

Ultrastructural Localization of Calsequestrin in Adult Rat Atrial and Ventricular Muscle Cells

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ABSTRACT The distribution of calsequestrin in rat atrial and ventricular myocardial cells was determined by indirect immunocolloidal gold labeling of ultrathin frozen sections. The results presented show that calsequestrin is confined to the sarcoplasmic reticulum where it is localized in the lumen of the peripheral and the interior junctional sarcoplasmic reticulum as well as in the lumen of the corbular sarcoplasmic reticulum, but absent from the lumen of the network sarcoplasmic reticulum. Comparison of these results with our previous studies on the distribution of the $\text{Ca}^{2+} + \text{Mg}^{2+}$ -dependent ATPase of the cardiac sarcoplasmic reticulum show directly that the $\text{Ca}^{2+} + \text{Mg}^{2+}$ -dependent ATPase and calsequestrin are confined to distinct regions within the continuous sarcoplasmic reticulum membrane.

Assuming that calsequestrin provides the major site of Ca^{2+} sequestration in the lumen of the sarcoplasmic reticulum, the results presented support the idea that both junctional (interior and peripheral) and specialized nonjunctional (corbular) regions of the sarcoplasmic reticulum are involved in Ca^{2+} storage and possibly release. Furthermore, the structural differences between the junctional and the corbular sarcoplasmic reticulum support the possibility that Ca^{2+} storage and/or release from the lumen of the junctional and the corbular sarcoplasmic reticulum are regulated by different physiological signals.

The sarcoplasmic reticulum is the intracellular membrane system that together with the sarcolemma and the transverse tubules regulates the calcium concentration in the cytoplasm of mammalian myocardial cells and thereby the state of contraction and relaxation in these cells (1–5).

Ultrastructural studies of mammalian myocardial cells with transverse tubules have shown that the sarcoplasmic reticulum is composed of at least three structurally distinct regions (6). These are the network sarcoplasmic reticulum, the junctional peripheral and interior sarcoplasmic reticulum, and the corbular sarcoplasmic reticulum. The network sarcoplasmic reticulum is composed of an anastomosing network of sarco-tubules that surrounds the myofibrils. The peripheral and interior junctional sarcoplasmic reticulum as well as the corbular sarcoplasmic reticulum are all continuous with the network sarcoplasmic reticulum but, in contrast to the network sarcoplasmic reticulum, contain electron dense material in their lumen. The obvious structural difference between the junctional (interior and peripheral) sarcoplasmic reticulum and the corbular sarcoplasmic reticulum is that the junctional sarcoplasmic reticulum is closely apposed to either the sar-

colemma or the transverse tubules to which it is physically connected by junctional processes called feet, whereas the corbular sarcoplasmic reticulum composed of corbular and cisternal expansions on the network sarcoplasmic reticulum is not closely apposed to either the sarcolemma or the transverse tubules.

It is generally agreed that in order to translate the event of depolarization of the myocardial sarcolemma into an increase in the cytoplasmic Ca^{2+} , which in turn initiates muscle contraction, Ca^{2+} must arrive in the cytoplasm from both the sarcoplasmic reticulum and the extracellular space (1–3). Accordingly, during relaxation some Ca^{2+} must be stored in the lumen of the sarcoplasmic reticulum, while some must be transported to the extracellular space. To understand the possible functions of the various structurally distinct regions of the cardiac sarcoplasmic reticulum with respect to the transport of Ca^{2+} to and from the myofibrils during the contraction–relaxation cycles, it is important to know the function and distribution of the sarcoplasmic reticulum proteins in the various regions of the complex membrane system.

The presence of a $\text{Ca}^{2+} + \text{Mg}^{2+}$ -dependent ATPase

(ATPase) in mammalian cardiac sarcoplasmic reticulum has been reported, and it is generally agreed that this protein, as in skeletal muscle, actively transports Ca^{2+} from the myofibril to the lumen of the sarcoplasmic reticulum when relaxation sets in (5). Ultrastructural localization of the ATPase in rat cardiac muscle by immunolabeling showed that the ATPase was uniformly distributed throughout the sarcoplasmic reticulum membrane except for the region of the interior and peripheral junctional sarcoplasmic reticulum where the sarcoplasmic reticulum is closely apposed to the transverse tubules and the sarcolemma, respectively (7). A recent study in which highly purified sarcoplasmic reticulum vesicles were obtained from canine ventricular muscle showed that the ATPase in cardiac muscle, as in skeletal muscle, is the major intrinsic protein of the cardiac sarcoplasmic reticulum (8).

Recently, we have identified, purified, and characterized calsequestrin from canine ventricular muscle tissue (9). In the same study, indirect immunofluorescence labeling showed that most of the calsequestrin was localized in the I-band region of rat and canine ventricular muscle cells, which suggests that most of the cardiac calsequestrin, like skeletal calsequestrin, is confined to the lumen of the portion of the sarcoplasmic reticulum that is in close apposition to either the sarcolemma or the transverse tubules (i.e., the lumen of the peripheral and interior junctional sarcoplasmic reticulum, respectively). This suggestion is supported by results reported by Jones and Cala (10) showing that a protein with biochemical characteristics corresponding to those of calsequestrin was present in the ryanodine-sensitive population of the isolated sarcoplasmic reticulum vesicles, but absent from the ryanodine-insensitive vesicles. Since ryanodine inhibits Ca^{2+} release from ryanodine-sensitive sarcoplasmic reticulum vesicles, these studies also implied that calsequestrin is localized near the Ca^{2+} release sites in the cardiac sarcoplasmic reticulum.

If calsequestrin is indeed confined to the lumen of the junctional sarcoplasmic reticulum, one would expect calsequestrin in myocardial cells without transverse tubules to be confined to the lumen of the peripheral junctional sarcoplasmic reticulum and absent from the sarcoplasmic reticulum in the interior regions of the myocardial cytoplasm. By immunolocalization, we have recently determined the distribution of calsequestrin in chick ventricular myofibers (11) and sheep Purkinje fibers (12), both of which lack transverse tubules. The results obtained showed as anticipated that calsequestrin was localized in the lumen of peripheral junctional sarcoplasmic reticulum; however, calsequestrin was also observed in the lumen of the corbular sarcoplasmic reticulum present in the I-band region of both of these types of myocardial fibers (13, 14).

To determine whether calsequestrin in mammalian cardiac muscle cells with transverse tubules is confined to the lumen of the interior and junctional sarcoplasmic reticulum or whether it, in addition to these sites, is also present in the lumen of the corbular sarcoplasmic reticulum recently reported to be present in mammalian cardiac muscle cells (15, 16), we have used affinity purified antibodies to canine cardiac calsequestrin to localize calsequestrin in adult rat atrial (few transverse tubules) and papillary muscle cells (many transverse tubules) by the indirect immunocolloidal gold labeling of cryoultramicrotomy sections at the ultrastructural level of resolution.

The results obtained showed that calsequestrin was present

in the sarcoplasmic reticulum membrane where it was confined to the lumen of the interior and peripheral junctional sarcoplasmic reticulum as well as in the lumen of the corbular sarcoplasmic reticulum but absent from the lumen of the network sarcoplasmic reticulum. In addition, calsequestrin was present in the lumen of the nuclear envelope.

MATERIALS AND METHODS

Preparation and Characterization of Antisera to Canine Cardiac Calsequestrin: Canine cardiac calsequestrin was purified according to the procedure of Campbell et al. (9). Rabbit antisera to canine cardiac calsequestrin were prepared and characterized as previously described by Jorgensen and Campbell (11). Affinity purified antibodies to cardiac calsequestrin was prepared by affinity chromatography of the rabbit antiserum to canine cardiac calsequestrin as previously described (17, 18). The cross-reactivity and specificity of the affinity purified antibodies used in the present study towards calsequestrin from rat ventricular muscle was demonstrated by indirect immunostaining of immunoblots of purified rat cardiac calsequestrin and extracts from rat ventricular muscle with a goat anti-rabbit gamma-globulin-peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA) as the secondary reagent, according to the procedure described by Towbin et al. (19). The details of this procedure were performed as previously described (7, 11, 20), except that affinity purified antibodies to calsequestrin from canine ventricular myocardium ($1 \mu\text{g}/\text{ml}$ in 0.15 M NaCl , 10 mM Tris-HCl pH 7.2, and 3% bovine

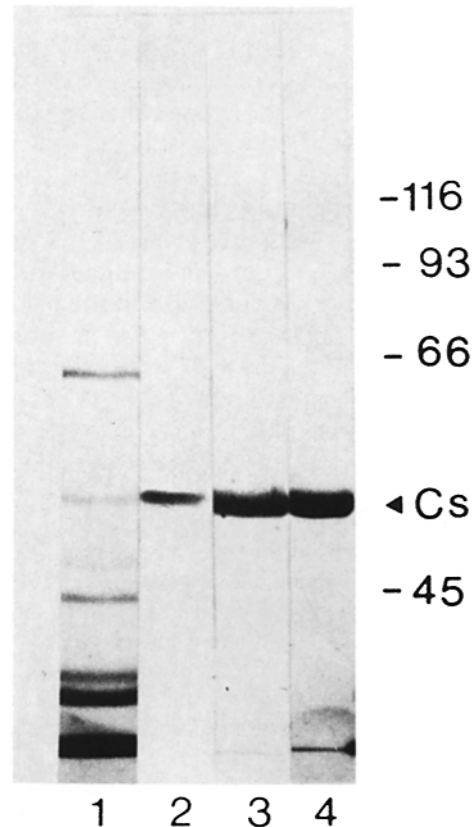


FIGURE 1 Specificity of the affinity purified antibodies to canine cardiac calsequestrin towards calsequestrin in an extract from rat ventricular muscle. Lanes 1 and 4, Coomassie Blue staining patterns of proteins separated on a 7.5% polyacrylamide gel. Lanes 2 and 3, immunoblots of muscle proteins labeled with affinity purified antibodies to canine cardiac calsequestrin by the indirect horseradish peroxidase immunoassay (15). Lanes 1 and 2, $10 \mu\text{g}$ of an ammonium sulfate extract from rat ventricular muscle. Lanes 3 and 4, $5 \mu\text{g}$ of calsequestrin purified from rat ventricular muscle. Cs, calsequestrin. Numbers on the right side of the figure represent mol wt $\times 10^{-3}$ of molecular weight standards.

serum albumin [BSA]) was used as the primary antibody reagent.

Dissection, Fixation, and Cryoultramicrotomy of Ventricular Muscle: Adult female Wistar rats (Charles River Breeding Laboratories, Inc., Canada) were killed by cervical dislocation. The papillary muscles from the left ventricle and bundles of atrial myofibers from the left auricle were quickly dissected and fixed as previously described (7). Subsequently, the bundles of fixed tissue were washed, infused with sucrose, and 80–100-nm thick cryoultramicrotomy sections cut as previously described (7, 20).

Indirect Immunoelectron Microscopic Labeling: Qualitative immunoelectron microscopic labeling of cryoultramicrotomy sections of fixed rat ventricular muscle fibers with affinity purified antibodies to canine calsequestrin was carried out as previously described (11, 20), except that a goat anti-rabbit gamma-globulin-colloidal conjugate (5–10 nm) (Janssen Pharmaceutic Inc., New Brunswick, NJ) was used as the secondary reagent in the labeling procedure.

The primary antibody reagent, affinity purified rabbit antibodies to canine cardiac calsequestrin, was used at 10 µg/ml in phosphate-buffered saline, pH 7.4 (PBS) (11). The secondary antibody reagent, a goat anti-rabbit gamma-globulin-colloidal conjugate (5–10 nm) was used at 0.5 mg/ml PBS. To assess nonspecific labeling of the ultrathin frozen sections, rabbit gamma-globulin purified from preimmune rabbit serum (10 µg/ml PBS) was substituted for the affinity purified antibodies to calsequestrin in the indirect immunolabeling procedure.

After the immunolabeling, the ultrastructural detail in the ultrathin frozen sections was positively stained by osmication, dehydration, and postembedding in LR white acrylic resin (London Resin Co. Ltd., Basingstoke, Hampshire, England) as described by Keller et al. (21).

RESULTS

Antibody Specificity

The ability of the affinity purified antibodies to canine myocardial calsequestrin to bind specifically to calsequestrin from rat ventricular muscle was demonstrated by the indirect immunoperoxidase staining on immunoblots as shown in Fig. 1. The affinity purified antibodies were bound to a single band at 55,000 D when tested against an extract from rat ventricular muscle. The electrophoretic mobility of the 55,000-D band corresponds to that of calsequestrin purified from either canine (9) or rat ventricular myocardium. This finding strongly suggests that the antibodies to canine myocardial calsequestrin bind specifically to calsequestrin in the rat myocardium.

Immunoelectron Microscopic Labeling

RAT ATRIAL MUSCLE CELLS: Longitudinal cryoultramicrotomy sections of fixed adult rat atrial muscle tissue were labeled with antibodies to canine cardiac calsequestrin by the indirect immunocolloidal gold staining technique. Examination of the distribution of the gold particles in my-

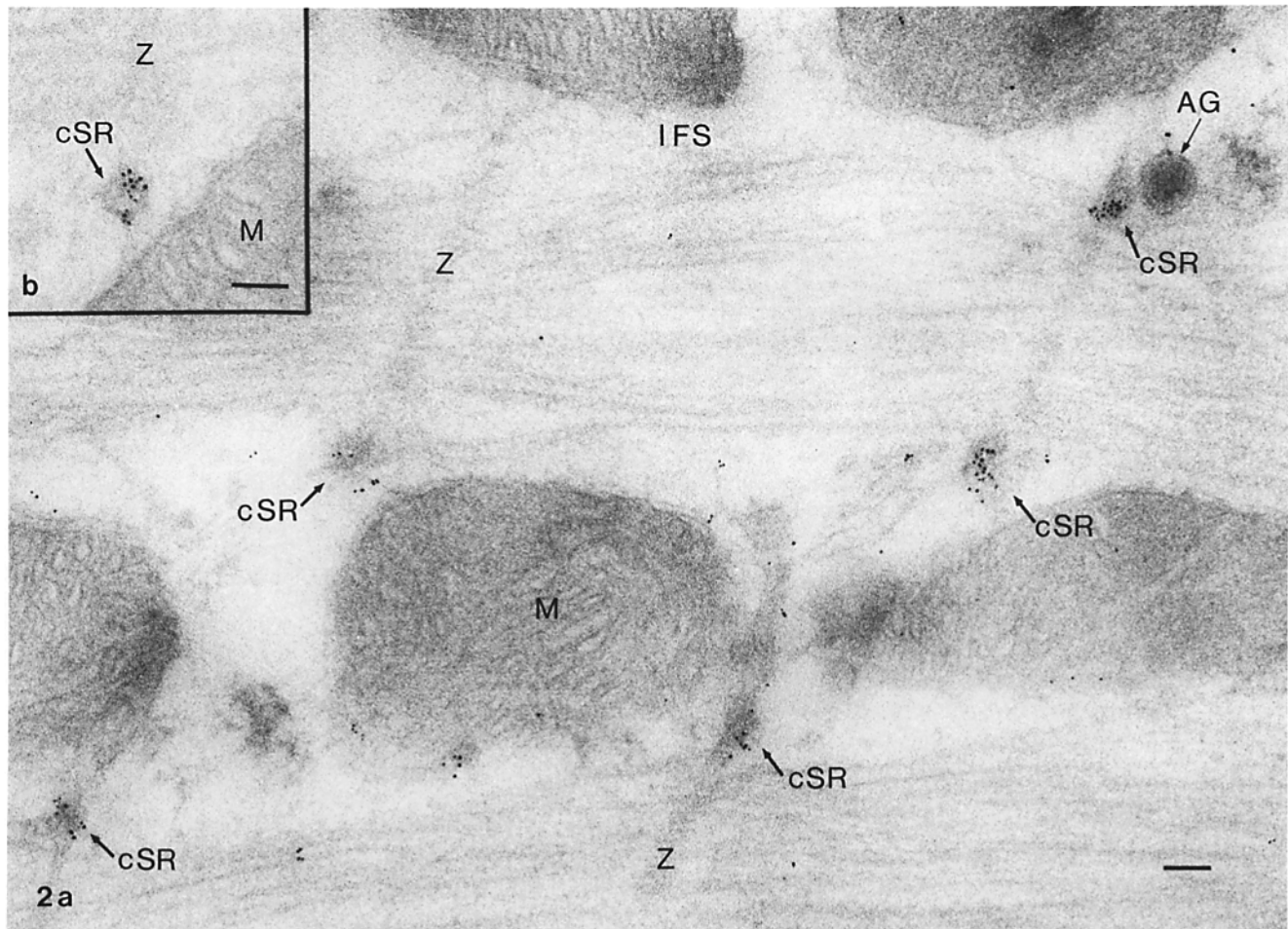


FIGURE 2 Electron micrographs of longitudinal ultrathin frozen sections of rat atrial muscle labeled with affinity purified antibodies to cardiac calsequestrin by the indirect immunocolloidal gold staining technique described in the Materials and Methods section, and showing interior regions of atrial muscle cells. Gold particles were present in discrete foci (cSR) (a) sometimes observed to be membrane bound (b) and mostly located close to the Z-line (Z) in the interior regions of the cell. The network sarcoplasmic reticulum present in the inter-fibrillar spaces (IFS) were only labeled at the level of the background. M, mitochondria. AG, atrial granule. Bar, 0.1 µm.

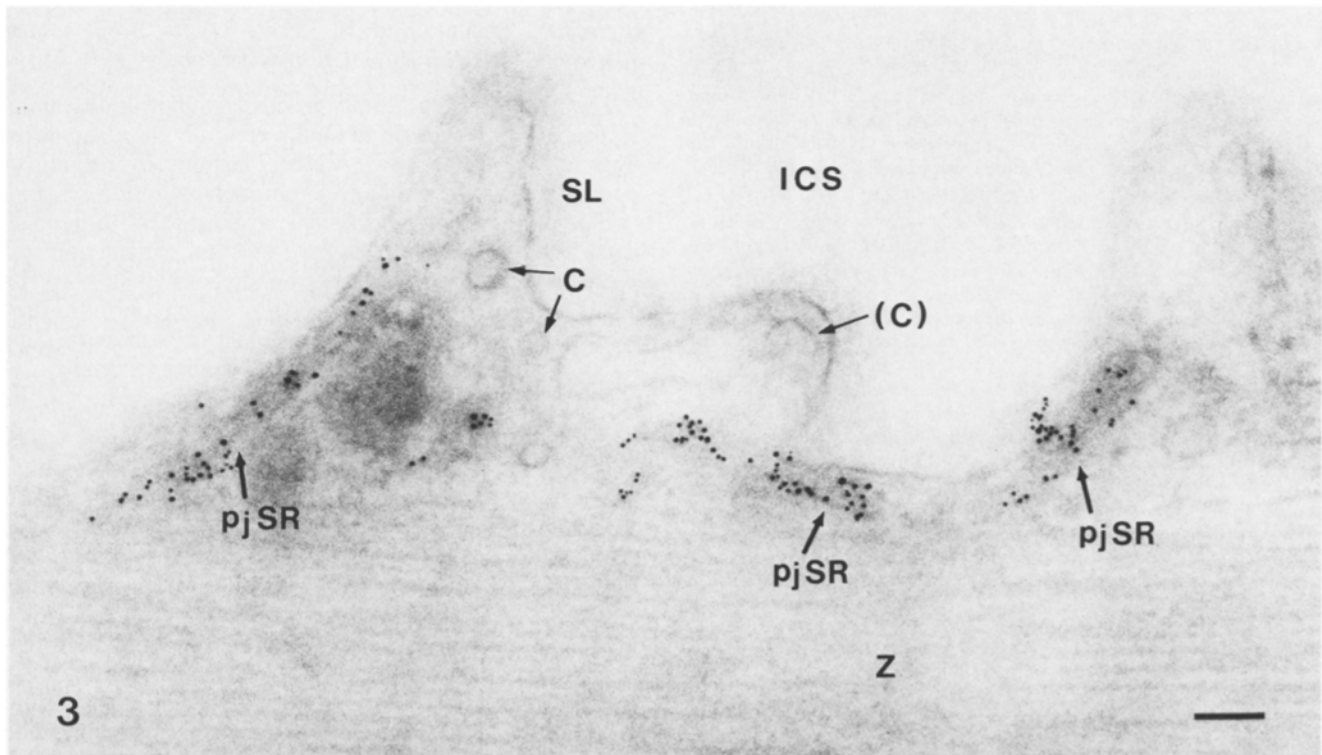


FIGURE 3 Electron micrograph of a longitudinal ultrathin frozen section of rat atrial muscle labeled with affinity purified antibodies to cardiac calsequestrin. Gold particles were present over the lumen of peripheral junctional sarcoplasmic reticulum (*pjSR*). The sarcolemma (*SL*) and the caveolae (*C*) were only labeled at the level of the background. *ICS*, intercellular space. *Z*, Z-line. Bar, 0.1 μm .

ocardial cells showed that the particles were localized over the lumen of the interior junctional sarcoplasmic reticulum (*ijSR*, Fig. 4), the peripheral junctional sarcoplasmic reticulum (*pjSR*, Fig. 3), as well as in discrete foci localized close to the Z-line in the interior regions of the muscle cytoplasm (*cSR*, Fig. 2*a*). In cases of optimal membrane visualization, the gold-labeled foci in the Z-line region was seen over the lumen of vesicular structures (Figs. 2*b* and 4). By contrast, the sarcolemma (*SL*, Fig. 3), the transverse tubules (*T*, Fig. 4), the mitochondria (*M*, Figs. 2, *a* and *b*, and 4), the interfibrillar spaces (*IFS*, Fig. 2*a*) where the network is present but not consistently visualized, the atrial granules (*AG*, Figs. 2*a* and 4), and the myofibrils were labeled only at the level of the background (Fig. 10).

RAT PAPILLARY MUSCLE CELLS: Longitudinal cryoultramicrotomy sections of fixed adult rat papillary muscle were labeled with antibodies to canine cardiac calsequestrin by the indirect immunocolloidal gold staining technique. Examination of the distribution of the gold particles in the myocardial cells showed that most of the particles were, as in the atrial myocardial cells, confined to the lumen of the sarcoplasmic reticulum where they were present over the lumen of the interior junctional sarcoplasmic reticulum (*ijSR*, Figs. 5, 7, 8*a*, and 8*c*) and the peripheral junctional sarcoplasmic reticulum (*pjSR*, Figs. 5, 6*a* and *b*, and 8*e*). Furthermore, clusters of gold particles were present in discrete foci and over the lumen of vesicular structures (*cSR*) most of which were localized close to the Z-line in the interior regions of the muscle cytoplasm (Figs. 5, 7, 8*b*, and 8*d*). In cases of optimal membrane visualization, it was clearly demonstrated that the gold-labeled vesicular structures in the Z-line region are continuous with the unlabeled network sarcoplasmic reticulum

(Fig. 8*d*). Occasionally a cluster of gold particles could be observed in the interfibrillar spaces where the network sarcoplasmic reticulum is localized (*cSR*, Fig. 8*b*). However, in general the network sarcoplasmic reticulum (*nSR*, Figs. 5, 7, 8*a*, and 8*b*) was only labeled at the level of the background (Fig. 10). Similarly, the sarcolemma (*SL*, Figs. 5, 6, and 8*e*), the transverse tubular membrane (*T*, Figs. 5, 7, 8*a*, and 8*c*), the mitochondria (*M*, Figs. 5, 7, and 8*b*), and the myofibrils were labeled only at the level of the background (Fig. 10). Finally, gold particles were densely distributed over the lumen of the nuclear envelope (*NE*, Fig. 9).

DISCUSSION

The results presented in this study show that calsequestrin in adult rat atrial and papillary myocardial cells in situ is localized in the lumen of the interior junctional sarcoplasmic reticulum, some but not all vesicular structures present at the cell periphery as well as at interior sites of the myocardial cytoplasm. It is very likely that the calsequestrin-containing membrane-bound structures closely apposed to the sarcolemma correspond to the peripheral junctional sarcoplasmic reticulum. Conclusive proof would require the visualization of the junctional processes (feet) between the sarcolemma and the calsequestrin-containing vesicles in the subsarcolemma region.

Since calsequestrin localized in the vesicular structures in interior regions of the cytoplasm is mostly confined to the interfibrillar spaces in the I-band region but, with few exceptions, absent from vesicular structures in the A-band region, it is likely that these vesicles correspond to the specialized extensions on the free sarcoplasmic reticulum called corbular

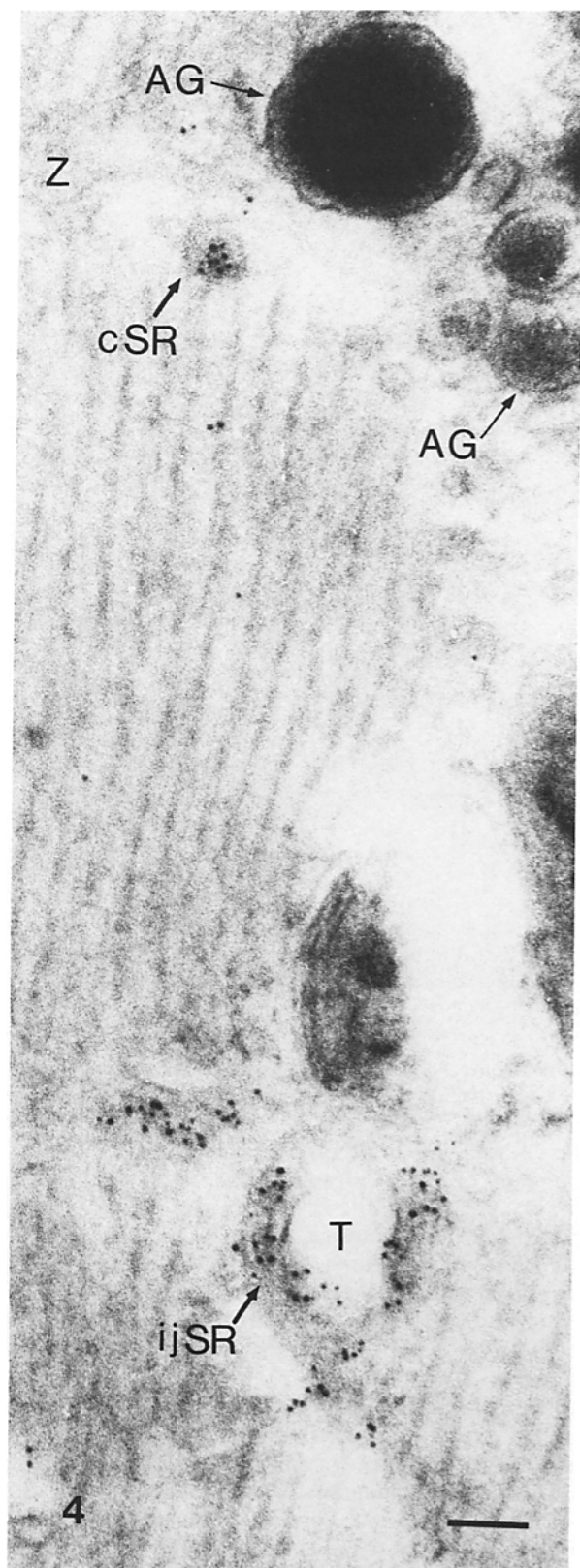


FIGURE 4 Electron micrograph of an ultrathin frozen section from the interior region of an atrial muscle cell labeled with affinity purified antibodies to calsequestrin. Gold particles were present over vesicular structures (cSR) located close to the Z-line (Z) as well as over the lumen of interior junctional sarcoplasmic reticulum (ijSR). Atrial granules (AG) and transverse tubules (T) were only labeled at the level of the background. Bar, 0.1 μ m.

sarcoplasmic reticulum which was first observed by Sommer in myocardial cells without transverse tubules (13). More recently, similar structures have also been shown to be very prominent in the I-band region of the rat atrial (22) and ventricular myocardial cells (15, 16). The possibility that the calsequestrin-containing vesicular structures present in the I-band region of the papillary myocardial cells correspond to corbular sarcoplasmic reticulum rather than to oblique cuts of interior junctional sarcoplasmic reticulum is supported by a recent study in which the ultrastructural localization of calsequestrin was determined in chicken myocardium by immunoelectron microscopy (11) as well as in sheep Purkinje fibers (12). The results obtained showed that calsequestrin was present in the lumen of both the peripheral junctional sarcoplasmic reticulum and the corbular sarcoplasmic reticulum. Since both chicken myocardial cells and sheep Purkinje fibers lack transverse tubules, it is very unlikely that the calsequestrin-containing, membrane-bound structures in the I-band region of these cells could be closely apposed to the sarcolemma and thus correspond to peripheral junctional sarcoplasmic reticulum.

Thus, it appears that calsequestrin in both atrial and papillary myocardial cells from adult rat heart is present in the lumen of the peripheral and interior junctional sarcoplasmic reticulum as well as the corbular sarcoplasmic reticulum while absent from the lumen of the network sarcolemma reticulum. Comparison of these results with our previous immunoelectron microscopic studies of the distribution of the ATPase of the sarcoplasmic reticulum (7) shows directly that the ATPase and calsequestrin are localized within distinct regions of the continuous sarcoplasmic reticulum membrane.

The calsequestrin present in the lumen of the nuclear envelope, a component of the rough endoplasmic reticulum, might represent newly synthesized calsequestrin since ribosomes are often associated with the outer nuclear membrane.

The localization of calcium (23–25) and calsequestrin to the lumen of the terminal cisternae of skeletal muscle (26–30) as well as the Ca^{2+} binding properties of calsequestrin (31) suggest that calsequestrin provides the major site of Ca^{2+} sequestration in the sarcoplasmic reticulum. The results presented here together with previous ones (11, 12) suggest that cardiac calsequestrin and thereby presumably Ca^{2+} storage sites in the corbular sarcoplasmic reticulum might be common to all mammalian and avian myocardial cells irrespective of whether transverse tubules and thus interior junctional sarcoplasmic reticulum is present or absent. The likely significance of the calsequestrin-containing corbular sarcoplasmic reticulum is supported by preliminary estimates of the relative distribution of calsequestrin in the different regions of the sarcoplasmic reticulum of atrial and ventricular rat muscle cells. Thus, it was observed that ~40–50% of the calsequestrin in the interior regions of the atrial and ventricular muscle cells, respectively, was localized in the lumen of corbular sarcoplasmic reticulum, while the remaining was localized in the lumen of the interior junctional sarcoplasmic reticulum (Jorgensen, A. O., and K. P. Campbell, unpublished results).

These results support the possibility that Ca^{2+} storage in and/or release from these structurally different regions of the cardiac sarcoplasmic reticulum occur in response to different stimuli during the excitation–contraction–relaxation cycle. Thus, Ca^{2+} release from the lumen of the peripheral and interior junctional sarcoplasmic reticulum might be directly

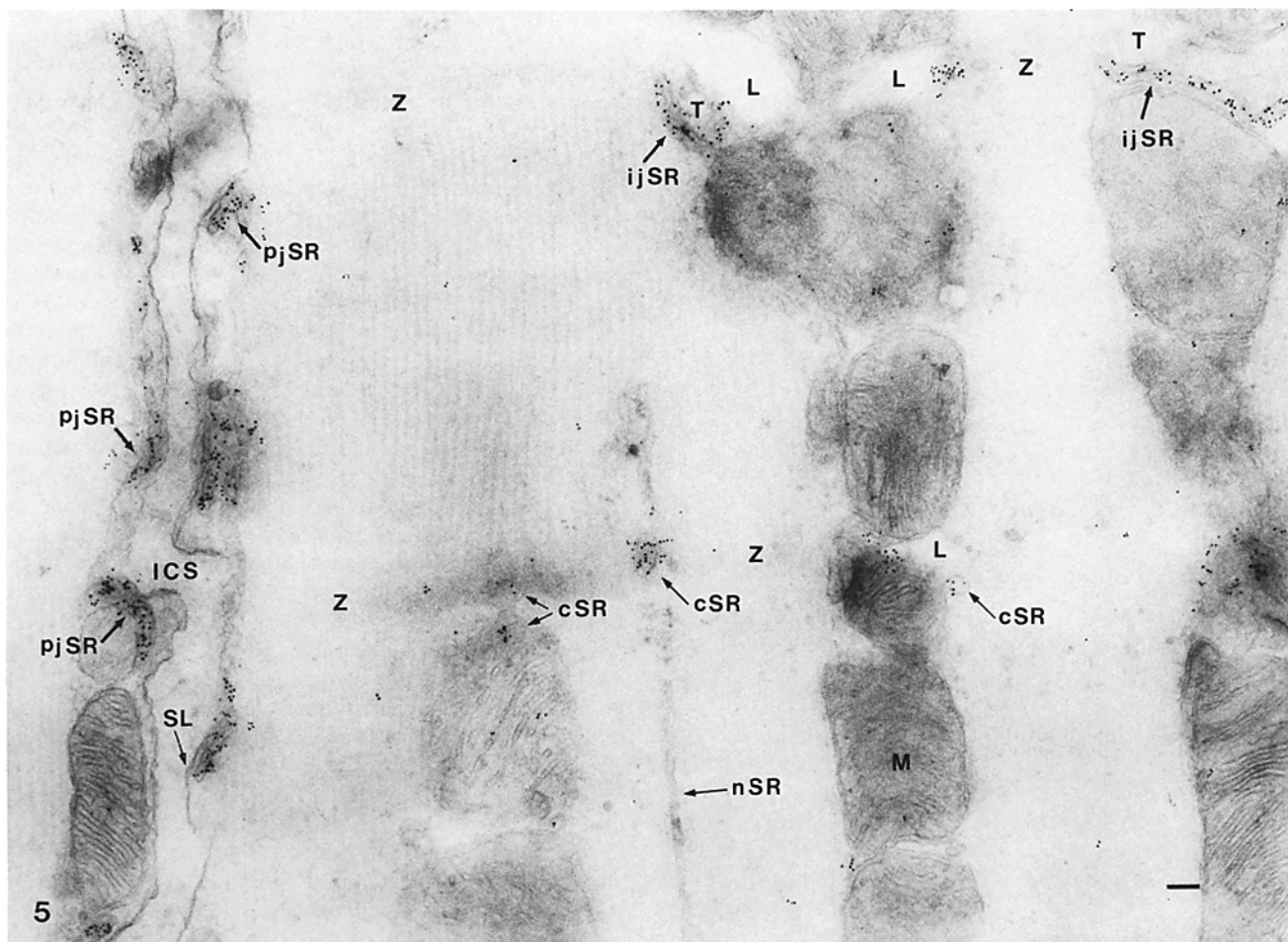


FIGURE 5 Electron micrograph of a longitudinal ultrathin frozen section of rat ventricular muscle labeled with affinity purified antibodies to canine cardiac calsequestrin. Gold particles were present over the lumen of interior junctional sarcoplasmic reticulum (*ijSR*), peripheral junctional sarcoplasmic reticulum (*pjSR*), and vesicular structures (*cSR*) close to the Z-line (Z) in the interior regions of the cell. The network sarcoplasmic reticulum (*nSR*), the transverse tubules (T), and the sarcolemma (SL) were only labeled at the level of the background. M, mitochondrion. ICS, intercellular space. L, extracted lipid. Bar, 0.1 μ m.

triggered by depolarization of the sarcolemma, whereas Ca^{2+} release from the corbular sarcoplasmic reticulum, which cannot be directly stimulated by the depolarization event, might be triggered by a diffusible agent. Fabiato (1) has shown that Ca^{2+} can induce Ca^{2+} release from the sarcoplasmic reticulum of mechanically skinned cardiac muscle cells and thus trigger muscle contraction even if the skinned cells lack junctional sarcoplasmic reticulum (e.g., dog Purkinje fibers and pigeon myocardial cells). Thus, the results presented in this paper are consistent with the idea that Ca^{2+} triggers Ca^{2+} release from the calsequestrin-containing corbular sarcoplasmic reticulum.

Studies of the distribution of Ca^{2+} in the myocardial sarcoplasmic reticulum of guinea pig ventricular muscle in situ (32, 33) and isolated rat and rabbit ventricular muscle cells by X-ray microprobe analysis (34) showed that Ca^{2+} accumulates in the lumen of the interior junctional sarcoplasmic reticulum during relaxation. One of these studies (33) suggested that Ca^{2+} can accumulate in both the interior junctional and the Z-retic sarcoplasmic reticulum (perhaps corresponding to the corbular sarcoplasmic reticulum) during muscle relaxation. Furthermore, it appeared that the relative distribution of Ca^{2+} in these two different regions of the sarcoplasmic reticulum varied with the frequency with which the myocardial cells were stimulated, thus implying that the accumula-

tion and/or release of Ca^{2+} from these sites might occur in response to different stimuli or be differently regulated.

Perfusion studies of $^{45}\text{Ca}^{2+}$ -labeled rat hearts suggested that there are at least two different Ca^{2+} storage pools in cardiac sarcoplasmic reticulum (35). Thus, while one Ca^{2+} pool sequesters Ca^{2+} at both normal and increased inotropic states of the heart and requires extracellular Ca^{2+} for the subsequent release of Ca^{2+} , another Ca^{2+} pool only sequesters Ca^{2+} at increased inotropic states of the heart (e.g., catecholamine stimulation) and does not require extracellular Ca^{2+} to release Ca^{2+} from this Ca^{2+} pool.

To determine whether these two differently regulated Ca^{2+} pools in the cardiac sarcoplasmic reticulum correspond to the calsequestrin-containing corbular and junctional sarcoplasmic reticulum of mammalian cardiac muscle cells, studies have been initiated to determine the distribution of Ca^{2+} in the different regions of the sarcoplasmic reticulum in hearts at normal and increased inotropic states by electrospectroscopy.

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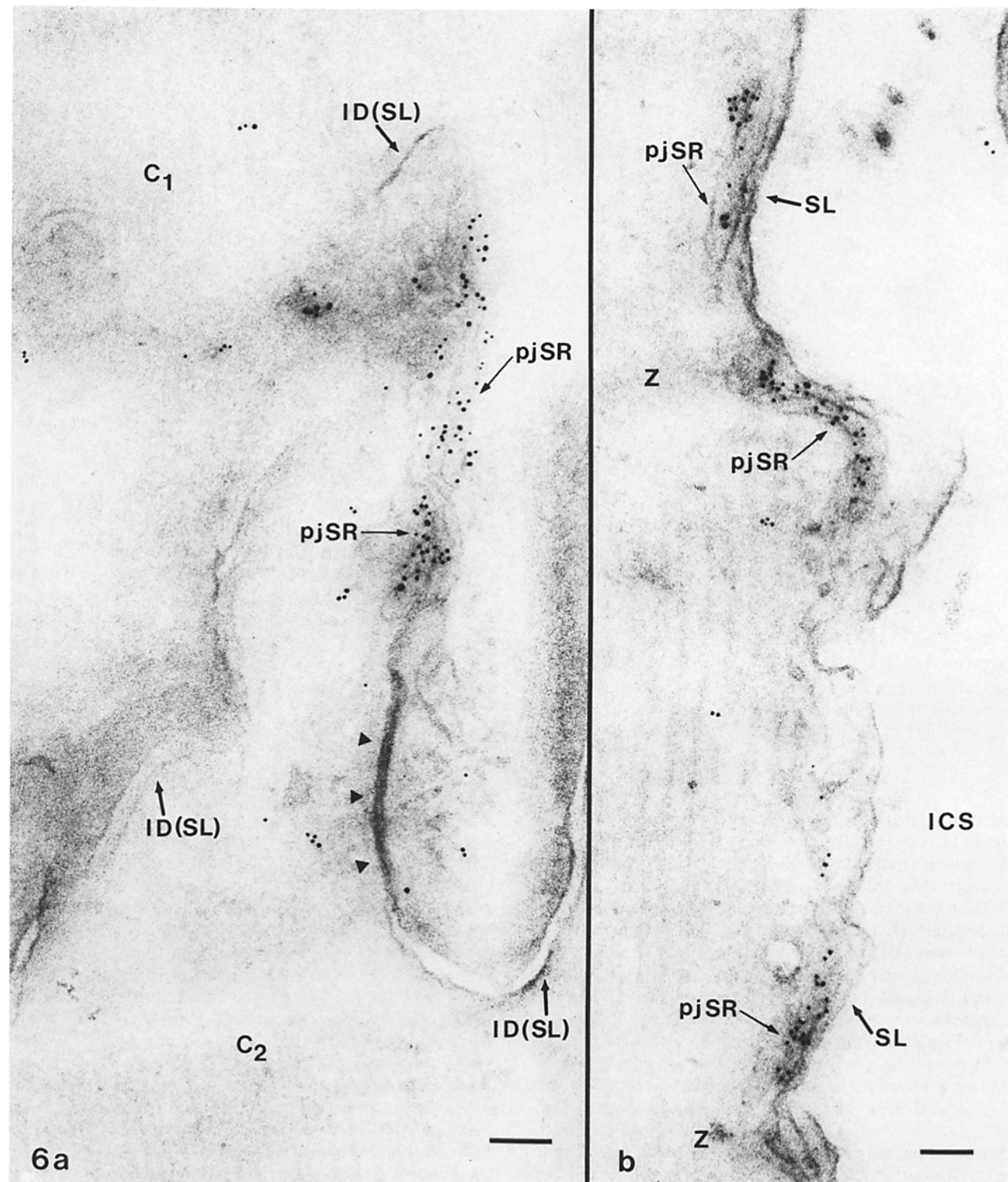


FIGURE 6 Electron micrographs of longitudinal ultrathin frozen sections of rat ventricular muscle cells labeled with affinity purified antibodies to calsequestrin and showing the peripheral region of these cells. Gold particles were associated with the peripheral junctional sarcoplasmic reticulum (*pjSR*) (a and b) while the sarcolemma [*SL* and *ID(SL)*] (a and b) was labeled only at the level of the background. Note that gold labeling over the lumen of the *pjSR* in close apposition to the intercalated discs [*ID(SL)*] were not observed in close vicinity to gap junctions (arrow heads). *ICS*, intercellular space. *Z*, Z-line. Parts of two different myocardial cells (*C*₁ and *C*₂) (a). Bar, 0.1 μ m.

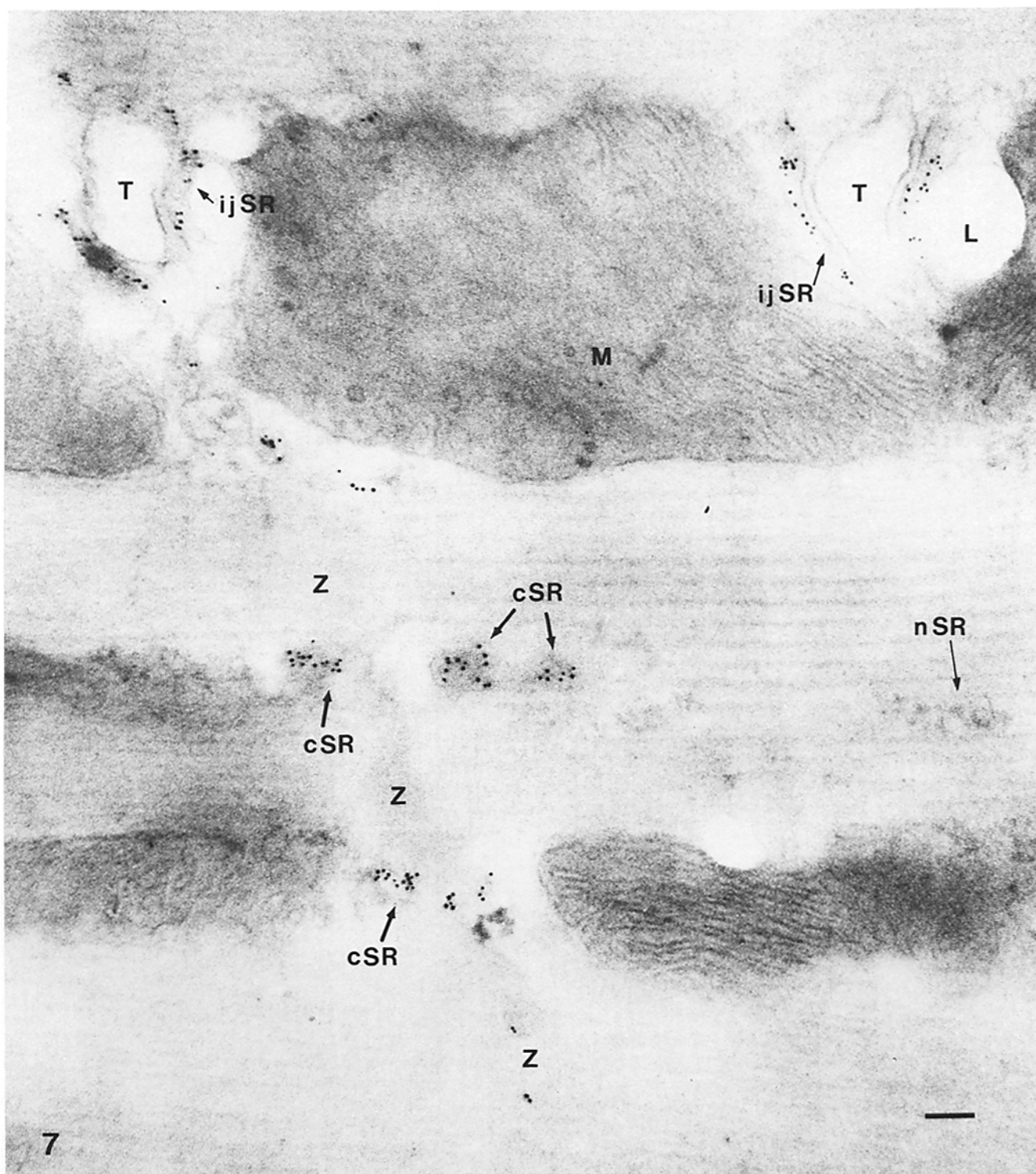


FIGURE 7 Electron micrograph of a longitudinal ultrathin frozen section labeled with affinity purified antibodies to calsequestrin showing an interior region of a rat ventricular muscle cell. Gold particles were present over the lumen of vesicular structures (cSR) located close to the Z-line (Z) and over the lumen of interior junctional sarcoplasmic reticulum (ijSR). The transverse tubular membrane (T) and the network sarcoplasmic reticulum (nSR) were labeled only at the level of the background. M, mitochondrion. L, extracted lipid. Bar, 0.1 μm.

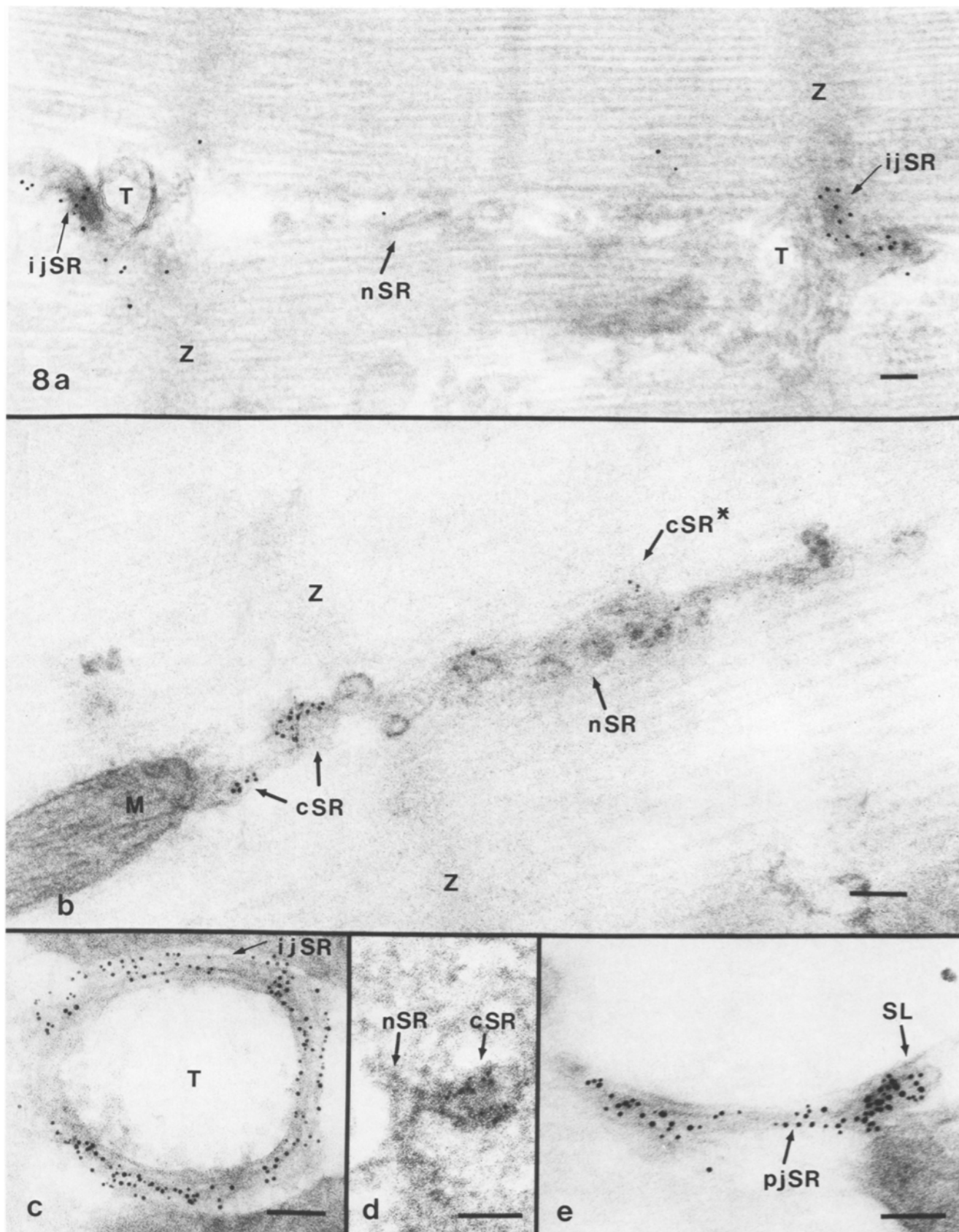


FIGURE 8 Electron micrographs of longitudinal ultrathin frozen sections of rat ventricular muscle cells labeled with affinity purified antibodies to calsequestrin. Gold particles were present over the lumen of the peripheral junctional sarcoplasmic reticulum (*pjSR*) (e), the interior junctional sarcoplasmic reticulum (*ijSR*) (a and c), and the corbular sarcoplasmic reticulum (*cSR*) (b and d) close to the Z-line (Z) (a and b). The transverse tubular membrane (T) (a and c) and the network sarcoplasmic reticulum (*nSR*) (a, b, and d) were with few exceptions (*cSR** in b) only labeled at the level of the background. *M*, mitochondrion. *SL*, sarcolemma. Bar, 0.1 μm .

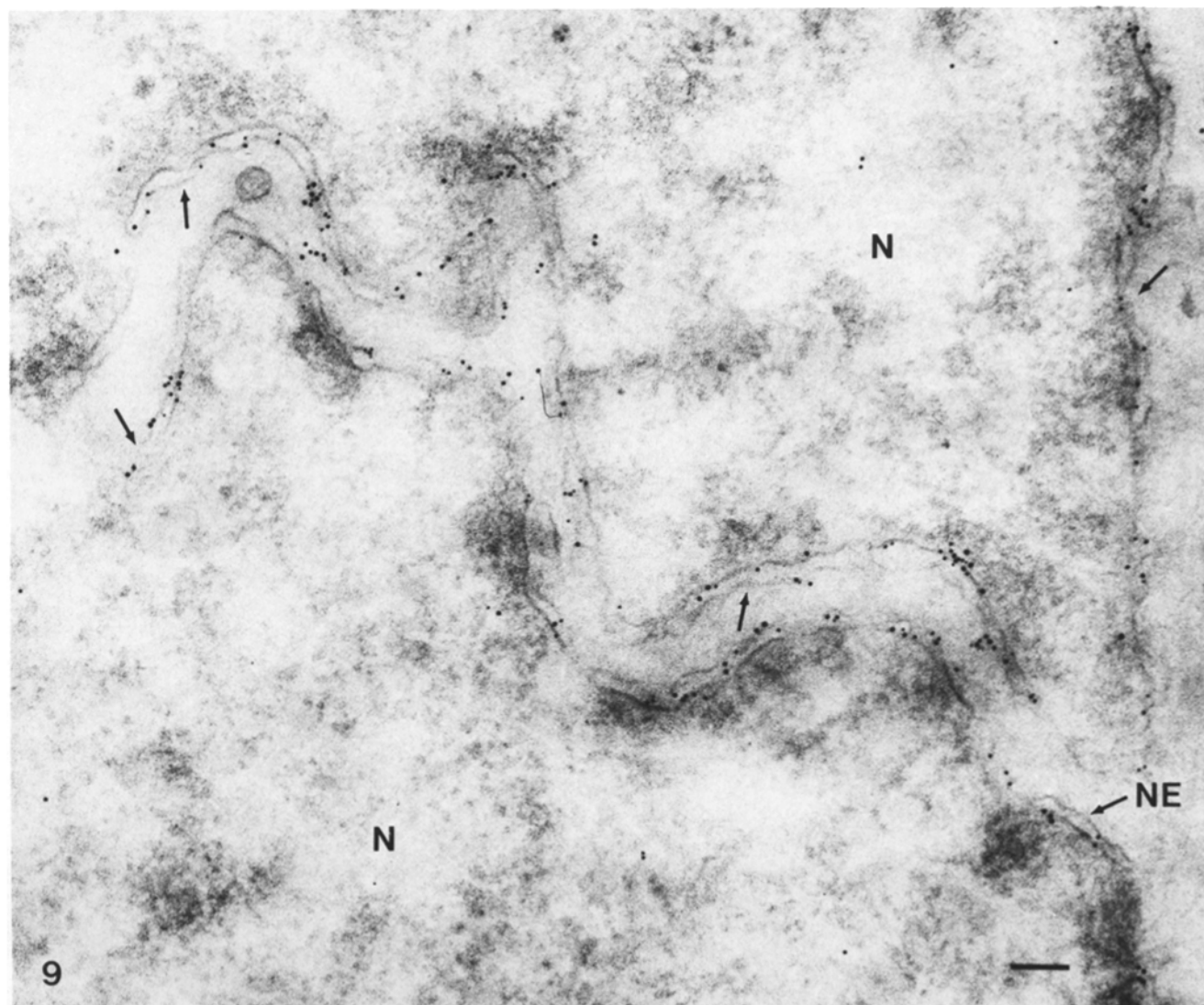


FIGURE 9 Electron micrograph of a longitudinal ultrathin frozen section from a rat ventricular muscle cell labeled with affinity purified antibodies to calsequestrin showing parts of the nuclear (N) and perinuclear region of a ventricular myocardial cell. Gold particles were densely distributed over the lumen of the nuclear envelope (NE and arrows). Bar, 0.1 μ m.

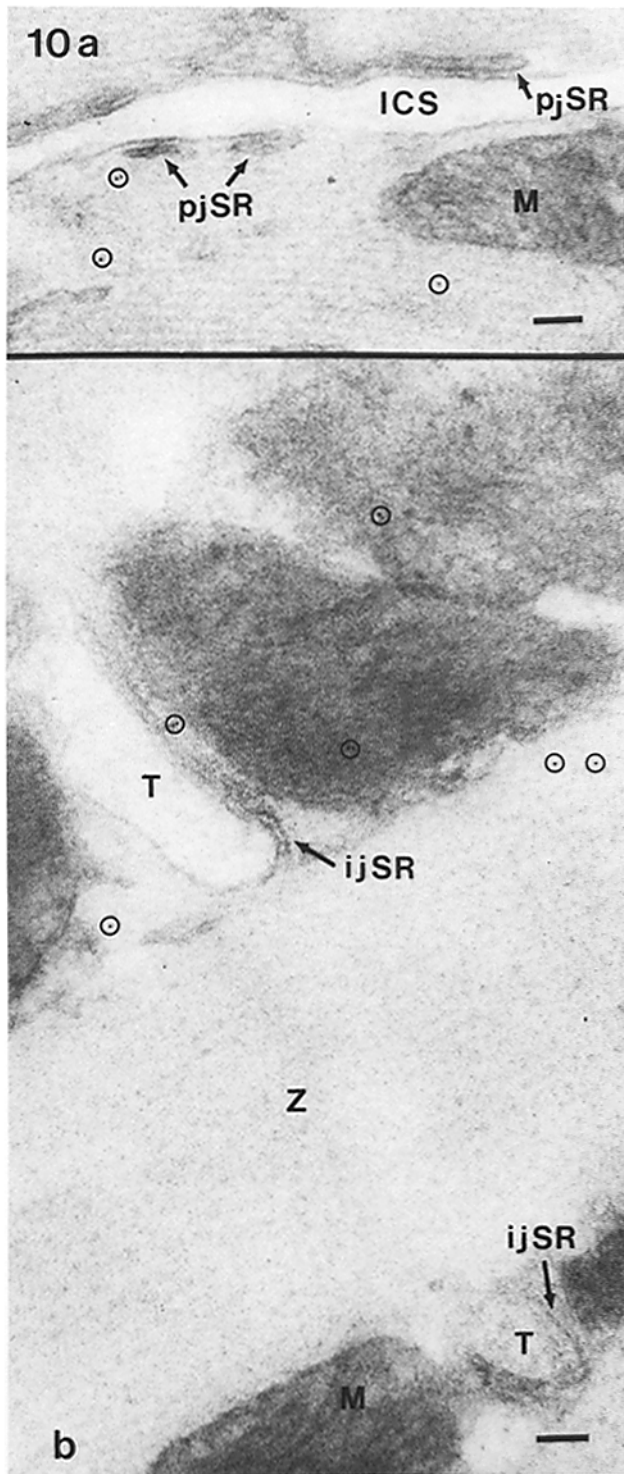


FIGURE 10 Electron micrographs of longitudinal ultrathin frozen sections from rat ventricular muscle labeled with normal gammaglobulin. Note that very few gold particles were observed on the sections including the peripheral (*pjSR*) (a) and the interior junctional (*ijSR*) (b) sarcoplasmic reticulum as well as the Z-line (Z) (b). ICS, intercellular space. M, mitochondrion. T, transverse tubule. Bar, 0.1 μm .

for ultrathin embedding of immunolabeled frozen sections available to us while their manuscript (reference 25) was in press.

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