The Anatomy of the Sarcoplasmic Reticulum in Vertebrate Skeletal Muscle: Its Implications for Excitation Contraction Coupling

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The sarcoplasmic reticulum *in situ* is an intricate tubular network that surrounds the contractile material in striated muscle cells. Its topographical relationship to other intracellular components, especially the myofibrils, is rather rigidly maintained by a cytoskeleton which enmeshes Z line material and sarcoplasmic reticulum and, ultimately, is anchored at the plasmalemma. As a result, the two main components of the sarcoplasmic reticulum, the junctional SR and the free SR, retain their typical location in the A band region and in the I band region, respectively. The junctional SR, which is thought to be the site for calcium storage and release for contraction, is, thus, always well within one micron of the regulatory proteins associated with the actin filaments. The junctional SR, a synonym for terminal cisterna applying to both skeletal and cardiac muscle, is generally held to be involved in the translation of the action potential into calcium release, mainly because of the close topographic apposition between the junctional SR and the plasmalemma, especially in skeletal muscle. This attractive structure-function correlation is challenged by the observation that in bird cardiac muscle 80% of the junctional SR is spacially far removed from plasmalemma, the site of electrical activity. This anomalous topography is not in conflict with the notion that translation of the action potential into calcium release may be accomplished by a diffusible transmitter substance, *e.g.* calcium. Any hypothesis dealing with this problem must account for the anatomy of the bird heart.

Anatomy and topography are very useful tools to gain insight into both structure and function of cell constituents. They define the structure and geometric relationship of intracellular compartments and, thus, provide insight into the possible separation of concurrent or sequential functional events that otherwise might interfere with eachother. Anatomy and topography have made valuable contributions also to the study of the sarcoplasmic reticulum (SR) of striated muscle, both in confirming and, indeed, predicting functional interactions. For example, and given the existence of the calcium pump, the very geometry, topography and distribution of the SR in striated muscle appears quite suitable, *ipso facto*, to accomplish with consummate speed and efficiency the removal of calcium from the myofibrillar compartment during muscle relaxation. Analogously, the unique, close apposition between certain SR compartments (the junctional SR) and the plasmalemma almost inescapably invites the conclusion that this membranous junction is, *ipso facto*, the anatomical site at which the action potential (demonstrably carried by the plasmalemma) is translated into the release of calcium (demonstrably contained within the SR).

In the following I shall give a brief account of the *in situ* anatomy of the SR in striated muscle, drawing on comparative data coming from a variety of animals and from cardiac *vs.* skeletal muscle [1 – 8]. In the discussion I shall emphasize some comparative features that counsel caution toward accepting some of the widely held premises concerning structure-function correlations in excitation-contraction-coupling (ECC).

Terminology and Dimensions

Figure 1 defines the terminology used in this communication. It emphasizes structural homology between cardiac and skeletal muscle without obscuring existing differences.

Quantitative morphometric data are available in a number of reports in the literature [2, 3, 9 – 12].

The Overall Geometry of the SR

The SR is a network of tubules which is wrapped around the contractile material of striated muscle fibers (Figs. 1, 2, 3). The quantity of the SR is, if on-

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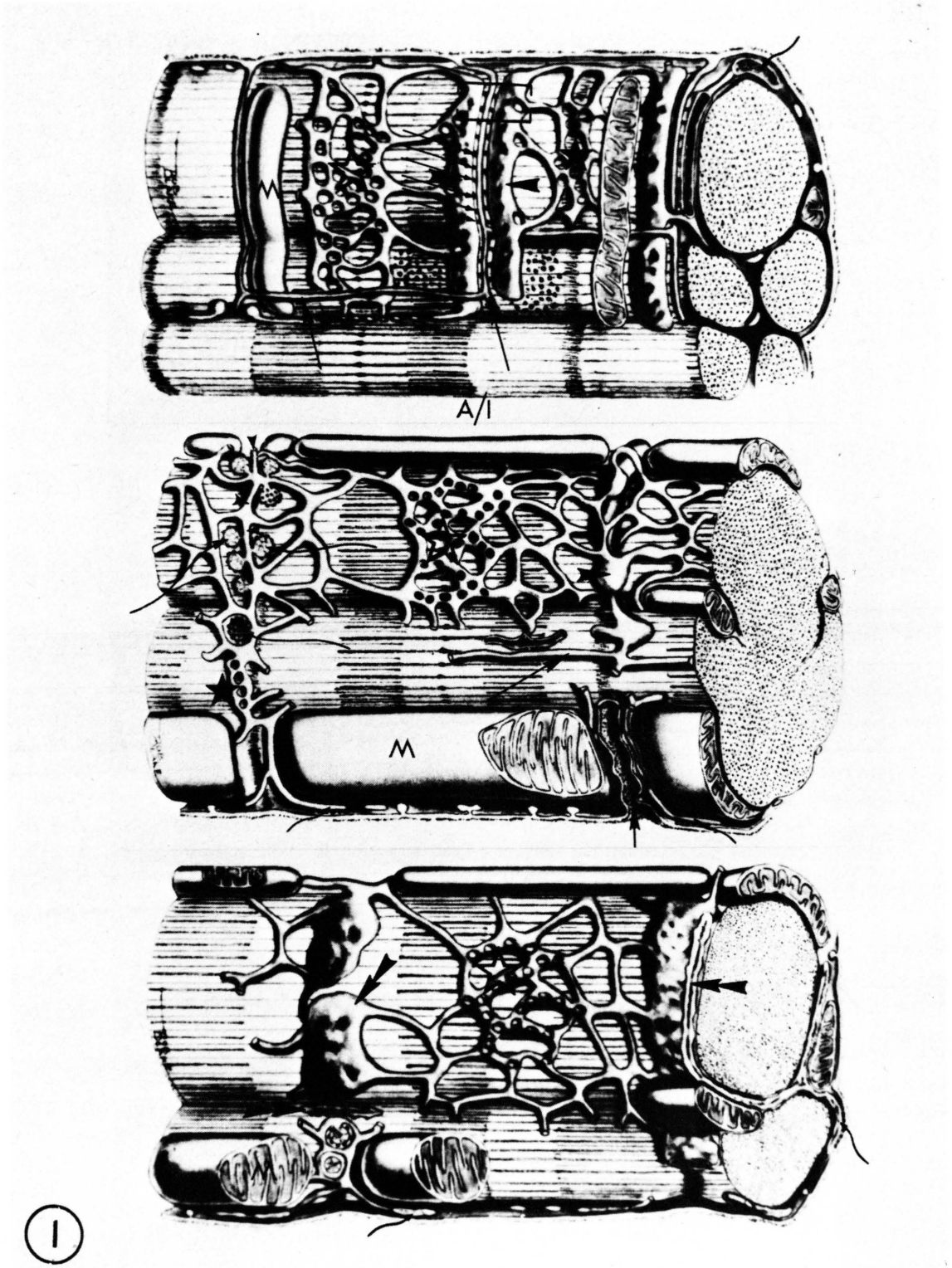

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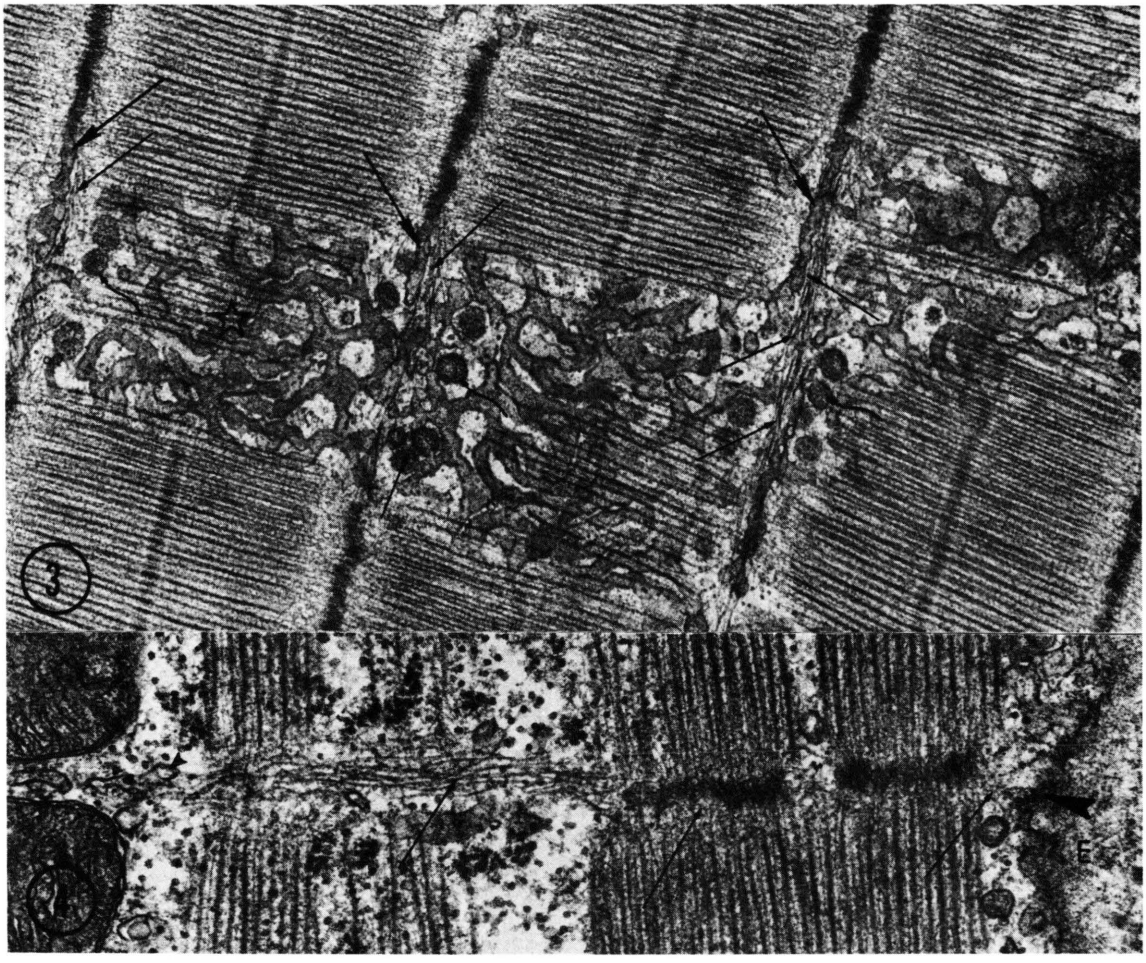

Fig. 3. Opossum cardiac muscle. The SR of almost three sarcomeres is exposed in a grazing section showing the M rete (open star), corbular SR (curved arrows), the Z tubules (large straight arrows), and the anchor fibers (thin straight arrows) that maintain the overall topography of the SR within the sarcomeres. Note the junctional processes at the upper border of the corbular SR indicated by the thin straight double arrow (Courtesy Am. Physiol. Soc., 1979).

Fig. 4. Opossum cardiac muscle. The anchorfibers of the cytoskeleton extend from the plasmalemma (large arrow head), coursing around the Z line by grazing its surface (thin arrows), onto some SR tubules (small arrow head). Extracellular space = E. × 50000.

◁ Figs. 1 and 2 see pages 666 – 667.

Fig. 1. Schematic drawing of skeletal (top), mammalian cardiac (middle) and bird cardiac (bootom) muscle. The fiber segments show the triad in skeletal muscle (brackets), interior junctional SR (large arrow heads) forming interior couplings with transverse tubules (straight arrows), peripheral junctional SR (small curved arrows) forming peripheral couplings with the surface plasmalemma, junctional processes (thin straight arrows), corbular SR (large curved arrows), extended junctional SR (double arrow heads), the M retes with fenestrations (large open stars), the Z retes with fenestrations (small black stars), and the Z tubules (Small arrow heads) at Z lines. Mitochondria = M. A/I junction = A/I (Courtesy J. Cell Science, 1978).

Fig. 2. Mouse skeletal muscle. SR and transverse tubules contrasted with lanthanum. The illustration shows the overall geometry of the SR. Round brackets indicate the M rete of the free SR, square brackets the Z rete with the fenestrations. The M retes are connected with the junctional SR (large curved arrows), that form triads (braces) with transverse tubules (large straight arrows), through a few longitudinal tubules (large straight double arrow). The junctional SR is separated from the transverse tubules by the junctional gap (thin double arrows) which is bridged by periodic feet, or junctional processes, that are quite well visible in the upper left corner. In that sarcomere one can also see a longitudinal branch of the transverse tubules. Free SR elsewhere = arrow head. Mitochondria = M. × 34000 (Courtesy J. Cell Biology, 1973).

ly roughly, correlated with the frequency and speed, excluding asynchronous muscles, with which a given muscle can beat. For example, the cricothyroid muscle of the bat [13], the telson muscle of the lobster [14], and the cardiac muscle of some small animals with fast heart rates (bat, bird, mouse) contain a large amount of SR, while tonic muscles and the hearts of some small vertebrates with slow heart rates (frog) have less. It should be noted, however, that a rigorous stereologic assessment of the relative quantity of SR between the different types of skeletal muscle, fast vs. slow etc., has not been reported in the literature. Although urgently needed, such studies are quite hard to do because of the difficulty of obtaining pure fiber populations and the difficulty to distinguish, rigorously, between free SR on the one hand, and non-junctional free transverse tubules (TT), on the other. Some illustrations of slow muscle fibers in the literature clearly show a very extensive SR network [1].

The SR is a continuous network of tubules displaying a repetitive topographic pattern from one sarcomere to the next which is maintained by a cytoskeleton that provides a quasi scaffold made up of bundles of intermediate filaments to which Z lines and SR are attached [15–17] (Fig. 3, 4). The Z lines, in turn, are attached to the plasmalemma which acounts for the Z-grooves that give striated muscle the scalloped surfaces, especially at short sarcomere lengths. The cytoskeleton also explains the invariant topography of the SR network. The rigidity with which this topography is maintained across classes signifies its probable importance for muscle function.

The SR has two main components, the junctional SR (JSR) and the free SR which are anatomically and topographically distinct but are continuous with each other through their membranous envelopes and lumens (Figs. 1, 2).

The Topography of the Junctional SR (Terminal Cisterna)

The *JSR* is attached to the plasmalemma (exceptions are the extended junctional SR, EJSR, and the corbular SR, CSR, of cardiac muscle; see below). It forms so-called peripheral couplings with the surface plasmalemma (syn. dyad) and so-called interior couplings (as dyads, triads, pentads) with the TT in both skeletal and cardiac muscle [2]. Peripheral couplings are usually located near Z lines at the plasmalemma.

EJSR of bird cardiac muscle is different from JSR only by its topography, not by its anatomy; bird cardiac muscle has no TT (Fig. 5) [2]. The EJSR is always located in the interfibrillary spaces at Z lines. It is far removed from the plasmalemma. The volume fraction of the total JSR (JSR and EJSR) in bird cardiac cells equals that found in the mouse heart [18]. Both hearts have similar heart rates and sizes. But 80% of the JSR in birds is EJSR; *i.e.*, not connected with plasmalemma. Only the remaining 20% is JSR attached to plasmalemma forming peripheral couplings.

CSR shares all fundamental anatomical features with the JSR of striated muscle [2, 19]. Topographically it is, however, a relative of EJSR in birds because it, too, lacks plasmalemmal contact. CSR occurs also in mammalian cardiac muscle cells that have no TT, *e.g.* in the conduction cells and in many atrial cells. It is also present in those selected regions of common ventricular working cells that lack TT. CSR is budding from, EJSR is intercalated into the Z rete of the free SR which is located around the Z line (see below). In all striated muscles, the JSR and its derivates (CSR and EJSR) are always well within 1 μm of the I band filaments and its regulatory proteins.

The Topography of the Free SR

The *free SR* consists of all membranous envelopes of the SR, including the nuclear envelope, that are free of differentiated JSR, EJSR or corbular SR (Figs. 1, 2). The free SR has three regions of different geometry in most striated muscles. The Z rete, the M rete (fenestrated collar), and the longitudinal tubules. The Z rete is a network of SR tubules that are arranged across the Z line connecting the JSR of adjacent triads when the TT (and, thus, the triads) are located at the A-I junctions, as in most skeletal muscles. In that case, the Z retes may be very extensive (13). In muscles in which the TT are at the Z line, *e.g.* bird pectoral muscle and all cardiac muscles with TT, the Z rete is absent or, at best, rudimentary. In cardiac muscle cells that lack TT, the Z rete is represented by the Z tubules which wrap around the Z lines [20] (Figs. 1, 3). The Z and M rete (fenestrated collar, ref. 3) contain fenestrae of even diameters (about 40 nm) in both cardiac and skeletal


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muscle [2]. This geometry provides a substantial surface to volume ratio of the SR in this region. The connections between the M rete and the JSR are usually a few longitudinal tubules. However, in many cases a widely interconnected network reaches as far as the JSR (Fig. 3). In many instances the cisterna of the JSR extends as far as the M line or beyond (Figs. 6, 7). This occurs only in skeletal muscle. In cardiac muscle one does find cisternal dilatations in the free SR in many places [19, 21] but these are never extension of the JSR.

Most electron photomicrographs of skeletal muscle show the JSR as a distended pouch connected to a markedly flattened cisterna, the intermediate cisterna [3, 22, 23] (Figs. 6–9), before joining the short longitudinal tubular connectors to the M rete. The flattening of this portion of the cisternal JSR is best explained by collapse of the wide cisternal space, a collapse that cannot occur in tubular SR nor at the edges of large cisternal spaces, because it would violate the minimal radius of curvature of the SR membranes. The pouch of the JSR remains distended, because it contains a large amount of granular material (Figs. 6–9). It is important to note that the intermediate cisterna usually does not contain granular material (Fig. 6), although such material is seen elsewhere in the free SR in addition to its prominent accumulation in the JSR pouch (Figs. 6, 8, 9). Sometimes, however, the intermediate cisterna is not collapsed (Fig. 6). In that case granular material, which can be contrasted with cationic dyes such as ruthenium red (Figs. 8, 9), extends from the JSR pouch far into the free SR. This suggests that at least some of the negatively charged granular material in the JSR is freely movable within the SR network, and that the geometry of the SR envelopes may be subject to geometric variations during the contraction-relaxation cycle. For example, it is possible that the free SR is capable of oscillations between an open and a collapsed state, as well as between a tubular and a cisternal configuration. It has been shown that ruthenium red and other cations succeed in totally obliterating the intermediate cisterna, leading to the formation of pentalaminate compound membranes in which the inner lamellae of the SR envelopes fuse to become one (Fig. 9) [22–25]. It has also been demonstrated that this total collapse of the SR membranes takes place only in cisternal SR (JSR and free SR) and that it is reversible [22]. A similar collapse is found in isolated SR membranes that had previously been forced into a lamellar array by partial drying. Whether this collapse is related to the time course of the contraction relaxation cycle, whether it is due to the action of specific ions (*e.g.* calcium) or due to osmotic phenomena, has not been sorted out. Since this collapse is seen also in quick-frozen isolated skeletal muscle fibers that had not been exposed to either fixatives or cryoprotectants [22], it is likely that the phenomenon, indeed, may be an *in vivo* one. If it were, the following consequences could be anticipated: 1. vectorial collapse of SR membranes could provide the *vis a tergo* to move SR contents, *e.g.* toward the JSR pouch and, 2. the obliteration of the lumen of the intermediate cisterna would result in electrical isolation of the JSR from the free SR. Whether either is of physiological significance remains to be seen. A similar collapse of SR membranes has not been observed in cardiac muscle [2].

The Anatomy of the Junctional SR

The *junctional SR* (*JSR*) consists of a SR cisterna which in skeletal muscle is dilated. It is terminal in skeletal muscle (terminal cisterna) in that it ends at the TT, flattening the latter over the contact distance, and only occasionally making contact with a similar cisterna on the opposite side of the TT. In cardiac muscle the JSR tends to have numerous connections with the opposing JSR across a TT, as well as with the free SR of adjacent sarcomeres. Thus, compared with skeletal muscle, it is not terminal in that sense. The JSR in cardiac muscle may form complete collars around a TT [2]. Cardiac JSR is not dilated. Rather, it has a diameter corresponding to the average diameter of the free SR tubules (about 40 nm).

Fig. 5. Finch cardiac muscle. Parts of 6 muscle cells show extended junctional SR away from the plasmalemma (straight arrows) the morphology of which is identical to the junctional SR forming peripheral couplings with the plasmalemma (curved arrows). Intercalated disc = ID. × 22 500. Inset: Higher magnification of extended junctional SR (straight thin arrows) at Z lines (asterisks) showing "central membrane" (large arrows). The junctional processes are mainly out of the plane of the section. The junctional SR of a peripheral coupling (curved arrow) is displayed in tangential section at the plasmalemma bordering the extracellular space (E). × 130 000.


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The JSR forms dyads (couplings) and triads (two couplings across a TT) in both cardiac and skeletal muscle.

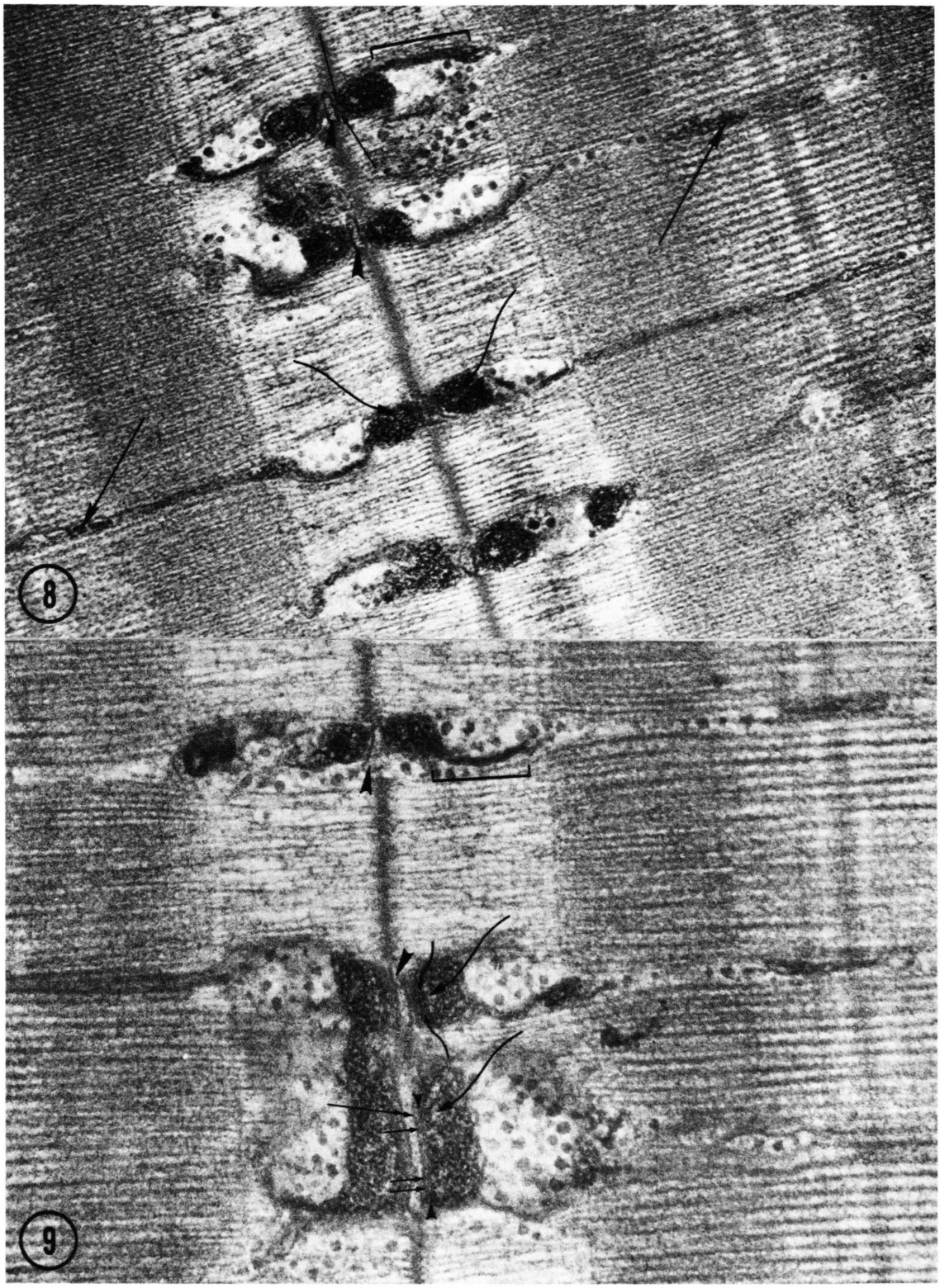
The membranous envelope of the JSR of both cardiac and skeletal muscle has two components that can be distinguished by freeze-fracture. One part of the JSR envelope has a freeze-fracture morphology indistinguishable from that of the free SR. The other part, the junctional face proper, which is the JSR membrane subjacent to the junctional processes (see below), contains fewer intramembranous particles, many of which are much larger than those found in the free SR. The intramembranous anatomy of the JSR's junctional face is especially well exposed in freeze-fracture preparations of the EJSR in bird cardiac muscle which consist almost completely of junctional faces [2, 26]; in bird hearts TT are absent and, thus, cannot divert the plane of fracture away from the junctional face which, annoyingly, they do when present. In skeletal muscle, periodic groups of particles have been demonstrated on the E face of the TT opposite periodic E face pits in the junctional membrane of the JSR [5, 7], but the particles and pits are not strictly complementary by number or location. In cardiac muscle the particles in the TT are absent, but pits on the E faces of the junctional membranes of the JSR, EJSR and CSR have been shown to occur [19, 26]. Recently, freeze fracture preparations through the junctional face of the JSR in a skeletal muscle have revealed periodic elevations of roughly cone-shaped geometry matching the JP lattice of junctional processes [27].

Junctional processes (feet; JP) of the JSR are one of the morphological features that define the JSR. They are about 20 nm apart from each other and are on the cytoplasmic side of the junctional faces of the JSR. The JSR of skeletal muscle has one such face toward the TT. Cardiac JSR, too, thas JP always on the side facing the TT, but often also on the other side of the JSR which faces open cytoplasm. When facing the TT or surface plasmalemma, the JP sometimes make contact with the plasmalemma, at other times they stop short of it. Occasionally, filamentous adhesions bridge the gap between JSR and plasmalemma (the junctional gap) out of register with the JP. The junctional gap varies in width from, say 15 nm to 25 nm, but usually the junctional face of the JSR and the apposed TT membrane are kept rather rigidly parallel. The JP of skeletal muscle have been described in great detail [5, 7, 27] and are identical to those seen in cardiac muscle [2, 18]. In cardiac muscle they are most prominent in hearts that are capable of very fast rates, such as in the mouse, bat and bird. There are indications, that the JP have hollow cores [2, 28, 29], and that they are attached to TT. Tracer experiments are not in conflict with the notion that cone-shaped cores of the JP are continuous with the lumen of the JSR and, indeed, with the lumen of the TT [29]. However, no direct unequivocal connection between the two adjacent lumens has ever been established! Indeed, there is strong morphologic evidence that the SR and the extracellular space are and remain separate compartments [4]. In skeletal muscle the occurence of JSR/TT bridges (pillars), not necessarily identical with the JPs, has been shown to increase after muscle stimulations [30]. When one embeds muscle in urea/glutaraldehyde which avoids lipid solvents during embedment, no membrane bridges between JSR and TT have been discovered so far [2]. Abnormal accumulations of JSR in back-to-back arrangement forming multilamellar arrays have been found in cardiac muscle [44]. These formations, in addition to EJSR and CSR, suggest that JSR is a genuine structural differentiation rather than being merely the result of SR/plasmalemma interaction.

Junctional granules (JG) refers to electron-dense material in the JSR of cardiac and skeletal muscle.

Fig. 6. Frog skeletal muscle. The junctional SR forms a triad with a transverse tubule (arrow head). On one side, the junctional SR forms a pouch (P) which turns into a flattened portion, the intermediate cisterna (bracket) that further to the right opens up into the free SR. On the other side the junctional SR seems to be widely open to the rest of the free SR (curved arrow). The intermediate cisterna does not contain granular material while the pouches of the junctional SR and the free SR do. ×39000 (courtesy J. Cell Biology).

Fig. 7. Frog skeletal muscle (freeze fracture preparation). This fracture has exposed the SR of two sarcomeres from one triad to the next. In this case (cf. Fig. 2) the SR is in its cisternal form (star) with the exception of some fenestrae (thin straight arrows) remaining in the center of the sarcomere and some tubules (large straight arrows). The junctional SR is distended in the form of pouches (P) forming a triad with the transverse tubule (arrow head). Because the entire free SR is cisternal and collapsed, there is no line of demarcation between the free SR and the intermediate cisterna. ×41000.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In cardiac muscle they consist of hatched linear electron densities ("central membrane"; Fig. 5, inset; ref. 2) that bisect the JSR without notably distending it (Fig. 5). In skeletal muscle, the JG are contained in a pouch-like distension of the JSR (Figs. 6–9). The granular material is apparently highly negatively charged as it stains heavily with cationic substances such as alcian blue, ruthenium red and cetylpyiridinium chloride [25] (Figs. 8, 9). Granular material of the type found in the JSR is also sparsely distributed throughout the free SR, especially in the M rete (Fig. 8). The "central membrane" in the JSR of cardiac muscle has its equivalent in skeletal muscle in the form of the "coextensive line" [31, 32]. It consists of electron densities in parallel with the junctional membrane of the JSR that periodically make contact with the inside of the junctional face of the JSR, but without being in register with the JPs on the cytoplasmic side of the junctional face. When uranyl acetate is introduced into the SR network [29] acting as a negative stain, the "coextensive line" cannot bei discerned. This suggests that the "coextensive line" represents very loosely aggregated substances (proteins?) between which uranyl acetate can readily diffuse more or less randomly, thus producing more homogeneous electron scattering in the absence of a persuasive structural order. Cations added to net anionic proteins can lead to considerable conformational changes in such proteins. Ruthenium red may amplify the visibility of the "coextensive line" even as it distorts its geometry. It causes electron-lucent spaces to appear between the "coextensive line" and the rest of the JSR [2] (Fig. 9). In many preparations, ruthenium red stains only granular material close to the junctional face of the JSR, leaving an electron-lucent halo in the periphery [32]. These morphological findings suggest that the JG are negatively charged material, part of which is attached to the luminal aspect of the junctional membrane, and part of which is elsewhere in the JSR and free SR (see below).

The nature of the JG continues to be uncertain. Since the granular material clearly is highly negatively charged, it may represent calsequestrin. In fractionated isolated SR preparations calsequestrin elutes in the heavy fraction which contains much JSR [33, 34]. The light fraction, which is composed mainly of free SR, contains little calsequestrin. Fluorescent anti-calsequestrin antibody experiments show calsequestrin to be localized mainly in the regions of the JSR of traids at the A-I junction [35, 36] and in the JSR of triads at the Z line [36]. Fluorescence may also occur in the regions of the M rete [36], that is in a location in which ruthenium red-stained granular material often is found in the SR of skeletal muscle. The M rete is also the region showing the greatest density of free SR membranes. Electron microscopic immunocytochemistry with peroxidase-labeled anti-calsequestrin antibodies has revealed that most of the reaction product is located over the regions of the JSR, but also along all the membranous envelopes of the free SR [36]. The JSR lumens often were partially empty. At the moment, the precise nature of the JG has not been established, neither has that of the "coextensive line". Once antibodies against the high affinity calcium-binding protein become available, it would be interesting to see where they localize.

Histochemically, the JSR, free SR and corbular SR have been shown to contain phosphatase activity, with many nucleoside tri- and diphosphates serving as substrates [2]. Recent cytochemical studies suggest that the so-called basic ATPase of SR vesicles localizes at the plasma membrane, especially at the TT [37]. TT membranes always contaminate isolated SR fractions [38]. Calcium has been localized with the

Fig. 8. Frog sekeletal muscle treated with ruthenium red. The junctional granules are seen within the pouches of the junctional SR (curved arrows) that form triads with the transverse tubules (arrow heads). Vague connections between the junctional face of the junctional SR and the transverse tubules across the junctional gap are seen (thin straight arrows); these are the junctional processes. The region of the intermediate cisterna is completely collapsed. Junctional granules are also seen in the free SR as far as the M rete (large straight arrows). In the lower part of the picture is a double pouch on the right which is filled with granular material. ×47 000.

Fig. 9. Frog skeletal muscle treated with ruthenium red. The granular material in the junctional SR is separated into two portions by electron-lucent regions (large curved arrows). One of them (opposed small curved arrows), the "coextensive line" is close to the junctional face (between opposed small arrow heads) from which bridging densities (short thin arrows), the junctional processes, emanate toward the membrane of the transverse tubule (double thin arrow). Transverse tubules = large arrow heads. Intermediate cisterna = bracket. ×47 000.

electron microscope in the form of calcium oxalate to the entire SR, including the JSR, the calcium component having been ascertained by microprobe analysis [2, 39]. The JSR in frog skeletal muscle is the preferred site of filipin-cholesterol complexes (FC) for reasons unknown [23]. In preparations treated with filipin, open connections between JSR and TT have been found. They do not occur in cardiac muscle under similar experimental conditions, and neither mouse nor bird cardiac muscle show any predilection of FC for JSR. The occurence of FC in skeletal muscle SR *in situ* compares well with the magnitude of cholesterol discovered biochemically in isolated SR membranes. Although the predilection of FC for the JCR/intermediate cisterna in frog skeletal muscle is striking, the filipin experiments in skeletal and cardiac muscle have not helped in elucidating the anatomical relationship between JSR and the plasmalemma.

Concerted efforts have been made more recently to gain insight into the dynamics of ion movements in muscle and to correlate these movements with ultrastructural substrata [40]. Earlier, radioautographic investigations intimated that there was displacement of calcium from the *Z* line region to the A band in tandem with the contraction-relaxation cycle [41]. Much needed sophistication and rigor has been introduced into these structure-function correlations by use of techniques and instrumentation such as electron probe x-ray microanalysis and electron energy loss microanalysis. Suffice it to say, that such studies have shown, among others, that Ca^{2+} is indeed released from the JSR during tetanus, and that the total amount of Ca^{2+} released agrees with the predicted amount bound to troponin and parvalbumen [40]. While these experiments underscore the importance of the JSR as the store for that calcium which is to be released for the contractile event, *per se*, the tentative conclusions drawn from them are not immediately applicable to cardiac muscle as yet. In that muscle the possible relationship between extra- and intracellular Ca^{2+} on the one hand, and the contractile process on the other is much more complex, it seems.

Discussion

In recent years much attention has been focused on the mechanism involved in excitation-contraction-coupling (ECC), especially the mechanism by which electrical activity at the plasmalemma (the action potential) is translated into calcium release for contraction. Electrical records have been interpreted to show a voltage dependent charge transfer at the plasmalemma which, according to one scheme [42], is translated at the coupling into a transformational change across the JP that effects the JSR and results in calcium release. Another scheme suggests that the charge transfer across the plasmalemma leads to current invasion of the JSR resulting in calcium release [43]. Both schemes need to surmount major obstacles. In one case, transformational changes across the junctional gap must be explained. In the other, the problem remains as to how the bulk of the SR membranes is electrically isolated from the plasma membrane, if only at the instance of electrical transmission. In any case, in both schemes the close apposition, if not fusion (however transitory) of JSR with the plasmalemma is *conditio sine qua non.*

The central role of the coupling for ECC finds support in the fact that couplings occur in all skeletal and cardiac muscles of vertebrates so far studied with only minor variations in topography and geometry. However, there are at least two examples which demonstrate the JSR also occurs in the absence of plasmalemmal contact, namely mammalian and bird cardiac muscle. In these two classes of animals the anatomy of the JSR in cardiac muscle is fundamentally identical to the JSR in vertebrate skeletal muscle. In bird cardiac muscle, however, 80% of the total JSR (in the form of EJSR) is not attached to, indeed, is far removed from plasmalemma by several micra in most instances. In mammalian cardiac muscle CSR occurs far removed from the plasmalemma in many regions of such cells that do not have TT, and in all cells that have no TT at all (conduction cells and many atrial cells). Consequently, any hypothesis concerning the translation of the action potential into calcium release during ECC must account for this anomaly within the generally accepted scheme of things. EJSR is not likely to be a nonsense remnant of phylogeny: In chickens it is much less prominent and less differentiated than in passerine birds. These two orders of birds are also distinguished by vastly different heart rates, perhaps analogous to the differences between frog and mammalian hearts concerning their respective heart rates and JSR differentiation; JSR is more prominent and better differentiated in the fast beating mammalian as opposed to the slower beating frog heart muscle.

The anatomy of bird cardiac muscle SR suggests that the EJSR (like JSR in other striated muscles) probably serves as the store and release-site for calcium. This is supported by its topography (JSR, EJSR and CSR are always well within 1/2 to 1 μm from the regulatory proteins of the I band) and by anatomical analogy with the JSR of skeltal muscle for which pertinent data from microprobe analysis exist. Whether the JSR has anything to do with the translation of electrical activity at the plasmalemma into calcium release is another matter. It should be recalled that the JP are usually referred to as attachment points between JSR and plasmalemma, although JP are also present at EJSR and CSR where the JP terminate freely in the cytoplasm [2, 44]. Nevertheless, the contact between JSR and plasmalemma seems to be a rather tight one. It is often preserved even in tissue revealing major distortions due to fixation. Indeed, even frationation procedures preserve JSR/plasmalemma contacts [45]. But while the JSR seems to be bonded tightly to the plasmalemma, it is not at all clear whether that bonding is accomplished by the JP. Rather, it is more likely due to another site unrelated to JP, which in the absence of plasmalemma simply remains unoccupied, or attaches to something else, such as the cytoskeleton.

It could be argued that in bird cardiac muscle the translation of the action potential takes place at the peripheral couplings all the same, whence it is conducted through the free SR to the EJSR deep inside the muscle cells. This would require the propagation of a signal along the free SR. Studies on skeletal muscle SR make it unlikely that the SR membranes can sustain a membrane potential, much less propagate one [46]. The propagation of signals other than electrical is conceivable but such signals remain to be discovered.

The very topography of the JSR in bird cardiac muscle, therefore, favors the translation of an action potential into calcium release to occur through a transmitter substance. There is a large body of evidence showing that calcium release can be triggered by calcium [47]. Indeed, in bird cardiac muscle, which has no TT, intracellular diffusion is facilitated by a relatively small cell diameter. In mammalian cardiac muscle cells, the disadvantage of cell diameters in excess of twice that of bird cardiac muscle cells is overcome by the presence of TT. The anatomy of cardiac muscle cells of lower vertebrates (frog, lizzard) is also not in conflict with the notion that the JSR is a source of calcium for contraction. Cardiac cells of these animals have peripheral JSR only, but the cell diameters are much smaller than those even in the bird. Moreover, the fast beating heart of the lizzard has vastly more JSR (at peripheral couplings) than the much slower beating heart of the frog. Within this context it should be recalled that the total JSR (interior at TT plus peripheral at surface plasmalemma) in the mouse heart occupies exactly the same cell volume fraction that the total JSR (peripheral JSR and EJSR) occupies in cardiac muscle cells of birds. Both hearts have similar sizes and heart rates.

A better understanding of the calcium release mechanism initiated by electrical activity at the plasmalemma must await biochemical studies on isolated JSR, and morphological studies of the couplings during stimulation. The former will tell us something about the properties of the proteins and other substances in this particular portion of the SR. The morphological studies hopefully might show structural alterations in tandem with the time course of the contraction-relaxation cycle, and analogous to the spectrum of membrane alterations that are now possible to be studied with ultra-rapid freezing techniques following electrical stimulation. At the moment there is no objection to the notion that calcium release from the JSR may actually take place by a sudden transitory rupture of the JSR envelope, perhaps even in the form of vesicular quanta. Such events should be detectable provided they take no less than a millisecond or two.

In any event, I should like to reiterate the importance of the bird's heart in our attempts to understand structure-function correlation in ECC and, by paraphrasing senator Cato from ancient Rome, I should like to close with the admonition: Ceterum censeo avem sanguineam esse memorandam.

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