The Collapse of the Sarcoplasmic Reticulum in Skeletal Muscle*

Joachim R. Sommer, Nancy R. Wallace, and Wilhelm Hasselbach

Duke University Medical Center, the VA Hospital, Durham, and Max-Planck-Institut für Medizinische Forschung, Heidelberg

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When various cations, including Ca^{2+}, are in the fixative, both sarcoplasmic reticulum (SR) of whole skeletal muscle and isolated SR vesicles collapse to form pentalaminate "compound membranes" that result from the apparent fusion of the lumenal lamellae of the membranous envelope of the SR. The process may be reversed by subsequently soaking the tissue in 1 M NaCl. An identical morphological phenomenon is observed in unfixed quickly frozen isolated frog skeletal muscle fibers, the cation in that case coming from endogenous sources. The hypothesis is advanced that the collapse is an *in vivo* process mediated by the sequestration of Ca^{2+} after contraction. The resulting obliteration of the SR lumen would have the effect of displacing the SR contents into the junctional SR, as well as electrically isolating the free SR from the junctional SR during relaxation. As a consequence, resistive coupling between the plasmalemma and the junctional SR becomes a plausible mechanism for the translation of the action potential into Ca^{2+} release, since the bulk of the SR membrane capacitance would now remain separated from the plasmalemma during relaxation.

Introduction

The present investigations were undertaken to study the formation by cations of external compound membranes [1] in the sarcoplasmic reticulum (SR). Quinton and Philpott [2] have demonstrated that the plasmalemmas of adjacent gallbladder epithelial cells exposed to polylysine fuse to form a pentalaminate membrane between the two cells. Howell [3] extended this observation to a topologically equivalent internal membrane system, the SR of skeletal muscle, by using the cation ruthenium red. We enquired whether the formation of the pentalaminate membranes 1. is confined to the so-called intermediate cisterna [4] of the SR as Howell had suggested, 2. is reversible, 3. occurs in the presence of cations other than ruthenium redespecially Ca^{2+}, 4. is present in unfixed quick frozen and freeze-substituted isolated muscle fibers and, 5. is seen also in isolated SR vesicles *in vitro*. The overall question of interest is whether the formation of the pentalaminate membranes in the SR of skeletal muscle is representative of an *in vivo* collapse of SR membranes mediated by the sequestration of calcium by the calcium pump of the SR. Such a collapse, properly timed within the contraction-relaxation cycle, would have important physiological consequences for excitation-contraction coupling.

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Reprint requests are to be adressed to Dr. Joachim R. Sommer, Department of Pathology, Duke University Medical Center, Durham, North Carolina 27710, U.S.A.

Materials and Methods

Whole muscle in situ

Isolated small bundles of frog skeletal muscle (*M. sartorius*) consisting of about 1 to 5 individual muscle fibers from *Rana pipiens* were used. The frogs were pithed and the sartorius was dissected and clamped in situ. The muscle was cut and put into 3% glutaraldehyde buffered with 0.1 M cacodylate and containing, respectively, no cation, 10 mM $BaCl_2$, 10 mM $BaCl_2$, 10 mM $LaCl_3$, 10 mg/100 ml volume alcian blue (Allied Chemical), 10 mg/100 ml volume cetylpyridinium chloride (Sigma), or 10 mg/100 ml volume ruthenium red. After about 15–30 min very small bundles of fibers were isolated and left in the fixative with or without cations from 6 to 72 h at room temperature. Thereafter the small bundles were inspected to see if they were stained, at least in the case of RR and AB [3]. All fiber bundles were briefly rinsed in distilled water, in block stained with 4% uranyl acetate in water for 60 min [5, 6], post fixed in 2% osmium tetroxide for 1 h, and embedded in Epon 812 [7] after dehydration in ascending concentrations of ethanol and finally, propylene oxide. Alternate samples of small bundles of muscle fibers were exposed to ruthenium

red in glutaraldehyde for 12 h and then soaked in 1 M NaCl for 96 h after which the bundles were embedded for electron microscopy. Longitudinal and transverse sections were cut with a diamond knife on an LKB Ultratome. The sections were stained on collodion and carbon-covered copper grids with lead citrate [8] (and in some cases, with uranyl acetate and lead), for 10 min and viewed at 60 kV with a Jeolco 100B electron microscope fitted with an objective aperture of 0 μm in diameter.

Rapid freezing

Single fibers approximately 30 nm in diameter from *M. semitendinosus* or *M. sartorius* of *Rana pipiens* and *Xenopus laevis* were isolated in amphibian Ringer's solution [9] and wrapped around prongs cut into the ends of wooden applicator sticks. The applicator sticks were rapidly dipped by hand into Freon 22, traveling about 3 inches through the coolant within a fraction of a second. The coolant was prepared in the following manner (personal communication, A. V. and A. P. Somlyo). Freon 22 was cooled with liquid nitrogen to between -162 and -166 °C in a 250 ml Erlenmeyer flask under constant stirring with a magnetic stirring rod. Dry nitrogen was blown over the mouth of the flask. The applicators with the isolated muscle were kept in the coolant for about 30 seconds and then quickly transferred into test tubes containing a mixture of 1% osmium tetroxide in dry acetone kept at -80 °C in a freezer for freeze substitution which was continued for 12 h. The test tubes were then rapidly brought to room temperature. The applicator sticks with the fibers were transferred into 2% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2 and left there for 60 min at refrigerator temperature. After a rinse in distilled water the fibers were removed from the applicator sticks and put into 4% uranyl acetate in water for 60 min, after which the tissue was dehydrated and embedded in Epon.

Preparation of isolated SR

SR of skeletal muscle of the rabbit was isolated according to the technique by Hasselbach and Makinose [10]. The procedure of creating lamellar layers of flattened SR vesicles followed the regimen

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Fig. 1. Frog sartorius muscle fixed in glutaraldehyde. Control. The sarcomere stretches from z line to z line (z). The thick myosin filaments (m) form the A band (A) that shows the M line in the center (M). The thin actin filaments form the I band (I). The M rete is a dense network covering the central third of the A band and often shows fenestrations (small arrows). The M rete is connected to the (junctional sarcoplasmic reticulum JSR; curved arrows) by a few longitudinal tubules (small arrow heads) the distal (close to the JSR) portion of which is referred to as intermediate cisterna (braces). The bulk of the SR, the free SR (FSR), extends from the JSR on one side of the sarcomere to the JSR on the other. JSR contains junctional granules (asterisks), is attached to the transverse tubules (T) by junctional processes (thin curved arrows) and forms couplings with portions of the transverse tubules (small square brackets). Two couplings form a triad with a transverse tubule (large square bracket). On the right, two portions of the JSR (curved double arrows) contain junctional granules (asteriscs) and are separated by a small empty tubule (large arrow head) which clearly relates to the collapsed tubules in corresponding locations in Figs 2 and 7. gl = glycogen. 45,000×.

Fig. 2. Longitudinal section through frog sartorius muscle exposed to ruthenium red in glutaraldehyde. The JSR (curved arrows) apposed to the transverse tubule (T) contains darkly stained granular material (junctional granules), the granules being of rather uniform size. Attached to the JSR is the region of the intermediate cisterna of the free SR (brace) next to which, inside the cytoplasm, is glycogen (gl). The intermediate cisterna shows a pentalaminate compound membrane. Some junctional granules (double curved arrows) are separated from the remainder of the JSR as if squeezed off by another interspersed compound membrane. SR granules are also seen in tubules of the M rete of the free SR (arrows) which corresponds to the location of the SR granules in the transverse section of Fig. 4. A = A band. I = I band. z = Z line. H = H band. L = L lines or pseudo-H. m = M line. 50,000×.

Fig. 3. Same preparation as in Fig. 2, but with alcian blue substituted for ruthenium red. Note the dark staining of the junctional granules within the JSR (curved arrow) and the SR granules (arrow) in the free SR of the M rete (brace). Arrow head = pentalaminate membrane in the region of the intermediate cisterna of the free SR. The M rete at the fenestrations is open. In this as in most other illustrations of this page, the transverse tubules (T) appear like flattened dumbells with the luminal aspects of the transverse tubular membranes almost being at the point of touching in the center of the profiles. m = M line. T = transvers tubule. 60,000×.

Fig. 4. Preparation as in Fig. 2. Two tubules of free SR are collapsed and fuse into a single fused tubule in the general location of the intermediate cisterna (brace). The junctional SR (curved arrows) contains junctional granules and is attached to the transverse tubule (T) forming two couplings (brackets), one each on either side of the transverse tubule. The two couplings make a triad (large brace). 72,000×.

Fig. 5. *Xenopus laevis* semitendinosus muscle. Quick-frozen isolated fiber without fixation, nor addition of extraneous cations nor cryoprotectants. That part of the free SR which roughly represents what is known as the intermediate cisterna is limited by the straight arrows. The free SR in the region of the intermediate cisterna is collapsed to form the characteristic pentalaminate membrane. Note the very small size of what appears to be a transverse tubule (T). In many instances transverse tubules cannot be discerned (*cf.* Fig. 13). my = thick filament. M = M line. z = Z line. 126,500×.

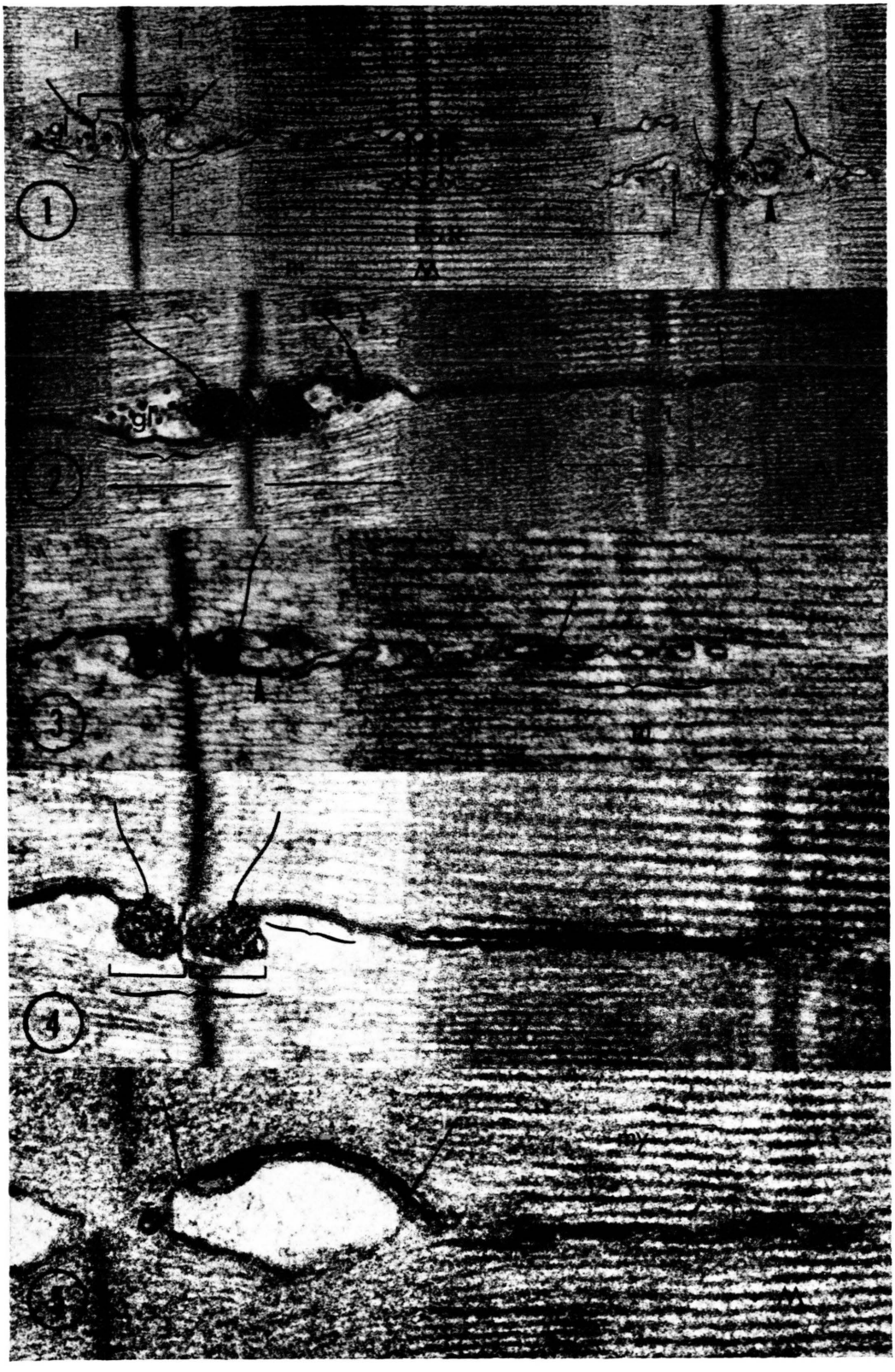
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Fig. 6. Transverse section through frog sartorius muscle exposed to ruthenium red in glutaraldehyde. The light band in the center is the I band (I) containing Z discs (z), JSR (curved arrows) and collapsed intermediate cisternae (double arrows) which are part of the free SR. Note the dark granular material (junctional granules) in the JSR. The free SR shows multiple stretches of collapse in the I band, A band and M rete region (arrows). Elsewhere the free SR is patent (small arrows heads). Mitochondrion = large arrow head. 21,500×.

of Stromer and Hasselbach [11, 12]. Briefly, SR vesicles were put into 12 ml polyallomer tubes fitting a Beckmann SW 41 rotor. The optimal protein concentration for through and through fixation of the pellet was determined to be about 0.5 mg protein per tube. The tubes contained also 0.4 mM histidine, pH 7.0, 0.1 M KCl and 10% glycerol. The tubes were spun at 35,000 rpm for 60 min. The bottom of the tubes was plugged with a plastic stopper having a flat surface. That surface was covered with a disc of aluminum foil, which, in turn, was covered by a millipore filter. After centrifugation, the millipore filters with the pellets were put into a desiccator for about 12 h at room or refrigerator temperature with phosphopentoxide as drying agent. There were no striking differences between the preparations dried at the two temperatures. Approximately 60 – 70% of water is removed by the drying process. After drying, aliquots from the pellets were exposed to Karnovsky's solution [13] containing either no cations, or one each of the different cations in amounts mentioned above respectively. The tissue was then embedded for sectioning after one to 60 days of fixation (with no apparent difference in results between time intervals).

Ferritin labeling

A ferritin-mercury phenyldiazonate (Hg-ferritin) was made as previously described [14] with minor modifications. 6 ml of the Hg-ferritin were added to about 1 ml isolated SR vesicles (20 mg protein per ml) and thoroughly mixed by shaking. The suspension was left in the refrigerator over night and then centrifuged at about 3,000 rpm in a Sorvall RC 34 rotor for 30 min. The resulting brown pellet was twice resuspended and washed in 0.1 M KCl containing 0.4 mM histidine at pH 7.0. The final almost water clear supernatant was discarded. The washed ferritin labeled vesicles (the brown pellet) were resuspended in 0.1 M KCl containing 0.4 mM histidine at pH 7.0 and added, 0.5 mg protein each, to tubes for centrifugation. Centrifugation, drying, fixation and embedding were carried out as described above.

Results

Observations on whole skeletal muscle fibers in situ

Tissue fixed in glutaraldehyde containing either ruthenium red, alcian blue or cetylpyridinium chloride showed collapse of the SR membranes (Figs 2, 3, 4, 6 – 10). That is to say, the inner lamellas of the SR envelope appeared to fuse thus obliterating the SR lumens and forming a pentalaminate structure in which the central dense line was slightly thicker than one but thinner than two tightly apposed lamellae of the unit membrane of the SR envelopes. Most of the SR membranes, including the nuclear envelope (Fig. 8), participated in the collapse, although the lumens of several stretches of SR tubules remained open (Fig. 6). The most widespread collapse occured with ruthenium red, alcian blue and cetylpyridinium chloride in that order. In the presence of the smaller cations, Ca^{2+}, La^{3+}, and Ba^{2+} only a very few small regions of collapse were found. Sometimes the free SR was collapsed along the entire length of the sarcomere, with the exception of the fenestrated portion of the M rete (Figs 2, 3). The junctional SR never took part in the process, although the presence of large SR pockets containing granular material made the junctional SR sometimes appear to be divided into two parts (Figs 1, 2, 7). Small aggregates of granular material were occasionally seen also in the free SR, predominantly in the region of the fenestrations of the M rete, at the center of the sarcomere (Figs 2, 3, 9, 10). In some regions individual SR tubules were found to be much narrower than usual (approximately 10 to 15 nm in diameter) and filled with granular material (Fig. 2). The morphology of the junctional granules varied with the cation used. With ruthenium red the granules were coarse but rather discrete individually (Fig. 2). With alcian blue and cetylpyridinium chloride the granules appeared more confluent to form homogeneous electron-dense masses of irregular shape (Fig. 3).

In order to determine whether the collapse of the SR membranes could be reversed after the application of ruthenium red, the cells were exposed to 1 M NaCl for about 12 h. Under these conditions the SR was found widely patent. Ruthenium red crystals were seen outside the SR tubules mainly over the I band and at the center of the sarcomeres (Fig. 11), and the junctional SR was free of dense staining, although some granular material often remained.

In the absence of cations in the fixative collapsed SR was found only in minute segments (no more then 10 nm in length) and then only once in numerous sections.

The dimensions of the pentalaminate structures were similar to those reported by Howell for these seen in the intermediate cisterna [3]. The central

lamina of the pentalaminate structure did not exhibit further substructure by high resolution electron microscopy.

Isolated SR preparations

The labeling of the outer lamellae of the vesicle envelopes by ferritin permitted the identification of the precise topography of the lamellar repeat. The preparations with and without ferritin labeling showed the same features concerning membrane appositions. As was observed in the *in situ* preparations of whole muscle fibers, the formation of pentalaminate membranes between the inner lamellae of the SR envelope occurred only in the presence of cations in the fixative. In the absence of cations in the fixative, flattened well-fixed vesicles with patent lumens were seen in the upper portions of pellets containing from 3 to 6 mg protein. The deeper portions of these pellets were usually poorly fixed. Nevertheless, in these deeper portions of the pellets close scrutiny occasionally revealed pentalaminate membranes. In pellets containing less vesicle protein (between 0.1 to 0.5 mg), the entire well fixed thickness of the pellets showed open vesicle lumens when cations were left out of the fixative. Pelleted vesicles without subsequent drying never flattened. Such pellets contained many vesicles whose contents were intensely electron dense when they were fixed in the presence of ruthenium red, alcian blue or cetylpyridinium chloride (data not shown).

The pattern of wavy, elongated flattened vesicles was often interrupted by rounded, curly regions (Fig. 16). As previously noted [11, 12], the circumference of the flattened vesicles suggested fusion of several vesicles. In many preparations containing cations in the fixative, the pentalaminate membranes opened up to enclose oval spaces filled with very electron-dense granular material (Fig. 17), especially in cases in which ruthenium red or alcian blue had been added to the fixative. Transitions from open vesicle lumens to narrow slits approximately 2 to 4 nm wide and, finally, fusion of the luminal lamellae of the vesicle membranes were often seen.

Quick freezing of single isolated muscle fibers

To determine if unfixed isolated muscle fibers might display SR collapse, isolated skeletal muscle fibers were quick frozen. We obtained very good tissue preservation in many preparations without evidence of ice crystal formation or disruption of conventional muscle geometry. The SR showed many regions of collapse, including the nuclear envelope. The resulting pentalaminate membranes were identical in dimensions to those observed in the preparations obtained with cations in the fixative. In all preparations there were several SR regions that had patent lumens. The central dense line of the pentalaminate structures often had lateral extensions resembling arrow heads (Fig. 12).

Discussion

Our data show that profound structural changes in the geometry of the SR of skeletal muscle are caused by cations both in whole muscle fibers *in situ* and in isolated SR vesicles *in vitro*. These changes consist in the formation of pentalaminate membranes by apparent fusion of the opposing inner lamellas of the SR envelope [15 – 18]. The entire SR takes part in the process, rather than only intermediate cisternae. The obliteration of the SR lumens resulting from the formation of pentalaminate structures is seen with a number of cations, including Ca^{2+}, which plays a central role in the contraction-relaxation cycle. Our data further show that the

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Fig. 7. Longitudinal section through frog sartorius muscle exposed to ruthenium red in glutaraldehyde. The light bands are the I band (I), the dark bands the A bands (A) in which additional bands can be made out (H, m). The triads (square bracket) are formed by two couplings, each composed of JSR (curved arrows) and a part of the membrane of the transverse tubule (double arrow). The free SR adjoining the JSR, referred to as intermediate cisterna (arrow heads), is often collapsed to form a pentalaminate compound membrane (see below for higher resolution). The SR adjacent to fenestrations proper is not collapsed. Sometimes the junctional SR appears to be divided into two portions that are separated by an area of collapsed membrane (straight arrows). 19,500×.

Fig. 8. Preparation as in Fig. 2. The nucleus (N) is surrounded by an envelope which is part of the SR, and so participates in the formation of JSR (curved arrows), which along with the transverse tubule (T) is part of the two couplings forming a triad. The nuclear envelope is partly collapsed (arrow) and partly patent (arrow head). 98,000×.

Fig. 9. Preparation as in Fig. 3. The pentalaminate appearance of the collapsed SR (arrows) is clearly shown in the region of the M rete of the SR at the M line (M) but there are also patent SR segments (arrow heads). The double arrow at the bottom right points to an SR tubule in the region of the M rete that contains electron dense granular material (SR granules). 71,000×.

Fig. 10. Same preparation as in Fig. 3 except that alcian blue was substituted for ruthenium red. Note the prominence of SR granules in the free SR profiles (arrow). There is no SR collapse in the picture. mit = mitochondria. A = A band. I = I band. 27,000×.

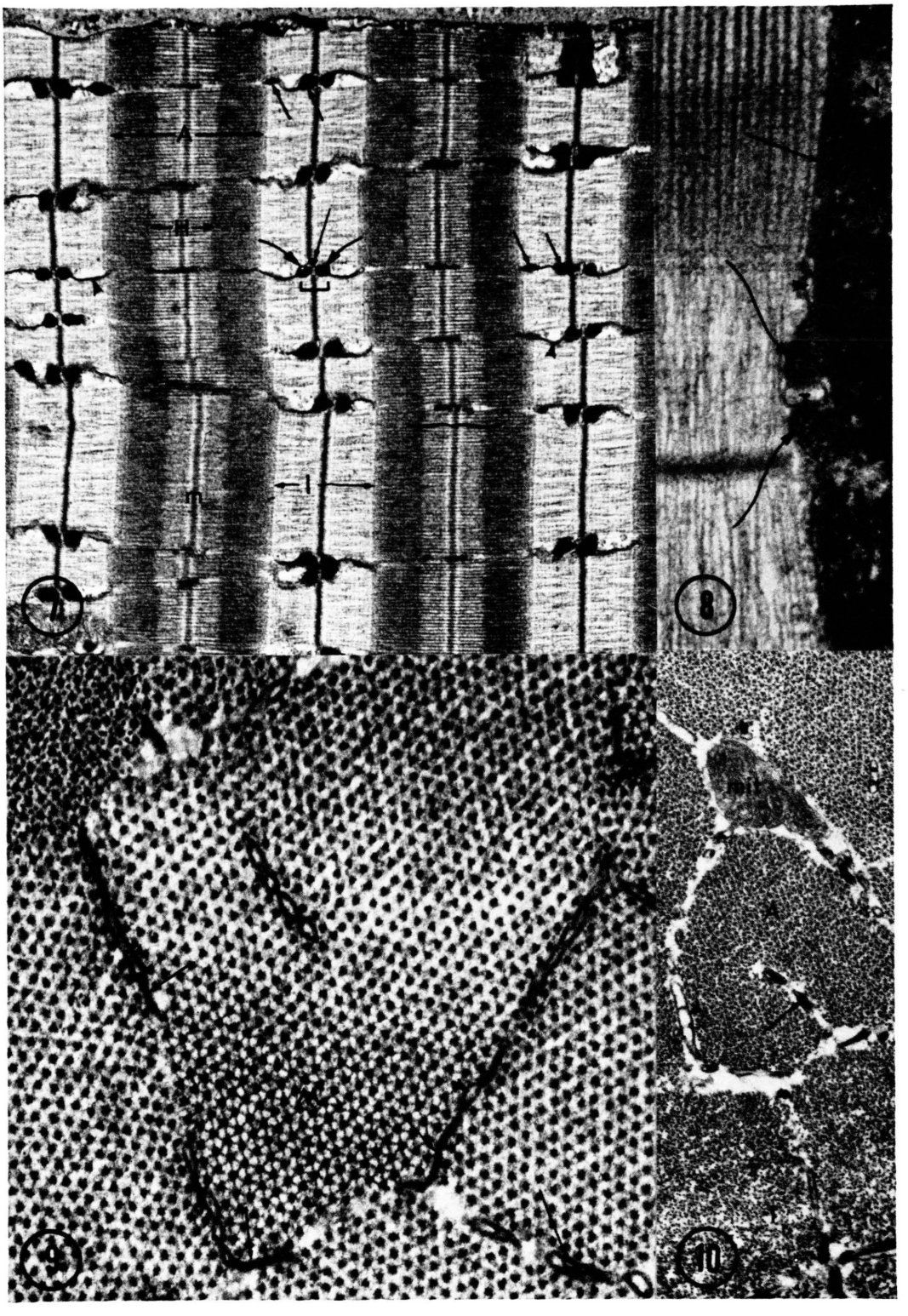
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Fig. 11. Preparation as in Fig. 1 except that the treatment with ruthenium red was followed by soaking the tissue for 4 days in sodium chloride before routine processing for electron microscopy. Note the rather sharply circumscribed localization of the crystals of ruthenium red. They are especially prominent in the I band (braces) and in the center of the sarcomere following the outlines of the H band (bracket). The precipitates in the I bands and in the nucleus are made up of larger crystals, and larger aggregates of these crystals than those in the center of the sarcomere. The free SR, including the regions of the intermediate cisternae, is widely patent (arrow head). The JSR (arrow) does not contain a large amount of dark staining material (ct. Fig. 1). 5,500×.

formation of pentalaminate structures due to ruthenium red is reversible even in fixed tissue, in that the SR lumens become patent again when ruthenium red is displaced by soaking the tissue in 1 M NaCl [16]. The fact that only very small areas of the SR are found collapsed with cations such as Ca^{2+}, La^{3+}, and Ba^{2+} is consistent with the possibility that these small cations may be readily washed out by various steps in the embedding procedure, thus permitting the reopening of the SR tubules. This possibility is further strengthened by the observation that the deeper layers of thick pellets of isolated SR vesicles show pentalaminate membranes, presumably due to endogenous Ca^{2+}, while in the absence of cations in the fixative the upper layers of the same pellet have no pentalaminate membranes.

From these findings we advance the hypothesis that the SR is capable of *in vivo* collapse which is mediated by Ca^{2+} [15]. As Ca^{2+} is removed from the myofibrillar compartment after contraction and having reached a certain threshold concentration, the SR membranes collapse, starting at the center of the sarcomere, and their contents are pushed toward and, ultimately, into the junctional SR at the Z lines (frog skeletal muscle). As long as the Ca^{2+} concentration in the junctional SR stays high the free SR membranes remain collapsed, at least at the very junction between free SR and junctional SR, thus preventing reflux from the junctional SR into the free SR. The SR collapse cannot be observed in muscle fibers prepared conventionally because the SR membranes return to their orthodox, *i. e.* open geometry during fixation. There is some evidence that Ca^{2+} is displaced toward the Z lines during the relaxation phase [19], *i. e.* that phase of the contraction-relaxation cycle when the SR would be collapsed. There is also very good evidence that rearrangements of membrane geometry to an apparently stable endpoint may take place during fixation [20].

The quick freezing experiments were performed in an effort to obtain evidence that would shed light on the *in vivo* geometry of the SR. Although the deformations of ultrastructure generated by different fixatives, *e. g.* osmium tetroxide *vs* glutaraldehyde, have been well recognized for several years, minor structural alterations proceeding during fixation have escaped detection until the introduction of effective quick freezing methods that seem to stop these deformations instantly [20]. Employing one of these techniques we have demonstrated that the SR membranes in isolated skeletal muscle fibers form pentalaminate compound membranes [17] indistinguishable from those obtained with a variety of cations *in situ* and *in vitro*. It is important to note that in each of the quick freeze preparations stretches of patent SR coexist with collapsed SR, making it unlikely that freezing and/or the freeze substitution, *per se*, cause the membrane collapse. A similar SR collapse has been seen by Heuser (personal communication).

In contrast to the in situ preparation of whole muscle, in isolated SR vesicles Ca^{2+} in the fixative causes widespread formation of pentalaminate membranes indistinguishable in form and extent to those encountered with ruthenium red and alcian blue treatment of isolated vesicles *in vitro* [18], as well as in whole muscle *in situ*. The difference between the widespread SR collapse due to Ca^{2+} *in vitro* as opposed to the very spotty one *in situ* may be related to two major aspects in which the *in vitro* preparation differs from that *in situ*. First, *in vitro* the SR vesicles are very large and consequently have very large opposing surfaces for tight apposition and, secondly, the formation of pentalaminate membranes is preceeded by partial dehydration. The possible importance of the displacement of water is underscored by the extensive SR collapse found in quick frozen isolated muscle fibers in which endogenous Ca^{2+} is the only cation present, and in which water in the form of ice is removed by the process of freeze substitution prior to fixation.

Our hypothesis that pentalaminate compound membrane formation is a dynamic *in vivo* aspect of the contraction-relation cycle is supported by the following additional observations and considerations. The junctional SR (terminal cisterna), of skeletal muscle is always distended with granular material. It presumably represents the highly negatively charged protein calsequestrin, since this protein is recovered mainly from the heavy fraction (the junctional SR) of isolated SR vesicles separated in sucrose density gradients [21]. The contrast of the granular material in the junctional SR and elsewhere is considerably enhanced by cations, especially ruthenium red, alcian blue and cetylpyridinium chloride (Figs 2, 3, 10), showing that highly negatively charged material is present in the SR [23]. That no structural organization of either the contents of the junctional SR or at the junction between junctional SR and intermediate cisterna is visible when

the SR *in situ* is negatively stained [22], suggests that the proteinaceous content of the SR is fluid. Our hypothesis would predict that if the entire SR is filled with soluble calsequestrin, this protein would be pushed into the cisternal space of the junctional SR due to the collapse of the SR during relaxation. During fixation the soluble protein would be congealed and, thus, prevented from returning into the free SR even as the membranes of the SR return to their orthodox (open) geometry during fixation. The typical presence of granular material in the vicinity of the fenestrations (approximately 20 to 30 nm in diameter) of the M rete may find its explanation in the fact that here many membrane profiles are at the minimum radius of curvature permitted by the molecular nature of the membranes. That is to say, a geometric situation exists at the fenestrations analogous to that of isolated SR vesicles which cannot form pentalaminate membranes without prior alterations of their geometry by drying, *i. e.* without first having been flattened. The occasional sequestration of the SR by pentalaminate membranes into pockets containing granular material (Figs 1, 2, 7) is consistent with the notion of a quasi peristaltic wave traveling along free SR tubulus *in vivo*. Our hypothesis would also predict the process of SR collapse to be reversible. We have presented some evidence for that by showing that the SR can return to its orthodox appearance when fibers treated with ruthenium red are exposed to 1 M NaCl, and by showing that the superficial portions of isolated SR pellets fail to show pentalaminate structures when they were rehydrated with fixative containing no cations.

The evidence presented here supports the hypothesis that Ca^{2+} accumulation by the SR induces the membranes of the SR to collapse. One of the consequences of the SR collapsing from the center of the sarcomere toward the junctional SR during relaxation would be the displacement of all SR contents into the junctional SR. Another important consequence of the collapse of the SR membranes would be the electrical isolation of the free SR from the junctional SR. According to the hypothesis, this isolation would occur during relaxation and persist in the state of rest. Under such conditions it is possible that the action potential is translated into Ca^{2+} release by resistive coupling since, for the duration of the occlusion, the greater part of the membrane capacitance of the SR membranes is not in parallel with the plasmalemma. Instead, the action potential would encounter only the added capacitance residing in the membranous envelope of the junctional SR.

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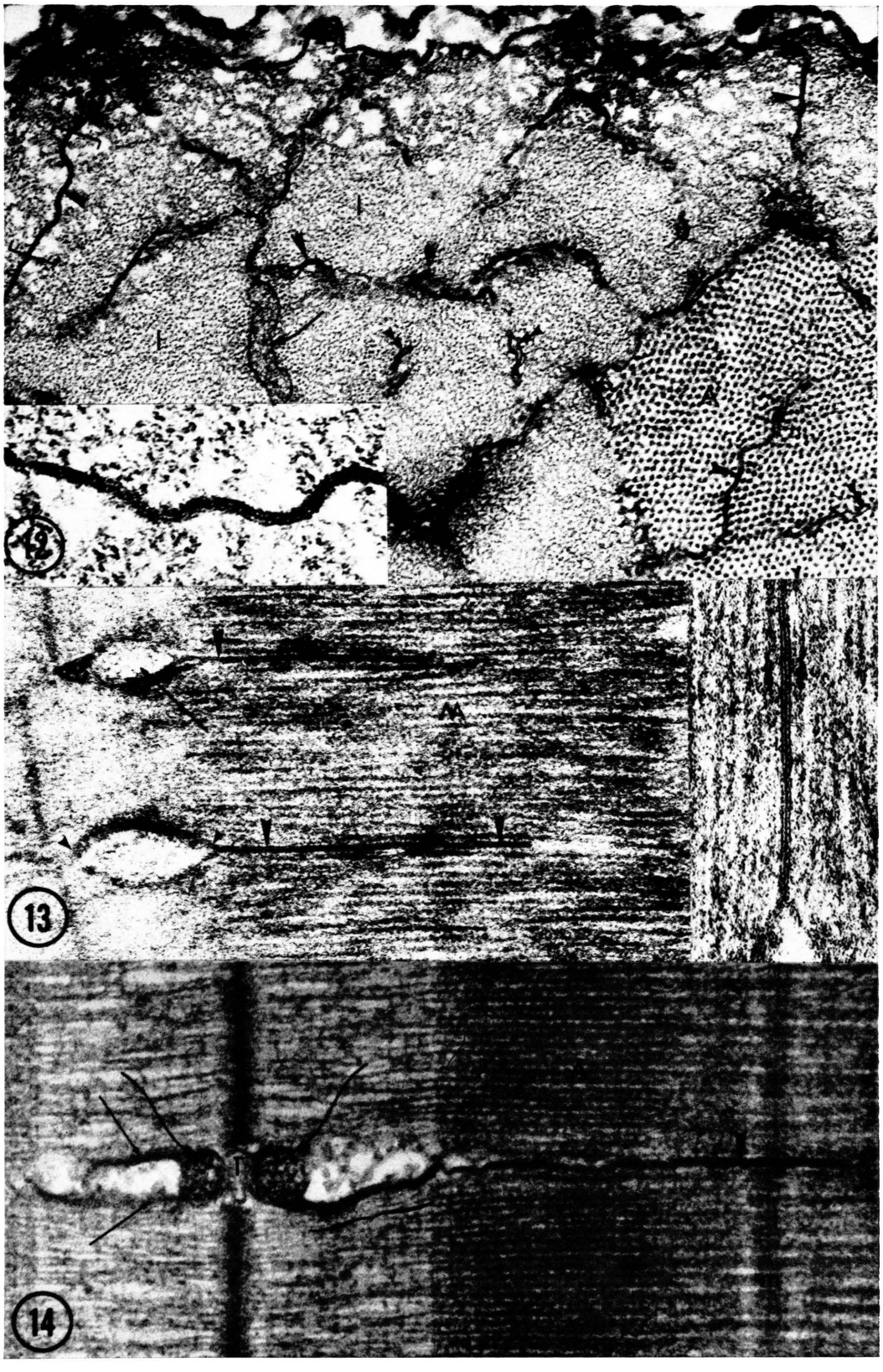
Fig. 12. Preparation as in Fig. 5 except that the tissue was sectioned in a transverse plane. The actin filaments of the I band (I) and myosin filaments of the A band (A) are well preserved. The SR is largely collapsed forming pentalaminate compound membranes (arrow heads) although in a few spots the SR is found patent (double arow heads). The JSR (curved arrow) contains granular material but is not distended. The inset shows the compound membrane between the brackets in higher magnification. Note the marked granularity of the individual lamellae and the arrow head configuration of the central fused membranes. 37,000×. Inset: 106,500×.

Fig. 13. Preparations as in Fig. 5. I band, thick myosin filaments and the Z line (Z) can be made out. The M line is vaguely identifiable; the letter M indicates the approximate center of the sarcomere. The free SR is collapsed to form pentalaminate compound membranes (large arrow heads) that extend across the center of the sarcomere. Inset shows higher magnification of the pentalaminate membrane. 55,000×. Inset: 100,000×.

Fig. 14. Preparations as in Fig. 2 (ruthenium red). The free SR has formed a continuous pentalaminate membrane beginning at the JSR and reaching across the M line (m) in the center of the sarcomere. The M rete in this instance has no fenestrations which is not unusual. The pentalaminate membrane is identical in structure to that in the quick frozen tissue (Fig. 13). The JSR (curved arrow) of the coupling to the left of the Z line (z) is approached by two longitudinal tubules of the free SR that are in the position of so-called intermediate cisternae and whose membranes apparently have fused into pentalaminate structures (straight arrows). T = transverse tubules. I = I band. A = A band. 90,000×.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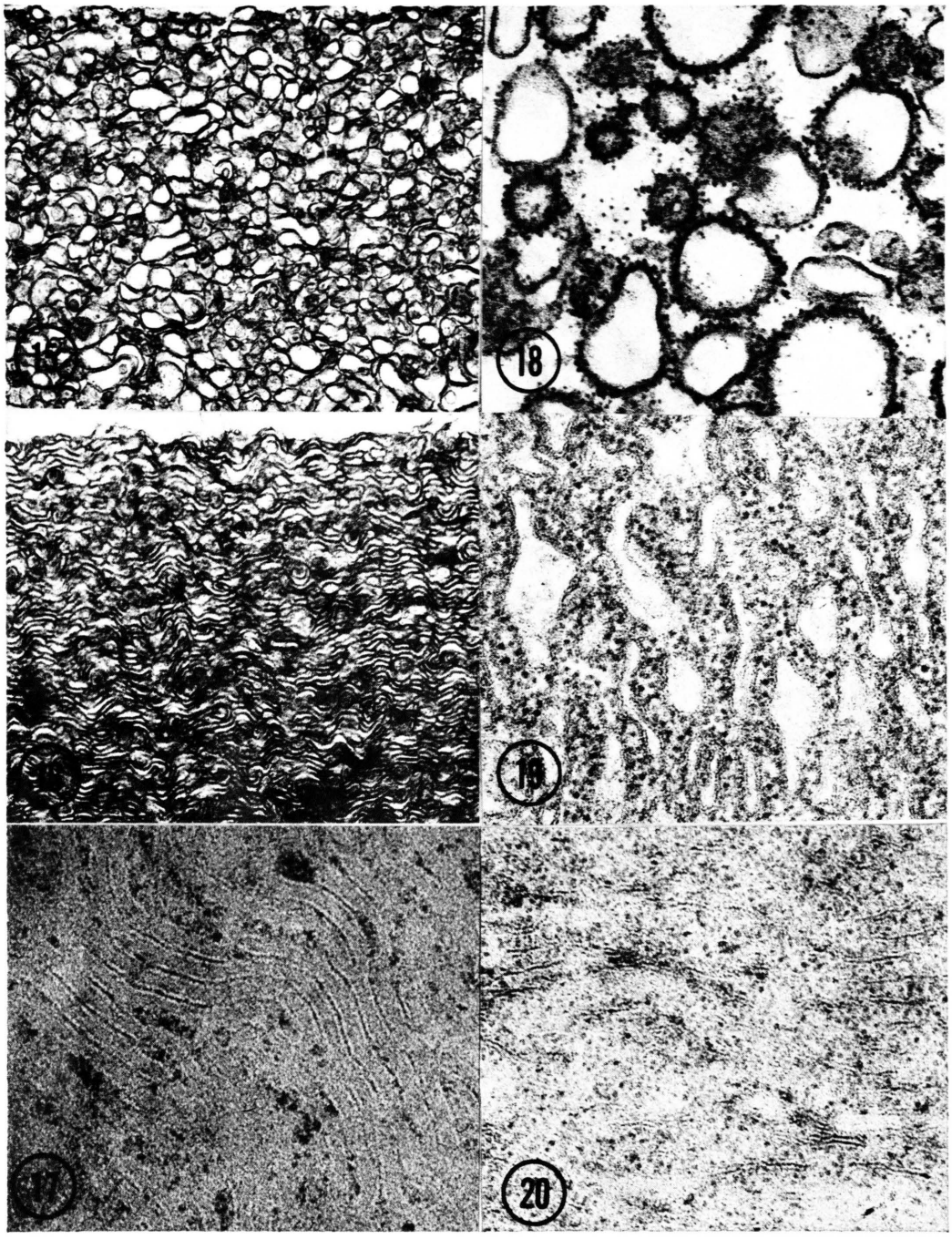

Fig. 15. Isolated SR vesicles. Pellet fixed in Karnovsky's solution. The vesicles are rounded and have a patent lumen. 8,500×.

Fig. 16. Isolated SR vesicles. Pellet partially dehydrated and fixed in Karnovsky's solution without added cations. The vesicles are flattened forming wavy lamellae. The lumens of the vesicles are patent. 6,900×.

Fig. 17. Isolated SR vesicles. Pellet partially dehydrated and fixed in Karnovsky's solution that contained ruthenium red. The flattened vesicles are further compounded to form pentalaminate structures that are separated by broader zones. The thick membrane forming the center of the pentalaminate structure represents the fusion of the inner lamellae of the membranous vesicle envelope (see Fig. 20). Occasionally the pentalaminate structure opens up to reveal accumulations of electron-dense granular material. Some of these accumulations are rounded and oval-shaped not unlike the junctional SR appears in situ. These isolated vesicles contained pooled the heavy and light SR fraction. 120,000×.

Fig. 18. Isolated SR vesicles. Pellet fixed in Karnovsky's solution after the vesicles had been labeled with ferritin on the outside. 27,000×.

Fig. 19. Isolated SR vesicles. Pellet partially dehydrated and fixed in Karnovsky's solution with added cations. The vesicles are flattened with patent lumens. The outside of the vesicles is identified by the location of the ferritin. 55,000×.

Fig. 20. Isolated SR vesicles. Pellet partially dehydrated and fixed in Karnovsky's solution that contained 5 mM Ca^{2+}. The flattened vesicles are further compounded to form pentalaminate structures. Ferritin particles are on the outside of the vesicles and, thus, identify the topography of the lamellar repeat. 55,000×.

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