STUDIES ON THE ENDOPLASMIC RETICULUM

III. Its Form and Distribution in Striated Muscle Cells

By KEITH R. PORTER, Ph.D., and GEORGE E. PALADE, M.D.

(From The Rockefeller Institute for Medical Research)

Plates 85 to 94

(Received for publication, November 30, 1956)

Previous papers in this series (1, 2) as well as numerous other reports on cell fine structure have provided evidence for the existence of a finely divided vacuolar system in the cytoplasm of all animal cells thus far examined except mature erythrocytes. The reticular character of the system and its exclusion from the thin cortical layer (ectoplasm) of the cytoplasm, as first observed in cultured cells (3, 4), account for the name "endoplasmic reticulum." More recently, in electron microscope studies of thin sections, the term has been applied to small tubular or vesicular elements of the cytoplasm that are limited by a single continuous membrane and have a homogeneous, structure-free content. In the majority of cells, such elements make up a system that is continuous and reticular in form. By its presence the cytoplasm is divided into two phases: (*a*) a constantly continuous phase, the cytoplasmic matrix, containing such resolvable components as ribonucleoprotein (RNP) particles and fibrous elements of several kinds, and (*b*) the inner phase of the membrane-limited reticulum, in general also continuous, but certainly discontinuous at times, and showing usually no resolvable elements. In some cells the system is abundantly represented; in others sparsely so. Its form, volume, and distribution tend to be characteristic for all cells of a particular type so that it becomes a feature of cellular differentiation. The system has also been observed to show local differentiations (2, 6) and to develop special associations with other components (*e.g.*, the RNP particles (5) and the nucleus (6)). Since it is reasonable to look to them for clues to the functions of the system, these latter associations seem especially significant. Some attention is therefore being given, in this and subsequent studies, to their occurrence, form, and behavior.

There are various indications to be found in published accounts of the structure of striated muscle that an equivalent of the endoplasmic reticulum is present in this as in other types of cells, and that it may show a pattern of distribution relative to the myofibrils. For example, Bennett and Porter (7), in a report on the fine structure of striated muscle of the domestic fowl, directed attention to an interfibrillar component—in some instances vesicular—localized opposite the Z or N bands of the myofibrils. Similar elements were later depicted

in the sarcoplasm of insect muscle (8) and more clearly in muscle of *Amblystoma* larvae (9, 10) and the gracilis muscle of the mouse (11).

These miscellaneous and preliminary observations have failed, however, to give more than a suggestion of the pattern the system adopts with respect to the myofibrils. In this regard, in fact, these recent observations are perhaps not so useful as older, somewhat neglected light microscope studies on muscle. As Bennett (11) has recently emphasized, certain of the images of gold- or silver-impregnated reticula described and depicted in papers by Thin in 1874 (12), Retzius (13), Cajal (14), and Veratti (15), among others, are readily identified with the reticular component seen at present in electron micrographs. The images of Veratti (15) are outstanding in showing patterns of organization, usually repeating in each sarcomere, but varying greatly from one type of muscle (and organism) to another. The thought is inescapable that the variation bears some relation to the special functional properties of the muscle under investigation.

The studies mentioned suggest therefore that the sarcoplasm of the striated muscle fiber also contains an equivalent of the endoplasmic reticulum which bears some unique and interesting structural relations to the myofibrils. This leads to the reasonable deduction that in its function the system may represent an important factor in the contractile phenomena these cells display. Possibly its limiting membrane conducts excitatory impulses from the sarcolemma to reactive bands in the myofibrils throughout the muscle fiber (11, 13). Possibly it provides to specific regions of the myofibrils a supply of essential metabolites. More detailed morphologic studies alone are not likely to define the significance, if any, of those various possibilities but they can, depending on the precision of the structural relationship discovered, give direction and impetus to experimental studies aimed at discerning function.

The present investigations have attempted, then, to fill in some of the structural information on this system which, for lack of equally suitable material or techniques, previous studies have not supplied. A small variety of muscles have been examined and from among these, muscle cells of the myotomes of *Amblystoma* larvae have received the major attention, for the reason that they show the reticulum to excellent advantage.

Materials and Methods

The observations to be presented have come from three sources: the caudal myotomes of *Amblystoma* larvae, the heart atrium and ventricle, and the skeletal muscles (sartorius,[1] obliqui, adductores, diaphragm, and tongue muscles) of the laboratory rat.

The larvae of *Amblystoma punctatum* were grown in the laboratory from early embryonic stages collected on Long Island, N. Y. When they had grown to a length of 12 to 15 mm.

[1] Though referred to as separate muscle, the sartorius of the rat is not a distinct anatomical entity but is closely united with the gluteus maximus and tensor fasciae femoris.

they were fixed by immersion for two hours in a 1 per cent solution of OsO_4 buffered at pH 7.8 with veronal acetate. The tails were then removed, washed, dehydrated, and embedded in *n*-butyl methacrylate, as described many times elsewhere. Sections of various thicknesses from 40 to 80 mμ were taken for examination.

The myocardium and the skeletal muscles of the rat that were studied were excised with the animal under ether anesthesia without attempting, in most instances, to control the extent of contraction. (The only specimens fixed in the extended condition were dissociated bundles of sartorius muscle.) They were then fixed in 1 per cent OsO_4 either in acetate veronal buffer (pH = 7.6) or in buffer with sucrose[2] added to give a final total osmolar concentration of ~ 0.3 M. The specimens were subsequently treated essentially as the *Amblystoma* tails.

Micrographs were taken with a modified RCA microscope (EMU 2c) at magnifications between 5,000 and 10,000 diameters and thence enlarged photographically.

OBSERVATIONS

I. Skeletal Muscle of Amblystoma Larvae

A. The Myofibrils:

The myofibrils in this muscle require for present purposes only a brief description. They seem not to differ in most fundamental respects from myofibrils of other vertebrate muscles for which there are now several descriptions in the literature (16, 7, 17, 18).

The sarcomeres are between 1.5 and 1.7 μ long. They show distinct A, H, I, and Z bands (Figs. 1 and 2). The A band is perhaps unusually long, being four-fifths of the sarcomere's length. Its limits are indistinct in Fig. 1 because the myofibrils shown are in an early stage of contraction. It is more distinctly set off from the I band in Fig. 2. The H band in Fig. 2 appears as a less dense stripe bisecting the A band, and shows no M band, whereas in Fig. 1 the pattern is slightly different, in that the H band shows a characteristically dense M band through its center. The I band in both images (Figs. 1 and 2) is, as usual, less dense than the adjacent A, and is bisected by a thin but distinct Z band. In no case does this telophragma or Z line resemble a membrane in the usual sense, nor is there any clear continuity of the Z lines across the sarcoplasm between adjacent myofibrils. Each myofibril, as is well known, is a bundle of myofilaments which, where relatively well defined as in the A band, have a diameter of ~ 110 A.

B. The Interfibrillar Sarcoplasm:

The less dense areas between the myofibrils in these micrographs are the regions occupied by the sarcoplasm. This is continuous at the cell margins with the subsarcolemmic or peripheral sarcoplasm (Fig. 3) and near the nuclei with the perinuclear sarcoplasm. In these muscle cells it contains at least four resolvable elements: the mitochondria or sarcosomes, lipide inclusions, a scattering of small dense particles, and the vesicular elements previously defined as components of the sarcoplasmic reticulum (7, 8, 10, etc).

[2] The use of sucrose to render the fixative more nearly isotonic has been shown by Dr. James Caulfield, working in this laboratory, to give substantially better fixation of a variety of tissues.

The *sarcosomes*, or mitochondria, in the *Amblystoma* cells are not unlike those encountered in other muscles. They are present in relatively small numbers and show no special association or disposition relative to the sarcomeres as has been reported for diaphragm and heart muscle (19, 20). The cristae are evident but not in sufficient numbers to pack tightly the mitochondria (see Figs. 2 and 3, and compare with Fig. 13). Where the plane of section is normal to the surface of the mitochondrion, a limiting membrane is evident and appears double in most instances (Figs. 2 and 3). (See Weinreb and Harman (21) and Harman (22) for a conflicting interpretation.) Lipide inclusions, characterized by a very high density after osmium fixation, are encountered in small numbers and usually in close association with the mitochondria (Fig. 2).

The small dense particles, which measure about 150 A in diameter and which, in this material, are interpreted as the muscle cell equivalent of those occurring more prominently in secretory cells (5, 9), are randomly distributed throughout the sarcoplasm. They show no special tendency to adhere to or associate in any way with other resolvable, formed elements of these muscle cells. It is assumed that they are RNP particles but it is recognized that definitive identification should await their chemical analysis after isolation, as has been done for liver and pancreas particles (23, 24).

The Sarcoplasmic Reticulum.—Longitudinal sections of these muscle fibers always show, in the interfibrillar sarcoplasm, numerous profiles of varying sizes, shapes, and densities. Where the plane of section is medial to the myofibrils these appear as in Fig. 1. A closer examination of such images provides some useful information on their form and disposition. Along the A band portion of the sarcomere these elements are usually long and slender (500 to 1,000 A in width) and extend, in some instances, in a row from one end of the sarcomere to the other. Occasionally the profile depicted appears as a single long unit (er_1, Fig. 1), but more frequently the continuity is interrupted. There seems always to be some evidence of such elements opposite the H band (*er*, Fig. 1). At each end of the sarcomere opposite the I bands, the profiles are consistently larger, especially in the dimension radial to the adjacent myofibrils, and seem to represent sections through dilated portions of the same sarcoplasmic system represented at other points along the sarcomere by similar but smaller units (er_2, Fig. 1). Where one such large unit is present, another of approximately equal size is found on the opposite side of the Z line. The opposing faces of the pair are flattened and separated by a dense region or space of constant width (500 A) which coincides precisely with the Z line of at least one adjacent fibril (Fig. 1). As a rule, all the profiles described occur in a single row in each interfibrillar space.

The individual elements of this sarcoplasmic component are seen, in some instances, to be limited by a sharp dense line, in others by a simple change in density, and in others by a relatively broad band of material, slightly more dense than the surrounding sarcoplasm. This latter image has the appearance

of a membrane passing obliquely through the section, and the others could be interpreted as images of membranes oriented either normal to or parallel to the plane of section. This interpretation together with the over-all similarity (in size, range, and apparent lack of content) to the endoplasmic reticulum of other cells suggests very strongly that the images referred to in Fig. 1 are profiles of a complex system of vesicles and tubules. Confirmatory evidence for this assumption will be derived from other micrographs.

It is obviously difficult to visualize the tri-dimensional form of many cell components from what is seen in almost two-dimensional thin sections, and for information on the third dimension it is sometimes necessary to resort to the laborious study of serial sections. Fortunately, however, for the microscopy of sarcoplasmic components, particularly those confined to layers of sarcoplasm between the myofibrils, this is not necessary because such layers lie in planes parallel to the long axis of the fiber and so must, in some instances, be included in the thickness of a longitudinal section. Where such a coincidence occurs (as shown in Fig. 2) the structural elements of the layer are seen in lateral view as distributed over the surface of the fibril. The image of this aspect in combination with the sectional views provided in Figs. 1 and 3 supply a complete three-dimensional image.

In Fig. 2, myofibrils crossing the upper right and lower left corners of the micrograph are sectioned more or less in a medial longitudinal plane and the intervening sarcoplasm contains profiles similar to those illustrated in Fig. 1. In the region between these more prominent myofibrils, only a few myofilaments are in evidence and these have been shaved off the surface of one or more adjacent myofibrils. Beside these, therefore, the 50 mμ (estimated) section contains a layer of interfibrillar sarcoplasm with its structural elements. These include, in addition to portions of two sarcosomes and a scattering of small particles, an irregular lacework of densities which in its organization shows some relation to the striae of the myofibrils. The reticular and continuous nature of the component, at least within the limits of one sarcomere, is obvious. In places (as at *X* in Fig. 2) where an individual element of the reticulum leaves the plane of section and is cut obliquely or transversely, it is seen to be round or elliptical and to have a membranous wall. Thus the predominant elements[3] of this reticulum are tubular or vesicular, as indicated also by the character of the profiles in Fig. 1. At the H band level these tubules run into cisternal structures (central cisternae) of irregular outline which coincide with the omnipresent profiles at this same level in Fig. 1. This part of the system seems, in each sarcomere, to be essentially continuous across the muscle fiber. At the I band level, or more precisely the A-I junction, as indicated in Fig. 1, the longitudinally oriented tubules connect (at *c* in Fig. 2) with dilated elements

[3] We have attempted to reserve the word "e'ement" for such structural units of the ER as vesicles, tubules, and cisternae.

which will be referred to as I band vesicles or terminal cisternae. These special differentiations of the system (er_2) have their longest axes oriented circumferentially to the myofibrils. The longer of them in this micrograph measure 0.5 microns, but this value is not better than an approximation because part of the unit is probably outside the plane of section.

As indicated in the description of Fig. 1, the terminal cisternae of one sarcomere invariably coincide (in position, size, and even alignment of margins) with equivalent units in the next sarcomere and just on the other side of the Z band. The terminal cisternae thus appear as paired structures. Their opposing faces are flattened, parallel, and are separated by a distance of about 500 A. In one case only a canalicular element was found to extend from one end of a terminal cisterna and proceed across the Z line past the opposing vesicle to connect presumably with the system at the next H band (see Fig. 7 and Text-fig. 1). But the occurrence of such a connection must be very rare to be encountered only once in the hundreds of fields examined. It seems, therefore, that in general the longitudinal continuity of the system throughout the fiber is to a very large extent interrupted at the level of each Z line.

Cross-sections of these same muscle cells, as shown in Figs. 3 and 4, provide additional views of the form and distribution of this component of the sarcoplasm. The section shown in Fig. 3 is sufficiently oblique to include in one field transverse images of the several bands of the sarcomere. The distribution of the levels represented is indicated along the margins of the micrograph. Within the lighter sarcoplasm between the myofibrils, profiles of the vesicular and tubular elements of the reticulum are prominent. At the H band level they tend to be in contact, thus forming a continuous membrane surface across the fiber at the level of each sarcomere. Where continuity is not evident it may be assumed that connecting elements are outside the plane of section. Relatively few profiles are present in the adjacent A band region (er_1), which indicates that only a few longitudinally oriented canaliculi are present in this particular part of this cell and which ties in as well with the small number of terminal cisternae (er_2) evident at the Z band level (compare with Fig. 4). These latter have their long axis in the plane of section and seem to extend along one face only of the adjacent myofibrils. Since, however, the section in its obliquity may not include the entire vesicle this latter point is perhaps to some extent illusory. In the region where the plane of section transects the Z band, the dense material at the same level between the paired I band vesicles is included (*iv* in Figs. 3 and 4).

Fig. 4 shows a transverse section that is confined very largely to the I band level of a fiber and consequently contains more I band vesicles. The picture is perhaps more descriptive of the typical distribution of those particular elements of the system than is that in Fig. 3. Apart from the greater number of I band vesicles shown, however, the information about them is similar. The vesicles constitute discrete units contiguous with one or occasionally two faces of a

myofibril. Variations in size and appearance result from the different planes or levels at which the vesicles are cut rather than from any inherent differences. The denser areas evident in some instances within the limits of the profiles represent the content of the dense space between paired units and show a small structural component (*iv*) to be considered in the following paragraphs.

Since the portion of the cross-section shown in Fig. 4 coincides with the Z band and adjacent levels of the I band over a relatively wide area, the micrograph includes profiles of all the I band vesicles in the adjoining sarcoplasm and should depict, in many instances, their extreme limits. Thus if the vesicles do not form complete rings around the myofibrils, as they indeed fail to, it may be concluded that at the level of the I band vesicles the reticulum is *not* continuous transversely across the fiber.

Local Differentiations of the Sarcoplasmic Reticulum.—Reference has already been made to an accumulation of dense material in the space between the I band vesicles, *i.e.*, the space regularly located in *Amblystoma* muscle opposite the Z line of the adjacent myofibril (Z_1 in Fig. 1). This space or region is limited on its two large faces (above and below) by the membranes of the flattened I band vesicles, while at the margins it opens on an adjacent myofibril or is continuous with the sarcoplasm. The two vesicles with the intervening space and its contents constitute a three-component structure which is being referred to as a triad.

When the content of the intervesicular space is examined in thin and favorably oriented sections it can be seen, as in Fig. 2, to contain a number of small circular profiles 200 to 250 A in diameter which appear to represent sections through small vesicular or tubular bodies to be described as intermediary vesicles.[4] Their size is in some cases very uniform and the arrangement regular (Fig. 7). In other instances (Figs. 2 and 8) they are less uniform, seem to be arranged in two levels and to be part of, or continuous with, the opposing face of one or both I band vesicles. Other views of these same bodies obtained from cross and longitudinal sections of the muscle show them to be small finger-like elements apparently consisting of a membrane enclosing a less dense center or lumen (Figs. 5 and 6). A longitudinal section through such an element is shown at *iv* in Fig. 6, and top views are shown in Figs. 3 to 5. In the latter image the magnification is sufficient to show the individual elements, though the density of the opposing faces of the I band vesicle, above or below in the preparation, prevents one obtaining a clearer view. They appear occasionally to be lined up more or less parallel to one another, as suggested by the regular arrangements depicted in Figs. 5 and 7. It is also evident in these micrographs that both margins of an array of such elements are never identical. Along one, they make a ragged or irregular line, whereas along the other side their ends seem to be

[4] In a preliminary report on *Amblystoma* muscle (10) these were referred to as intervesicular bodies. More recently acquired information recommends the present terminology.

more nearly in register. From the various appearances described it is tentatively concluded that the intermediary vesicles represent tubular evaginations from the opposing faces of the terminal cisternae or small vesicles that coalesce with the latter. It seems, also, that they make their connection in a row along one margin of the I band vesicles, thence are directed across the space (*i.e.*, right angles to the long axes of the I band vesicles) and occasionally for a short distance into the adjacent myofibril. They seem therefore to be polarized with respect to the adjacent myofibrils. The cross-section shown in Figs. 4 and 5 reveals further that these intermediary vesicles (*iv*) of the reticulum are not confined solely to the space between the terminal cisternae but may be present as well in the sarcoplasm between the myofibrils at the I band level. Whether they have escaped from the intervesicular space or have another origin is not indicated. As a rule the content of the intermediary vesicles appears denser than that of the adjoining cisternae.

Another differentiation of the reticulum is found at the H band level. Here, as described above, the reticulum appears in the form of irregular cisternae (central cisternae) which are flattened between the faces of the adjacent myofibrils. These are shown in profile in Fig. 1 (*er*) and in face view in Fig. 2. When the images of the cisternae in Fig. 2 and similar pictures are examined carefully, it can be seen that coinciding with the H band there are small (200 A in diameter) circular patches of lower density in the otherwise even density of the shadow. They seem to be confined to this region of the reticulum. They appear not to be fenestrae in the thin vesicles (because they are not limited by a thin line of greater density) but seem to represent thin spots in the membrane. Because of their small diameter and the greater thickness of the sections it is difficult if not impossible to see them in profiles of the reticulum where the limiting membranes are viewed edge-on.

C. The Peripheral Sarcoplasm:

Thus far the description has been devoted to the reticulum as encountered in the interfibrillar sarcoplasm. For formulating hypotheses regarding the function of the system it is important to know whether this interfibrillar development represents an hypertrophied form of some universally occurring division of the endoplasmic reticulum such as the Golgi component, and also whether the segmented pattern exists anywhere in the cell without associated myofibrils and so presumably for some purpose independent of myofibril functioning. Some attention has therefore been given to the membrane-limited elements of the peripheral and perinuclear sarcoplasm.

In the peripheral sarcoplasm, for example, there is, as a general rule, a population of vesicular elements. The vesicles vary in size for the most part within the 50 to 100 mμ range. They are not incorporated into any patterned system but seem to be free and randomly disposed within the narrow margin of the subsarcolemmal sarcoplasm. Some are attached to the inner surface of

the dense line defining the cell membrane, and in others the line limiting the vesicle is continuous with that limiting the cell, as though the vesicles were in the process of discharging their contents or budding off the inner surface to carry some of the environment into the cell's interior. This puts them in a class with vesicles showing a similar relationship to the cell membrane and found in endothelial and other types of cells (24).

In some instances it is apparent that more vesicular units are present opposite the Z bands of the peripheral myofibrils than elsewhere. Thus at the I band level in Fig. 8, a population of small elongate vesicles is continuous (forms a bridge) from the sarcolemma to the space between the I band vesicles, and it is further to be noted that the units making up the population are similar in size and character to the intermediary vesicles (*iv*). At the H band level, vesicles of an entirely different size, resembling the larger cisternal elements at this level, are present between the sarcolemma and the nearest fibril. Arrangements of this nature, have been seen only infrequently, and in no instance has any more patent connection than this been found running between the interfibrillar reticulum and the sarcolemma.

D. Perinuclear Sarcoplasm:

The nuclear envelope observed in these muscle cells consists of two membranes and an enclosed space (a perinuclear cisterna), essentially as found in all types of cells (25, 6). At infrequent points the outer membrane is continuous with the membrane limiting the sarcoplasmic reticulum where the latter lies close to the nucleus.

Otherwise the perinuclear sarcoplasm is located at the poles of the nuclei. It contains a randomly distributed population of large and small vesicles and stacks of closely packed, flattened vesicles making up the Golgi component. The small dense particles, present here in about the same concentration as elsewhere in the sarcoplasm, lie free and unattached to any of the membrane-limited units.

It is judged from these observations that the interfibrillar system is a special differentiation of the endoplasmic reticulum modified in relation to the sarcomere segments of the myofibrils and that it does not displace or represent a hypertrophied form of any other commonly occurring component of the cytoplasm.

Summary.—The foregoing information on the structure of the sarcoplasmic reticulum of *Amblystoma* muscle cells is summarized and interpreted in Text-fig. 1. Thus from the electron microscope evidence it is concluded that an elaborate lacework or reticulum of tubular and vesicular elements exists as a structural component of the interfibrillar sarcoplasm. This reticular component is essentially continuous and disposed in such a way that if the myofibrils were removed, it would appear as a fine honeycomb For convenience of consideration

it may be regarded as forming a sleeve around each myofibril, though obviously parts of any one sleeve are shared with surrounding myofibrils Along the length of the associated myofibril this lace-like sleeve shows repeating and characteristic patterns of structure which invariably coincide with certain segments of the myofibril it covers. Thus, opposite each H band there is a continuous central cisternal element which encircles the fibril. From it, slender canaliculi extend longitudinally to the ends of the sarcomere (*i.e.*, to the level of the I bands) where they join circumferentially oriented and somewhat dilated vesicles (terminal cisternae). These are of limited length and so do not form complete rings around the fibrils. They are paired with equivalent units in the next sarcomere and separated from them by a space of only 500 A in which are located small, intermediary vesicles. There are extremely few tubular connections (one is shown) across the Z band from the reticulum of one sarcomere to that of the next. Hence continuity along the length of the sleeve is largely interrupted at the Z band level.

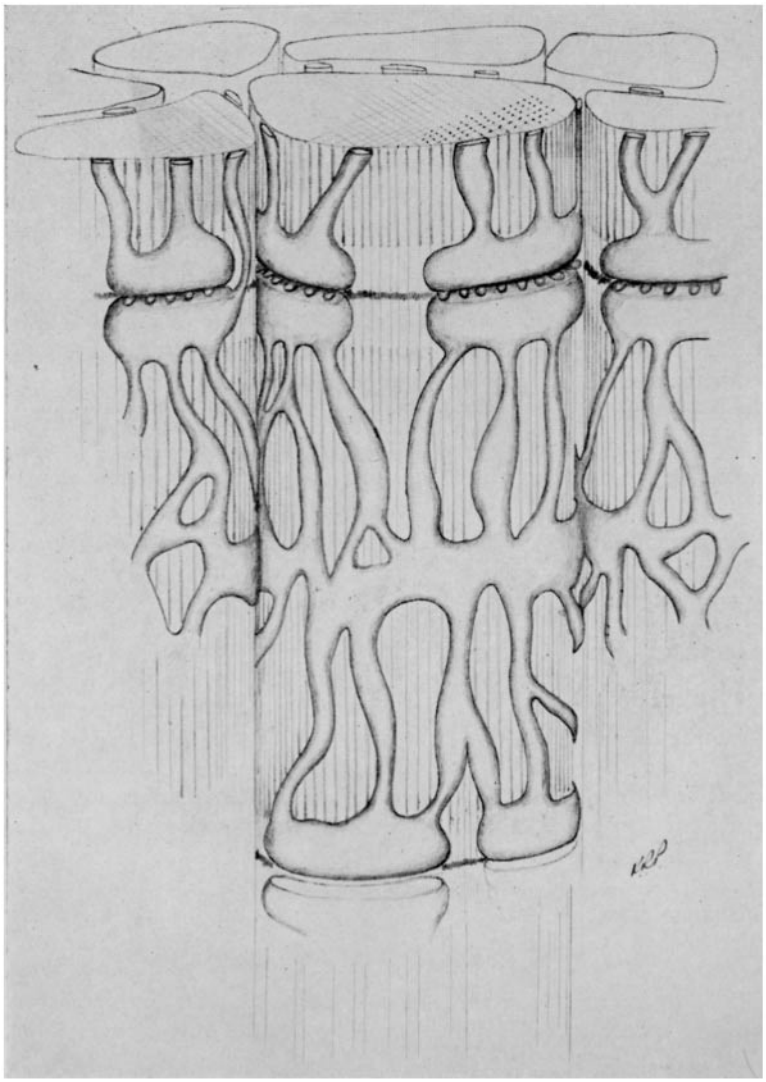
II. Skeletal Muscle of Rat

Other accounts of electron microscope studies of mammalian skeletal muscle have given only limited attention to the sarcoplasmic reticulum. Ruska (26), in some micrographs of the quadriceps of the mouse, noted slender tubular elements in the sarcoplasm between the myofibrils and regarded them as equivalent to the endoplasmic reticulum of other cells. Edwards *et al.* (27), in a study of several kinds of muscle including the gastrocnemius of the mouse, commented on the universal presence of the reticulum and mentioned certain differentiations more or less evenly spaced on opposite sides of the Z band and referred to as "reticular dilatations." Similarly located "double membranes" were observed in reptilian skeletal muscle by Robertson (28). Bennett (11), in a more extensive exploration of the system of membrane-limited channels between the myofibrils, pointed out "concentrations" of the sarcoplasmic reticulum opposite I bands. His micrographs appear to show that the reticulum is continuous along each sarcomere from Z band to Z band.

These observations make it apparent that in muscle fibers of mammals, as in those of the fowl (7), *Amphibia* (10), and insects and other forms (27), there is in the sarcoplasm a finely divided vesicular component forming a more or less continuous reticulum and that there is indeed here, as in the other muscles examined, a suggestion of local differentiations in the system showing some relation to the myofibril bands. It would perhaps not be important to present the following observations on skeletal and cardiac muscles of the rat if better preservation of the tissue had not provided improved images from which may be extracted more detailed information on the structure and organization of the system. With such expanding knowledge of details available, postulates on the function become more feasible, if not more convincing.

A. The Myofibrils:

The *myofibrils* of the sartorius muscle[1] show the characteristic banding of skeletal fibers and resemble, in most features, the equivalent in *Amblystoma* muscle.



TEXT-FIG. 1. A formalized interpretation of the sarcoplasmic reticulum and its relation to the myofibrils in fibers of *Amblystoma* myotomes. The drawing is meant to provide a three-dimensional image of the system and so summarize the observations made on the sections. A complete sarcomere segment of the system plus a third of another is represented on the central myofibril. A Z line and associated structures cross the upper part of the image. For other comment on the figure see text. × approximately 36,000.

The sarcomeres, in fibrils judged to be at rest length, measure about 2.0 microns. The A and I bands are distinct and the two are of nearly equal length (A = 0.94 μ, I = 1.06 μ). The H, which in *Amblystoma* muscle is well defined, is apparent in the sartorius muscle studied only as a band of slightly distinctive character across the A (Figs. 9 and 10). The I is bisected as usual by a narrow but dense Z band. Midway between the Z and the A-I junction it is possible to detect a region or band of slightly greater density (with very indistinct margins) which is taken to be the N band. Myofilaments are prominent in the A band and far less so in the I and Z bands.

B. The Interfibrillar Sarcoplasm:

The sarcoplasm is distributed in small quantities between the myofibrils and in greater amounts in peripheral and perinuclear regions of the cell. As in other muscles studied, the principal elements evident in the interfibrillar sarcoplasm are, in descending order of magnitude, the sarcosomes or mitochondria, lipide inclusions, the sarcoplasmic reticulum, and the small dense particles.

The *Sarcosomes.*—In sartorius (and in obliqui), two different types of muscle fibers, one endowed with numerous, and the other with few mitochondria, can be clearly distinguished. Fibers belonging to the first type are usually of small diameter and their numerous sarcosomes are regularly disposed in relation to the myofibrils. As seen in Fig. 9, mitochondrial profiles are preferentially located opposite the I bands of the sarcomeres (between the N and Z bands), an arrangement which results in the frequent formation of doublets or pairs symmetrically disposed in relation to the Z lines. Favorably oriented sections show that the long axes of these mitochondria are perpendicular to the direction of the myofibrils (Fig. 10). In three dimensions they correspond to long, tortuous, and branched sarcosomes which surround more or less completely each I band. The transverse mitochondria around each sarcomere are frequently connected by longitudinally oriented branches. Their profiles appear in continuous rows located here and there in the interfibrillar spaces. Each sarcosome has two membranes and numerous, tightly packed cristae disposed cross-wise in the longitudinally oriented organelles and lengthwise in those transversely oriented.

The fibers of the second type are of larger diameter. Their transversely oriented mitochondria are few in number, small in diameter, and have only a few longitudinal cristae. Mitochondrial pairing is less evident and many I bands do not have any associated sarcosomes. Longitudinal connections are exceptional. In the diaphragm practically all fibers belong to the first, mitochondria-rich type.

Lipide inclusions are frequent in fibers with numerous mitochondria where they normally occur scattered within the rows of longitudinally disposed sarcosomes. They are in intimate contact with the mitochondria and frequently show punctate zones of particularly high density.

The Sarcoplasmic Reticulum.—In longitudinal sections, that cut medially through the myofibrils, the interfibrillar sarcoplasm appears as narrow strips

of non-fibrous material containing, besides sarcosomes, a linear scattering of smaller profiles (Fig. 9). The orientation of the section shown in Fig. 9 and its relation to the myofibrils is similar to that depicted in Fig. 1. The interfibrillar profiles, as in the earlier figure, have shapes which vary from round to elongated, are limited by a dense line, and in most cases have centers of relatively low density. They are reasonably interpreted as images of sections through vesicular or canalicular elements of the sarcoplasm. It is quite apparent (Fig. 9), that the distribution of these profiles is uneven and that therefore the system of which they are a part varies in its form along the fibril. It can be noted further that there is a pattern of variation that repeats with each sarcomere. In this respect, this system is similar to that in the skeletal muscle of *Amblystoma*, except that the elements are smaller and their pattern of distribution partly different. Opposite the H bands there are uniformly present in this mammalian muscle clusters of small circular or ovoid profiles (*er*, Fig. 9). At this level therefore, the component represented is regularly present around each sarcomere and continuous across the fiber. Less consistently encountered in the interfibrillar sarcoplasm are long profiles extending from the H band cluster in either or both directions toward the I band level (as at er_1 in Fig. 9). These represent tubular elements of the reticulum, running parallel to the long axis of the myofibrils. They seem to end in a somewhat dilated element at a level just beyond the A-I junction (see arrows in Fig. 9). Even in cases in which long tubules are not present in the section, there is usually (but not always) a profile similar in size to the terminal dilatation at the level mentioned. This indicates that the longitudinal elements connect into a transversely oriented tubule running circumferentially around the fibrils. Such tubules are depicted in face view at er_2 in Fig. 10. In this latter image, as well as in Fig. 9, it is evident that this particular tubular structure is only one of a group of three or a triad. The adjacent, or middle one of the three, is usually smaller and appears sandwiched between the other two in a space that is remarkably constant in its width ($\sim$ 500 A). The third tubule of the group, on the Z line side of the triad, is continuous in its turn with canalicular elements of a finely divided and complex reticulum which extends in the narrow interfibrillar space past the Z band and on into the opposite side, *i.e.*, into the next sarcomere. Thus, around and among the myofibrils at the N band level of each sarcomere there is a complex structure consisting of three parallel, closely apposed tubules (a canalicular triad) which on one side connects with the tight reticulum at the H band level, and on the other side with a lacework of tubules (an extensively fenestrated cisterna) opposite the I and Z band levels. The frequency with which triad profiles are encountered in longitudinal sections of the muscle (arrows in Fig. 9) suggests that the structure is almost continuous transversely among the myofibrils and across the fiber.

Where an expanse of interfibrillar sarcoplasm is included in the thickness of

a section, a frontal or face view of the reticulum is provided, as in Fig. 10. This is comparable to the aspect shown in Fig. 2. The larger and more constant cisternae opposite the H band are shown at *er*. These connect by way of longitudinally oriented tubules (er_1) with the transversely oriented, elongate vesicle at er_2. This in turn is parallel with and spaced at 500 A from a similar vesicle which is continuous with a complex, difficult to analyze reticular system (er_3) extending along the I and Z bands to the equivalent element in the next sarcomere. In between the transversely oriented and parallel vesicles there are very thin vesicular elements (intermediary vesicles, *iv*).

It is regarded as noteworthy that the triad structure near the N band level is essentially the same as that present at the Z band in *Amblystoma* larval muscle, *i.e.*, it consists of two dilated circumferentially oriented tubules or vesicles with somewhat flattened, opposing faces and with several intermediary vesicles arranged usually in a single row between them. The outstanding differences are that the sartorius fiber has two such organizations for each I band, whereas the amphibian fiber shows only one, and that the intermediary vesicles are thin, flattened elements rather than finger-like bodies.

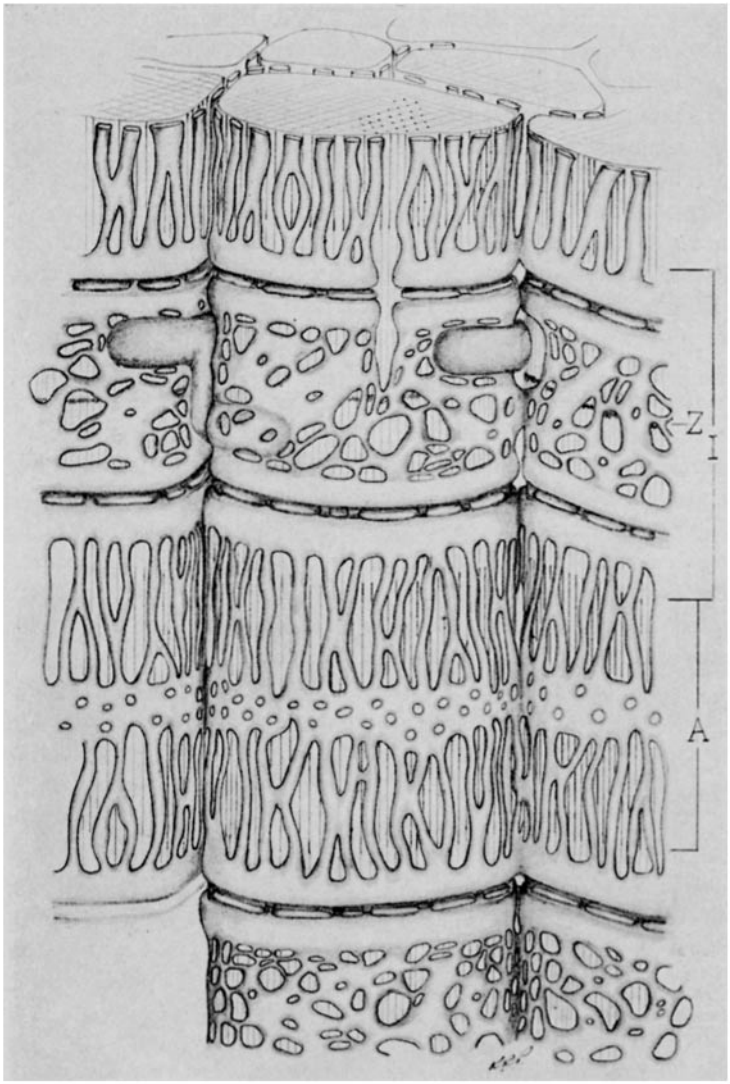
As mentioned above, the reticulum between the two triadic organizations along the I band is complicated and not readily analyzed. It bypasses the sarcosomes, where they are present (as at *bp* in Fig. 9), and occasionally is present on both sides of these bodies. Even in the absence of sarcosomes the system frequently consists of two reticular sheets at this level. As nearly as can be determined, the network is continuous through the Z band level, as depicted in Text-fig. 2. The delicacy of the structure plus the presence of sarcosomes and of large amounts of poorly defined granular material in the sarcoplasm has made more detailed analysis impossible at this time. A disposition similar to that described is encountered also in the diaphragm, the obliqui, the adductores femoris, and the tongue muscles.

C. The Peripheral Sarcoplasm:

The sarcolemma of sartorius fibers has the three component structure commonly encountered in muscle cells: (*a*) a dense line next to the sarcoplasm representing the plasma membrane, (*b*) external to it a thicker but much less dense layer of material referred to as the cuticular layer,[5] and (*c*) outside of all a thin feltwork of fine fibrils. It differs not appreciably from that limiting cardiac cells and pictured in Figs. 13 and 14.

Between the sarcolemma thus defined and the nearest myofibrils there is in

[5] The so called cuticular layer of the sarcolemma (Porter, *Anat. Rec.*, 1954, **118**, 342) has greater density than the methacrylate-embedding matrix and appears, therefore, to represent a layer of material, possibly mucoprotein, and not simply a space. From images such as these presented here and described elsewhere by Robertson (28) the layer is judged to have a minimal thickness of 200 to 300 A.



TEXT-FIG. 2. A schematic summary and interpretation of observations on the sarcoplasmic reticulum of rat sartorius muscle. A single myofibril occupies the center of the image with portions of adjacent myofibrils surrounding it. A complete sarcomere with associated reticulum is pictured as part of the central fibril. Sarcosomes, shown in less than the normal number, occupy well defined positions relative to the Z line. They are pictured with their long axes oriented circumferentially with respect to the fibril and covered or not by the close reticulum of the I band region. × approximately 32,000.

sartorius fibers a thin layer of sarcoplasm. Resolvable elements within it include sarcosomes and small particles. Just adjacent to the plasma membrane and sometimes continuous with it there are also many small vesicles, 30 to 50 mμ in diameter. These seem to be especially numerous in regions opposite the N bands of the adjacent myofibrils and therefore opposite the structures of the sarcoplasmic reticulum. In no instance encountered did these vesicles form the bridge depicted in Fig. 8, but their preferential distribution at these levels is unmistakable. In addition to the vesicles described, there are few elements ascribable to the endoplasmic reticulum in the peripheral sarcoplasm. Among them, elements bearing attached small particles are occasionally encountered.

In the perinuclear regions, the sarcoplasm is more abundant and contains, in addition to the clusters of mitochondria, stacks of tightly packed cisternae, similar to the Golgi complex described in other cells (29), compound vesicles, a few randomly disposed elements of the endoplasmic reticulum, and a scattering of small dense particles. The elongated and frequently indented nuclei are each surrounded by a perinuclear cisterna which bears a few attached particles on its outer membrane.

Summary.—The structure of the sarcoplasmic reticulum in the sartorius muscle[1] of the rat is interpreted and summarized in Text-fig. 2. The lacework of tubules and cisternae (the sarcoplasmic reticulum) surrounding the A band is similar to that found in *Amblystoma* muscle except that the elements are finer and more numerous and consequently form a more closely meshed reticular structure. Within the limits of the I band, however, the system shows pronounced morphological differences. First, the triad arrangement of the two circumferentially oriented and somewhat dilated channels with small intermediary vesicles lies not opposite the Z band but between the A-I junction and N band levels. Then also, this structure is repeated, making in all two triads within the limits of each I band. The intervening lacework which appears to be continuous along the I and over the Z line has no equivalent in the *Amblystoma* material.

Continuity in the sense of an uninterrupted membrane surface seems to exist in a transverse direction along the A band, particularly at the H level for each sarcomere. Continuity longitudinally, on the other hand, appears to be to a large extent interrupted at each triad. The circumferentially oriented triads are pictured also as discontinuous and the continuity of the intervening I band lacework transversely, though indicated in the figure, is not absolutely certain. Mitochondria are not shown in the average abundance observed in the micrographs, in order not to detract from the major purpose of the drawing.

III. Cardiac Muscle of Rat

Electron microscope studies of cardiac muscle previously reported have placed particular emphasis on the form and interrelationships of the myofibrils (30–32),

and the structure and disposition of the sarcosomes (33). In most instances these early observations were made on badly preserved material and, as a consequence, have limited value. This is, however, far less true of results reported more recently by Weinstein (34) from a study of chick heart. This investigator provides convincing evidence for detailed descriptions of myofibrils and of sarcosomes and their internal structure and mentions the sarcoplasmic reticulum as "a finely reticulated material, often vesicular in appearance." This latter component he reports as residing in the interfibrillar sarcoplasm and as being concentrated at the Z band level. He notes further that evidence of organization in the cardiac reticulum is less apparent than in skeletal muscle.

Additional features of fine structure found in thin sections of rat heart muscle are described in what follows.

A. The Myofibrils:

The myofibrils of rat cardiac muscle show a striation pattern essentially similar to that of skeletal muscle. The A bands of the uncontracted fibrils (Fig. 11) are long (1.4 μ) relative to the length of the sarcomere (1.8 μ) and in this respect resemble A bands of the *Amblystoma* fibrils described earlier. The I or isotropic bands, which appear considerably lighter than the anisotropic in Fig. 11, are bisected by a prominently dense Z band. A faintly defined H band marks the middle of the A. When contracted (Fig. 12) the fibrils show, as expected, striking structural changes. The sarcomere shortens to about 1.2 microns, the I band is *not* easily defined if indeed still present, and the H or M line is indicated only by slight thickenings in the myofilaments at a certain level midway in the sarcomere.

Unlike those of skeletal muscle, the myofibrils of cardiac fibers frequently branch and anastomose with other myofibrils so that the fibril content of the heart cell constitutes a more or less continuous mass.

The myofibrils, as in other muscles, are bundles of slender ($\sim$ 110 A) myofilaments which are readily identified in the A band, but less readily so in the I band. The relative numbers of filaments in the H and A bands is not shown distinctly enough to warrant comment.

B. The Interfibrillar Sarcoplasm:

The amount of sarcoplasm in the interfibrillar spaces is relatively small and unevenly distributed in cardiac fibers because of the branching of the myofibrils. Just beneath the sarcolemma and more especially in the central perinuclear region of the cell the amount is greater. The content of the interfibrillar sarcoplasm, in terms of resolvable units, is similar all over the cell, and sarcosomes, the sarcoplasmic reticulum, and small dense granules are most prominent.

The sarcosomes are exceptionally numerous in heart muscle fibers. They occupy, by virtue of their size, the larger interstices between the myofibrils and tend to form end-to-end columns which may extend over many sarcomere lengths. They are oriented for the most part with their long axes parallel to that of the fiber and

show some tendency to be confined individually within the limits of one sarcomere. There is obviously a large surface of contact between sarcosomes and adjacent myofibrils. They fail, however, to show in their distribution the uniform band association encountered in the sartorius muscle. Transversely oriented mitochondria, associated with the I bands, are frequently but less regularly encountered than in the mitochondria-rich fibers of skeletal muscles.

In their fine structure, cardiac sarcosomes show the characteristic features of other mitochondria. They are limited by two successive membranes (Fig. 12) and contain large numbers of cristae as expected in tissue cells with a high energy turnover (19). The number is indeed greater than encountered in mitochondria or sarcosomes of any other tissue. The cristae are oriented transversely or obliquely with respect to the long axis of the organelle (see *m*, Figs. 11 and 13) and possess a relatively complex morphology with evidence of branching and, more commonly still, fenestrations.

Lipide inclusions of various sizes are frequently found in close association with the interfibrillar sarcosomes.

The Sarcoplasmic Reticulum.—The interfibrillar sarcoplasm in cardiac muscle is usually confined to narrow spaces which are marked by rows of profiles seemingly representative of sections through small vesicles or slender tubules. These profiles range in shape from circular to oval to long ellipsoids and in their smallest dimension, representing the space between adjacent myofibrils, they may be as little as 200 A.

Within the limits of the A band, the distribution of these profiles is quite uniform, with greatest constancy of occurrence opposite the H band (*er*, Fig. 11). Where a sarcosome is present in the interfibrillar space, such small profiles are found on one or both sides of it.

At the I band level the profiles are frequently larger, describing some dilatation of the vesicles in this part of the system (er_2, Fig. 11). At this point they also depart from the form and orientation shown by the midsarcomere elements. The unit most commonly encountered here is frequently U-shaped, with the opening directed toward the Z line. Within the area enclosed by the arms of the U there may be an additional circular or oval profile which in some instances is continuous with the inner surface of the U-shaped element (*iv*, Figs. 11 and 12). This unit of two components will be referred to as a dyad. It is usually located opposite one-half of the I and not directly opposite the Z. Since it does not appear consistently in this position in images of medial longitudinal sections of myofibrils, it may be assumed that the dyad extends along only a short portion of any myofibril circumference. The pair is sometimes faced by a profile of similar size and orientation apparently belonging to the reticular system of the next sarcomere (near Z, Fig. 11) and such combinations of three members are equivalent to the triadic structures of the other muscles studied.

The morphological character of these various elements and their associations at the I and Z band levels is reminiscent of those encountered in the two types

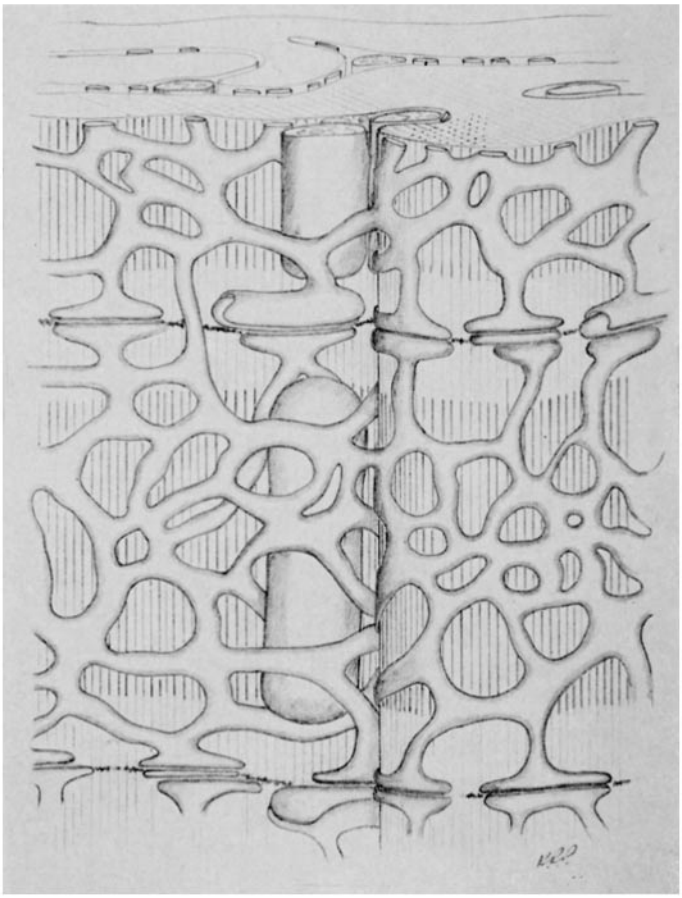
of skeletal muscle described above. Only dimensions and distribution are significantly different. The relatively uniform distribution of profiles opposite the A band suggests, *e.g.*, that the reticular structure departs somewhat from that of the skeletal muscle cells and that instead of mostly transverse or longitudinal components it may consist more of randomly oriented elements. This is borne out in micrographs of thin sections which include a thin layer of interfibrillar sarcoplasm (Fig. 13) and which therefore repeat the coincidence depicted in Fig. 2. In such instances it is evident that the system represented by scattered profiles in Fig. 11 is constructed of numerous canaliculi.

The distribution and direction of the component units are seemingly random in Fig. 13, but in fibers at rest length there is some differential organization. Thus the reticulum is tighter opposite the H band and extends distally into predominantly longitudinally oriented elements at the two I band extremities of the sarcomere (see Text-fig. 3). As shown in Fig. 13 (er_2) these latter elements run into transversely directed vesicles or cisternae opposite the I band—or where it would be in the rest-length fibril. The same figure shows further that the transverse elements run in combinations of two or three, representing the dyads or triads of the other images. The extremities of these where they face off are seemingly not so perfectly in register here as in the *Amblystoma* fibers. Likewise the intermediary vesicles (*iv*, Fig. 13) are not nearly so uniform in size. They may be small and more or less completely enwrapped by one of the distal or terminal vesicles or they may be large and almost free of this association. In some instances no intermediary vesicle can be identified. From the evidence available it appears that continuity of the system along the length of the fibrils is, in general, interrupted at the Z band level. Cross-connections may exist (as depicted in Text-fig. 3), but if so they do not occur with sufficient frequency to provide for their definite appearance in the numerous micrographs examined.

The small dense particles represent a third resolvable component of the interfibrillar sarcoplasm of heart cells. Many of these have indistinct margins and so are difficult to measure, but where most clearly defined they range between 100 and 150 A in diameter (Fig. 13). They occur most frequently in clusters which of necessity lie in the immediate vicinity of the membrane of the sarcoplasmic reticulum. They are not, however, attached to this membrane, but appear to be free in the cytoplasmic matrix.

The Peripheral or Subsarcolemmic Sarcoplasm.—As mentioned above, it is customary to find at the margins of these cardiac muscle fibers an expanse of sarcoplasm considerably in excess of that occurring between the myofibrils. This contains numerous isolated vesicles, of which some are in close contact with the plasma membrane of the sarcolemma (*pm*, Fig. 13; Fig. 14). It is assumed that the sarcolemma is a compound structure consisting of plasma

membrane, a homogeneous cuticular layer (*cu*, Fig. 13),[5] and externally, a finely fibrous sheath. Sarcosomes are also present. Structures of greatest interest in



TEXT-FIG. 3. A schematic drawing of myofibrils and associated reticulum as observed in cardiac muscle. The fibrillar material is represented by parallel lines which are drawn thicker in the A bands to distinguish these from the I bands. Portions of two sarcomeres are included in the figure (center) opposite the A bands. The reticulum is represented by rounded strands. One and one-third sarcomeres, separated by dense Z lines, are included in the image. For other comments on the figure see the text. × approximately 25,000.

the present connection extend from the I-Z band level of the myofibrils to the sarcolemma. They appear as slender membrane-limited units with one end inserted in the reticular system at the I band level and the other extending out to and along the inner surface of the sarcolemma. In contracted fibers of this nature (Figs. 13 and 14) they usually appear in pairs, one going to each side of

the sarcolemmic infolding (Fig. 14), but in Fig. 13, which was not selected to show this feature, only one such connection is evident (at arrow). The scalloped form of the sarcolemma which is always present in contracted regions of heart fibers, suggests that the extension of the sarcoplasmic reticulum serves, among other things, to anchor the sarcolemma to the Z band levels of the sarcoplasmic reticulum and associated myofibrils.

The central mass of perinuclear sarcoplasm contains sarcosomes, stacks of tightly packed cisternae (Golgi complex), compound vesicles, elements of the endoplasmic reticulum, including a few elements bearing attached particles, and a relatively large number of small dense particles.

Summary.—Text-fig. 3 is designed to summarize the observations made on cardiac muscle. The reticulum is represented by shaded strands, and the myofibrils it covers by parallel lines. Here as in the other muscles studied the system appears as a complex lacework of interconnected tubules and vesicles confined to the sarcoplasm between the myofibrils. Within the limits of each sarcomere the reticulum shows a similar pattern of structure, which, though not so sharply defined as in the skeletal fibers, is nevertheless discernible. For example, opposite the central part of the A band, the open areas in the reticulum are smaller than elsewhere so that the structure approaches that of the fenestrated cisternae observed at the H band level in sartorius fibers. The orientation of the strands of the reticulum in each sarcomere segment is random except opposite the I bands where they tend to be longitudinal relative to the fibril. The I band vesicles, in which these latter elements terminate, do not seem to form in cardiac muscle so constant or fixed a relationship with equivalent elements in the next sarcomere segment. The space between them varies (as opposed to the 500 A space in skeletal fibers) and their margins are not in perfect register. Likewise, the intervening elements, known as intermediary vesicles, number usually one per apposition and vary greatly in size and in their relation to the I band vesicles.

These observations indicate, as depicted in Text-fig. 3, that the reticulum in cardiac fibers is a continuous structure within the limits of each sarcomere and across the fiber. Thus transversely there is good membrane surface continuity at each sarcomere level. Longitudinally, the situation is less clear and although strands of the reticulum may cross the Z line and connect adjacent sarcomere segments (as shown in the drawing), such connections are not frequent.

DISCUSSION

Identification and General Disposition

The foregoing observations picture the sarcoplasmic reticulum, the principal interest of this report, as a complex tri-dimensional lattice (or network) constructed of membrane-enclosed spaces which have the form of slender tubules

or thin, flattened vesicles or cisternae. In their size, structure, and general lack of resolvable content, these are not essentially different from elements composing the finely divided vacuolar system of other cells. Hence it seems entirely reasonable, as suggested earlier (7), to associate the sarcoplasmic reticulum with the membrane-limited and frequently reticular system described in other cell types and so classify it as another form of the endoplasmic reticulum (see reference 44 for a description of other forms). Its presence in the muscles described here together with earlier descriptions of it in other muscles from other animals (8, 11, 26, 27) suggests that the system, in one pattern or another, is an ubiquitous component of striated muscle.

Though the bulk of attention in this report has been given to the elements of the reticulum located in the interfibrillar sarcoplasm, note has also been taken of the fact that the fibril-free subsarcolemmic and perinuclear regions of the sarcoplasm contain the usual complement of membrane-limited structures such as centrosphere vesicles, the Golgi complex, nuclear envelopes (or perinuclear cisternae), cortical vesicles just beneath the plasma membrane, and a few other elements ascribable to the endoplasmic reticulum. These last elements are distributed without any apparent order. The peripheral sarcoplasm fails to show any extensive parallel arrays of thin, particle-studded (rough surfaced) vesicles (such as are prominent in cells engaged in protein synthesis) or other exceptional configurations. It follows that the usual local differentiations of the endoplasmic reticulum are recognizable in muscle fibers and that the interfibrillar reticulum (which represents the bulk of the entire system) does not correspond to any of the already known local differentiations. Hence this reticulum associated so intimately with the myofibrils, represents a unique and distinctive differentiation of the endoplasmic reticulum (ER) and one that characterizes the striated muscle cell. In this sense it may be regarded as the muscle cell equivalent (homologue) of such specializations of the ER as the ergastoplasm of the pancreatic acinar cell. The detailed structure and distribution of these two are, of course, very different and, whereas the sarcoplasmic reticulum is apparently associated with the fibrous elements of the cytoplasmic matrix, the reticulum of the ergastoplasm is closely allied with the small dense particles of the matrix which are known to be high in ribonucleoprotein (5, 9, 23, 41).

Relation of Sarcoplasmic Reticulum to Myofibrils

Several earlier accounts of muscle structure, in which the reticulum was identified, have noted a certain regularity between the distribution of representative elements and the structure of the myofibrils (13–15, 7, 26, 11, 27, 34, etc.). The present observations amply confirm the existence of such a segmented and repeating pattern and provide for a fuller description of the structures involved. It seems important to place some stress on this relationship between these two components of the sarcoplasm because a knowledge of its details

makes more reasonable the assumption that the reticulum is intimately involved in the process of muscular contraction.

The structural signs of the repeating pattern in the reticulum are several. It has been observed, for example, in the case of each of the muscles examined that there is a special unit of structure at the middle of the A band or, in other words, at the H band level. Another repeating detail is found in the tendency for the tubular elements of the system to be oriented longitudinally at levels opposite the distal fourths of each A band. It is, however, opposite the I band, often close to the A-I junction that the system shows its most prominent local differentiation in relation to a specific part of the sarcomere. Here, somewhat dilated terminal cisternae face similar elements belonging to the next segment of the reticulum. The space between, in the skeletal muscles studied, is uniform at 500 A and represents a discontinuity between two successive segments. Within the space there is commonly another vesicular element which makes the whole a three-component structure, here referred to as a triad. In certain muscles (*e.g.*, sartorius) a special segment of the sarcoplasmic reticulum encompasses the I band and accounts for the presence of two triads per sarcomere segment. The arrangement might be correlated to the greater length of the I bands in such muscles.

The occurrence of the I band differentiation (the triad) seems fairly widespread, as judged from these observations and from other studies already published (7, 27, 28, 34). For example, Bennett and Porter (7) directed attention to "paired masses of sarcoplasmic reticulum . . . straddling the Z band" and opposite the N bands in skeletal muscle from the domestic fowl. Edwards *et al.* (27) noted paired dilatations approximately opposite the A-I junctions in fibers from the mouse gastrocnemius and flight muscles of Periplaneta, and it seems reasonable as well to interpret the "pairs of double membranes or tubules" which Robertson (28) reported opposite the A-I junction of skeletal fibers from the lizard, *Anolis*, as representing this same triadic differentiation. Apparently none of these studies was carried far enough to provide a picture of the finer structure of these differentiations, but their position relative to the fibril banding makes identification with the triads of the present study fairly certain.

The intermediary vesicles, possibly of considerable functional significance, are seen to vary considerably in the material of the present study. For example, in the fibers of *Amblystoma* larvae where the entire system shows a simple and possibly more primitive form, there are slender cylindrical bodies (250 A in diameter) arranged more or less parallel between the apposing terminal vesicles. The relation of these to the myofibril may be most intimate in that one end is frequently inserted in the Z band. In sartorius fibers, the elements similarly located have the same thickness (250 A) but are otherwise larger and more variable in size. In cardiac muscle, the departure from the *Amblystoma* type is

even greater. The triads usually possess a single, relatively large intermediary vesicle which bears a less constant relation to one or both of the associated terminal cisternae. Simpler arrangements, described as dyads, are frequent.

Some special significance probably attaches to the fact that these triadic differentiations are always located opposite the I band or some part of it, for in isotonic contraction it is the I band that disappears (35–37). Whether this results from the incorporation of the I band myofilaments into the A, as Huxley and Hanson have proposed (37), or from some other process, it does seem that the isotropic band is the especially "active" segment of the fibril and that associated structures must be involved in its activity.

Continuity in the Reticulum:

In the above description, continuity was repeatedly noted as a property of this or that part of the sarcoplasmic reticulum. Thus it was emphasized that the central cisterna opposite the H band is a continuous structure across the fiber mass in any one sarcomere segment of the system. The central cisterna is, in turn, continuous longitudinally with the terminal cisternae of the same sarcomere so that, in a roundabout way, these latter elements are all interconnected across the fiber even though they are not continuous laterally at their own level in the sarcomere, except perhaps in the sartorius fibers. It was also noted that along the length of the fiber the system is characterized mostly by discontinuities as defined by the spaces between opposing terminal cisternae or vesicles. There are occasionally slender tubules of the system which bypass the triadic differentiations and connect one sarcomere with the next but to judge from available evidence these must be relatively few in number and widely separated to be encountered so infrequently in the thin sections examined. Communication in terms of structural continuity appears therefore from the electron microscope evidence, to be relatively free across the fiber but more limited longitudinally.

Some of the best information available on this question of continuity comes from light microscopy of muscle done during the latter part of the last century. The microscopists of this period were able, with the aid of gold and silver impregnation procedures, to resolve sarcoplasmic structures possessing reticular form and repeating patterns of organization entirely similar to these now pictured by electron microscopy. Bennett (11), who has done the most to resurrect the earlier descriptions, ascribes to Thin (12) the first observations. Whether Thin's descriptions are to be thus interpreted or not, it seems certain that the figures of Retzius (13) and Cajal (14) which appeared a few years later represent the gross features of the system as now depicted. One of the best and most complete studies was published by Veratti in 1902 (15). He examined a wide variety of muscles from the major classes of animals and showed in excellent drawings repeating, lace-like patterns in register with the striations of the fibers. In

addition to lateral continuity within the system his figures show as well a few longitudinal connections between sarcomere segments and provide additional reasons for viewing the system as continuous throughout the whole muscle fiber.

It is important to emphasize in discussing this topic that more than continuity of membranes is being considered The sarcoplasmic reticulum, like the endoplasmic reticulum, is at least a two-component system consisting of the membrane and the material that occupies the space enclosed by this membrane. The reticulum is also a system closed towards the cytoplasmic matrix. The existence of its limiting membrane actually establishes two distinct phases in the ground substance of the cytoplasm, one surrounding the reticulum and the other enclosed in its cavities. These essential facts are not sufficiently considered by Sjöstrand and his coworkers (38–40) who, in the opinion of the authors, place undue stress on the membranes alone in their reports and in the nomenclature they employ. After using for a while the ambiguous term "double membranes" (for a critique see Palade (24)) they now propose a nomenclature (α, β, and γ cytomembranes) that is assumed to have the advantage of being "descriptive" (40). As indicated by many studies, a membrane cannot be considered as a structural cytoplasmic unit. "Independent" membranes with free edges have not, thus far, been observed in the cytoplasm. The common structural units are vesicles of various shapes and sizes. The endoplasmic reticulum is a complex of such interconnected vesicles. It seems probable that its internal phase is as important to the functioning of the system as the membrane limiting it. In any case the two must be regarded as inseparable if the integrity of the system they make up is to be maintained. It is appropriate, therefore, to use a terminology which excludes neither one from the thought that is being conveyed and which is, to some extent, descriptive of the general morphology of the system.

Relation of Reticulum with Other Cell Components

Mention has been made in the above account of observations, to connections found between the sarcoplasmic reticulum and the nuclear envelopes. These connections, which have not been studied beyond the point of simple recognition, involve continuity of the outer membrane of the nuclear envelope with that of the sarcoplasmic reticulum and consequently continuity between the enclosed cavities of each. In all this, then, the muscle cell seems to be similar to other tissue cells studied (6, 25).

The elements of the reticulum are surrounded in the interfibrillar sarcoplasm by varying numbers of small particles which, in their general morphology, are similar to those found in basophil cytoplasm and have been shown, in the case of liver and pancreas, to consist of ribonucleoprotein (23, 41). In the sarcoplasm, however, these dense particles are distinguished, as already noted (5),

by wider variation in size and shape and by the fact that they are not attached to the limiting membrane of the reticulum, as they are in many cell types, primarily in glandular cells with a high protein output. The sarcoplasmic reticulum appears, therefore, to be essentially free of attached particles and its close relationship with the latter may be due to the crowding of all non-fibrillar components of the cytoplasm into the narrow spaces that separate the myofibrils.

In the peripheral and perinuclear sarcoplasm, similar particles occur in clusters usually free of visible connections with the endoplasmic reticulum and only occasionally elements bearing attached particles are encountered. It can be concluded that in general the association of the endoplasmic reticulum with particles does not express directly a major function of this system and that for muscle function, especially, this association is not important. The sarcoplasmic particles may consist of ribonucleoprotein, as suggested by evidence obtained on other cell types, but their final chemical characterization should await their isolation from muscle tissue specifically.

The association of the ER with the cell membrane is similar to that described in many other cell types (*cf*, 24) and has comparable functional implications to be discussed in the next section. In the special case of muscle fibers, this association may explain the "connection" repeatedly described in light microscopy between the Z bands and the sarcolemma. The electron micrographs show that connections are actually established with the ER elements located at the level of the Z band, not with the Z band proper. They appear to be responsible, at least in the myocardium, for the deep infoldings of the cell membrane facing each Z line in contracted fibers.

Function:

There is unfortunately little in the nature of direct observation or experimental results to guide one in defining the functions of the sarcoplasmic reticulum, thus what follows will be largely speculative. That some functional relationship exists between the reticulum and the myofibrils is indicated, as mentioned earlier, by the close structural association of the two. In assigning functions to the reticulum, one is therefore naturally influenced by the requirements of the myofibrils for metabolites and appropriate excitation.

The morphological features that may be considered of importance for the general function of the endoplasmic reticulum are: the presence of two components (a limiting membrane and a content) in its construction; its continuity throughout the cell body, and its connections with the perinuclear cisternae at the inner limit of the cytoplasm and with the cell membrane at its outer margin. In muscle fibers, as in other cell types, the connections with the nuclear envelope establish, in every likelihood, continuity between the content of the sarcoplasmic reticulum and that of the perinuclear cisternae. Similarly small vesicular units resulting, it appears, from pinocytic activity at the level of the cell membrane may constitute continuous or discontinuous channels of transport be-

tween the cavities of the reticulum and the extracellular spaces. Thus the probably fluid inner phase of the reticulum, seems effectively continuous from the perinuclear cisternae to the cell's environment. It may be assumed that the composition of the phase occupying the cavities of the reticulum is intermediate between the external and internal (*i.e.*, the fluid component of the cytoplasmic matrix) environment of the cell.

The structural features and relations described encourage two fairly obvious thoughts regarding the function of the system. One is that the inner and probably fluid phase of the system serves to channel or direct diffusion of metabolites to all parts of the cell. Where the system is continuous it may be assumed that diffusion would keep the content of this finely divided "interiorized environment" of the cytoplasm more or less uniform throughout. Components of the cytoplasmic matrix, *e.g.*, myofibrils, with special metabolic requirements might have these satisfied by a more extensive development of the associated reticulum or by the differentiation of the limiting membrane to give regions of special permeability. In this concept, then, the reticulum is regarded as a kind of intracellular circulatory system with powers of adaptive response not unlike those possessed by its larger tissue analogue (44). In so far as diffusible materials alone provide for certain needs of the functioning myofibrils, the reticulum might be considered a good vehicle for their distribution.

In a second possible function to be ascribed to the system, the limiting membrane serves as an intracellular conductor. It was reasoned just above that the content of the reticulum might be kept similar to the extracellular environment by a brigade of pinocytic vesicles working across the peripheral or outer layer of cytoplasm. From this it would follow that the membrane limiting the reticulum separates the cytoplasmic matrix from a mixture and concentration of ions comparable to that in the external environment. In this role, then, it would resemble the plasma membrane limiting the cell and might be regarded as having similar structural and permeability properties. If now membrane potentials are maintained across the plasma membrane by selective membrane permeability or some other mechanism energized by metabolism within the cytoplasmic matrix, it is not unreasonable to assume that in a similar manner potentials are maintained across the membrane-limiting elements of the reticulum. Under this concept a wave of depolarization of the sarcolemma might be picked up by or transmitted to the sarcoplasmic reticulum and thence spread rapidly as an action potential along its limiting membrane to all parts of the sarcomere. Presumably lateral conduction would be along that part of the reticulum opposite the H band, thence along the longitudinal elements, where these are present, to the discontinuities in the triads opposite the I or Z bands. It is difficult to guess what events this might set in motion and observations on fibers in contraction are too meager thus far to provide any hints. Whatever equilibria are disturbed the series of events initiated may be assumed to lead to the contraction of the adjacent myofibril. Possibly a depolarization of the

membranes at the triad leads to a discharge or breakdown of the intermediary vesicles, and thus to the release of a trigger substance for the contraction of the myofibrils.

The two suggested functions for the sarcoplasmic reticulum are not mutually exclusive except in so far as they are both regarded as responsible for transmitting the excitatory impulse. For this the channeled diffusion which the system might provide would be inadequate for the same reasons that A. V. Hill (42) discarded the idea of any diffusible substance being responsible, namely that the rate of diffusion is too slow. He suggested, therefore, as one possibility that the stimulus must be transmitted throughout the matrix of the fiber to the myofibrils, especially the deeper ones, at much the same rate as over the sarcolemma. The sarcoplasmic reticulum would seem to be an ideal vehicle for this transmission. Recently, Huxley and Taylor (43), using microelectrodes and small stimuli, have provided evidence for a lateral transmission of excitation within the I band of a sarcomere and suggest that Krause's membrane (the telophragma or Z line) with its connection at the sarcolemma is responsible. The telophragma is not a membrane according to information obtained by electron microscopy and in the morphology depicted does not seem as well designed for conducting impulses as the system described in this present report. The fact that the sarcoplasmic reticulum is more developed and shows more elaborate differentiations in skeletal than in heart muscle, also favors the view that the system is more important for impulse transmission than for supplying of essential metabolites.

The idea that the sarcoplasmic reticulum might function as an internal conducting system has been suggested earlier by Bennett (11), who says that Retzius proposed it first.

Therefore, while the channels or spaces of the sarcoplasmic reticulum may provide for the diffusion of metabolites throughout the cell, its membranes would best serve as the conductor of the excitatory impulse. Presumably the changes in membrane permeability associated with depolarization, permitting the inflow and outflow of appropriate ions, would duplicate those of the plasma membrane. We are not unaware that these suggestions may be difficult to test directly but some effort will shortly be made in this direction. The minuteness of the vesicular elements of the reticulum (diameters of 1,000 to 2,000 A) would seem to preclude the use of microelectrodes in such investigations. Furthermore the introduction of any foreign bodies into the sarcoplasm would probably induce pronounced structural changes in this extraordinarily labile system. Thus some less direct approach to the problem is indicated.

SUMMARY

Several types of striated muscle have been examined by the technics of electron microscopy and the findings in myotome fibers of *Amblystoma* larvae,

the sartorius, and cardiac muscle of the rat are reported on in some detail. Particular attention has been given to structural components of the interfibrillar sarcoplasm and most especially to a finely divided, vacuolar system known as the sarcoplasmic reticulum. This consists of membrane-limited vesicles, tubules, and cisternae associated in a continuous reticular structure which forms lace-like sleeves around the myofibrils. It shows a definable organization which repeats with each sarcomere of the fiber so that the entire system is segmented in phase with the striations of the associated myofibrils. Details of these repetitive patterns are presented diagrammatically in Text-figs. 1, 2, and 3 on pages 279, 283, and 288 respectively. The system is continuous across the fiber at the H band level and largely discontinuous longitudinally because of interruptions in the structure at the I and Z band levels. The structure of the system relates it to the endoplasmic reticulum of other cell types. The precise morphological relation of the reticulum to the myofibrils, with specializations opposite the different bands, prompts the supposition that the system is functionally important in muscle contraction. In this regard it is proposed that the membrane limiting the system is polarized like the sarcolemma and that the corresponding potential difference is utilized in the intracellular distribution of the excitatory impulse.

BIBLIOGRAPHY

1. Palade, G. E., and Porter, K. R., Studies on the endoplasmic reticulum. I. Its identification in cells *in situ*, *J. Exp. Med.*, 1954, **100,** 641.
2. Palade, G. E., Studies on the endoplasmic reticulum. II. Simple dispositions in cells *in situ*, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 547.
3. Porter, K. R., Claude, A., and Fullam, E. F., A study of tissue culture cells by electron microscopy. Methods and preliminary observations, *J. Exp. Med.*, 1945, **81,** 233.
4. Porter, K. R., Observations on a submicroscopic basophilic component of cytoplasm, *J. Exp. Med.*, 1953, **97,** 727.
5. Palade, G. E., A small particulate component of the cytoplasm, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 59.
6. Watson, M. L., The nuclear envelope, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 257.
7. Bennett, H. S., and Porter, K. R., An electron microscope study of sectioned breast muscle of the domestic fowl, *Am. J. Anat.*, 1953, **93,** 61.
8. Edwards, G. A., and Ruska, H., The function and metabolism of certain muscles in relation to their structure, *Quart. J. Micr. Sc.*, 1955, **96,** 151.
9. Porter, K. R., Electron microscopy of basophilic components of cytoplasm, *J. Histochem. and Cytochem.*, 1954, **2,** 346.
10. Porter, K. R., The sarcoplasmic reticulum in muscle cells of *Amblystoma* larvae, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 163.
11. Bennett, H. S., Modern concepts of structure of striated muscle, *Am. J. Phys. Med.*, 1955, **34,** 46.

12. Thin, G., On the minute anatomy of muscle and tendon, and some notes regarding the structure of the cornea, *Edinburgh Med. J.*, 1874, **20,** pt. 1, 238.
13. Retzius, G., Zur Kenntniss der quergestreiften Muskelfaser, *Biol. Untersuch.*, 1881, **1,** series 1, 1.
14. Cajal, S. R., Observations sur la texture des fibres musculaires des pattes et des ailes des insectes, *Internat. Monatschr. Anat. u. Physiol.*, 1888, **5,** 205.
15. Veratti, E., Richercha sulla fine structura della fibra muscolare striata, *Mem. r. Inst. Lombardo sc. e lettere*, 1902, **19,** 87.
16. Hall, C. E., Jakus, M. A., and Schmitt, F. O., An investigation of cross striations and myosin filaments in muscle, *Biol. Bull.*, 1946, **90,** 32.
17. Huxley, H. E., Electron microscope studies of the organization of the filaments in striated muscle, *Biochim. et Biophysica Acta*, 1953, **12,** 387.
18. Hodge, A. J., The fine structure of striated muscle. A comparison of insect flight muscle with vertebrate and invertebrate muscle, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 361; 1956, **2,** No. 4, suppl., 131.
19. Palade, G. E., Electron microscopy of mitochondria and other cytoplasmic structures, *in* Enzymes: Units of Biological Structure and Function, (O. H. Gabler, editor), New York, Academic Press Inc., 1956, 185.
20. Hodge, A. J., Huxley, H. E., and Spiro, D., Electron microscope studies on ultrathin sections of muscle, *J. Exp. Med.*, 1954, **99,** 201.
21. Weinreb, S., and Harman, J. W., Phase and electron microscope studies of the interrelationship of cytochondria and myofibrils in pigeon breast muscle, *J. Exp. Med.*, 1955, **101,** 529.
22. Harman, J. W., The cytochondrion of cardiac and skeletal muscle, *Internat. Rev. Cytol.*, 1956, **5,** 89.
23. Palade, G. E., and Siekevitz, P., Liver microsomes. An integrated morphological and biochemical study, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171.
24. Palade, G. E., The endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.* 1956, **2,** No. 4, suppl., 85.
25. Hartmann, J. F., An electron optical study of sections of central nervous system, *J. Comp. Neurol.*, 1953, **99,** 201.
26. Ruska, H., Elektronenmikroskopischer Beitrag zur Histologie des Skelettmuskels kleiner Säugetiere, *Z. Naturforsch.*, 1954, **9b,** 358.
27. Edwards, G. A., Ruska, H., Souza-Santos, P., and Vallejo-Freire, A., Comparative cytophysiology of striated muscle with special reference to the role of the endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 143.
28. Robertson, J. D., Some features of the ultrastructure of reptilian skeletal muscle, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 369.
29. Dalton, A. J., and Felix, Marie D., A comparative study of the Golgi complex, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 79.
30. Beams, H. W., Evans, T. C., Janney, C. D., and Baker, W. W., Electron microscopic studies on the structure of cardiac muscle, *Anat. Rec.*, 1949, **105,** 59.
31. Kisch, B., and Bardet, J. M., Electron-histology of the heart, *Exp. Med. and Surg.*, 1951, **9,** 1.

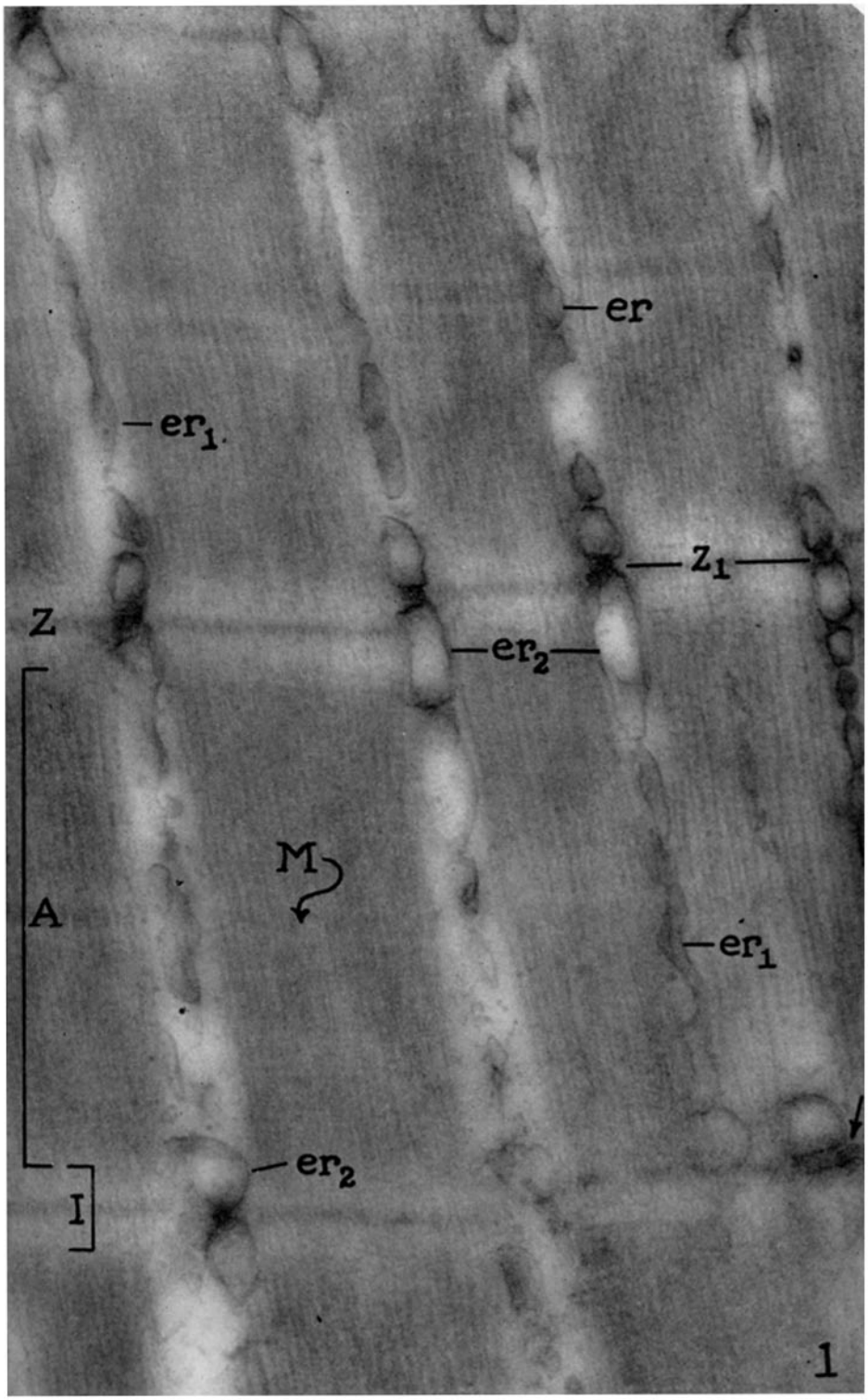
32. Price, K. C., Weiss, J. M., Hata, D., and Smith, J. E., Experimental myocardium of dogs with particular reference to histologic study by electron microscopy, *J. Exp. Med.*, 1955, **101,** 687.
33. Kisch, B., A vital part of the heart muscle. The sarcosomes of the heart, *Exp. Med. and Surg.*, 1952, **10,** 208.
34. Weinstein, H. J., An electron microscope study of cardiac muscle, *Exp. Cell Research,* 1954, **7,** 130.
35. Huxley, A. F., and Niedergerke, R., Structural changes in muscle during contraction; interference microscopy of living muscle fibers, *Nature,* 1954, **173,** 971.
36. Knappeis, G. G., and Carlsen, F., Electron microscopical study of skeletal muscle during isotonic (afterload) and isometric contraction, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 201.
37. Huxley, H. E., and Hanson, J., Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation, *Nature,* 1954, **173,** 973.
38. Sjöstrand, F. S., and Rhodin, J., The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy, *Exp. Cell Research,* 1953, **4,** 426.
39. Sjöstrand, F. S., and Hanzon, V., Membrane structures of cytoplasm and mitochondria in exocrine cells of mouse pancreas as revealed by high resolution electron microscopy, *Exp. Cell Research,* 1954, **7,** 393.
40. Sjöstrand, F. S., Electron microscopy of cells and tissues, *in* Physical Techniques in Biological Research, Volume 3, (G. Oster and A. W. Pollister, editors) New York, Academic Press Inc., 1956, 241.
41. Palade, G. E., and Siekevitz, P., Pancreatic microsomes, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 671.
42. Hill, A. V., The abrupt transition from rest to activity in muscle, *Proc. Roy. Soc. London, Series B,* 1949, **136,** 399.
43. Huxley, A. F., and Taylor, R. E., Function of Krause's membrane, *Nature,* 1955, **176,** 1068.
44. Porter, K. R., The submicroscopic morphology of protoplasm, *Harvey Lectures,* New York, Academic Press Inc., 1956, 175.

EXPLANATION OF PLATES

PLATE 85

FIG. 1. Micrograph of a longitudinal section through a muscle fiber contained in a caudal myotome of a 15 mm. *Amblystoma* larva. Myofibrils which run from top to bottom of the image fill most of the field. The bands A, I, and Z are so identified. The slightly lighter band bisecting the A band contains within its limits a dense stripe which is designated as the M band.

The membrane-limited structures between the myofibrils are regarded as portions of the sarcoplasmic reticulum. Some are always present opposite the H band (*er*), which suggests that at this level the system is continuous around and among the fibrils and across the fiber. Less constantly there are elongate profiles running from the H band toward the Z, as at er_1. These connect frequently with somewhat dilated vesicles located opposite the I band on one side of the Z (er_2). Two such vesicles seem to face off opposite the Z band and the material between as well as the opposing membranes are more dense and thicker than elsewhere (see Z_1). Where the section through this region is thinner or otherwise less dense it is possible to see minute small vesicular elements in the intervening space (arrow, lower right). In no instance in this micrograph does a profile of the sarcoplasmic reticulum continue across the Z band level into the next sarcomere. It appears therefore that the sarcoplasmic reticulum is segmented and that the segments coincide precisely with the sarcomeres of the fibrils. × 48,000.



(Porter and Palade: Studies on the endoplasmic reticulum. III)

PLATE 86

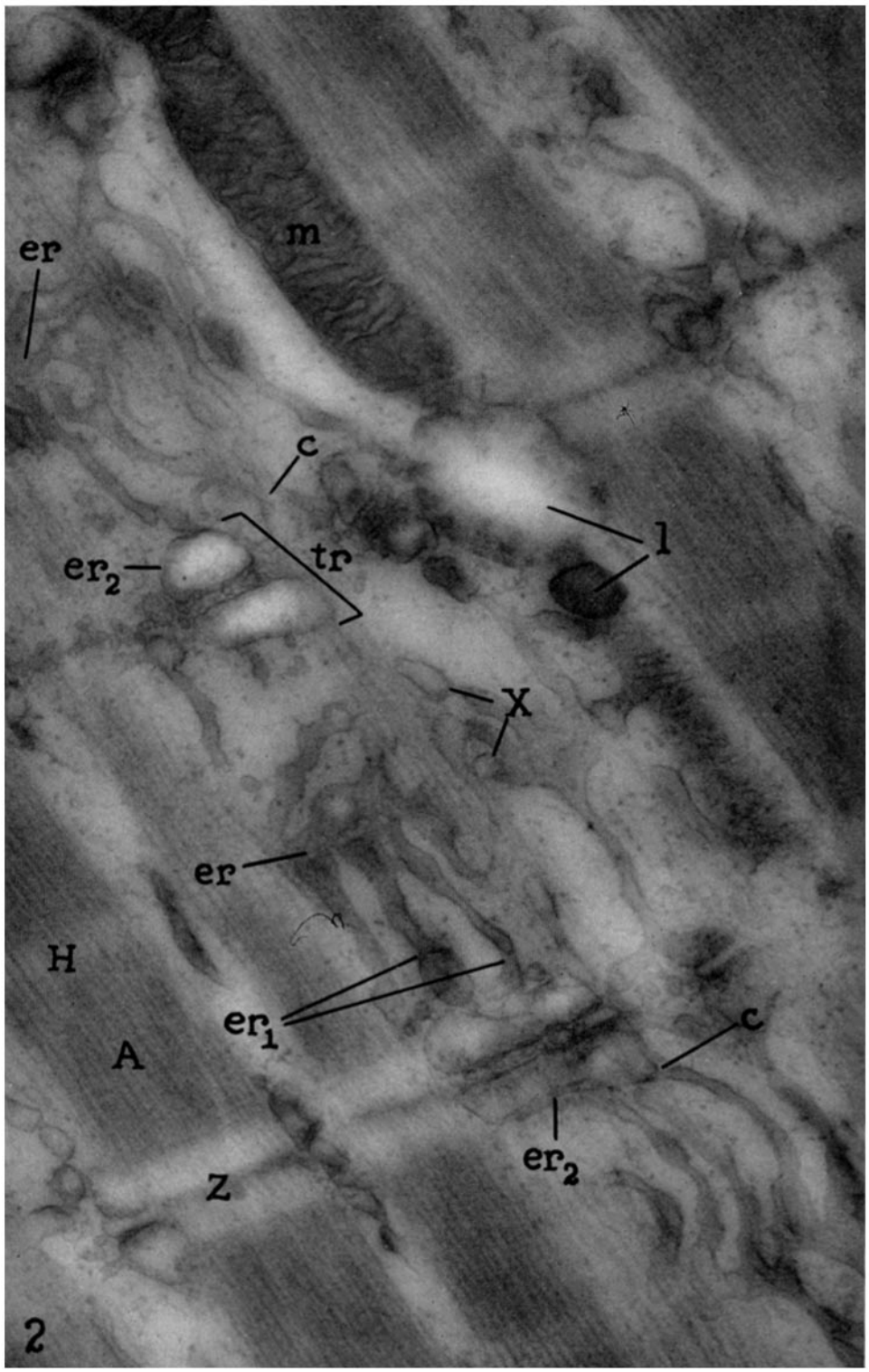
FIG. 2. A micrograph of a longitudinal section (same material as shown in Fig. 1) which includes in the plane of section a relatively broad expanse of the sarcoplasm between two myofibrils. The reticulum, as part of this sarcoplasm and thus caught within the section, is represented by the lacework of strands (canaliculi) and vesicles passing diagonally across the center of the figure.

This reticulum is organized with respect to any one sarcomere of a fibril in the following manner. Running along the A band and roughly paralleling the fibril axis are a number of canaliculi designated er_1. They are depicted in oblique or cross-section at *X*. At the H band level these run together into a common sinus or central cisterna (*er*) which is continuous circumferentially around and among the fibrils (sections through this cisternal unit are depicted in profile opposite the H band —at *er*— in Fig. 1). In the other direction, *i.e.*, toward the I bands or ends of the sarcomere, the longitudinally oriented canaliculi (er_1) are continuous at *c* with dilated vesicles (er_2) which have their long axes oriented transversely or circumferentially with respect to the fibrils. This general pattern is repeated in the two adjacent sarcomeres. Thus a sarcomere of this muscle, considered singly, can be said to have around it a bracelet of complex design but similar to that surrounding every other fibril sarcomere (Text-fig. 1). Considered as a whole, the sarcoplasmic reticulum is made up of segments which coincide in length and distribution with the sarcomeres (major bands) of the muscle fiber.

At the boundary between the sarcomeres, *i.e.*, at the I and Z bands, the dilated vesicle (terminal cisternae) of one sarcomere face equivalents in the next and such equivalents are essentially mirror images. The opposing faces are flattened and the membranes seem thickened (see at *tr*). A fairly uniform space (500 A) is maintained between the opposing faces of these vesicles and within the space are profiles of smaller vesicles. The two large opposing units and the intervening space, with smaller vesicular units contained, will be referred to as a triad (*tr*).

In general, the content of the reticulum is not more dense than the surrounding matrix of the sarcoplasm except along the inside surface of the opposing membranes in the triad where there appears to be a condensation of finely fibrous material.

Small circular areas of lower density, 200 A in diameter, are usually present in the wall of the central cisternae at the H band level. They may represent pores or thin places in the membrane (see center Fig. 2). A mitochondrion is indicated at *m* and lipide inclusions (one exploded) at *l*. × 48,000.



(Porter and Palade: Studies on the endoplasmic reticulum. III)

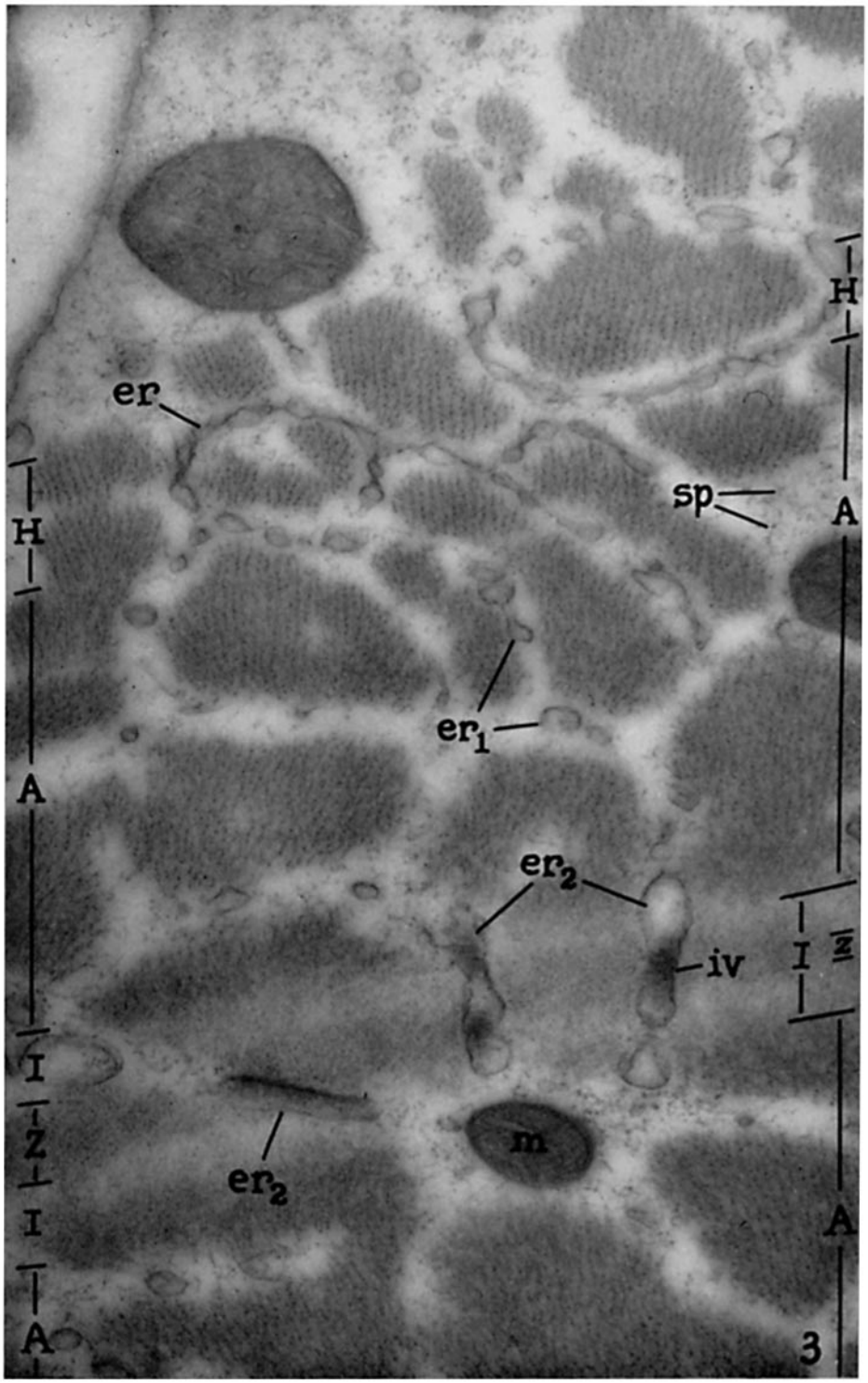
PLATE 87

FIG. 3. Transverse (but slightly oblique) section of part of a muscle fiber (material same as for Fig. 1). The obliquity of the section provides cross-sectional views of the fibrils at several band levels in adjacent sarcomeres. The limits of these bands are indicated by appropriate letters along the margins of the micrograph. The sarcolemma runs across the upper left.

The myofilaments are thickest and most clearly resolved at the H band, less distinct in the denser portions of the A band, and so fine and so disordered as to be invisible in the I and Z bands.

The sarcoplasmic or extrafibrillar material of the cell contains a few mitochondria (*m*) and a scattering of small particulates (*sp*), besides numerous profiles of the reticulum. The uneven distribution and morphology of these latter could be predicted from the preceding views (Figs. 1 and 2) of longitudinal sections. The number present at the H band level (*er*) forms a continuous chain and hence a membrane surface across this part of the micrograph. The fewer profiles evident in the adjacent part of the A band (er_1) are descriptive of the few longitudinal elements at this level. The oblong outlines and structures (er_2) at the I band represent top (or bottom) views of the larger, paired, I band vesicles of the system (also called terminal cisternae) which, with the small intermediary bodies, form a triad of vesicular units opposite this part of the fibril. It is to be noted that these I band vesicles do not form rings around the fibrils or anything like the continuous chain of vesicles apparent at the H band level.

Minute, dense, elongate bodies oriented across the long axis of the I band vesicles are the small intermediary vesicles (*iv*) seen in other aspect in Figs. 2 to 8. × 44,000.

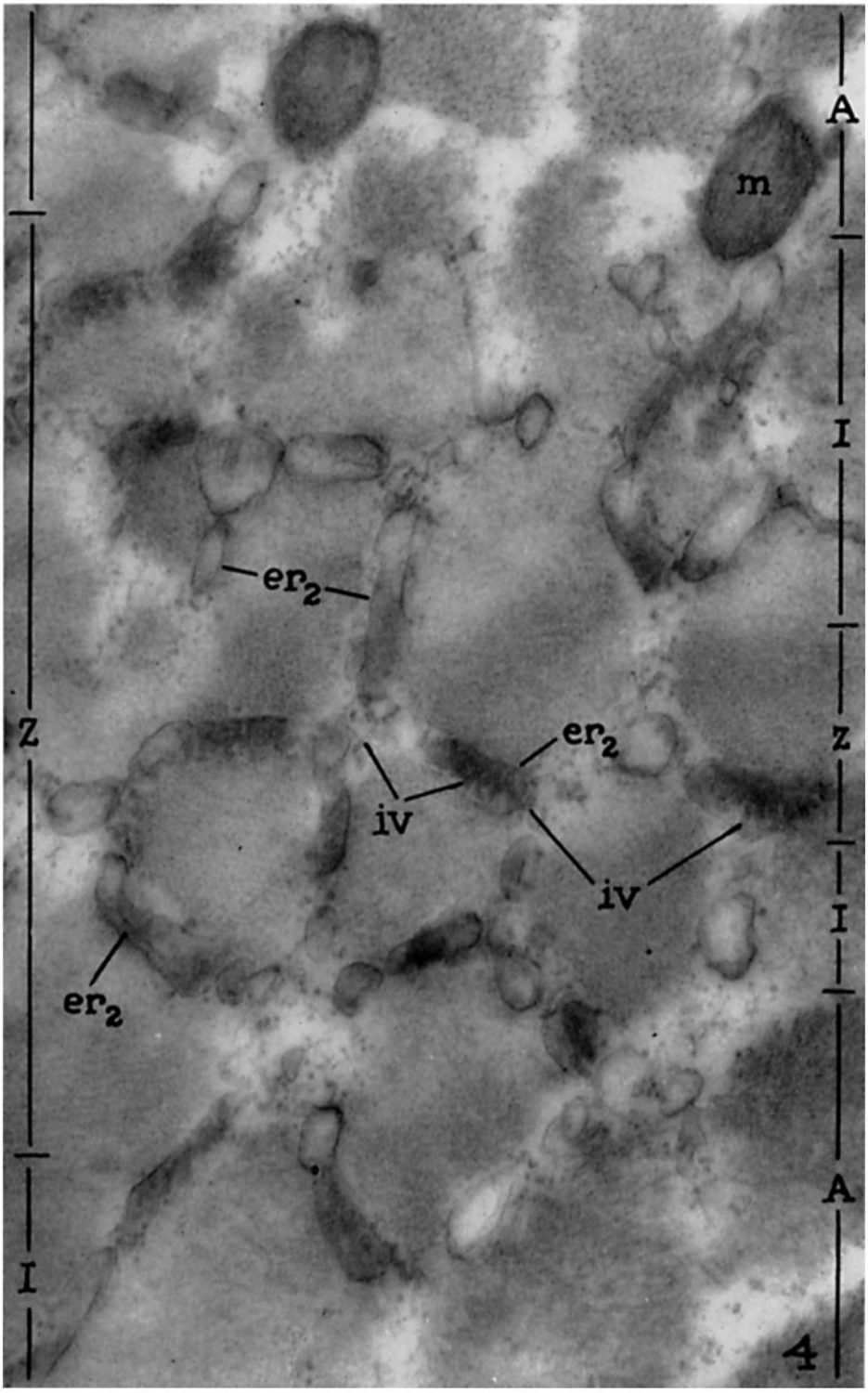


(Porter and Palade: Studies on the endoplasmic reticulum. III)

PLATE 88

FIG. 4. A cross-section of the central portion of a muscle fiber in which the plane of section and a large part of the field coincides with the Z band. Limits of this and other (A, I) bands are indicated along the sides of the figure. Profiles of numerous I band vesicles (er_2) of the sarcoplasmic reticulum are included in the section. The circumferential length of these ($\sim$ 0.5 μ) is greater than their radial dimension by a factor of 3 or 4. They are not long enough, however, to form complete rings around the myofibrils and as a consequence the sarcoplasmic reticulum is not continuous across the fiber at this level.

In some instances the section includes the space between the two opposing I band vesicles (Z_1 in Fig. 1) and so shows the intermediary vesicles (*iv*). These small finger-like bodies are fairly uniform in diameter ($\sim$ 200 A) but of varying lengths. They tend to have their long axes oriented radially with respect to the adjacent myofibrils or, in other words, normal to the long axes of the I band vesicles above and below. Along one side of the arrays the ends of the *iv's* are essentially in line, whereas across the other side, possibly representing their free ends the opposite is true. An occasional *iv* on this latter side intrudes among the myofilaments at the margin of the Z band. It thus appears that the arrays of these bodies are polarized relative to the contiguous myofibrils. A few bodies of similar character lie free in the sarcoplasm among the small dense particles which, in this material, are not attached to the membranous surfaces of the reticulum. A mitochondrial profile is designated by *m*. $\times$ 44,000.



(Porter and Palade: Studies on the endoplasmic reticulum. III)

PLATE 89

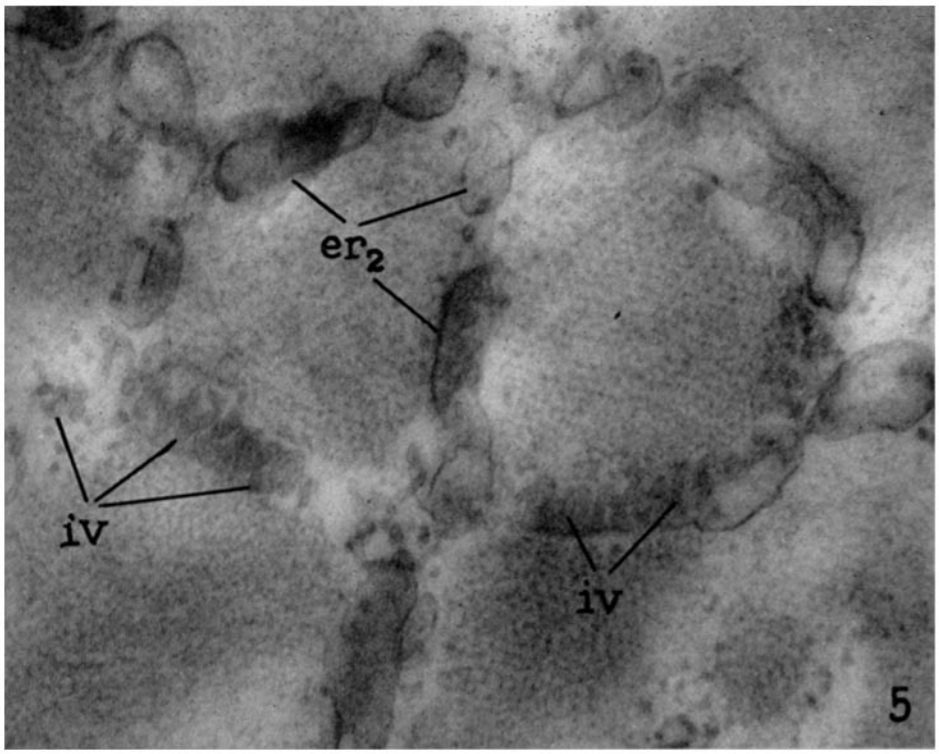
FIG. 5. Greater enlargement of a small portion of Fig. 4, to show to better advantage the interrelationship of the reticulum and the myofibrils at the Z band level.

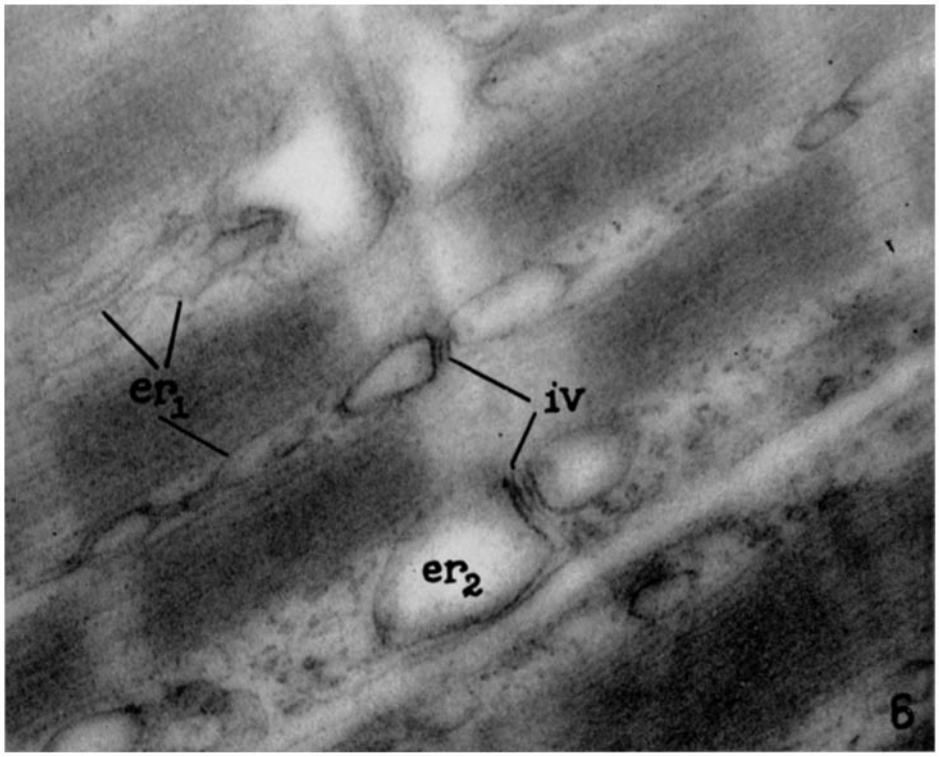
The cross-hatched appearance of certain areas in the figure describes the fine organization of the myofilaments in the Z band. Outside and around these areas are several profiles of I band vesicles (er_2). In three places in this image, the section includes arrays of intermediary vesicles (*iv*) which lie in the space between the I band vesicles (see Fig. 6) and opposite the Z band. Thus a top view of their arrangement is provided and comments on this included in legend for Fig. 4 apply here as well. Similar small finger-like bodies are present also outside the intervesicular space and free in the surrounding sarcoplasm. ×70,000.

FIG. 6. Micrograph of a longitudinal section of myofibrils (same *Amblystoma* larval muscle) designed to show profile of I band vesicles (er_2) and side view of intermediary vesicles (*iv*). For cross-sections of same see Fig. 7.

In this aspect the intermediary vesicles appear as slender tubular structures with long axis normal to that of myofibril. It is possible that the lower *iv* in the figure represents the major portion of one such body. Both ends are free. One is seen to be in close contact with the myofibril; the other—not here but in some cases—appears to be continuous with one or the other face of the opposing vesicle surfaces. Note the apparent thickened character of the membranes constituting these latter surfaces.

The myofibrils, running across the image from lower left to upper right, have extraordinarily light Z bands as though stretched. Profiles of longitudinally oriented tubules of sarcoplasmic reticulum are indicated at er_1 (see also Fig. 2). × 70,000.





(Porter and Palade: Studies on the endoplasmic reticulum. III)

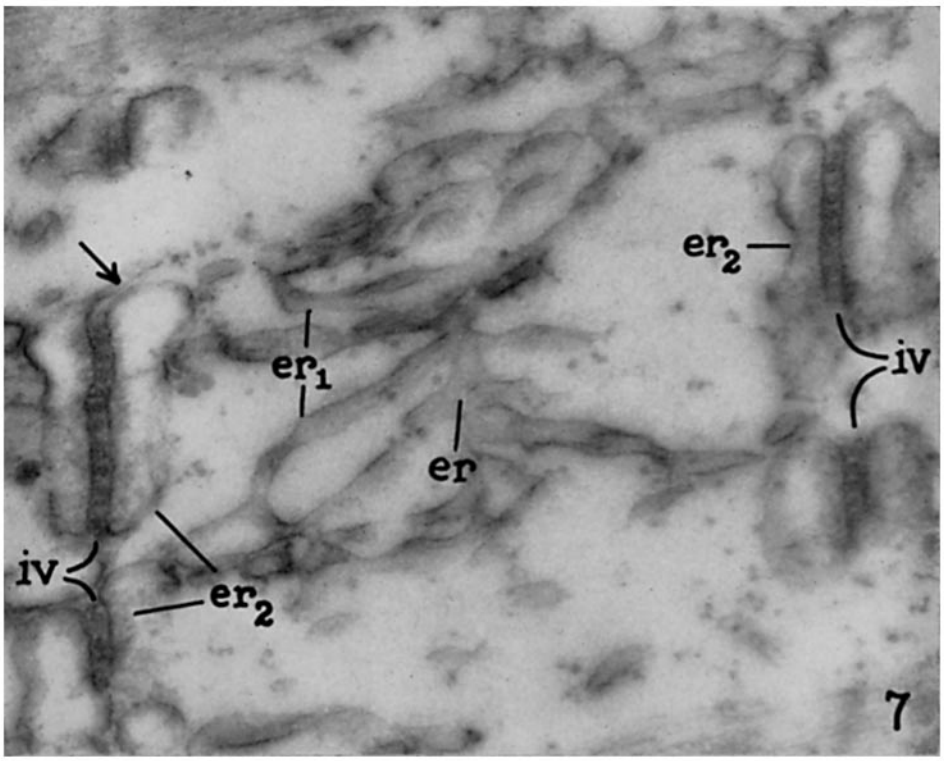
PLATE 90

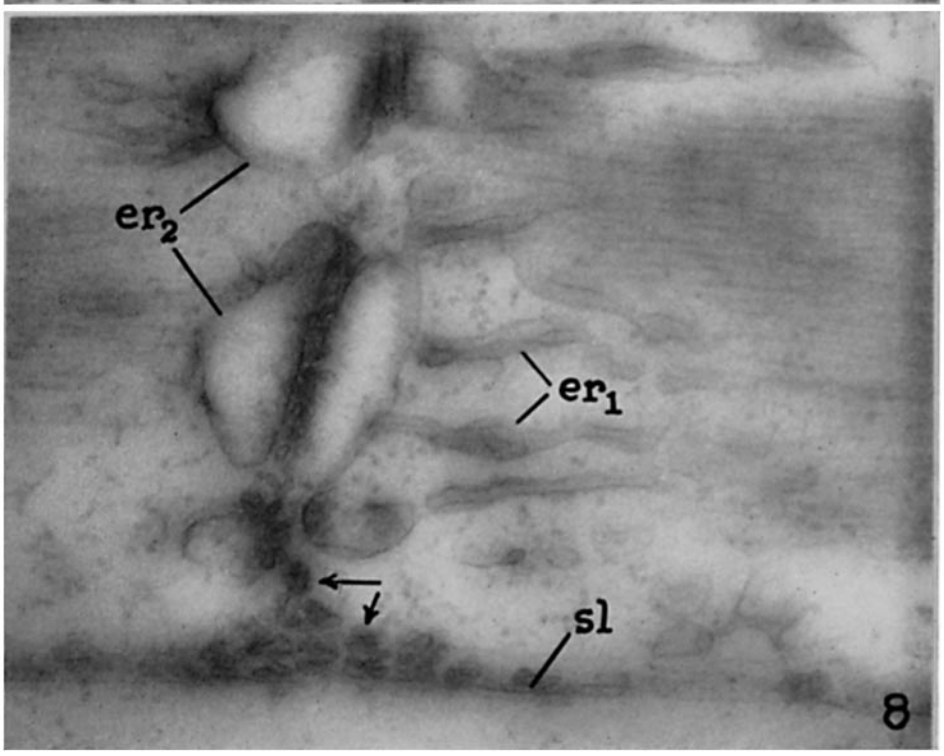
FIG. 7. Micrograph of an especially thin, longitudinal section of larval muscle, designed to show (1) the intermediary vesicles cut transversely and (2) their distribution between the I band vesicles. The various parts of a single sarcomere segment of the reticulum are labelled as before. The midsarcomere cisterna is at *er*, longitudinal tubular extensions at er_1, and dilated ends, or I band vesicles, at er_2. These latter face off as opposing vesicles of similar dimensions across a space of about 500 A. This contains numerous intermediary vesicles (*iv*) which, depicted as here in cross-section, appear round or ovoid and 200 A in diameter. They are evenly distributed along the length of the space. The opposing vesicular membranes which limit the space seem somewhat thickened and more prominent than the membrane limiting the rest of the vesicle, but this may reflect only the presence of some finely fibrous material along their internal surface.

A slender extension from one I band vesicle (at arrow) seems to traverse the Z band level and extend on into the next sarcomere. Such extensions from one sarcomere segment into another are apparently rare in this material, for this is the only definite instance encountered in numerous fields recorded.

Small particles present in the sarcoplasm seem to show no special affinity for the reticulum surface. × 63,000.

FIG. 8. Micrograph of a longitudinal section of larval muscle, depicting a relationship encountered between the sarcoplasmic reticulum and the sarcolemma (*sl*). Longitudinally oriented canaliculi of the reticulum are shown at er_1 and dilated terminal vesicles at er_2. The picture contains profiles of two typical triadic arrangements of such vesicles at the I and Z band levels. Extending from this level toward the sarcolemma there is a continuous bridge of these small vesicles which spreads out over the inside surface of the sarcolemma (arrows). They look not unlike intermediary vesicles. The suggestion is that this formation represents a stream or migration of such bodies to or from the sarcolemma. Other features of the micrograph are covered in other legends. × 65,000.





(Porter and Palade: Studies on the endoplasmic reticulum. III)

PLATE 91

FIG. 9. Micrograph showing a small portion of a longitudinal section of a rat sartorius muscle. *The myofibrils*, mostly cut medially, cross the figure along the short dimension. A light I band, bisected by a dense Z band, runs from upper left to lower right. A faint N band can be discerned in both halves of the I band and midway between Z and A. An equally indistinct H band bisects the A of the sarcomere on the left (*H*). The various bands are very nearly in perfect register across the fiber (top to bottom of figure) and their appearance characterizes the fiber as uncontracted at this level.

It is to be noted that the I band in this muscle is much longer relative to the A than in the *Amblystoma* larval muscle. It does in fact exceed the A by 1.2 times, whereas in the myotome fibrils the I was only a fraction (¼) the length of the A.

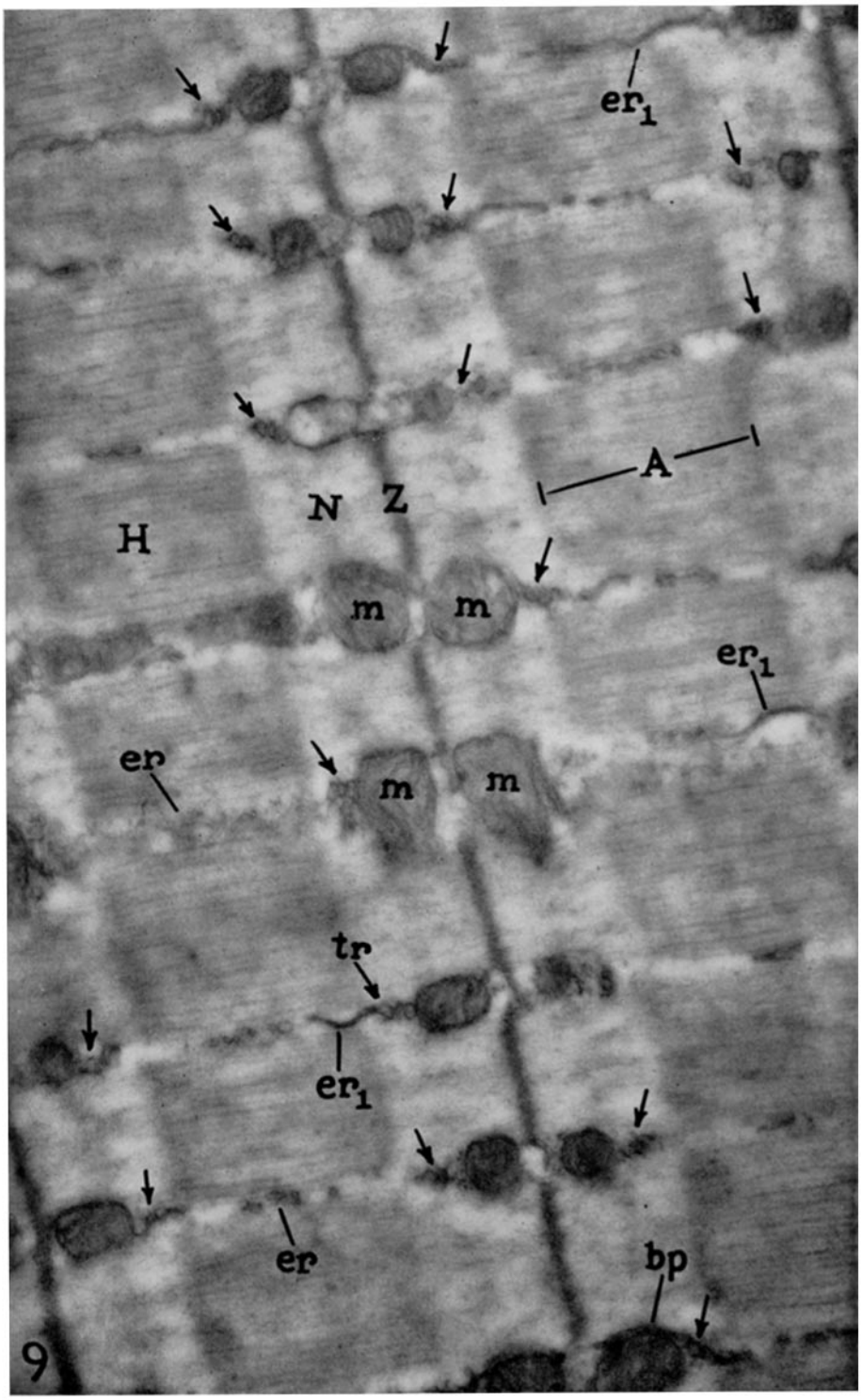
The myofilaments are evident in the A band but lost in confusion in the I band.

The sarcosomes, or mitochondria, (*m*) show in transverse section as paired bodies close to but on opposite sides of the Z band. Their long axes are oriented transversely with respect to the myofibrils. Only rarely are sections of the sarcosomes seen in the sarcoplasm opposite the A bands.

The sarcoplasmic reticulum is more finely divided than in the *Amblystoma* material but shows in its form and organization some of the same features. Small circular or ovoid profiles, uniformly present in some concentration opposite the H bands (see *er*) indicate the existence of a continuous structure at this level. Where the plane of section cuts this concentration somewhat obliquely (as at *er*, left center, and also right center) individual elements of the reticulum can be made out. From this level extending in both directions towards the I band, the section occasionally shows a profile of a longitudinally oriented tubule (see er_1 in three places). These end in a dilatation, as in Fig. 1, except that here this enlarged tip is just within the margin of the I band (arrows). From micrographs showing the system in other aspects (see Fig. 10) it has been learned that these dilatations represent cross-sections of long slender vesicles running circumferentially with respect to the myofibrils. It may be noted further (at arrows) that such dilated ends are only one of three profiles of apparently vesicular bodies in the same region of the sarcoplasm and that identical arrangements or triads are present in the same location in almost every instance (*tr* and arrows). If they were always present, then the structure represented would be regarded as continuous across the fiber at this level. They seem, however, not to be there in every case, hence the continuity must, in places, be interrupted.

The vesicle on the Z band side of the triad coincides in its dimensions with its opposite and in its turn is continuous with a reticulum (of small canaliculi constituting a fenestrated cisterna) which extends to the triad at the other end of the I band (see Text-fig. 2). Where sarcosomes are present at this level, the reticulum simply bypasses them on one or both sides (see at *bp*). It appears, from available evidence, that this I band portion of the reticulum is continuous across the Z line.

The two larger vesicles of the triad have between them a third vesicular element which corresponds to the intermediary vesicles seen in larval muscle. Here, in the sartorius, however, it seems not to be as finely divided as in the amphibian material. The individual units are of uniform thickness (200 A) but vary some in their longest dimension circumferential to the myofibrils. × 36,000.



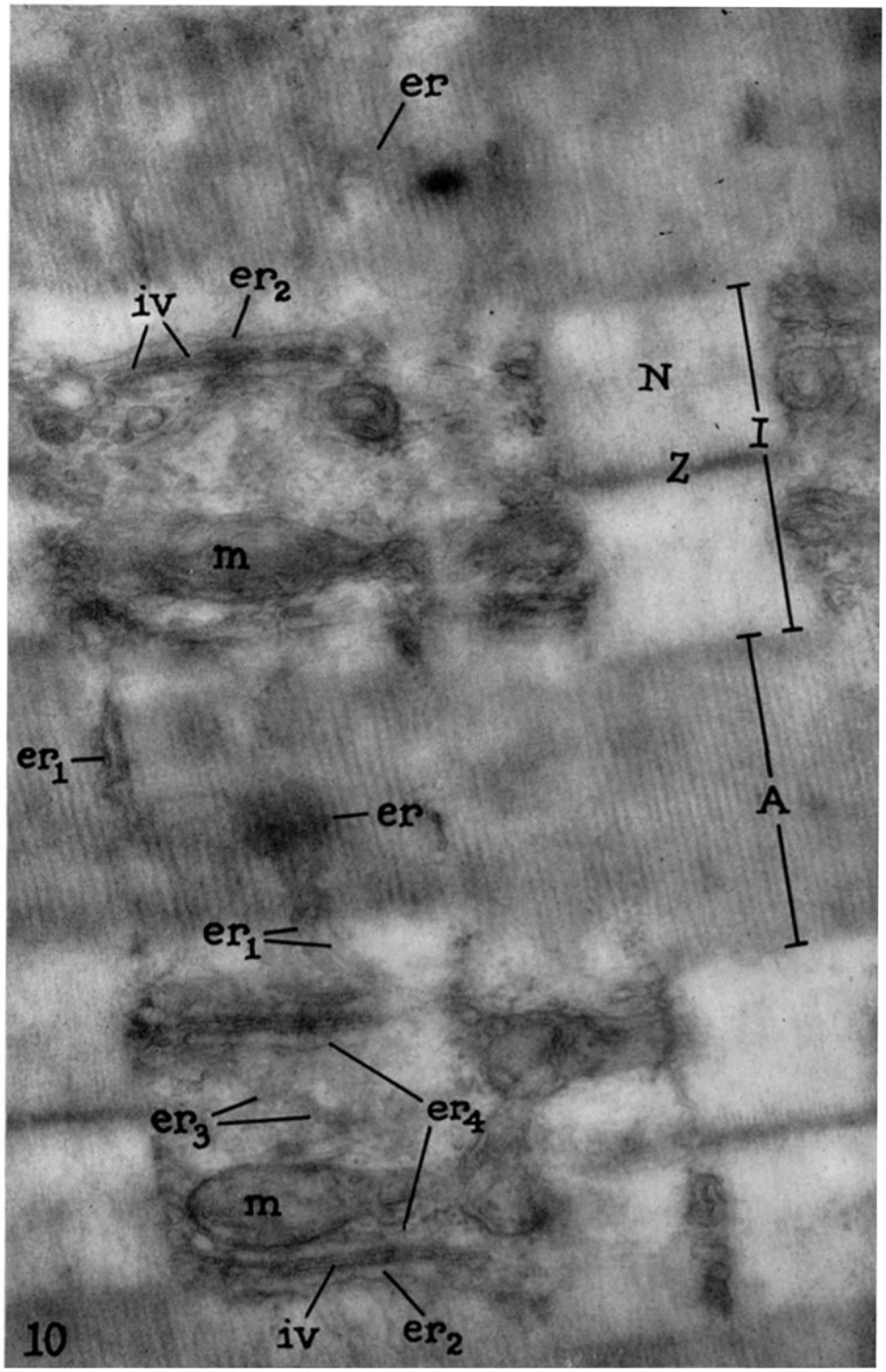
(Porter and Palade: Studies on the endoplasmic reticulum. III)

PLATE 92

FIG. 10. This image of a longitudinal section of rat sartorius supplements Fig. 9, in showing a portion of the sarcoplasmic reticulum in face view as it spreads over the surface of a myofibril (view comparable to that in Fig. 2). Since it is spread two-dimensionally in a curved plane, only fragments of it are present in a flat section.

Myofibrils and direction of orientation are obvious. A, I, Z, and N bands are indicated. *Sarcosomes* are marked *m*.

Er, as before, points to outlines of central cisternae found at H band levels of the sarcomeres. Canalicular elements extend from these longitudinally (see er_1) to transverse or circumferentially oriented elements, er_2. These are paired with similar elements, er_4, on the opposite side of a well defined space of uniform width (500 A). This latter space contains small elongate vesicles (*iv*) apparently unattached (in the material studied) to the two larger elements on either side. The whole is termed a triad. On the Z band side, er_4 is continuous with a compact reticulum of tubules (er_3) which extends across the Z band to the other end of the I band. Here it is continuous with a transversely oriented vesicle (er_4). Fine tubular elements and dense granules, apparently free in the sarcoplasm at the Z band level, resemble free elements at the same level in *Amblystoma* material. × 48,000.



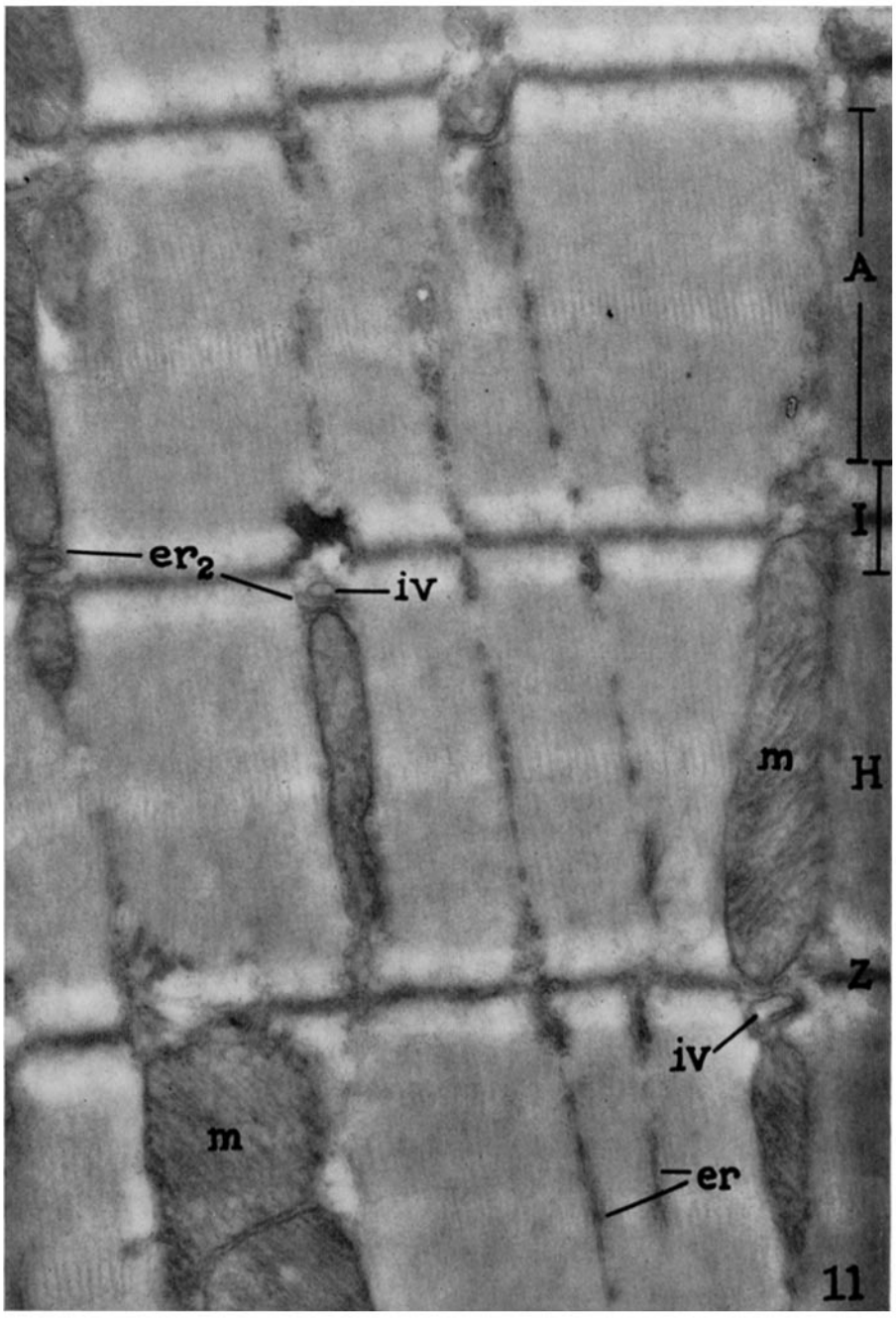
(Porter and Palade: Studies on the endoplasmic reticulum. III)

PLATE 93

FIG. 11. Micrograph showing a longitudinal section of rat cardiac muscle at rest length. The gross striations of the myofibrils are indicated on the right hand margin of the figure. Myofilaments are evident in the A bands but are characteristically less distinct in the I's. An anastomosis of fibrils is depicted at the lower margin of the micrograph.

The sarcosomes (*m*) appear to vary considerably in diameter but this is more a reflection of the off-center relation of the plane of section to the medial axis of the cylindrical organelle than to any inherent variation in diameter. Where the surface of the mitochondrion is normal to the plane of section a clearly defined double membrane is evident in the images. The inner of these two is continuous with the membrane-limited cristae which fill the mitochondria and which, in this case, are oriented obliquely to the long axis of the organelle.

In regions where the section cuts the myofibrils more or less medially, profile images of small interfibrillar elements are provided. Examined carefully these are seen to be line-limited, and descriptive of sections through slender vesicular or canalicular elements (*er*). They are distributed quite evenly along the length of the sarcomere and opposite the I bands connect with larger and more transversely oriented profiles of the same nature (er_2). These frequently occur in pairs (dyads), one sometimes partly surrounding the other (as at er_2), and about as frequently in threes (triads), as near Z. The central profile of the three, or the partially enclosed profile of the dyad, is referred to as the intermediary vesicle (*iv*) × 32,000.



(Porter and Palade: Studies on the endoplasmic reticulum. III)

PLATE 94

FIG. 12. Micrograph of a small portion of cardiac muscle section designed to show at higher magnification the so called dyad associations of vesicles found at the I-Z band levels in these cells. The muscle depicted is contracted. At *iv* a large intermediary vesicle is shown in section enwrapped by a thinner vesicular element. This latter seems in turn to be continuous with a canalicular unit of the general sarcoplasmic reticulum. This paired association (constituting a dyad) is repeated in the upper left. × 43,000.

FIG. 13. Micrograph of a portion of a longitudinal section of a cardiac fiber. The margin of the cell at the lower left is limited by the sarcolemma consisting of a dense line (representing the plasma membrane *pm*), a less dense layer of uniform thickness external to the *pm* and called the cuticular layer[5] (*cu*), and further externally a finely fibrous layer of indistinct limits. Myofibrils and Z bands are readily identified. Approximately 4 sarcomere lengths are shown in the micrograph.

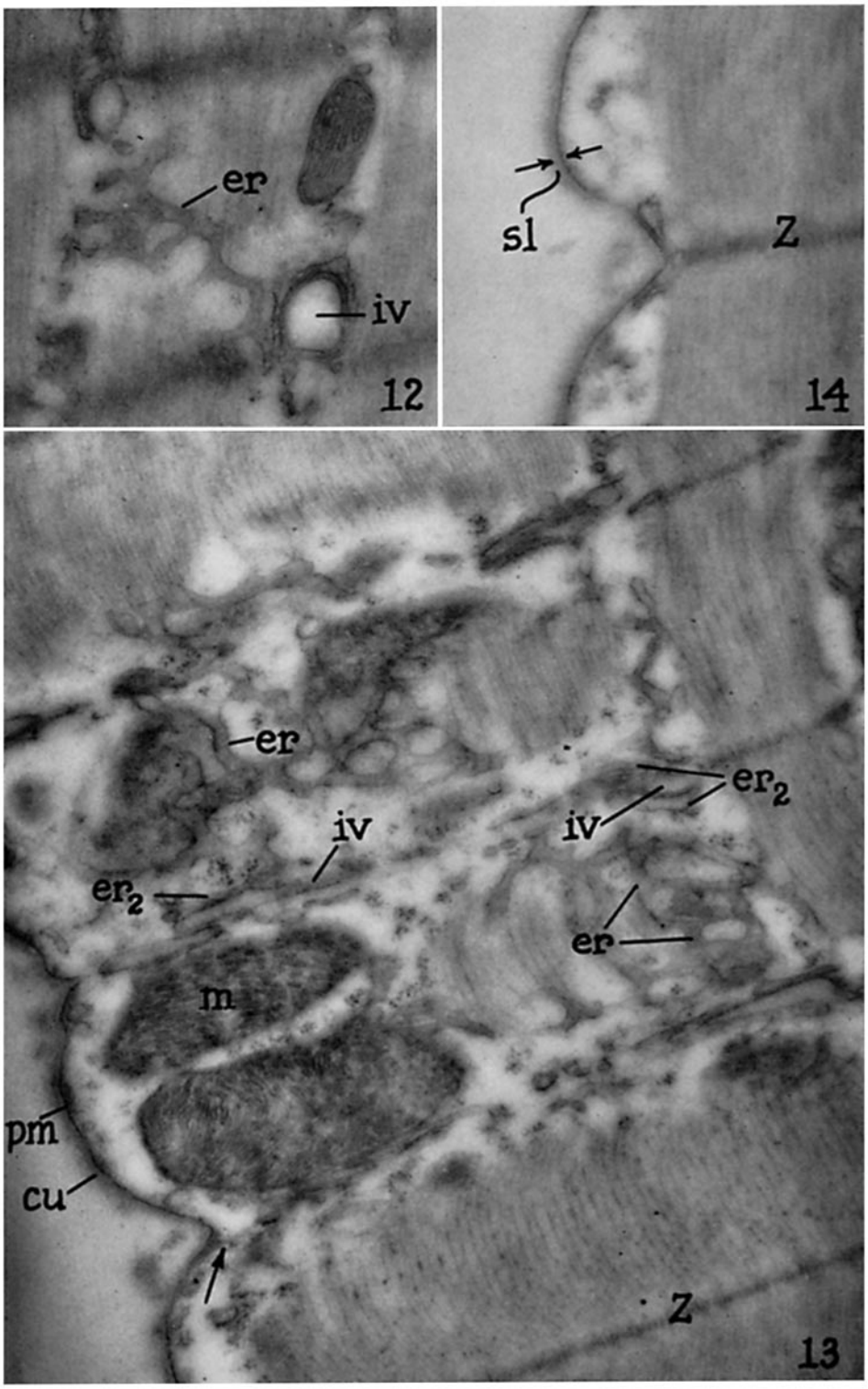
A pair of sarcosomes or mitochondria are shown in the section at the lower left. Thin shavings of mitochondrial surfaces are shown at two places in the next sarcomere above. The image marked *m* represents a section off the side of a mitochondrion, which means that the limiting membrane of the curved surface passes obliquely through the section. The shadow or projection of this is broad and grades off gradually in density to that of the surrounding sarcoplasmic matrix. Images such as this have led Harman and coworkers to describe mitochondria as gelatinous bodies devoid of membranes (21, 22). At one end of the mitochondrion, below the one marked *m*, the surface passes at right angles through the section and here a line of characteristic density and doubleness can be seen to limit the organelle. This is the membrane that investigators, experienced in interpreting electron micrographs of thin sections, have uniformly described as limiting mitochondria and sarcosomes.

The plane of section coincides here with a thin layer of interfibrillar sarcoplasm, thus providing a face view of the sarcoplasmic reticulum. At *er* the ribbon-like elements of the system form a reticulum opposite the mid region of the sarcomere. Toward the ends of the sarcomere the canaliculi represented are continuous with transversely oriented units which face off against similar units in the next sarcomere (er_2). In some places there is an intermediate element of the same character (*iv*) which generally appears free of connection with the vesicles on one or both sides of it. Clear-cut evidence that the reticulum of one sarcomere is structurally continuous with that of the next is found very infrequently, indicating that in general continuity is interrupted at the Z band level.

The subsarcolemmic sarcoplasm contains profiles of vesicular elements which seem to be randomly scattered without relation in most instances to the bands of the adjacent myofibrils. Only at the Z band level of contracted fibers is it common to encounter elongate elements extending from the Z line out to the inner face of the sarcolemma (see arrow and also Fig. 14). The sarcolemma seems to be held in at this point, giving the scalloped outline characteristic of contracted fibers.

Clusters of small dense particles are scattered about in the sarcoplasm without preference for other structures. × 32,000.

FIG. 14. A small region at the margin of a contracted cardiac fiber to show paired vesicular elements which appear frequently between the margin of the Z line or sarcoplasmic reticulum at this level and the sarcolemma (*sl*). The latter is always held in at the Z line level. × 40,000.



(Porter and Palade: Studies on the endoplasmic reticulum. III)