THE SARCOPLASMIC RETICULUM IN MUSCLE CELLS OF AMBLYSTOMA LARVAE

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PLATES 55 AND 56

In an earlier paper Bennett and Porter (3) described some observations on the morphology of striated muscle of the domestic fowl and, among other features, pointed out an interfibrillar component which they referred to as the sarcoplasmic reticulum. This appeared in electron micrographs then available as small scattered condensations of dense material, in some instances vaguely vesicular, localized either opposite the Z bands or as paired densities on opposite sides of the Z band, at the N bands or the A-I junctions. The system was defined as probably analogous to the endoplasmic reticulum of other cell types. By present standards the earlier pictorial evidence was somewhat less than convincing, although the preparations were of average quality for that time.

Recent improvements in fixation, microtomy, and microscopy are responsible for the improved images of the sarcoplasm which have since appeared in papers by Bennett (2), Edwards and Ruska (5), and Porter (8, 9). These more clearly depict the reticulum as a system of vesicles and tubules and give some indication that they are all linked together to form lace-like sleeves around the myofibrils. Bennett (2), in particular, has stressed that the system is similar in form and distribution to that recognized in gold-impregnated preparations by Thin (12) and later by Retzius (11) and Veratti (13). Certainly the special differentiations in the sarcoplasm at the level of the I and H bands shown in the remarkable figures of these earlier manuscripts are easily related to the electron microscope images of the reticulum to be described here and elsewhere (10).

Despite the excellence of some of the observations that have thus far been made on this component of the sarcoplasm (summarized by Bennett (2)) it is obvious that more might be learned about it from particularly favorable materials and preparations. Our prevailing interest in the endoplasmic reticulum has kept us watchful for the unusually "good" presentations of the system and so it was that we were attracted by its appearance in some thin sections of muscle cells constituting the myotomes of *Amblystoma* larvae.

These depict the system as developing some remarkable relationships with the myofibrils—relationships that may reasonably be considered to have some

significance in the contraction phenomena of the muscle. The observations on the system will be described more fully elsewhere along with related observations on the system in mammalian muscle (10).

Materials and Methods

Whole larvae (12 to 15 mm. long) of *Amblystoma punctatum*, grown in the laboratory from eggs collected locally, were immersed in 1 per cent OsO_4, buffered at pH 7.8 with veronal acetate. Fixation was continued for 2 hours, after which the embryos were washed briefly in water and dehydrated in ethanol. The tails were clipped from the embryos and embedded separately in *n*-butyl methacrylate polymerized at 45°C. with 2 per cent luperco. Sections were mounted on formvar, or, more recently, on carbon films (14), and examined in a modified EMU 2C.

OBSERVATIONS

Longitudinal sections of the myomeres of these tail tips reveal a structure more or less characteristic for uncontracted skeletal muscle. Slender, striated myofibrils run longitudinally in the fiber. The A bands are relatively long (1.2 μ) making up approximately four-fifths of the sarcomere length (Fig. 1). The typically less dense H band precisely bisects the A. The I band is correspondingly short (0.3 μ) and characteristically divided into two halves by a dense Z line. N and M bands are not shown. The myofilaments making up the myofibrils are easily discerned throughout the sarcomere but are most prominent in the H band. At the Z line, where myofilaments of successive sarcomeres join, a zigzag configuration results. In some sections a faint axial periodicity (230 A) can be seen in the myofilaments and associated A substance.

The sarcoplasm between the myofibrils contains at least three resolvable elements. One is particulate and resembles the small dense particles that Palade has described as occurring rather generally in the cytoplasmic matrices of many types of cells (6, 8). The sarcosomes or mitochondria constitute the second. The third, and the one with which this brief report is most concerned, is membrane-limited and vesicular in character. It is considered to be equivalent to the interconnected tubular or vesicular elements of the sarcoplasm observed by Edwards and Ruska in certain insect muscles (5) and by Bennett in the gracilis muscle of the mouse (2).

The distribution of these outlines or profiles of vesicles observed in longitudinal sections is uneven. A few small ones (diameter 50 to 100 mμ) are usually evident opposite the H bands, suggesting some concentration of vesicles or tubules at this level (at er_1 in Fig. 1). Much more prominent and larger paired vesicles occupy positions opposite the I bands on either side of the Z line and these will be referred to as the I band vesicles (as at er_2 in Fig. 1). Where the plane of section coincides roughly with the central axis of a fibril, the profiles of the adjacent vesicles represent medial vertical sections which depict radial and longitudinal dimensions. In this aspect these I band vesicles

are 100 to 150 mμ along the radial dimension. Where the plane of section is oriented tangentially to the myofibril and passes along the interfibrillar space or sarcoplasm (as at er_2—upper right in Fig. 1), the paired vesicles are shown in what might be called a frontal view (showing circumferential dimension) and here appear much broader, equalling in some cases the diameter of the average fibril (500 to 800 mμ). The height or dimension of these vesicles that extend along the fibril axis (longitudinal dimension) is evident in both aspects and varies considerably but is usually between 200 and 300 mμ. Finally, in cross-sections of the muscle cells, where the plane of section coincides with the I and Z bands, these I band vesicles appear in transverse section (as at various places marked er_2 in Fig. 2). In this aspect the radial and circumferential dimensions are again evident.

Since, in the longitudinal section (Fig. 1), profiles of these I band vesicles are not uniformly present between adjacent myofibrils, it is evident that they do not encircle the fibril. This is shown as well by the cross-sectional image (Fig. 2), where the discontinuity is clear, and it is seen that the vesicles are confined to different faces or sides of the myofibril. A similar study of the membranous profiles at the H band level (not shown in transverse aspect in these figures but see reference 10) discloses that here, on the other hand, the system tends to be continuous across the muscle cell; continuous in the sense that the membranes enclosing the vesicles and tubules are in contact, if indeed the enclosed space is not patent.

Tubular or canalicular elements of this system, running lengthwise in the fiber and connecting the H and I band vesicles, are frequently seen even to better advantage than at er_3 in Fig. 1. Thus each sarcomere segment of the muscle cell may be pictured as possessing a three dimensional network of vesiculated strands around and among the myofibrils. At the midplane, or H band level of the sarcomere, the system appears to be continuous transversely and from this level canalicular extensions run in both directions longitudinally along sides of the myofibrils to special foot-like enlargements at the level of the adjacent I band.

Reference to Fig. 1 shows that the I band vesicles of adjacent sarcomeres adopt a special relationship opposite the Z lines. They appear to develop in all cases two opposing flat surfaces which are separated by a space of remarkably uniform width (500 A). It is as though the two vesicles were under some internal pressure to meet but were kept apart by mutually repulsive forces between their external surfaces, or by some intervening material. The space between them is located in *Amblystoma* muscle precisely opposite the Z line of the adjacent myofibrils. This relationship has been found to be constant in these preparations and such minor deviations as have been found are regarded as induced by the procedural manipulations or localized contractions of the myofibrils.

The space between the two opposing I band vesicles, which often appears denser than the surrounding sarcoplasm is found in thinner sections to contain small circular or oblong outlines or profiles (*b*, Fig. 1). These are approximately 200 A in diameter and appear as sections through minute vesicles or tubules. Reference to the cross-sections of the muscle at the Z band level (Fig. 2) provides another view of these same structures and in this they appear as tiny finger-like bodies (at *b*, Fig. 2). In favorable preparations it can be seen that these show a tendency toward parallel arrangement with their long axes normal to the long circumferential axes of the I band vesicles above and below them and thus directed toward an adjacent myofibril. It may be noted further from the cross-section (Fig. 2) that these small, intervesicular bodies extend, in some instances, beyond the margin of the I band vesicle and occasionally seem to penetrate the myofibril for a short distance. They appear to do this on one side only, as though the whole unit, I band vesicles and intervening bodies, were polarized with respect to one of the two adjacent myofibrils. A few small tubular elements of the same (200 A) dimensions appear free in the sarcoplasm opposite the Z band and outside the limits of the I band vesicles.

The facing membranes of the I band vesicles and the limiting membranes of the intervesicular bodies are, in most instances, notably prominent (Fig. 1) being $\backsim$100 A thick. To what extent their thickness and density relate to their structural thickness or to their osmiophilia is not clear, but they certainly differ in one or both respects from the other membranes of the sarcoplasmic reticulum, which are not more than one-half as thick.

It would, of course, be valuable, for functional correlations, to be able to follow events at the opposing faces of the I band vesicles during contraction and recovery of the myofibrils. From preparations of this material currently available the picture is obscure. During contraction the precise arrangement apparent in the resting state, as depicted in Fig. 1, is frequently, but not always disrupted; the intervesicular bodies disappear from view, and the paired I-band vesicles lose prominence. In the recovery phase, following the contraction wave, they all appear again and seem abruptly to assume the state depicted in Fig. 1. Whether they are actually displaced and then quickly restored or simply changed into another form is not at present known. There is some evidence to suggest that the intervesicular bodies are at times continuous with the opposing surfaces of the I band vesicles and should perhaps be regarded as minute digitations from them (see later report (10)).

One additional feature of the reticulum deserves mention in this preliminary account and that is that the vesicles and connecting tubules seem, on the whole, to be singularly devoid of organized structural content. The single exception is shown by the I band vesicles, where, next to the internal surfaces of the dense membranes, there is condensation of finely fibrous material.

The above are, then, the principal features of the sarcoplasmic reticulum and its local differentiations in the muscle cells of this material. In muscle cells of other animals and other types its form and distribution may be quite different, as shown, for example, by the light microscope studies of Veratti and as known to us from other material. In all cases, however, thus far observed it seems to comprise tubules and vesicles connected to form a lacework around and among the myofibrils. Regular and stabile connections between the sarcolemma and the reticulum have not been noted in these preparations, though at times there are intervening populations of vesicles at the H and Z band levels. No connection with the nuclear envelope has been observed. Continuity within the reticulum in a transverse direction at the H band of each sarcomere seems established, but continuity in the same sense along the length of the muscle cell is in question.

DISCUSSION

The systems of interconnecting vesicles and tubules depicted here are readily related by their distribution, size, and character to the sarcoplasmic component observed earlier in fowl muscle (3) and subsequently in mouse and insect (2, 5). Differences in general prominence and organization of the system exist among different muscles, but the ubiquity of a finely divided vacuolar system in the sarcoplasm seems established. Especially is this true if, as seems entirely justified, some of the older light microscope observations of Thin (12), Cajal (4), Retzius (11), Veratti (13), and others are accepted as referring to the same system. What they saw following silver and gold impregnation procedures certainly coincides in distribution and general form with the system described above. It must, however, be admitted that direct confirmation of this correlation on identical muscles would be desirable.

As pointed out earlier (3), the general features of the system justify one's referring to it as the muscle cell equivalent of the endoplasmic reticulum encountered in the cytoplasms of all cells. Here it is organized most highly with respect to the myofibrils and presumably performs a role in contraction and recovery of the myofibrils. In other types of cells, organization is also evident but in quite different relation to the cell and its other components. In each instance, and the number is constantly increasing, the form and organization are found to be similar in cells performing similar major functions (7).

The most striking feature of this reticular organization encountered in these muscle cells is perhaps that found at the level of the Z bands. Here two I band vesicles, which connect back through tubular extensions to the H band system of vesicles, show flat opposing surfaces. A definite and constant space is retained between these surfaces which interrupts the longitudinal continuity of the reticulum. The fairly precise coincidence of these differentiations relative to the Z bands (where isotonic shortening occurs) of the myo-

fibrils obviously suggests a functional relation to myofibril contraction. Of further interest in this regard is the presence within the space of discrete, finger-shaped bodies (intervesicular bodies) with one end directed toward and sometimes embedded in one of the two adjacent myofibrils. Whether these minute bodies are discharged into the myofibril and surrounding sarcoplasm at the moment of stimulation is not known, but bodies of similar shape and size are found in the immediate vicinity. Possibly they are present and so oriented to transfer the excitation to the fibril at the time of contraction. This suggestion implies that the reticulum is involved in distributing excitatory impulses among the myofibrils, an implication that rests solely on the structural features of the system. For example, the fact that it is the only structure with evident continuity across the muscle fiber, and with longitudinal extensions to the Z band, makes it logically the pathway for lateral conduction of the impulse regarded as essential for the synchronous contraction displayed normally by the myofibrils of a fiber (1, 2). Then also, the fact that the membrane limiting the system is in no obvious feature different from the plasma membrane of the cell makes it reasonable to assume that across it a potential may exist equivalent to that across the cell membrane. On the basis of such assumptions it is conceivable that a depolarization of the sarcolemma at any point could set off a sympathetic wave of depolarization of the membranes in the adjacent sarcoplasmic reticulum and that the impulse might travel along the membranes wherever they go. Thus it could move freely in a lateral direction and flow longitudinally *via* the extensions to the I band vesicles. At this point events of significance in contraction may be initiated by the impulse hitting the "barrier" represented by the special gap or break in the continuity of the system.

This is clearly only one of the several roles this unusual system may perform for the muscle cell, and it is regrettable that so little factual information is available. It should be noted that the content of the system is usually less dense to the electron beam than the surrounding matrix. This implies, among other things, that the continuous internal phase may be more highly solated, relatively free of large molecules, and perhaps available for more rapid diffusion of metabolites than any other part of the sarcoplasm. Thus energy-rich materials might be channelized and rapidly transported by the system to the Z band, where presumably they may be utilized in contraction.

SUMMARY

Electron microscopy of thin sections of muscle fibers in myotomes of *Amblystoma* larvae has revealed the presence of a complex, membrane-limited system of canaliculi and vesicles which form a lace-like reticulum around and among the myofibrils. This seems to correspond to the sarcoplasmic reticulum of the earlier light microscopists and the endoplasmic reticulum of other cell

types. The elements constituting the reticulum are disposed in a pattern which bears a constant relation to the bands of the adjacent myofibrils and is therefore repeated in each sarcomere. At the H band the system is transversely continuous but not so at other levels. Longitudinally continuity is interrupted at the Z bands where large vesicles belonging to adjacent sarcomere segments of the system face off on opposite sides of the band. The opposing faces of these vesicles are flat and separated by a space of more or less constant width, in which are located small, finger-shaped vesicles. In view of these and other close structural relationships with the myofibrils it seems appropriate to assign to the system a role in the conduction of the excitatory impulse.

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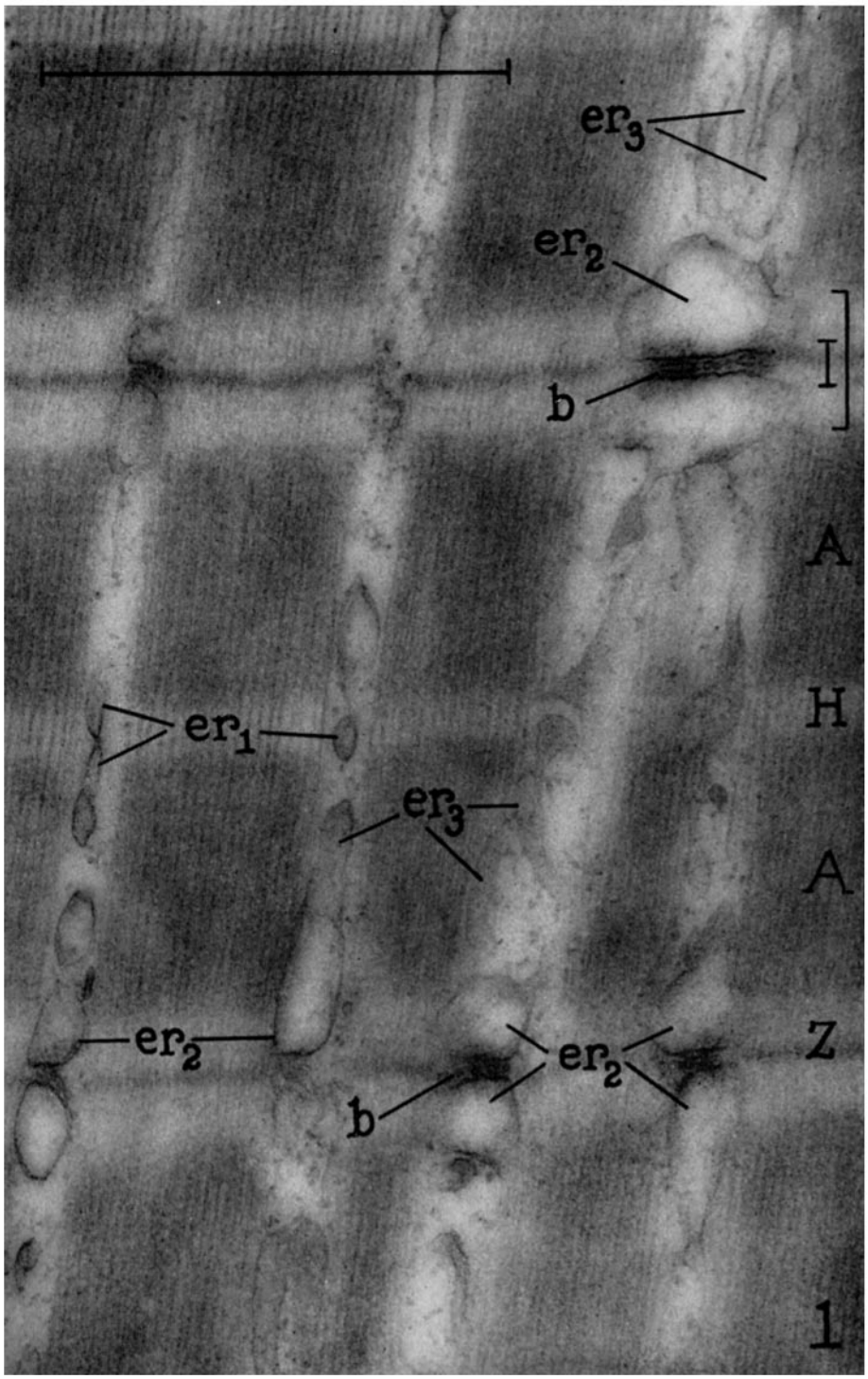
EXPLANATION OF PLATES

PLATE 55

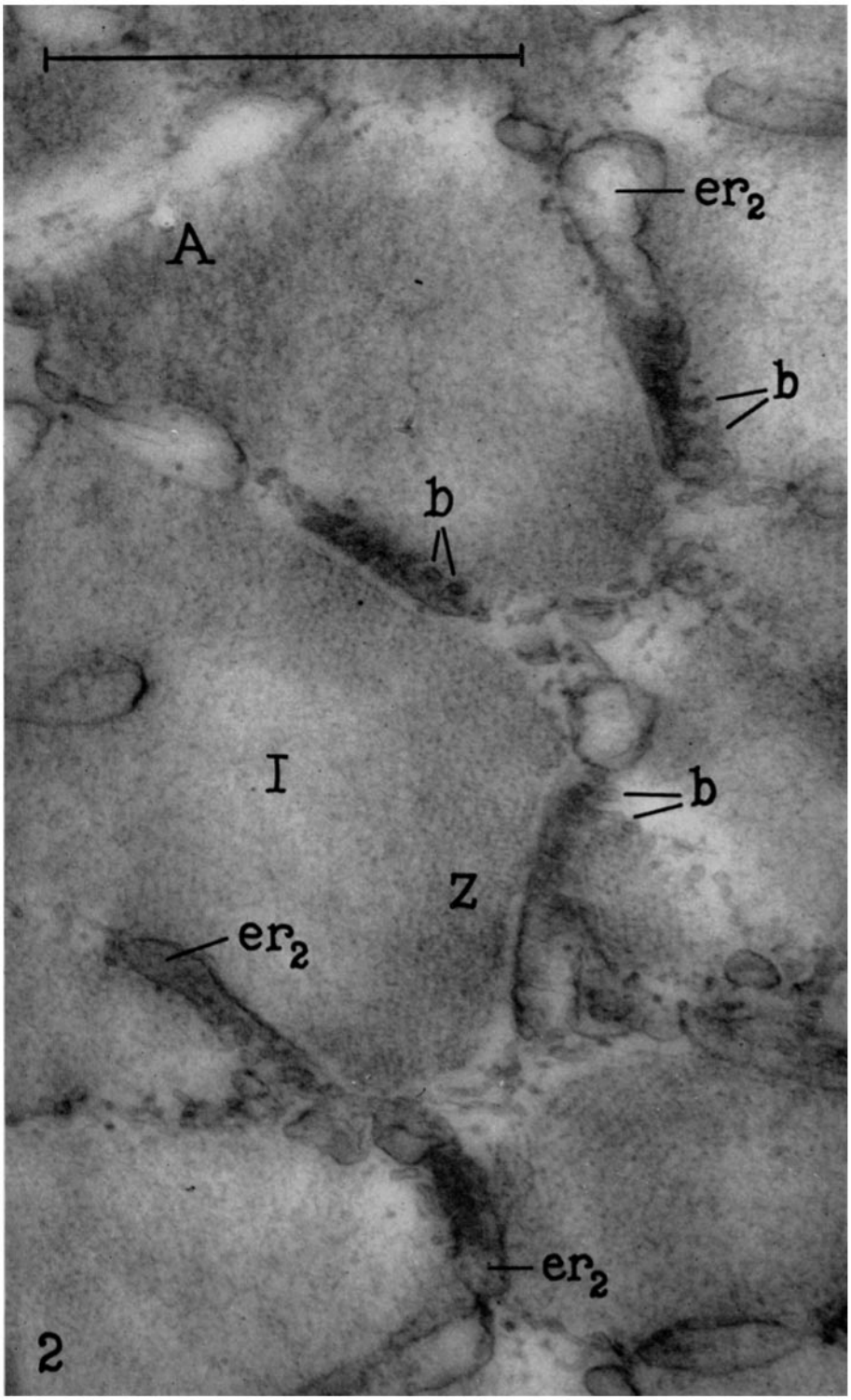
FIG. 1. Micrograph of a longitudinal section of muscle cell in myotome of *Amblystoma* larvae. The well known bands, A, H, I, and Z are indicated by appropriate letters. Profiles of sarcoplasmic reticulum are commonly present opposite H bands, as at er_1. Larger vesicles, at er_2, are paired at I and Z band level. In frontal view they appear as at er_2, upper right. The structures at er_3 are canalicular members of the reticulum running lengthwise the fibril and connecting the I band vesicles with the laterally continuous elements of the system at the H band level. At the Z band the opposing surfaces of the I band vesicles flatten out and define a space of uniform height in which lie minute finger-shaped bodies, *b*. × 65,000.



(Porter: Sarcoplasmic reticulum in *Amblystoma* muscle cells)

PLATE 56

FIG. 2. Cross-section of same type of muscle cell from another animal. The section is slightly oblique and so includes portions of Z, I, and A bands, as marked. At the Z band level the section contains profiles of the I band vesicles, er_2. Where the space between these is included in the section, as at *b*, it is possible to see the finger-shaped intervesicular bodies. The long axis of these is oriented normal to the longer, circumferential axis of the I band vesicles above and below them. × 68,000.



(Porter: Sarcoplasmic reticulum in *Amblystoma* muscle cells)