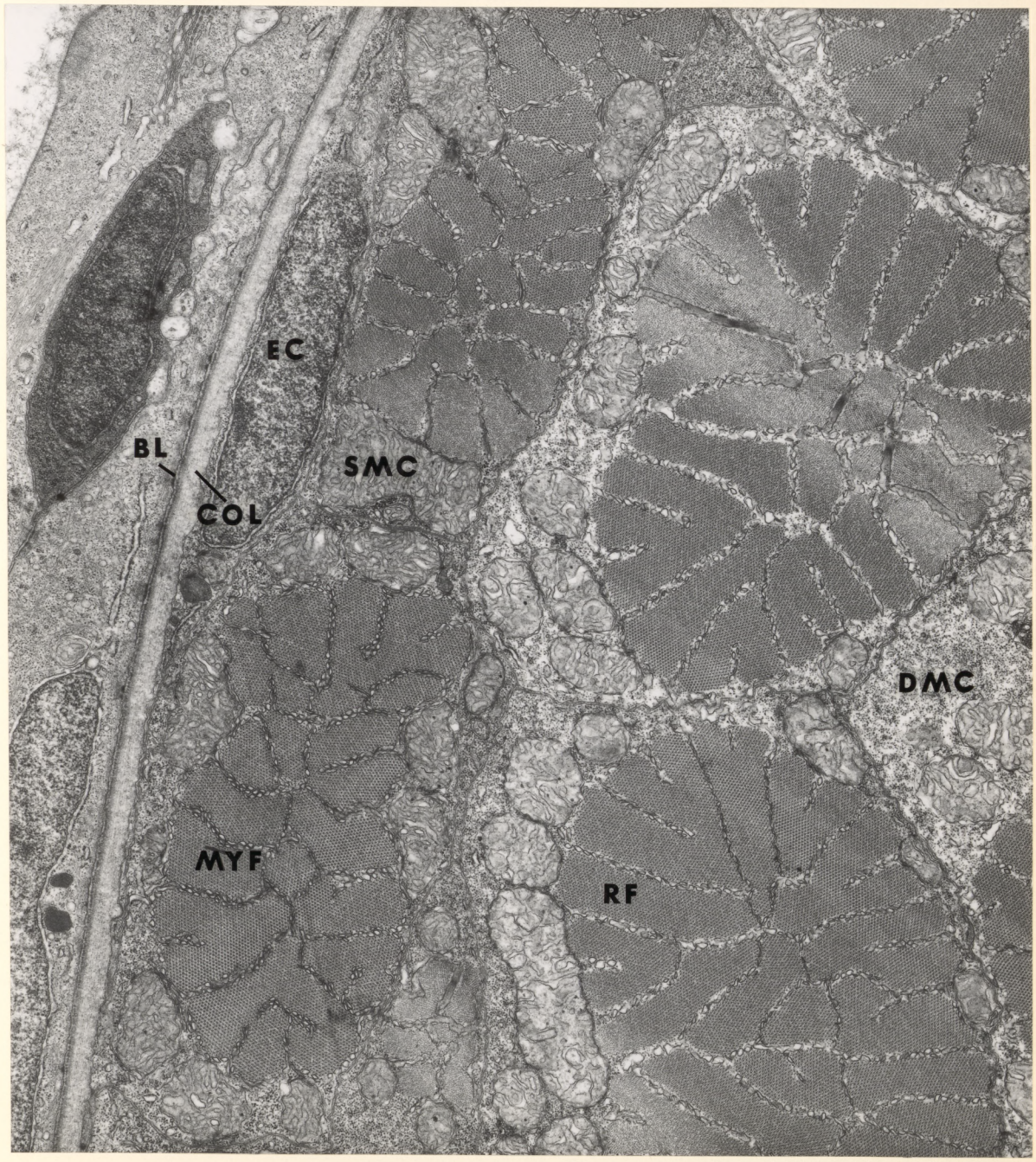

EC
BL
COL
SMC
DMC
MYF
RF

Figure 34. Larval superficial muscle cells are shown in cross-section.

a) One of 5 serial sections showing a portion of a superficial muscle cell containing a centriole (C). Gaps are frequent between adjacent external cell processes (EC) at this stage. Electron dense material (arrows) is often found between the epidermal basement membrane (BM) and the cell membranes of the external cells and superficial muscle cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 25,600 X.

b) Centrioles (C) are also found in external cells. Patches of electron dense material (arrow) are visible in some regions between the external cell processes and superficial muscle cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 25,600 X.

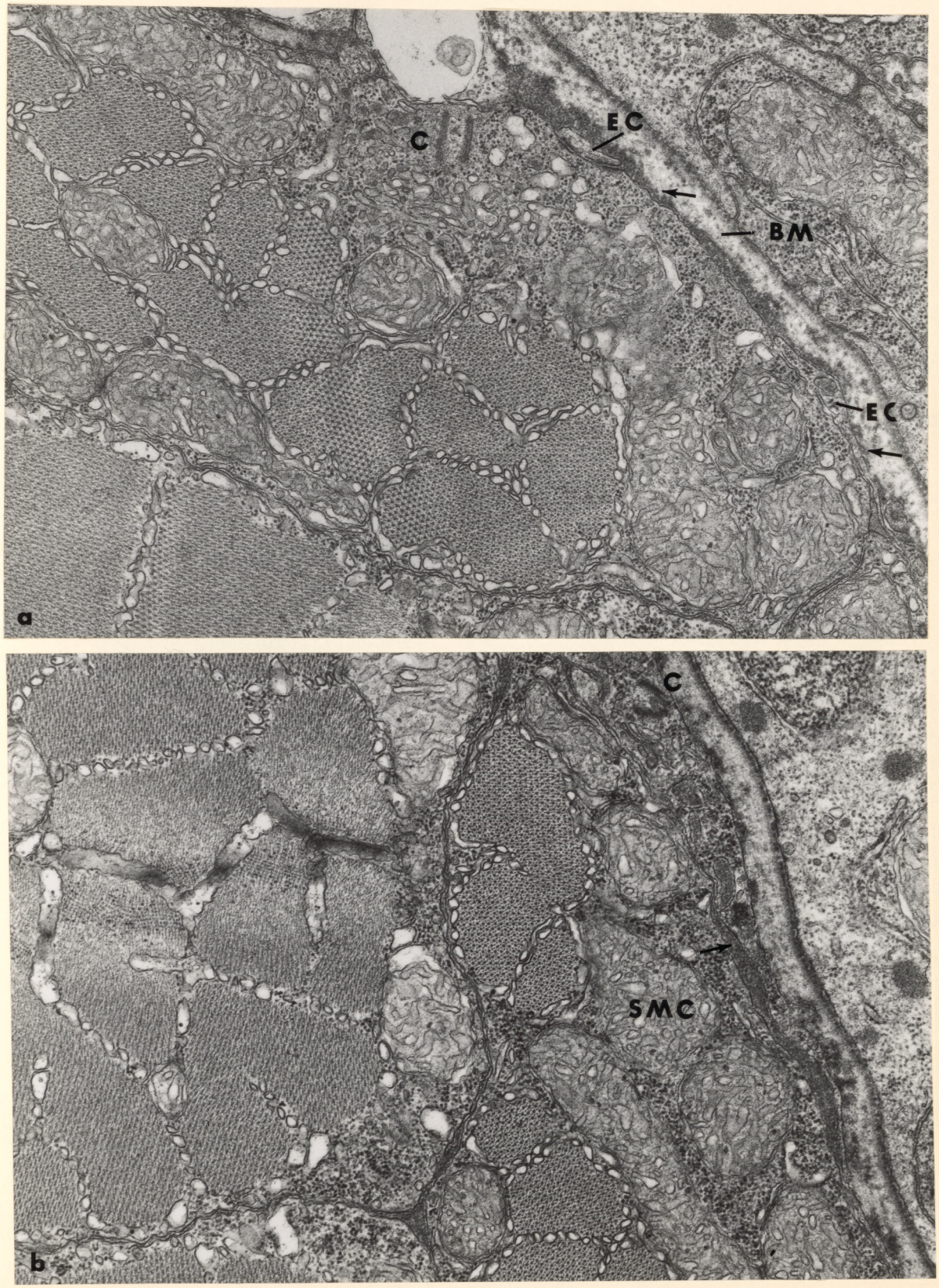

C
EC
BM
EC
a
C
SMC
b

Figure 35. Cross-section of somite No. 18 (larval stage) illustrating a difference in the arrangement of the sarcoplasmic reticulum (SR) in the superficial muscle cells (SMC) as compared with that in the deep muscle cells (DMC). The longitudinal cisternae in the superficial muscle cells are rather uniform in shape, and are frequently arranged in double rows (arrows) between adjacent myofibrils at the A-band level. Those of the deep muscle cells vary greatly in size and shape, and are more often aligned in a single row between fibrils (arrow pair). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 13,100 X.

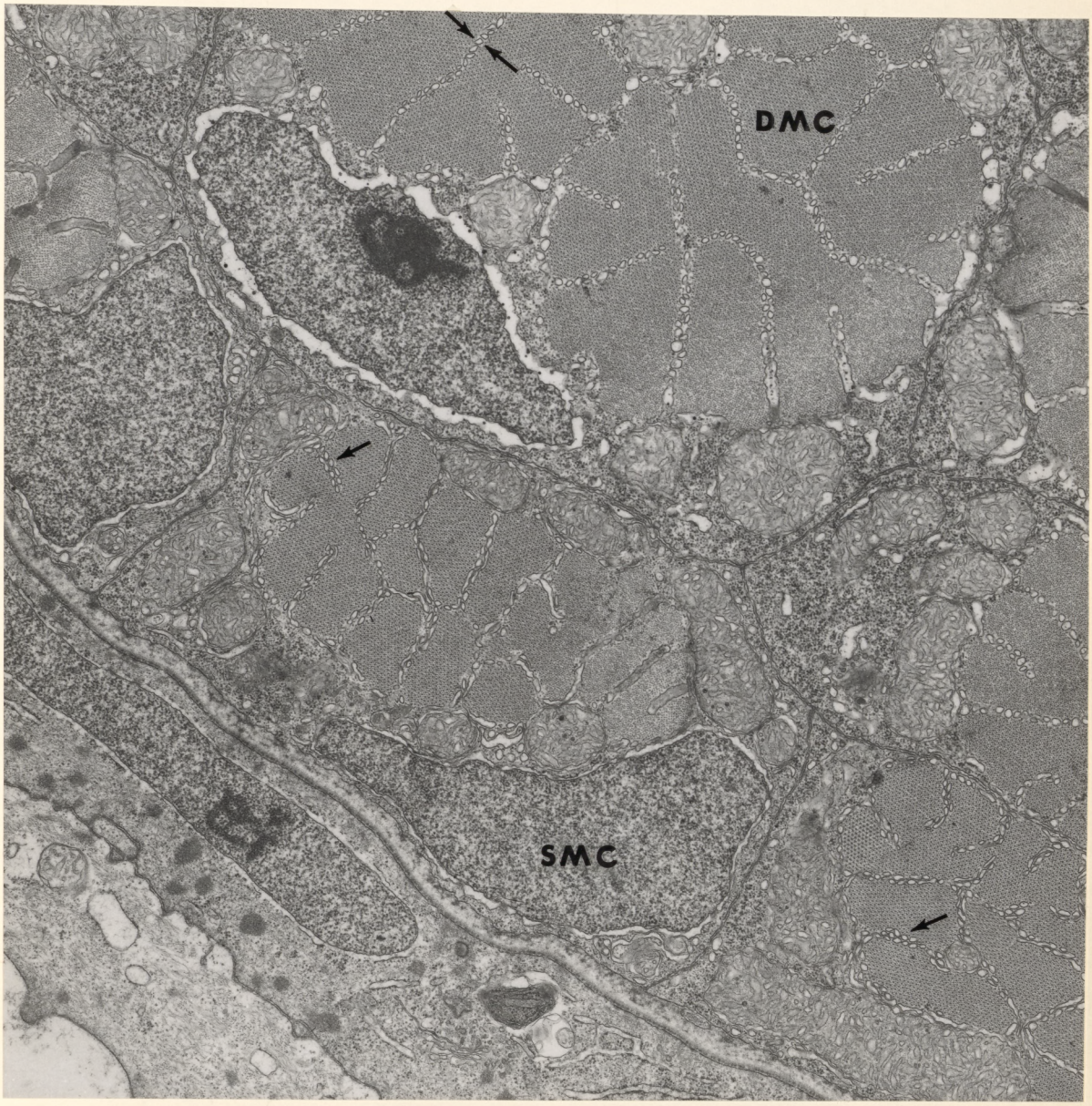

DMC
SMC

Figure 36. Portions of several deep muscle cells from somite No. 19 of a larva. They contain small ribbon-shaped myofibrils (RF) which almost completely fill the cell, forcing the cytoplasm and most of the organelles toward the periphery. Nuclei (N) are found in various positions within the cells. Patches of electron-dense material (arrows) are common between adjacent muscle cells, but no continuous layer of such material is present around individual cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 13,100 X.

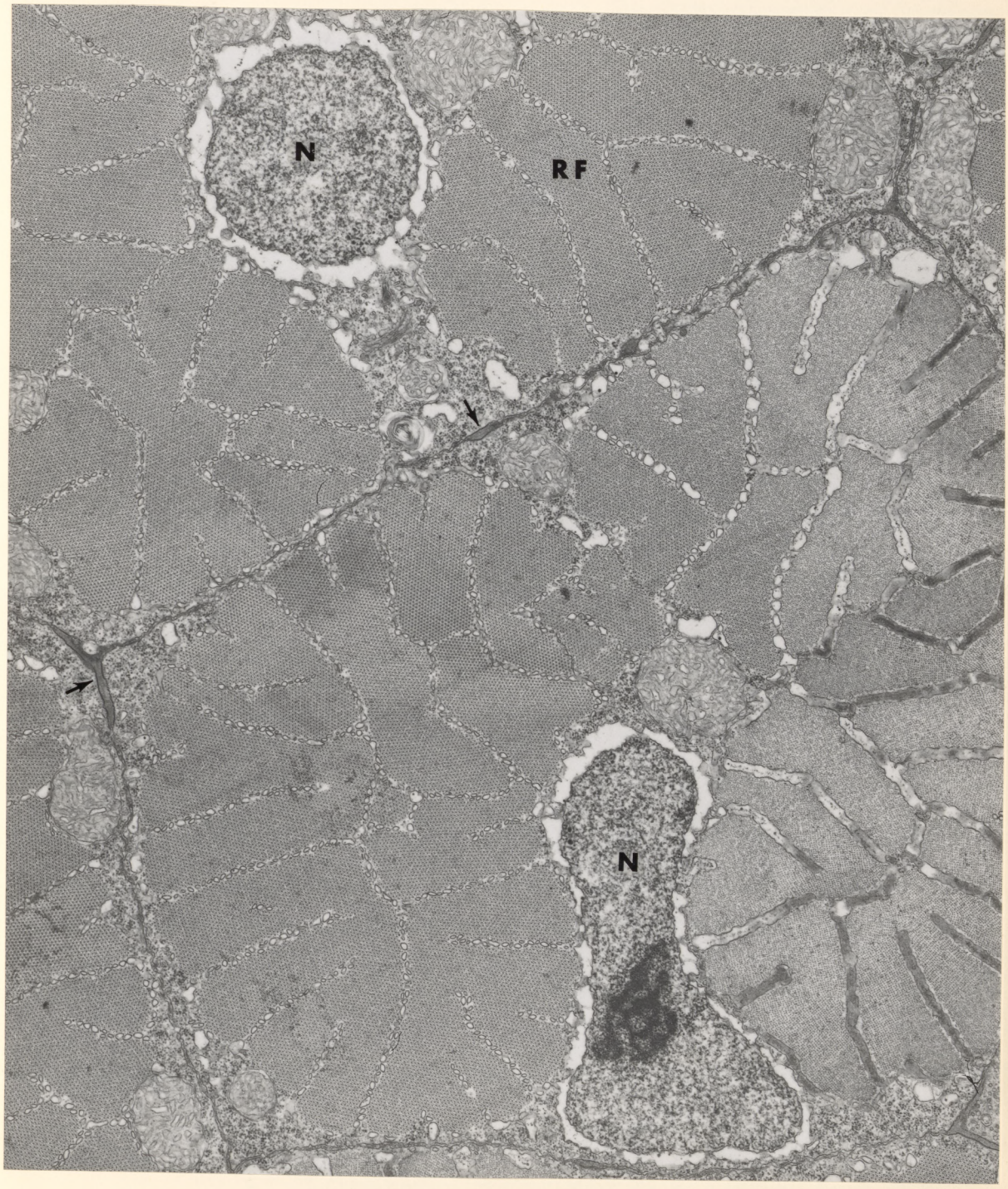
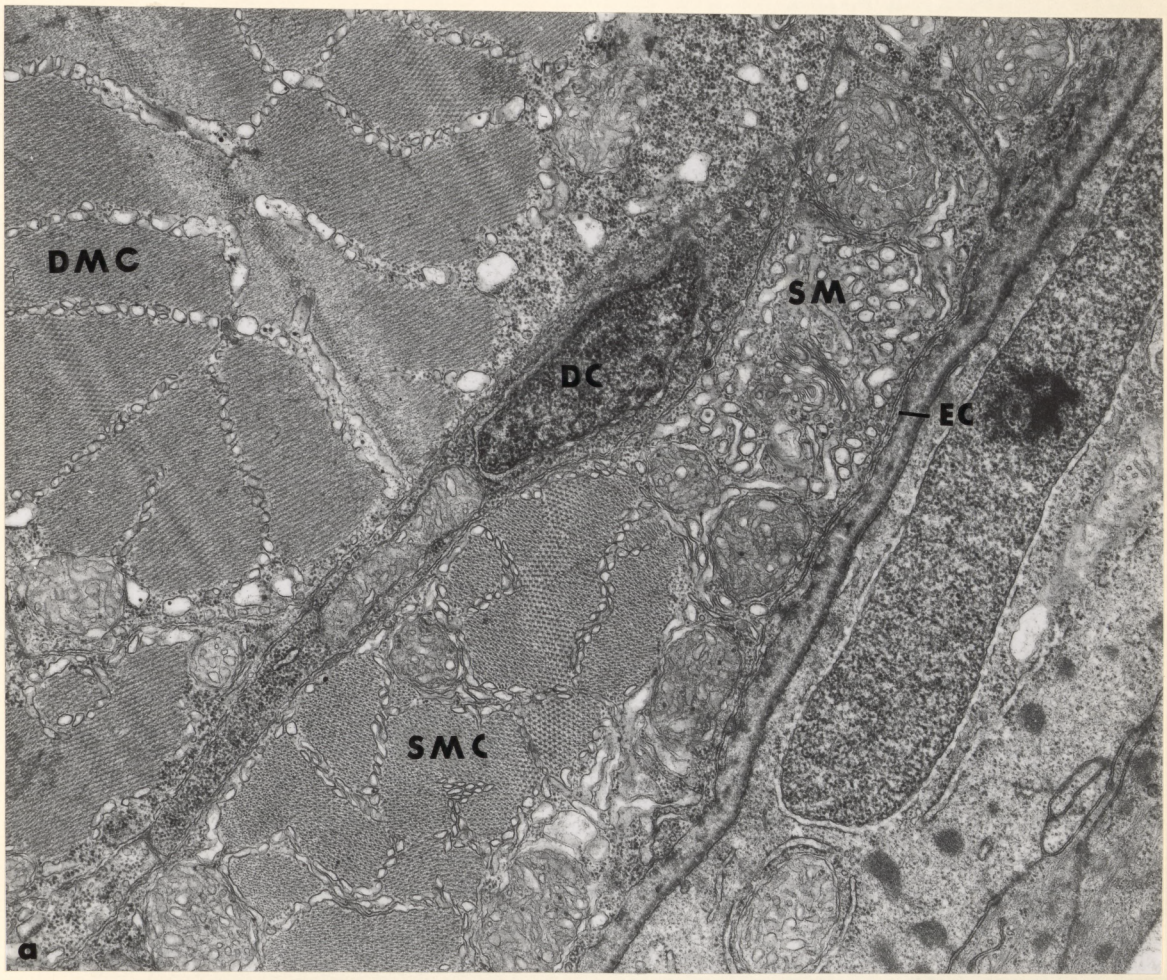

N
RF
N

Figure 37. Cross-sections of somite No. 18 from a larva.

a) Accumulations of smooth surfaced membranes (SM) are more frequently observed in superficial muscle cells than in the deep muscle cells. A dark cell (DC) is situated subjacent to a superficial muscle cell. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 16,700 X.

b) Dark cells have tentatively been identified as muscle cells based on this single observation of myofilaments (MF) within such a cell. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 34,600 X.


DMC
SM
DC
EC
SMC
a


MF
DC
MF
b

Figure 38. Horizontal section from a larva. Confluence of the longitudinal cisternae of the sarcoplasmic reticulum (SR) is seen over the H-band region in a superficial muscle cell. Terminal sacs (TS) are adjacent to the T-tubule (T). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 30,800 X.

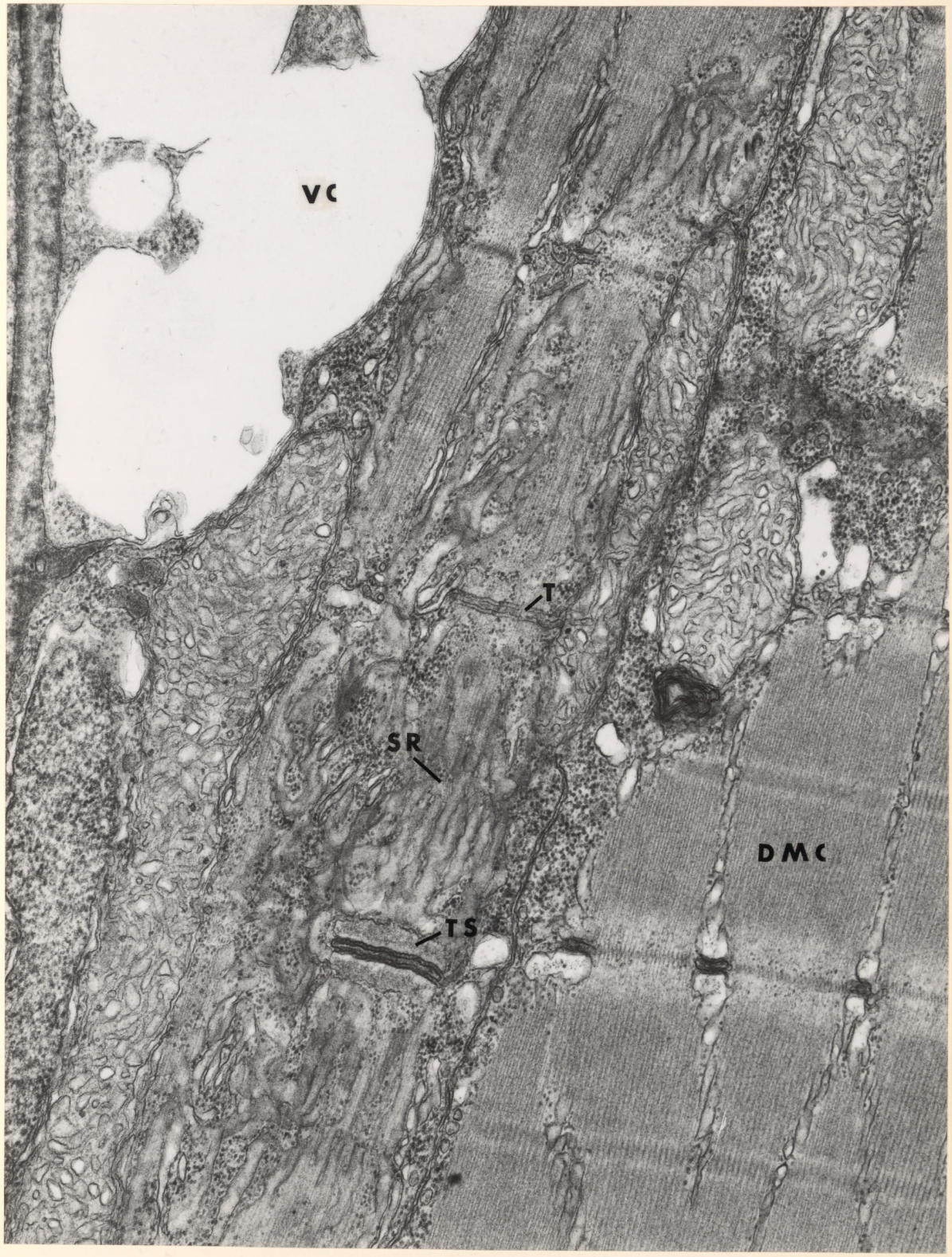
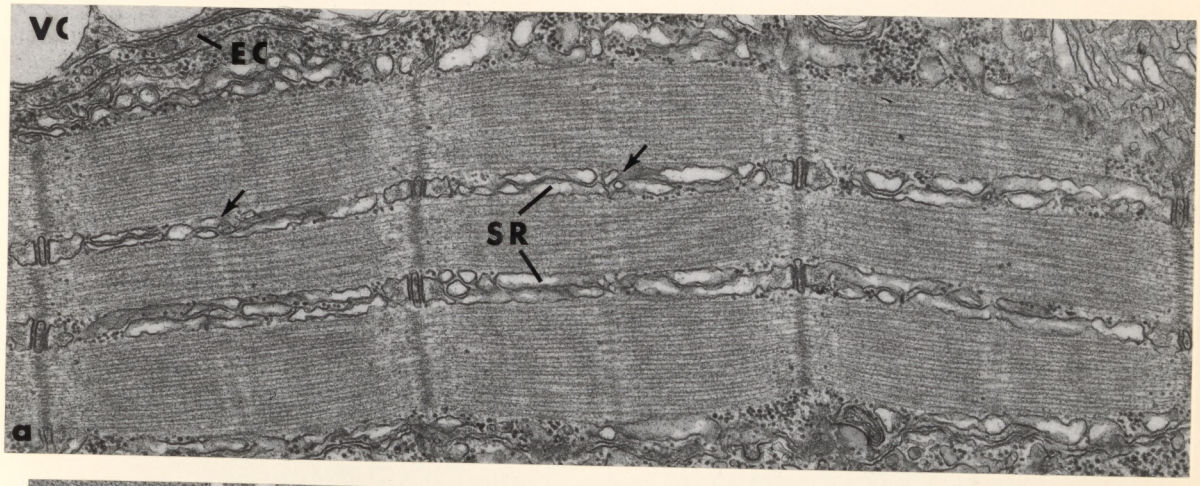

VC
T
SR
DMC
TS

Figure 39. a) Horizontal section containing a portion of the myofibrillar bundle in a superficial muscle cell of a larva. Transverse anastomoses of the numerous longitudinal cisternae of the sarcoplasmic reticulum (SR) over the H-band is indicated by the presence of cisternal cross-sections there (arrows). Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 28,200 X.

b) Portions of several deep muscle cells from a larva and the myoseptum between somites Nos. 18 and 19 are seen in cross-section. Long cellular processes (arrows) of unknown origin are present within the myoseptum, but short cell processes and specialized attachments characteristic of the 33-somite embryo are not visible. Neuronal profiles (A) between deep muscle cells are located both near the myosepta or deep within the muscle mass (see Figure 39c). They may be separated from the muscle cell at some points by a space containing electron dense material. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 13,300 X.

c) Portions of two axons (A) are seen between deep muscle cells near the spinal cord. They are separated from one muscle cell by a uniform space containing electron dense material (arrows), but are more closely apposed to other muscle cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 16,000 X.


VC
EC
SR
a

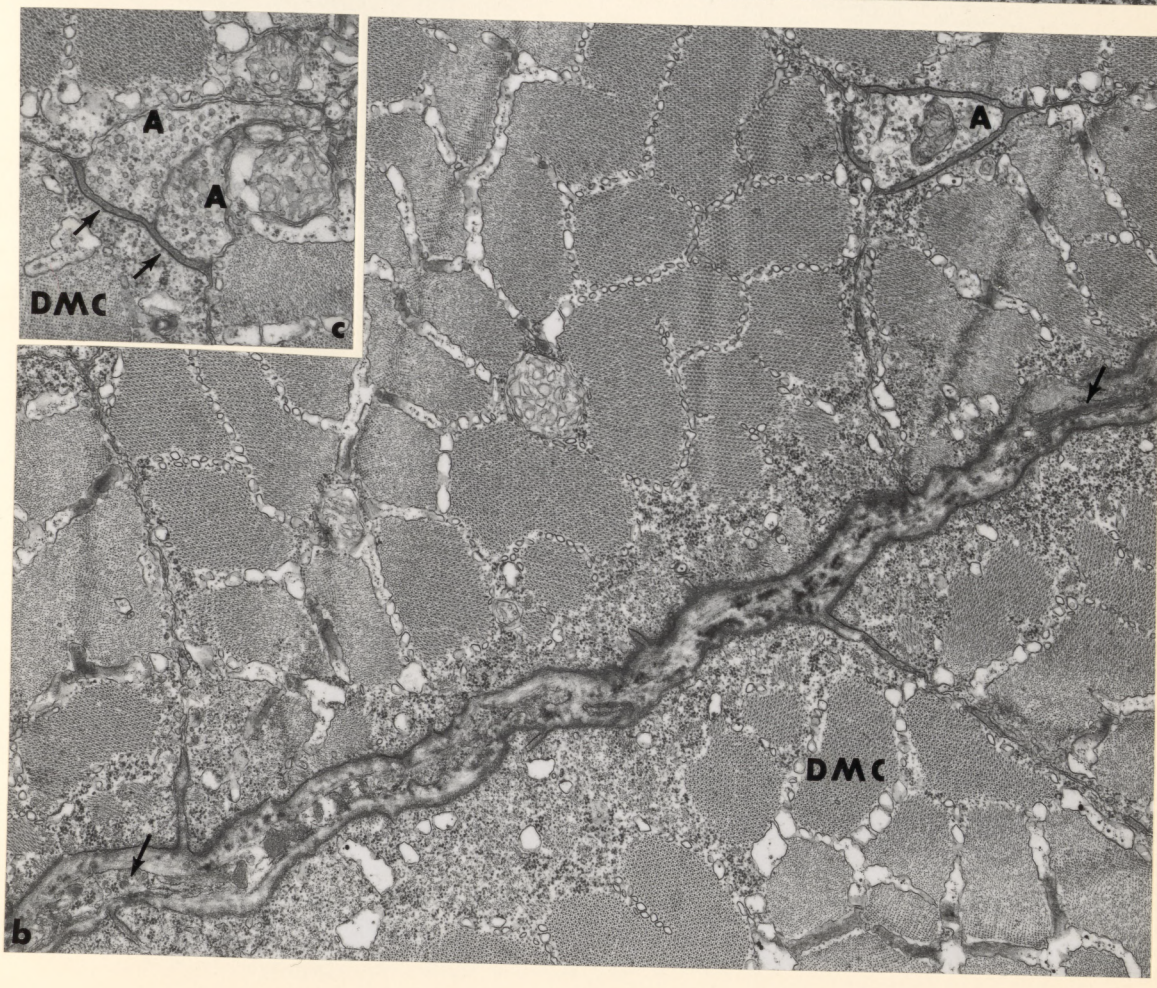

A
A
DMC
c
A
DMC
b

Figure 40. a) The lateral end of a myoseptum separating two mid-body somites in a larva is seen as it merges with the epidermal basement membrane (BM). Axons (A) associated with the superficial muscle cells (SMC) are located at the ends of these cells. Axons associated with deep muscle cells (DMC) are often, but not exclusively, found near the myosepta. Long mitochondria (M) are often aligned parallel to the myofibrils in superficial muscle cells. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 15,800 X.

b) A portion of a superficial muscle cell (SMC) near the myoseptum (MS). A distinct external lamina (BL) is seen when the plane of section is normal to the muscle cell membrane. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 7740 X.

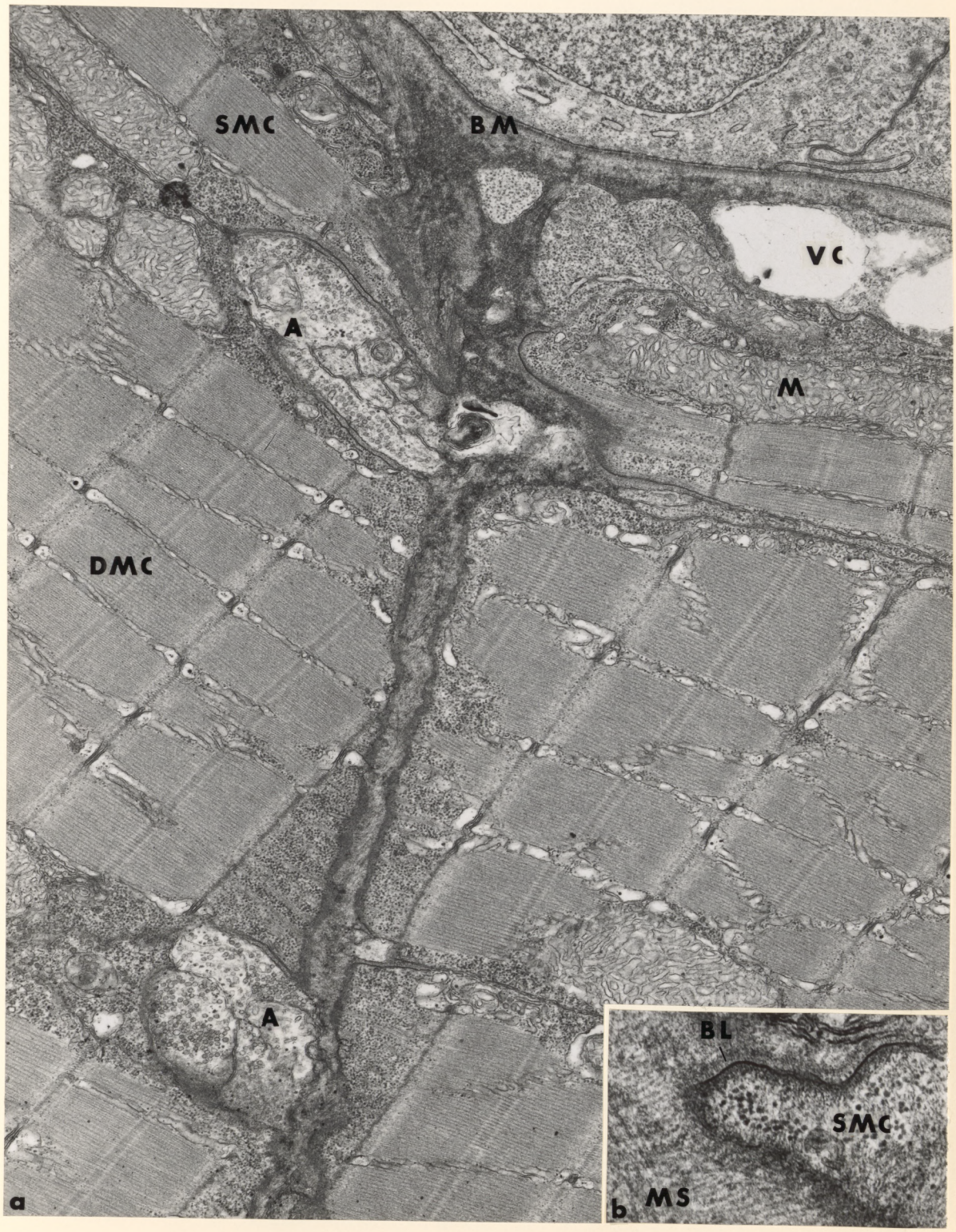

SMC
BM
VC
A
M
DMC
A
a
BL
SMC
MS
b

Figure 41. Portions of 2 axons (A) are seen between adjacent deep muscle cells near the myoseptum (MS) (larval stage). The region indicated by the arrow may be a neuromuscular junction. Groups of thin (and occasionally thick) filaments (MF) pass from the last recognizable Z-bands of the myofibrils toward the myoseptum. The length of these groups apparently may be any proportion of the sarcomere length. The appearance of the sarcoplasmic reticulum in the deep fibers as seen in horizontal section is indicated at the lower left. Glutaraldehyde-formaldehyde fixation. Stained with uranyl acetate and lead. Magnification approximately 21,800 X.

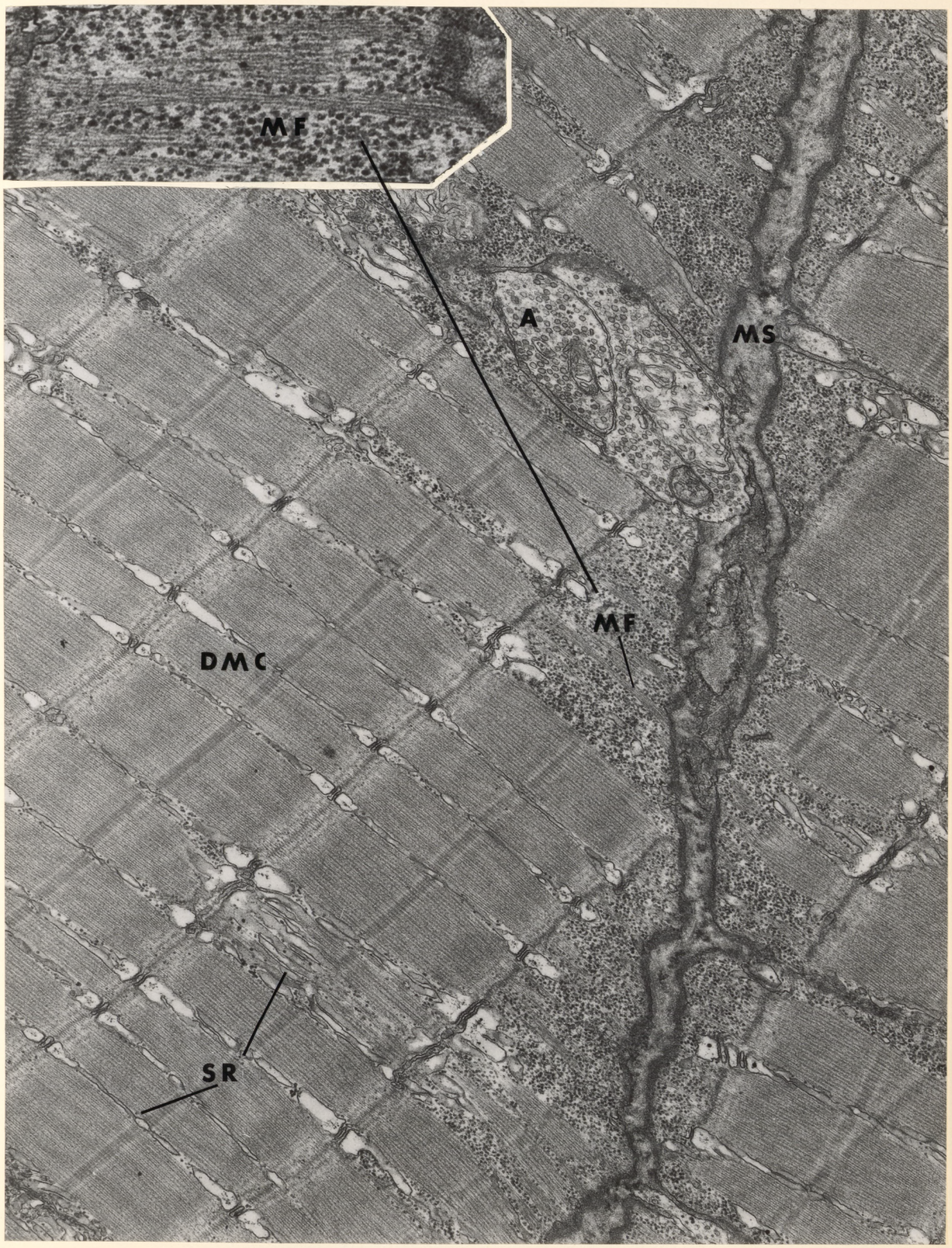

MF
A
MS
MF
DMC
SR

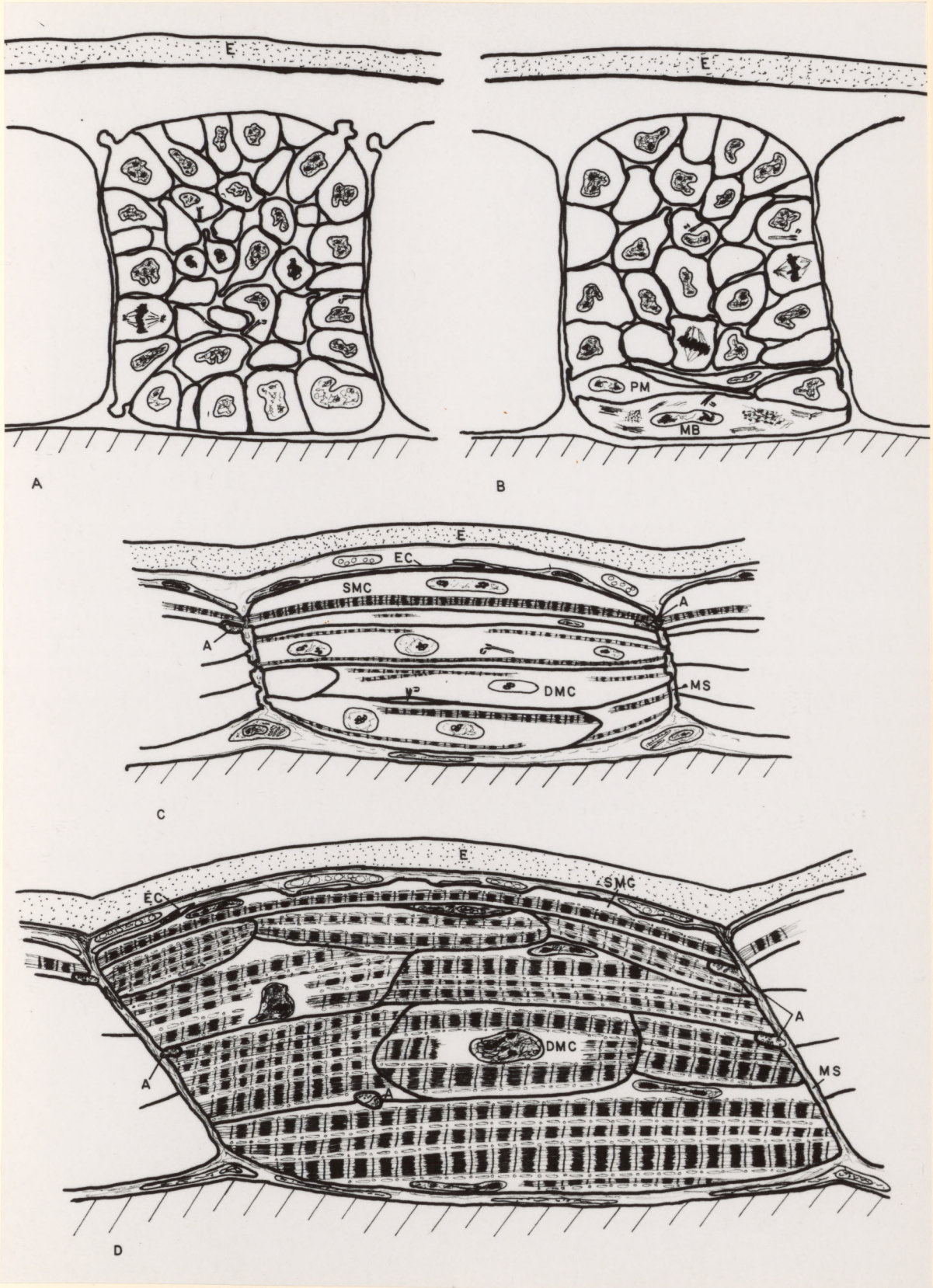
Figure 42. Schematic representations summarizing certain important features of a typical midbody somite at each developmental stage examined as seen in horizontal section at the level of the spinal cord (ruled lines). Age given in time after fertilization. Approximate scale: 1 cm = 10 μ.

a) 20-somite embryo (24 hrs.). The peripheral cells are more closely apposed than those at the center. Intercellular spaces are prevalent, but no distinct cavity is evident. The cells retain the ultrastructural appearance of the unsegmented mesoderm. Numerous flagella and mitotic figures are seen. No muscle cells or presumptive myoblasts are present.

b) 25-somite embryo (26 hrs.). The general shape of the somite remains unchanged, but at least a single layer of uninucleate myoblasts (MB), containing bundles of myofilaments and small myofibrils, has formed at the medial surface of the somite. Elongated, presumptive myoblasts (PM) are seen lateral to the myoblasts.

c) 33-somite embryo (40-45 hrs.). Several layers of deep muscle cells (DMC) and a single layer of superficial muscle cells (SMC) form the myotome. Flattened external cells (EC) form a cellular layer separating the superficial muscle cells from the epidermis (E). Superficial muscle cells are characterized by a single, centrally located nucleus and a large myofibrillar bundle medially. Deep muscle cells contain numerous small myofibrils around the periphery and up to 3 nuclei in the central cytoplasmic core. Smaller cell profiles are commonly seen subjacent to the superficial muscle cells. Small cellular processes and specialized cell attachments are prevalent across the developing myoseptal region (MS). The first axonal profiles (A) seen in the myotome occur characteristically medial to the anterior ends of the superficial muscle cells.

d) Recently hatched larva (7 days). A single layer of superficial muscle cells (SMC) and several layers of deep muscle cells (DMC) are still present. Both cell types contain well developed myofibrils. Portions of external cells (EC) are still present along the external surface of the myotome, but they no longer form a continuous cellular layer. Smaller muscle cells (some quite dense) are most common subjacent to the superficial muscle cells. Essentially no intersomitic contact across the well developed myosepta (MS) by means of cell processes and cell attachments is observed. Axonal profiles (A) and presumed neuromuscular junctions occur in relation to both the superficial and deep muscle cells.


E
A
E
PM
MB
B
E
EC
SMC
A
A
DMC
MS
C
E
EC
SMC
A
DMC
A
MS
D

VITA

Robert Earle Waterman was born in Watertown, Wisconsin on June 10, 1940, the son of Arthur and Margaret Waterman. He attended public schools in Fort Atkinson, Wisconsin. A Bachelor of Arts degree in Biology was awarded by Lawrence College, Appleton, Wisconsin in 1962. From 1962 to 1967 he was a graduate student in the Department of Biological Structure at the University of Washington School of Medicine, serving as a teaching and research assistant. He was supported by a Woodrow Wilson Fellowship (1962-1963), USPHS Training Grant 5T1-GM 136 (1963-1965) and a USPHS Predoctoral Fellowship (1965-1967). He was married January 29, 1966 to Anne Margarete Roosen-Runge. They have one daughter, Erin Margaret, born December 16, 1966.