SPHEROIDAL BODIES IN THE JUNCTIONAL SARCOPLASMIC RETICULUM OF LIZARD MYOCARDIAL CELLS

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ABSTRACT

The sarcoplasmic reticulum (SR) of lizard (*Anolis carolinensis*) myocardial cells has been examined, with particular attention being paid to the structural details of the peripheral couplings (junctional SR). Spheroidal bodies are present within the opaque core of junctional SR; these can be seen both in sections made *en face* and in sections cut to show the apposition of the junctional SR with the sarcolemma. Opaque junctional processes extend between the sarcolemma and the peripheral junctional SR. The myocardial cells in addition contain some SR cisternae deep within the cells which also possess opaque cores composed of spheroids. Although the significance of the junctional SR spheroidal bodies is unknown, it is thought that they could act as a matrix on which enzymes such as calcium-specific ATPase may be located.

INTRODUCTION

It has become obvious only recently that the myocardial cells of nonmammalian vertebrates are not necessarily lacking in many characteristic organelles of mammalian heart. For example, even in frog heart in which investigators long were unable to demonstrate substantial sarcoplasmic reticulum (SR), abundant smooth-surfaced tubules continuous with structures which resemble junctional SR of higher vertebrates can be found under optimal conditions of fixation (Page and Niedergerke, 1972). The avian myocardium shows a high degree of development of the SR (Jewett et al., 1971). Even though transverse (T) tubules are absent in bird heart, cisternae (termed "extended junctional SR" [EJSR]) can be found deep within the cell which are morphologically similar to the junctional SR contacts with the T system of mammalian heart (Jewett et al., 1971; Jewett et al., 1973). Specialized membrane appositions between cells (gap junctions) have been noted in hearts of birds and of other lower vertebrates (Jewett et al., 1971; Martínez-Palomo and Mendez, 1971; Scott, 1971). Myocardial cells of the iguanid lizard *Anolis* are highly advanced (Forbes and Sperelakis, 1971) with respect to those of some other reptiles that have been studied, such as the boa constrictor (Leak, 1967). Well-developed SR and short T tubule-like structures have been demonstrated in the heart of the soft-shelled turtle, *Amyda* (Okita, 1971). The present further investigation of the SR of *Anolis* heart demonstrates a peculiar internal substructure of the junctional SR as well as the presence of some EJSR.

MATERIALS AND METHODS

Adult lizards (*Anolis carolinensis carolinensis* Voigt) were maintained in terraria at 25°–31°C. Each ani-

mal was decapitated and thoracotomized, and fixative solution was injected directly into the beating heart (heart rates ranged from 40 to 140 beats/min). The fixative consisted of 2.6% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.4) with 9% (wt/vol) sucrose added. Small pieces of ventricular and atrial tissues were fixed an additional 4 h, washed in cacodylate buffer (12% sucrose added), and post-fixed 2 h in 1% phosphate-buffered osmium tetroxide (Millonig, 1962). The tissues were stained 30 min *en bloc* in a saturated aqueous solution of uranyl acetate, dehydrated in a series of alcohols, passed through propylene oxide, and embedded in Epon 812 (Luft, 1961). Thin (500–900 Å)[1] sections were cut with diamond knives, collected on copper grids, and stained 2 min with saturated uranyl acetate in 50% acetone and 45 s with 0.4% lead citrate (Venable and Coggeshall, 1965). The sections were examined in an Hitachi HU-11E-1 electron microscope operated at 75 kV. The microscope routinely was calibrated against a replica of an optical grating. For precise measurements of intracellular structural dimensions, micrographs were taken at high magnification, and the instrument then was calibrated at that magnification. Measurements were made with vernier calipers on micrographs printed at 3.5–7 times enlargement (usually from ×200,000 to 400,000).

RESULTS

The general morphological features of *Anolis* myocardial cells have been described previously (Forbes and Sperelakis, 1971). In that study, the SR was characterized as a highly developed system of anastomosing, smooth-surfaced tubules which formed networks on the surfaces of the myofilamentous masses ("myofibrils") (Fig. 1). The SR networks sometimes are arranged in a double layer as can be seen in Fig. 1. In addition to the relatively electron-lucent tubules of network SR, the SR system also is composed of junctional SR, an integral component of peripheral couplings. The junctional SR consists of expanded SR tubules with opaque interiors in close apposition to the sarcolemma (Fig. 1). Couplings in *Anolis* heart are not limited to any particular level of the sarcomeres in contrast to bird heart in which the couplings are restricted to the Z-line levels (Jewett et al., 1971).

Transverse sections of *Anolis* myocardial cells (Fig. 2) indicate that the intracellular volume of each cell is occupied mainly by the myofibrils, mitochondria, and nucleus. In general, very little SR is found deep within the cell; instead, most of it is located on the external surfaces of the myofilamentous masses, just under the sarcolemma (Fig. 2).

In sections which graze the cell surface, the network SR sometimes can be seen to expand into lucent cisternal regions having perforations (fenestrations) extending through them (Fig. 3). Similar fenestrations have been found in chicken ventricular cells (Jewett et al., 1973) and in amphibian skeletal twitch fibers (Franzini-Armstrong, 1963; Peachey, 1965).

Continuity between network SR and junctional SR cisternae often can be demonstrated (Figs. 4, 5). In the numerous peripheral couplings of junctional SR with sarcolemma, opaque bodies, termed "junctional processes" by Sommer and Johnson (1970), bridge the gap between the two (Fig. 4). The processes often are distributed at approximately equal intervals along the length of the junctional SR cisterna as in the case of mammalian myocardial cells (Fawcett and McNutt, 1969). Although in mammalian couplings the junctional SR lumen contains a more-or-less linear opacity termed either the "central density" (Walker et al., 1971) or "junctional granules" (Sommer and Johnson, 1970), the junctional SR contents of *Anolis* instead form a more extensive opaque "junctional core" surrounded by an electron-lucent rim (Figs. 4–11) as previously noted (Sperelakis et al., 1973). In some instances, the junctional core continues into the region of the SR which veers away from the sarcolemma (Fig. 6). In side-view sections through the disk-shaped couplings, the junctional core can be resolved in thin sections into a series of spheroidal opacities (Figs. 7–11). These often appear to be enclosed in an envelope composed of material ar-

[1] As has been discussed by Jewett et al. (1971), the visualization of such features as the junctional processes and junctional granules of couplings is limited by the thickness of the sections. That is, in a 600–700 Å section, an *en face* view of a coupling will most often include all the components (junctional processes, junctional granules, and cisternal membranes) belonging to the junctional SR, as well as contributions from myofilaments and/or sarcolemma. The superposition of these structures creates a complex pattern from which it is difficult to interpret structural arrangement. In the case of the *Anolis* peripheral coupling, whose total thickness is greater than 700 Å, the thinner sections (gray to light silver) obtained of couplings cut *en face* have a greater potential for revealing the architecture since many of the sections will include only a portion of the components of the junctional SR.

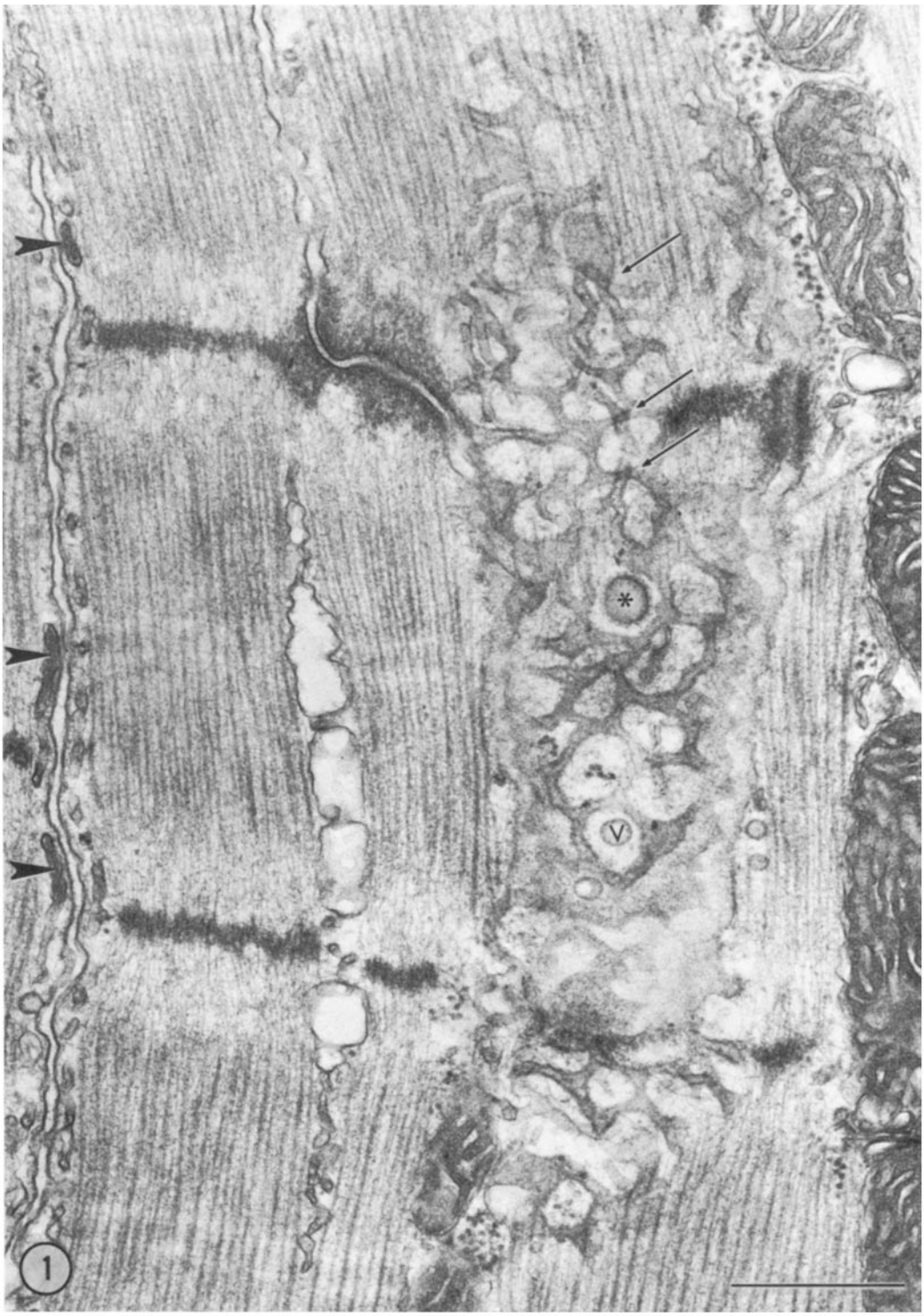

FIGURE 1 Longitudinal section of *Anolis* ventricular myocardial cells. SR forms an intricate network on the face of the myofilamentous mass at the right-hand side of the micrograph; this network SR is present in a double layer at some points (arrows). That this portion of the section is near the cell surface is indicated by the presence among the SR tubules of both surface vesicles (*V*) and a bristle-coated vesicle (*). At the left side junctional SR makes contact with the sarcolemma at three places, forming peripheral couplings (arrowheads). These couplings are not limited to the Z-line level, as opposed to the case in avian heart. × 51,500; scale bar = 0.5 μm.

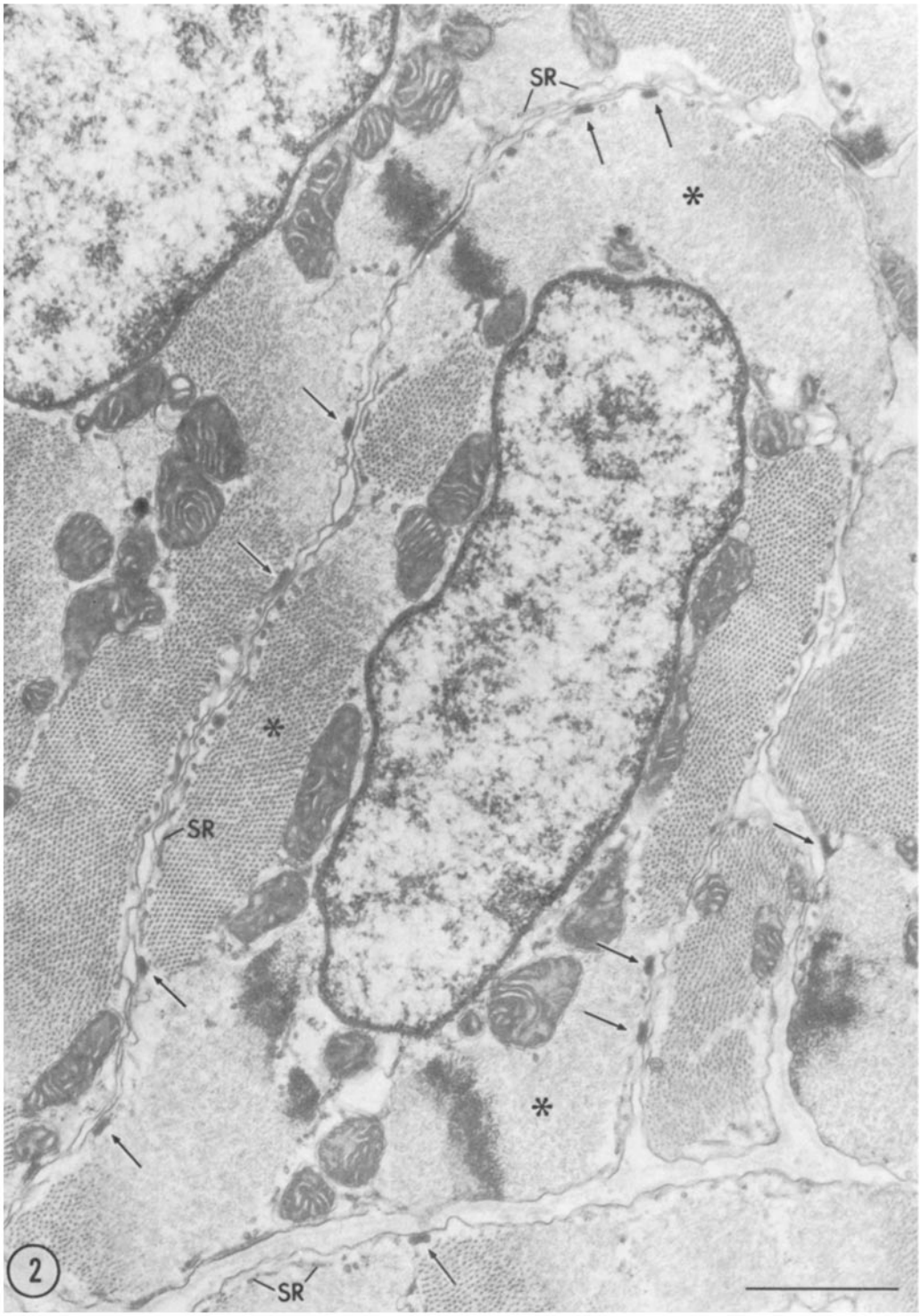

FIGURE 2 Transverse section through *Anolis* myocardial cells. The small diameter of such cells is apparent; the greater part of each cell's volume is occupied by several myofilamentous masses (*), mitochondria, and the nucleus. The sarcoplasmic reticulum (*SR*) is located, for the most part, at the periphery of the cell, just underneath the sarcolemma. Numerous peripheral couplings (arrows) are formed between the sarcolemma and junctional SR. × 22,500; scale bar = 1 μm.

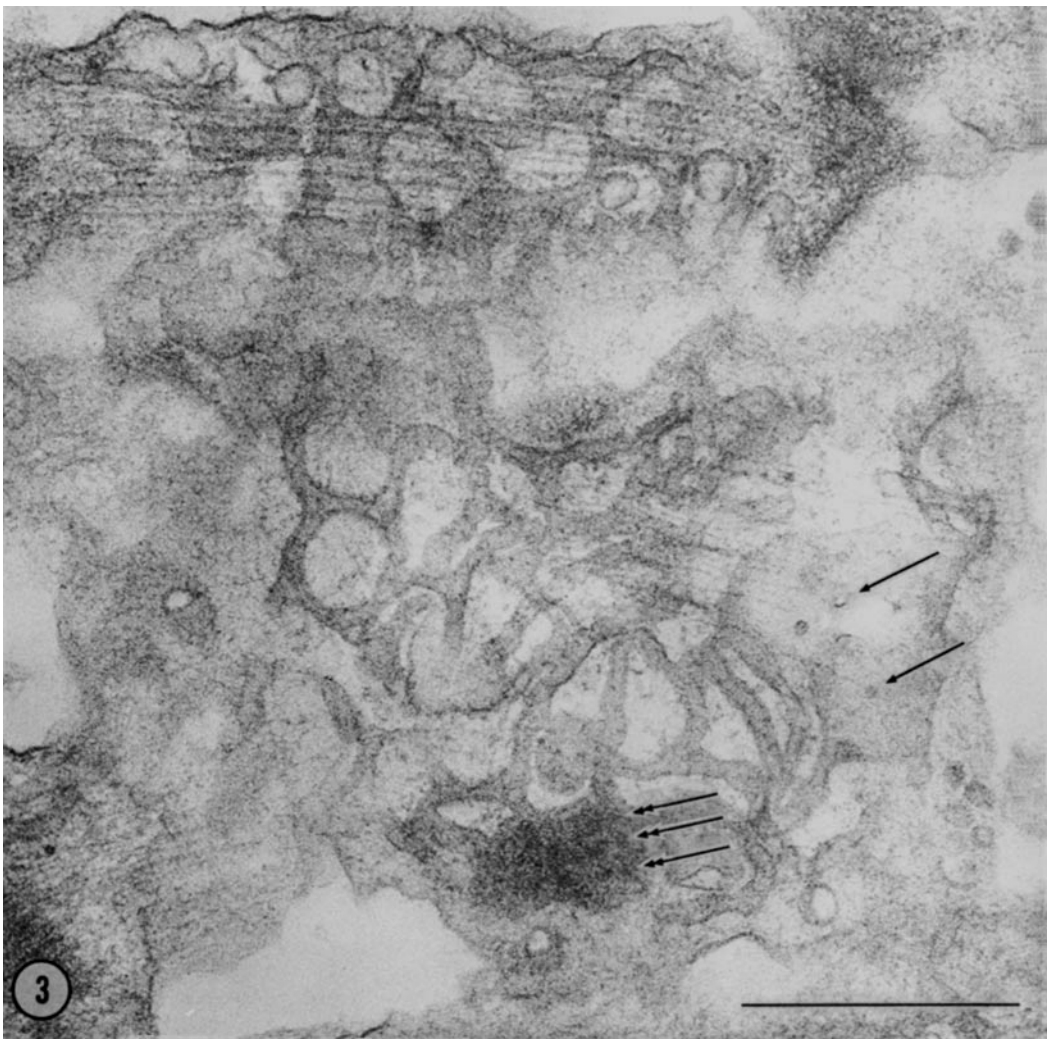

FIGURE 3 Grazing longitudinal section. The network SR merges both into a cisterna of junctional SR (lower center) and, at the right side, into a flattened cisternal structure. In this latter structure are perforations (single-headed arrows) resembling those in the fenestrated collar of amphibian fast twitch skeletal fibers. Along the long axis of the junctional SR cisterna (which is cut *en face*) there appear three rows of opacities (double-headed arrows). × 73,500; scale bar = 0.5 μm.

ranged in a unit membrane-like configuration (Figs. 10, 11).

The junctional SR of *Anolis* is much thicker than that found in mammals and birds. Although the gap between the apposed membranes (therefore the maximum length of the junctional processes) is about the same as that of mammals (ca. 150 A), the thickness of the junctional SR is ca. 570 A for *Anolis* as compared to ca. 340 A for mammals (unpublished observations on 2–3-wk old guinea pig hearts). Junctional SR of bird heart (Jewett et al., 1971) is about as thick as that of the guinea pig.

In some sections cut tangential to the cell surface, the network SR tubules of *Anolis* connect into round or ovoid expansions (approximately 2,500–3,000 A in diameter). These cisternal structures contain material which often varies in electron opacity from one cisterna to another (Fig. 12). Close examination of such cisternae

reveals the presence within them of circular bodies (Figs. 13, 14). It appears that these cisternae are junctional SR seen in *en face* section, that is, a section which grazes the surface of the myofibrils and cuts through the SR cisternae. Figs. 13 and 14 represent sections through junctional SR spheroids at different levels. Since the SR and some myofilaments are superimposed in Fig. 13, it is likely that the circular opacities visible within the junctional SR represent the "tops" of the spheroids, i.e., the portions farthest from the sarcolemma. In Fig. 14, the absence of myofilaments and presence of surface vesicle profiles, as well as the greater diameter of the spheroids, indicate that the junctional SR cisterna here is sectioned in a plane about midway through its thickness. The failure to observe their profiles in more than two successive serial sections indicates that views such as shown in Figs. 13–15 are indeed *en face* sections through flattened cisternal structures. These results argue against the profiles representing tubules of network SR continuous with membranes of mitochondria, as reported in dog heart (Bowman, 1967).

Although SR is rarely encountered deep within the cell, tubules of network SR occasionally are found here, and these are continuous with expanded regions containing electron-opaque material similar to that of the junctional core of peripheral junctional SR (Fig. 16). Such cisternae, because of their location, may represent EJSR such as found in bird heart (Jewett et al., 1971).

In the grazing longitudinal section illustrated in Fig. 17, the paucity of myofilaments and the presence of vesicular profiles indicate that the opacities seen here perhaps represent the junctional processes superimposed on the intracisternal spheroids. This interpretation is supported by the presence of "bull's-eye" patterns in the centers of some of the circular profiles (Fig. 18). This suggests that a positional correspondence may exist between junctional spheroids and junctional processes as is indicated also in side views of couplings (e.g., see Fig. 8).

Our proposed scheme of three-dimensional relationships between the myofilamentous masses, sarcolemma, network SR, and junctional SR is illustrated diagrammatically in Fig. 19.

DISCUSSION

It is clear that SR is present in some form in myocardial cells of lower vertebrates. Inadequate fixation procedures may have hampered many previous efforts to demonstrate the SR, and it was not until recently that a fairly well-developed system of SR tubules and peripheral couplings was demonstrated in frog heart by Page and Niedergerke (1972).

Junctional granules (or the central density) form an approximately linear array of opacities within myocardial junctional SR of mammals (Johnson and Sommer, 1967; Walker et al., 1970, 1971), birds (Sommer and Johnson, 1969; Jewett et al., 1971), turtle (Hirakow, 1970), frog (Page and Niedergerke, 1972), *Necturus* (Hirakow, 1971), and newt (*Notophthalmus*) (Forbes, unpublished observations). Little information is available on the SR of fish heart, other than that subsarcolemmal cisternae are present in goldfish (Yamamoto, 1967).

Cytochemical examination indicates that the junctional SR of mammalian myocardial cells is the site of intense enzymatic activity, including nucleoside diphosphatases (Ferrans et al., 1969), 5′-nucleotidase (Rubio and Berne, 1970), and Ca^{++}-, Mg^{++}-dependent ATPase (Rostgaard and Behnke, 1965). The presence of Ca^{++} in SR tubules has been documented (Legato and Langer, 1969; Yarom et al., 1972; Shiina and Mizuhira, 1970). Although the junctional SR and network SR are morphologically continuous, their enzymatic activities are discontinuous. For example, ouabain-sensitive ATPase activity (i.e., Na^{+}-, K^{+}-ATPase) is found throughout mouse heart network SR; however, intense ATPase activity unaffected by ouabain (i.e., Ca^{++}-, Mg^{++}-ATPase) occurs mainly in junctional SR (Forbes and Sperelakis, 1972). Because of the Na^{+}:Ca^{++} exchange reaction (Baker et al., 1969), Ca^{++} sequestration could be brought about throughout the SR by either type of ATPase.

The junctional granules within junctional SR cisternae of myocardial cells may somehow be related to the Ca^{++}-, Mg^{++}-ATPase activity. The junctional granules which sometimes seemed to be limited by unit membranes in *Anolis* heart could form the matrix on which enzymes are located and/or to which Ca^{++} is bound. Similarly, the junctional SR contents in mammalian heart have on occasion been resolved into structures resembling unit membranes, sometimes appearing to fuse with the membrane of the junctional SR cisterna (Walker et al., 1971). Since SR apparently is the homologue of endoplasmic reticulum in nonmuscle cells, and since membrane-

like or tubular structures are often observed within the confines of the endoplasmic reticulum (Valeri et al., 1971; Thake et al., 1971; Baringer and Swoveland, 1972; Deutschländer, 1972), the unit membrane-like envelopes seen associated with some junctional SR spheroids in *Anolis* are not necessarily unique.

Because most of the SR tubules in the *Anolis* myocardial cell are located at its periphery and because of the great numbers of peripheral couplings, we conclude that most of the opaque structures continuous with network SR, as seen in grazing longitudinal sections, represent *en face* views of junctional SR located at the surface of the cell. However, certain profiles seen deep in transversely cut cells indicate that a small amount of junctional SR is not associated with the sarcolemma of *Anolis* myocardial cells. The function of such junctional SR is unknown. Avian hearts, such as those of the hummingbird and finch, contain relatively great amounts of this internally located junctional SR. These avian myocardial cells thus are roughly equivalent to mammalian cells, both having peripheral and internal junctional SR, but the avian cells lacking a T system. The small cell diameters and profuse distribution of SR potentially can combine to bring about rapid Ca^{++} movements, thus enabling the extremely high heart rates attainable in these birds (up to 1,000 beats/min). A much lower incidence of internal junctional SR is found in the slower beating myocardial cells of the chicken (Jewett et al., 1973) and the lizard. The general ultrastructure of the SR suggests that a closer phylogenetic relationship than previously realized may exist between this lizard and the birds.

The geometrical arrangement of junctional processes is poorly understood in cardiac muscle but is more clear in skeletal muscle. In skeletal muscle, two rows of processes (termed "SR feet" or "dimples") extend between each terminal cisterna and the T tubule of the frog sartorius triad (Franzini-Armstrong, 1970); a similar pattern is demonstrable in mammalian twitch fibers (Forbes, unpublished observations). Four or more rows of junctional processes may be present on each terminal cisterna in newt skeletal muscle (Kelly, 1969; Kelly and Cahill, 1969). In inverte-

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FIGURES 4–11 Features of peripheral couplings formed by junctional *SR* and the sarcolemma.

FIGURE 4 Network SR (*N-SR*) continuous with junctional SR (*J-SR*). In contrast to mammalian junctional SR, the specialized regions of *Anolis* SR are expanded into cisternal structures, the interiors of which contain an opaque core. Opaque structures ("junctional processes," shown by arrowheads) extend between the sarcolemma (*SL*) and the membrane of the junctional SR cisterna. × 155,000; scale bar = 0.1 μm.

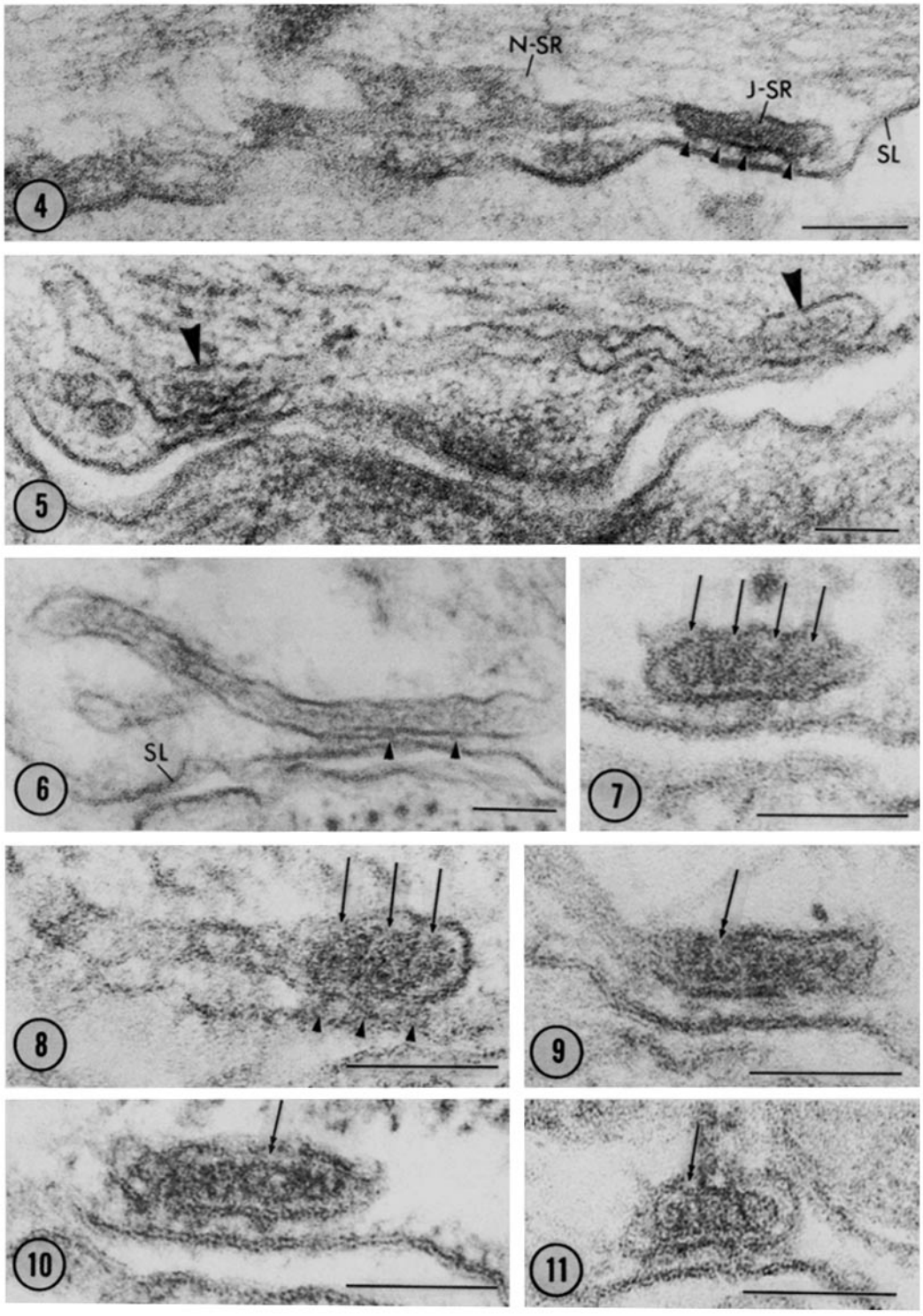
FIGURE 5 Two cisternae of junctional SR form couplings (arrowheads) with the sarcolemma. The cisternae, whose interiors contain opaque "junctional cores," are connected by a tubule of network SR whose lumen is relatively electron lucent. × 122,000; scale bar = 0.1 μm.

FIGURE 6 An example of "continuous" extended junctional SR (see Jewett et al., 1971). Junctional SR forms a coupling (junctional processes indicated by arrowheads) with the sarcolemma (*SL*), but the opaque junctional core also is present within the portion of the SR which does not come into close contact with the sarcolemma. × 122,000; scale bar = 0.1 μm.

FIGURE 7 The internal junctional core in this example of junctional SR is composed of four recognizable spheroidal bodies (arrows) arranged linearly along the long axis of the cisterna. × 225,000; scale bar = 0.1 μm.

FIGURE 8 Three junctional spheroids (arrows) compose the junctional core in this section. Below each of the spheroids is a corresponding junctional process (each indicated by arrowhead). × 225,000; scale bar = 0.1 μm.

FIGURES 9–11 In each of these couplings, the junctional spheroids (Fig. 9) or the majority of the entire junctional core (Figs. 10, 11) appear to be enclosed by structures resembling unit membranes (double-headed arrows). × 225,000; scale bar = 0.1 μm.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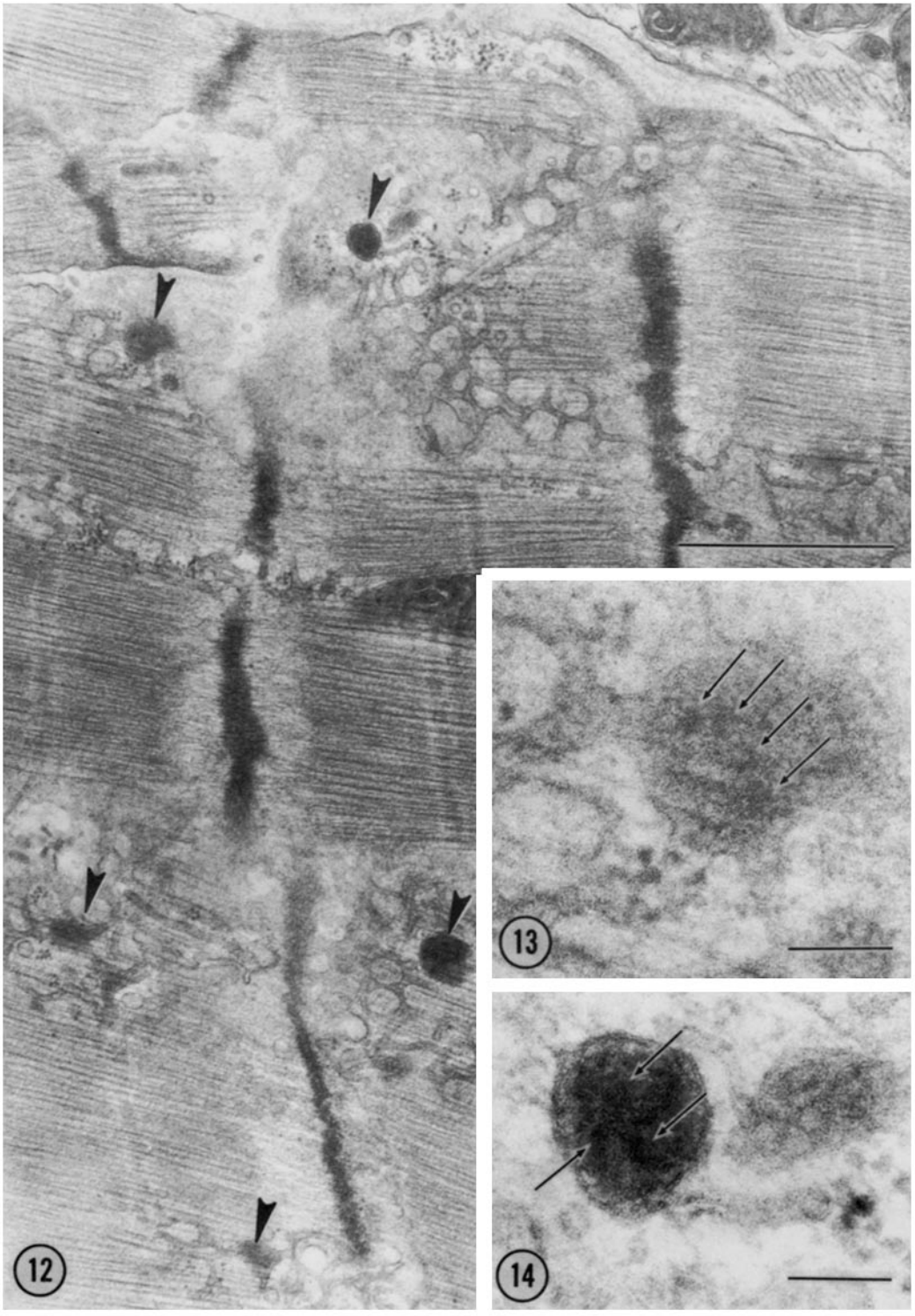
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brate skeletal muscles, *en face* sections reveal that the junctional processes form a crosscross or checkerboard pattern (Sherman and Luff, 1971; Fourtner and Sherman, 1972). Grazing sections indicate that these junctional SR cisternae are discoidal or platelike expansions apposed to the T tubules. In this respect, the invertebrate skeletal junctional SR is more similar to its counterparts in vertebrate myocardium, rather than to the flattened terminal cisternae of vertebrate skeletal muscle. As discussed, the thickness of mammalian and avian couplings in cardiac muscle is insufficient to allow a clear view of the junctional processes.

One proposed three-dimensional concept of the junctional processes, in both skeletal and cardiac muscles, is that of hollow cones (Kelly, 1969) representing extensions of the junctional SR which can be filled from within the SR (Sommer and Jewett, 1971). *En face* sections of avian junctional SR (Jewett et al., 1971) show circular profiles representing sections through the processes. The junctional SR membrane associated with junctional processes usually is scalloped, the peaks of the scallops corresponding to the position of the processes. Although the scalloping effect is not present in *Anolis*, grazing sections reveal similar circular profiles (Fig. 18), thus supporting the "hollow cone" model for the junctional process. Possible interconnection between the lumina of the SR cisterna and the junctional processes is suggested by the one-to-one correspondence often seen between junctional spheroids and junctional processes in *Anolis* (Fig. 8). However, there appears to be no continuity between the extracellular space and junctional SR, because colloidal lanthanum hydroxide does not enter the *Anolis* SR (Forbes and Sperelakis, 1971). Electron-opaque tracers also do not enter mammalian (Forssmann and Girardier, 1970; Sperelakis and Rubio, 1971) or invertebrate (Forbes et al., 1972) myocardial junctional SR or the SR of smooth muscle (Devine et al., 1973). Apparent continuity between the extracellular space and the SR has been documented, however, in amphibian skeletal muscle (Birks and Davey, 1969, 1972; Rubio and Sperelakis, 1972). This property has been postulated to allow electrical continuity across the coupling (triad), permitting the action potential to travel from the sarcolemma inward via T tubules and to invade the SR, causing calcium stored there to be released into the myoplasm. It appears that in cardiac muscle much of the Ca^{++} required to elicit contraction enters the myoplasm across the sarcolemma (and T-Ax tubules when present) via slow ionic channels (Reuter and Beeler, 1969). If so, then the network SR and perhaps the junctional SR function mainly in the sequestration of Ca^{++} to permit relaxation (cf. Langer, 1971). On the other hand, there is evidence that some Ca^{++} may be released from the SR to initiate contraction and, if so, the signal for this release could be transmitted across the peripheral couplings (perhaps via the junctional processes) and result in a membrane potential change in the junctional SR and network SR.

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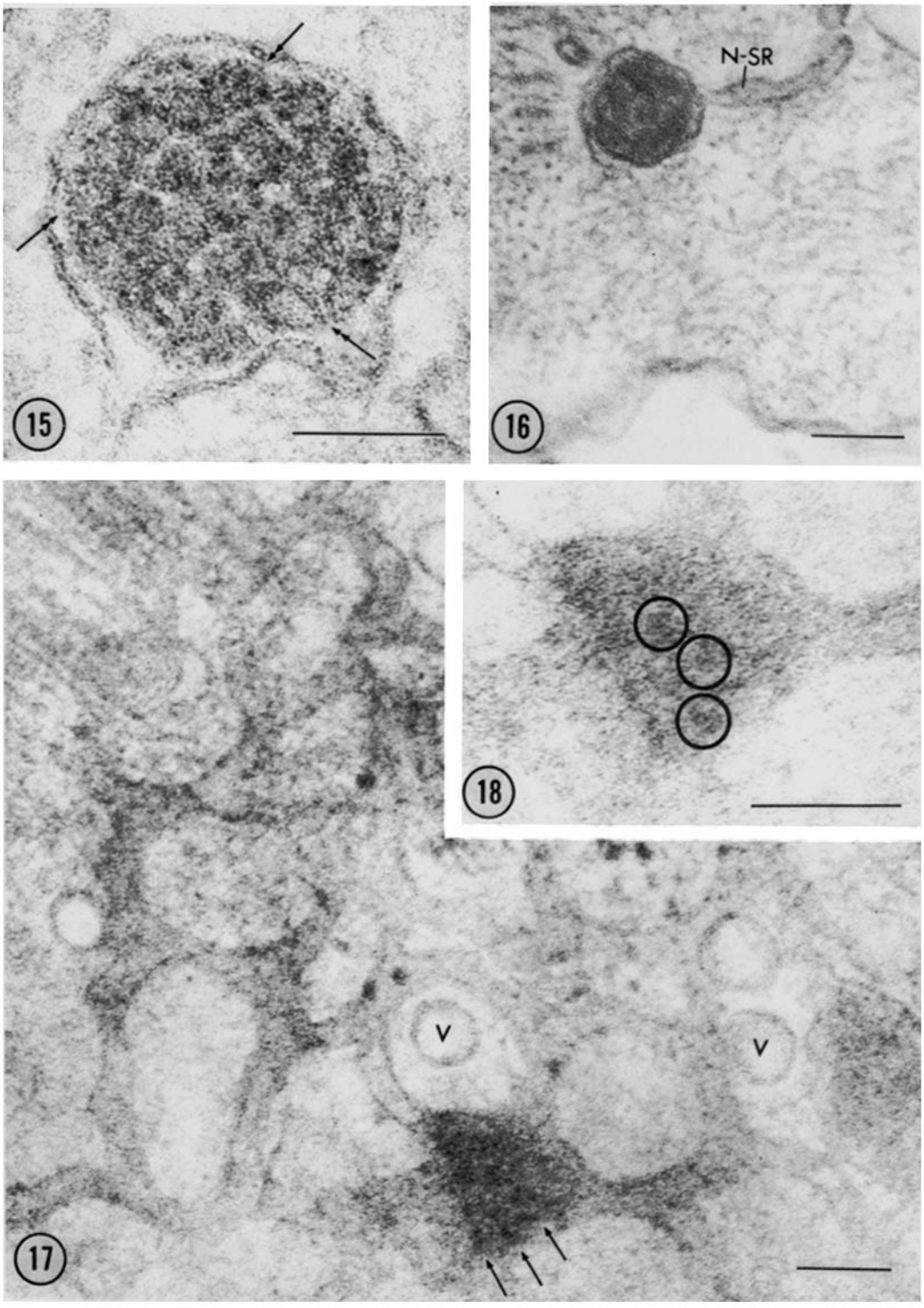
FIGURE 12 Grazing longitudinal section showing elements of SR cut *en face*. The relatively electron-lucent tubules of network SR are continuous with opaque cisternae of junctional SR (arrowheads). Note that the junctional SR in this field is found at various points along the A bands, rather than being confined to the Z-line level as in bird heart. × 32,000; scale bar = 1 μm.

FIGURE 13 Enlargement of the upper left cisterna of junctional SR shown in Fig. 12. Myofilaments are visible in this profile, as well as a number of opaque circular bodies (arrows). × 152,000; scale bar = 0.1 μm.

FIGURE 14 Enlargement of the two upper right cisternae of junctional SR shown in Fig. 12. The lower cisterna has an electron-lucent rim; its opaque interior is composed of circular structures (arrows). × 152,000; scale bar = 0.1 μm.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N-SR
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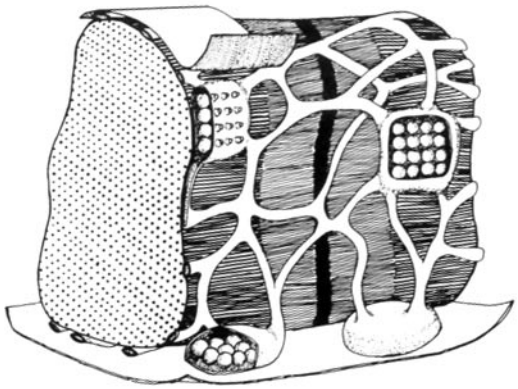

FIGURE 19 Three-dimensional view of a myofilamentous mass from an *Anolis* myocardial cell. Network SR on the surface of the mass merges into four expanded cisternae of junctional SR, three of which are shown in cutaway view. The sarcolemma has been removed over most of the surface of the myofilamentous mass, but at the bottom of the diagram it is shown pulled away slightly from the surface in order to display the upper surfaces of the two junctional SR cisternae forming peripheral couplings there. The intact junctional SR cisterna (lower right) is depicted as an ovoid structure, although other examples (upper right) may present a more rounded, discoidal profile. In cutaways, the spheroidal bodies are shown as being arranged in rows, with corresponding rows of junctional processes (upper left cisterna) overlying them. Viewed end on, the cutaway of the upper left junctional SR cisterna and the associated tubule of network SR is the equivalent of side view sections such as seen in Fig. 8.

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FIGURE 15 High magnification of junctional SR cut *en face*. The opaque spheroids which form the junctional core are particularly evident, and unit membrane-like structures (double-headed arrows) are associated with the spheroids. × 225,000; scale bar = 0.1 μm.

FIGURE 16 A cisterna of junctional SR having its long axis perpendicular to that of the myofilaments. Its junctional core is made up of opaque spheroids, as is the case in peripheral couplings, but the transverse section indicates that the junctional SR is not in contact with the sarcolemma. *N-SR*, network SR. × 137,500; scale bar = 0.1 μm.

FIGURE 17 Grazing longitudinal section along the cell surface, as indicated by the presence of surfaec vesicles (*V*). Elements of network SR merge into a cisterna of junctional SR containing opaque spheroids; some of the spheroids appear to be arranged into rows (arrows). × 139,500; scale bar = 0.1 μm.

FIGURE 18 Enlargement of the junctional SR cisterna shown in Fig. 17. Superimposed on the junctional spheroids are circular profiles (circled) which may represent sections through junctional processes. × 225,000; scale bar = 0.1 μm.

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