Ca^{2+}-Sequestering Smooth Endoplasmic Reticulum in an Invertebrate Photoreceptor. I. Intracellular Topography as Revealed by OsFeCN Staining and *In Situ* Ca Accumulation

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ABSTRACT Two ultrastructural approaches were used in photoreceptor cells of the leech, *Hirudo medicinalis*, to (*a*) investigate the intracellular topography of the smooth endoplasmic reticulum (SER) and (*b*) identify among the various subregions of the SER those which might function as Ca-sequestering sites. When the cells are prefixed with $CaCl_2$-containing glutaraldehyde and postfixed with osmium tetroxide-ferricyanide (OsFeCN), only a part of the total SER is specifically stained. The stained SER cisternae include the submicrovillar cisternae (SMC), subsurface cisternae (SSC), the nuclear envelope, Golgi-associated SER, paracrystalline SER, and SER associated with glycogen areas. An extensive tubular SER cisternal system always remains unstained.

When the cells are permeabilized by saponin and subsequently incubated with Ca^{2+}, MgATP, and oxalate, the SMC (Walz, 1979, Eur. *J. Cell Biol.* 20:83-91), the SSC and the nuclear envelope contain electron-opaque Ca-oxalate precipitates indicating their ability to function as an effective Ca^{2+} sink. The results show that the very elaborate SER in this photoreceptor cell includes many functionally heterogeneous subregions. Of special physiological significance are those components (SMC and SSC) which are effective in Ca^{2+}-buffering in the immediate vicinity of the plasma membrane.

The smooth endoplasmic reticulum (SER) is a prominent organelle in photoreceptor cells of invertebrates (see reference 43 for review) and in rod inner segments of vertebrates (see reference 22 for review). In both vertebrate and invertebrate photoreceptors there is a great diversity in the structure and intracellular topography of SER elements. We do not know the functional capabilities of the various SER elements nor do we have any idea whether, or to what extent, the morphological diversity among certain SER subregions reflects functional heterogeneity among these subregions.

In vertebrate photoreceptors the SER has been implicated (see reference 22 for review) as being involved in the synthesis, storage, and/or turnover of lipids destined for the renewal of the photoreceptive membrane. Similar considerations have been put forward for invertebrate photoreceptors (see reference 43 for review). Recently, there appeared experimental evidence (23, 34, 41) indicating a possible involvement of certain SER elements in photoreceptors in buffering the concentration of ionized calcium (Ca^{2+}_{in}).

The contribution of a Ca^{2+}-sequestering SER to the regulation of Ca^{2+}_{in} is of special importance in photoreceptor cells of invertebrates. It has been proposed that a light-induced increase in Ca^{2+}_{in} (5, 6, 30) is one factor controlling light adaptation (4). This hypothesis is strengthened by physiological experiments in which Ca^{2+} or the Ca^{2+} chelator, EGTA, have been injected intracellularly into photoreceptor cells of *Limulus* (27, 28), the honey bee (1), and the squid (35). These studies show that the effects of intracellular injections of Ca^{2+} mimic important aspects of light adaptation (see also reference 15).

Furthermore, the studies of Fein and Lisman (17) and Fein and Charlton (15) show that local illumination of *Limulus* ventral nerve photoreceptors leads to local adaptation, and local injections of Ca^{2+} locally desensitize the receptor. These experiments show that the cell is able to perform a precise spatial regulation of Ca^{2+}_{in}.

It has been hypothesized (12, 29) that submicrovillar cisternae of the SER might be a possible source and sink for Ca^{2+} mobilized by illumination. Submicrovillar cisternae (SMC) of the SER are present in most invertebrate photoreceptor cells. Synonymously, these structures have been called perirhabdomal cisternae (9), subrhadomere cisternae (26), and palisades (24).

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In a recent study (41) it was shown that SMC in the photoreceptor cells of the leech are able to accumulate Ca^{2+} at the expense of ATP. The present paper extends this earlier work, in giving a full account of the intracellular topography of the various SER elements, and tries to identify, among these, all those elements that might participate in the buffering of cytoplasmic Ca^{2+} levels. The results to be presented also address one of the questions mentioned initially, in that they demonstrate the functional heterogeneity among various subregions of the SER.

The accompanying paper (38) reports on basic functional properties of the Ca^{2+}-sequestering SER in the photoreceptor cells of the leech. Some results of the present paper have been published previously in abstract form (39, 40).

MATERIALS AND METHODS

Animals

Hirudo medicinalis L. were obtained from commercial dealers. The leeches were kept in aquaria illuminated by fluorescent light (12 h light : 12 h dark) and maintained at 16°C.

Conventional Electron Microscopy

The pigmented eye cups were quickly excised from the animals and immediately transferred into 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. In this fixative, excess tissue was dissected away from the eye cups, which were finally bisected to further decrease the block size. After 1-h prefixation at room temperature, the specimens were washed in buffer and postfixed for 1 h in 2% OsO_4 in 0.1 M phosphate buffer. The specimens were then washed in distilled water and stained en bloc with 0.5% aqueous uranyl acetate for 1 h. Subsequently, the eye cups were dehydrated in ethanol, passed through propylene oxide, and embedded in Spurr's (36) resin.

OsFeCN Postfixation/Staining

The excised eye cups were prefixed for 1 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, containing 5 mM $CaCl_2$. They were then washed in the same buffer and postfixed in a mixture of 1% OsO_4 and 0.8% potassium ferricyanide ($K_3Fe[CN]_6$), buffered with 0.1 M cacodylate buffer to pH 7.4. The specimens were then washed in buffer and further processed as described above.

In one control preparation, $CaCl_2$ was omitted from the primary fixative. Another control was prepared without uranyl acetate staining.

Sections were stained with uranyl acetate and lead citrate or left unstained. All micrographs of the OsFeCN-treated material were made from unstained sections with a Philips EM 301 electron microscope using an accelerating voltage of 80 kV.

Ca^{2+} Accumulation In Situ

The eye cups were dissected in physiological saline of the following composition (mM): NaCl 113.5, KCl 4.3, $CaCl_2$ 1.8, maleic acid 10, Tris (hydroxymethyl) aminomethane 10, pH 7.4.

The experimental protocol for electron microscope (EM) demonstration of ATP-dependent Ca^{2+} uptake by SER differs slightly from that reported previously (41). The photoreceptor cells were first permeabilized by treating the bisected eye cups for 10–15 min in a lysis medium of the following composition (mM): KCl 100, $MgCl_2$ 10, ATP·Na_2 5, K_2EGTA 2, histidine 20, sucrose 40. The pH of this medium was adjusted with KOH to 7.0, and 500 μg/ml saponin was added immediately before use.

Saponin can be used to disrupt cholesterol-rich membranes (such as the plasmalemma) without injuring cholesterol-poor membranes (e.g. the muscle sarcoplasmic reticulum) (see references 2, 10, 11, 41, for details).

After permeabilization of the cells, the preparation was treated for up to 1 h with a loading medium of the following composition (mM): KCl 75, potassium oxalate 25, $MgCl_2$ 10, ATP·Na_2 5, histidine 20, sucrose 40, K_2EGTA 1, CaEGTA 4. The pH was adjusted with KOH to 7.0.

The concentration of free Ca^{2+} in the loading medium was calculated (see companion paper [38] for details) to be $\sim 8.5 \times 10^{-7}$ M.

Preparation for electron microscopy was performed as described previously (41). All sections were investigated unstained with a Philips EM 301 electron microscope operated at 40 kV.

RESULTS

Intracellular Topography and Morphology of the SER

The photoreceptor cells of the leech (Fig. 1) contain an elaborate SER (Figs. 2, 3) (25, 41). The organizaton of the prominent submicrovillar cisternae (SMC) of the SER (Fig. 2) has previously been described in detail (41).

Between and beneath the palisadelike arranged SMC and in more centrally located portions of the cytoplasmic cortex of the leech photoreceptors extends a system of tubular SER cisternae (Figs. 2, 3). Figs. 2 and 3 show, however, that the dense packing of organelles in these cells makes it difficult to trace out a defined SER element when the cells are prepared for electron microscopy by standard procedures.

In contrast, when cells are prefixed in $CaCl_2$-containing glutaraldehyde and postfixed with OsFeCN, defined SER elements are selectively stained. In accordance with Forbes et al. (13) and Hepler (21) it was observed that the OsFeCN method leads (*a*) to an electron-dense staining of the lumina of the SER cisternae (Figs. 4–6, 8, 10 *a*, *b*, and 11–13), and (*b*) to an enhanced contrast of membrane leaflets (Fig. 10 *a*, *b*). As the micrograph (Fig. 3) of the control preparation shows, the presence of $CaCl_2$ in the primary fixative was essential for the SER densification to occur.

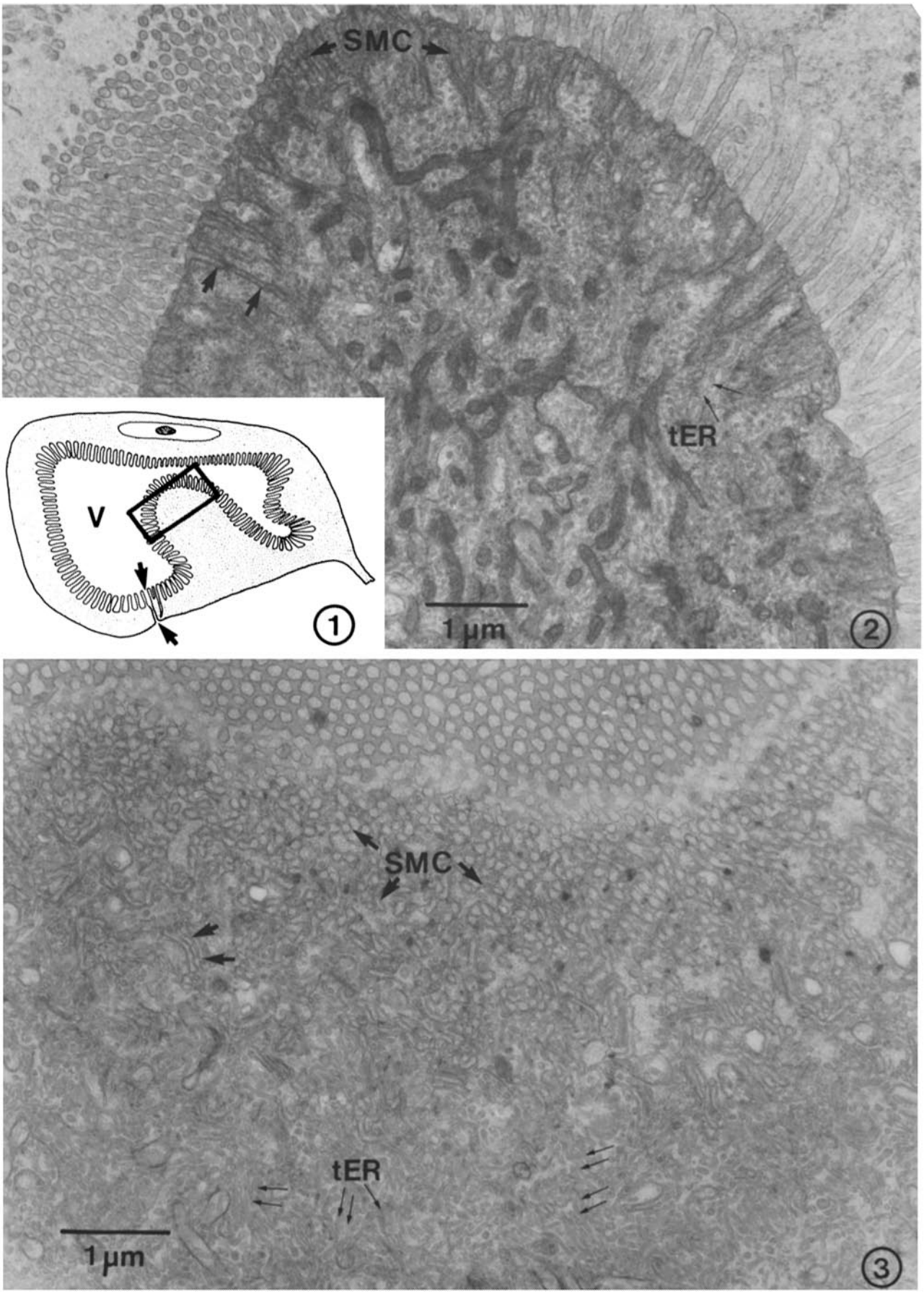
The effect of other divalent cations in promoting the OsFeCN staining of the SER cisternal lumina has not been tested in the present study. However, Forbes et al. (13) reported that substitution of Mg^{2+}, Mn^{2+}, Sr^{2+}, Ba^{2+}, and La^{3+} for Ca^{2+} in the primary fixative also produced staining of the sarcoplasmic reticulum of muscle cells.

The OsFeCN method allows one to distinguish between the following SER elements (see Fig. 15 for a summary of the results): The low-power electron micrograph of Fig. 4 demonstrates that the SMC stain heavily with the OsFeCN method. The selective contrast shows up this highly organized membrane system which underlies the entire photoreceptive (microvillar) membrane. This is clearly shown in Fig. 4 (asterisk)

FIGURE 1 Schematic representation of a photoreceptor cell of the leech, *Hirudo medicinalis*. The cell's cytoplasm surrounds a vacuole-like cavity (*V*). The microvilli of the photoreceptive membrane protrude into the "vacuole." The arrows label one of the clefts which connect the "vacuole" to the intercellular space. The rectangle labels approximately the section of Fig. 2.

FIGURE 2 Submicrovillar region of a cell prepared by standard procedures. The micrograph illustrates the palisadelike arranged submicrovillar cisternae of the smooth endoplasmic reticulum (*SMC*, thick arrows) and the more centrally located tubular ER cisternae (*tER*, thin arrows). The dense packing of organelles makes it difficult to trace out a defined SER element. × 18,000.

FIGURE 3 Submicrovillar region of a cell prepared by the OsFeCN method but without $CaCl_2$ additions to the primary fixative. The micrograph illustrates the tremendous amount of smooth endoplasmic reticulum in this region of the cell. Due to the lack of $CaCl_2$ in the primary fixative, no SER element became stained by OsFeCN. Labeling as in Fig. 2. × 21,000.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tER
V
1
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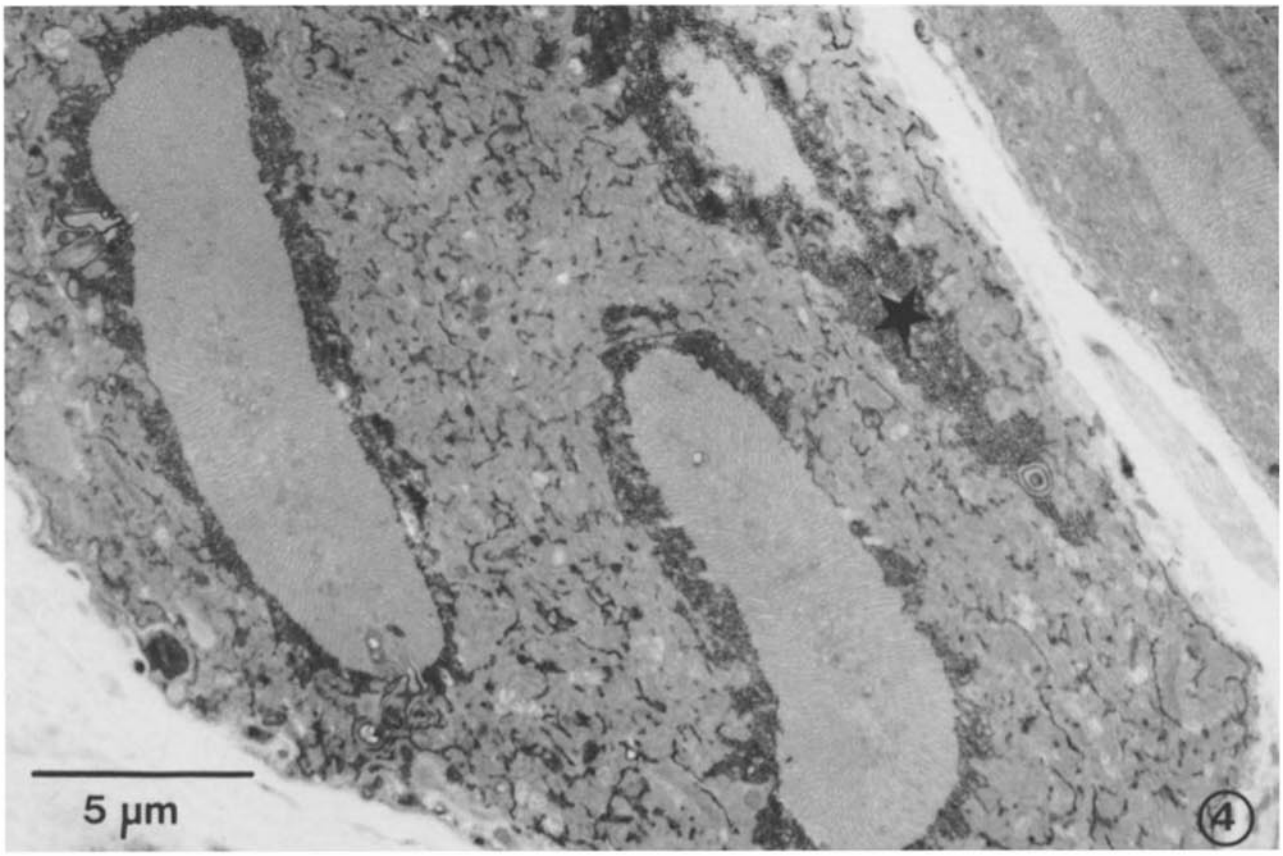


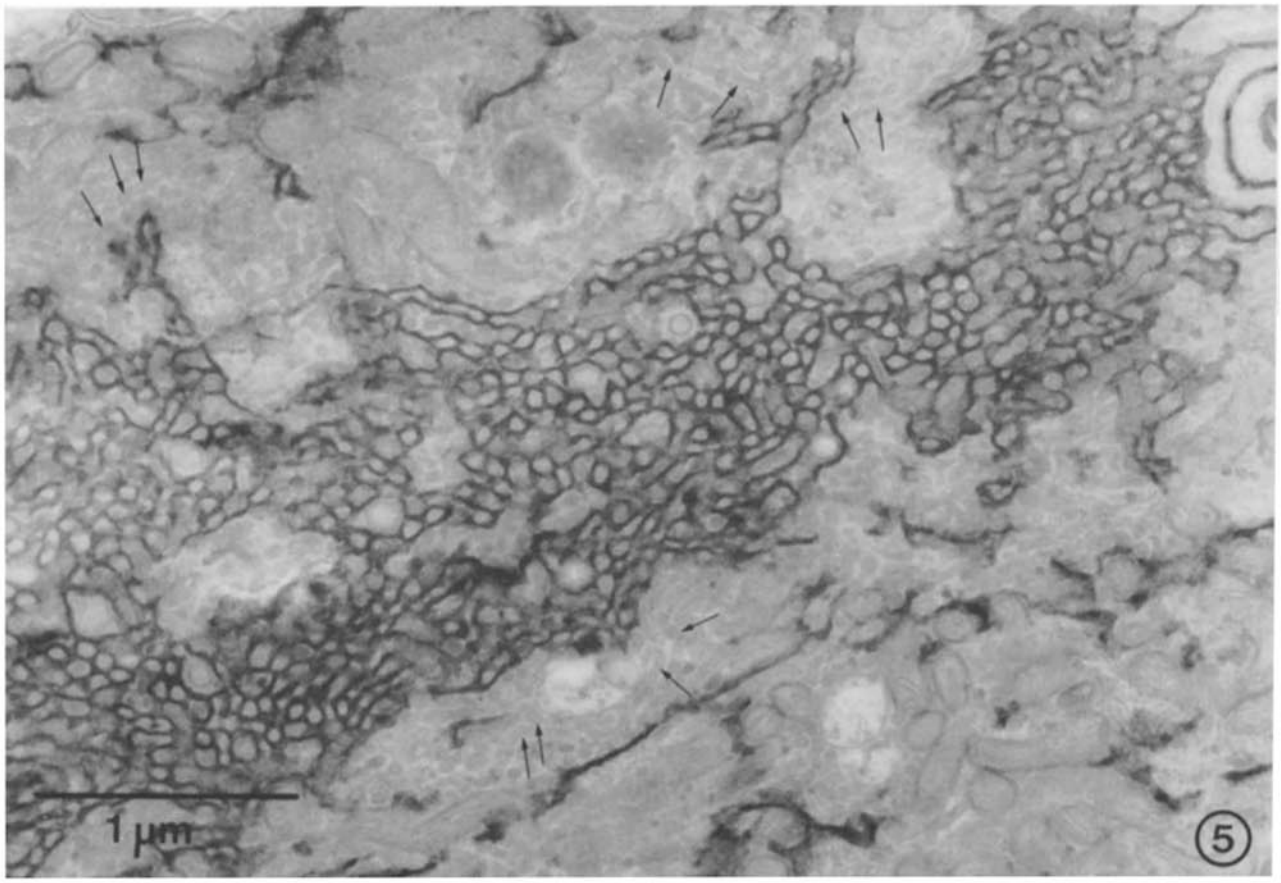
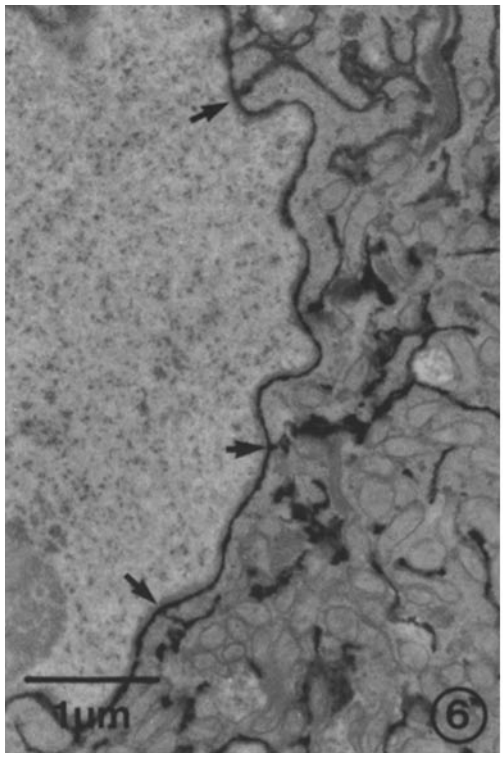


FIGURE 4 Low-power electron micrograph of a cell stained by OsFeCN postfixation. The section cuts the "vacuole" at three very peripheral sites. The OsFeCN method stains specifically the SMC and some SER elements in the bulk of the cell. The star labels a site where the section plane passes through the submicrovillar region perpendicular to the longitudinal axis of the microvilli. Fig. 5 shows this region at higher magnification. × 6,000.

FIGURE 5 Enlarged view of the region which is marked by the asterisk in Fig. 4. The OsFeCN method stains only the lumen of the submicrovillar smooth ER cisternae. The smooth tubular ER cisternae (arrows) remain unstained. × 35,000.



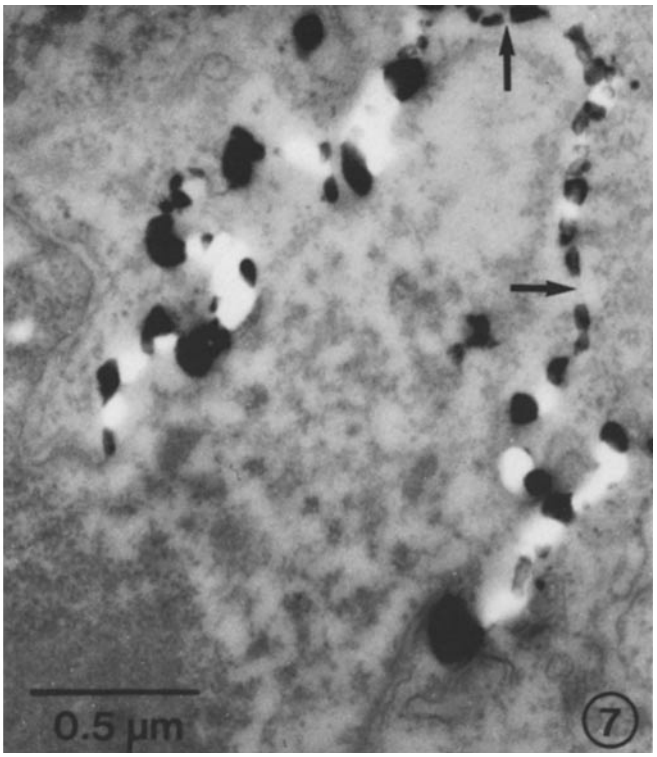


FIGURE 6 Nuclear region of a cell stained by the OsFeCN method. The micrograph illustrates that the nuclear envelope-endoplasmic reticulum continuum (arrows) is positively stained. × 18,000.

FIGURE 7 Nuclear envelope of a cell which was permeabilized by saponin treatment and subsequently incubated in a medium containing Ca^{2+}, MgATP, and oxalate. Calcium oxalate precipitates within the perinuclear cisterna show that this part of the ER is capable of actively accumulating Ca^{2+}. × 45,000.

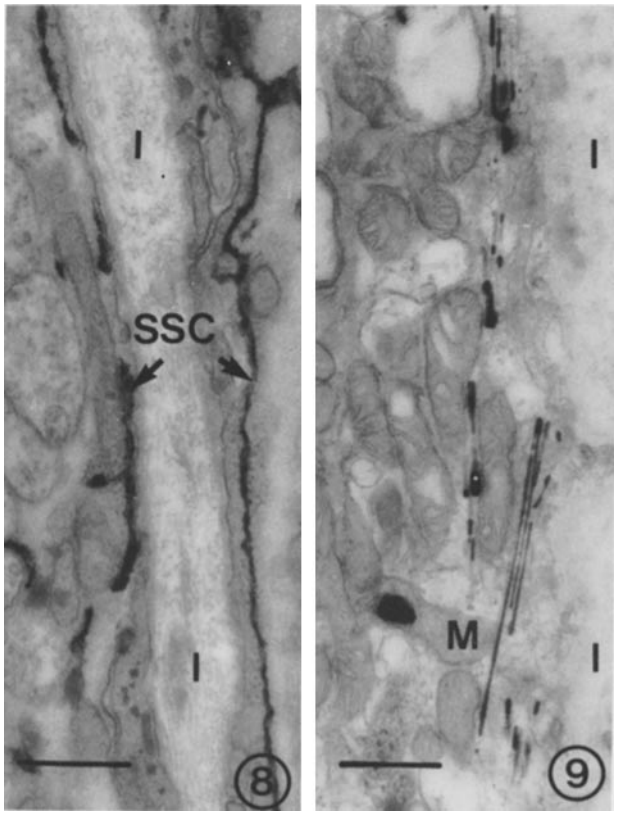


FIGURE 8 Periphery of two adjoining cells prepared by OsFeCN fixation/staining. The intercellular space is labeled *I*. In both cells, surface cisternae are closely juxtaposed to the nonreceptive plasmalemma and stained positively. Bar, 0.5 µm. × 30,000.

and Fig. 5, where the section plane runs through the cell just underneath, and almost perpendicular to, the microvilli.

In addition to the SMC, the whole cytoplasm contains numerous reticular elements which are selectively stained (Fig. 4). These are not isolated SER elements but parts of an interconnected cisternal network with confluent lumina as shown in Fig. 11. A stained SER cisterna (labeled by arrows) originates from the SMC, continues into the cytoplasm adjacent to a Golgi stack, and runs into the paracrystalline SER.

The paracrystalline SER either consists of a bundle of parallel tubules (micrograph not shown) or is differentiated as illustrated in Figs. 11 and 13. Paracrystalline SER of this kind is found in most (every?) photoreceptor cells of *Hirudo*, mostly close to the nucleus.

The perinuclear cisterna is part of the SER system which stains with the OsFeCN method. Continuities between the smooth perinuclear cisterna and cytoplasmic SER cisternae are frequently observed (Fig. 6).

The distribution of OsFeCN-stained SER is also related to glycogen areas in several distinct regions of the cell. Fig. 11 shows how such a glycogen region is interspersed with SER cisternae.

Important elements of the OsFeCN-positive SER system are those cisternae which form typical subsurface cisternae (SSC) closely juxtaposed to the nonreceptive plasma membrane (Fig.

FIGURE 9 Periphery of a cell which was permeabilized by saponin and subsequently incubated in a loading medium containing Ca^{2+}, MgATP, and oxalate. Ca oxalate precipitates have formed close to and almost parallel with the nonreceptive membrane due to active Ca^{2+} uptake by subsurface cisternae as they are illustrated in Fig. 8. (*I*) intercellular space, (*M*) mitochondria. Bar, 0.5 µm. × 27,000.

8). Fig. 10*a* shows that the SSC are continuous with SMC. In the illustrated example they are continuous through a concentric cisternal specialization (compare Figs. 10*a* and *b*). This specialization is closely associated with the plasmalemma of the clefts which connect the "vacuole" to the intercellular space (see also Figs. 1, 15).

Not all SER elements within photoreceptor cells of the leech stain with the OsFeCN method. Fig. 5 shows that most smooth tubular cisternae also labeled in Figs. 2 and 3 are not stained. The question of whether there exists physical continuity between the OsFeCN-positive and negative SER cisternae can not, as yet, be answered. However, the observation that these cisternae are never stained, even when they are closely associated with OsFeCN-positive cisternae (Fig. 5), suggests that the

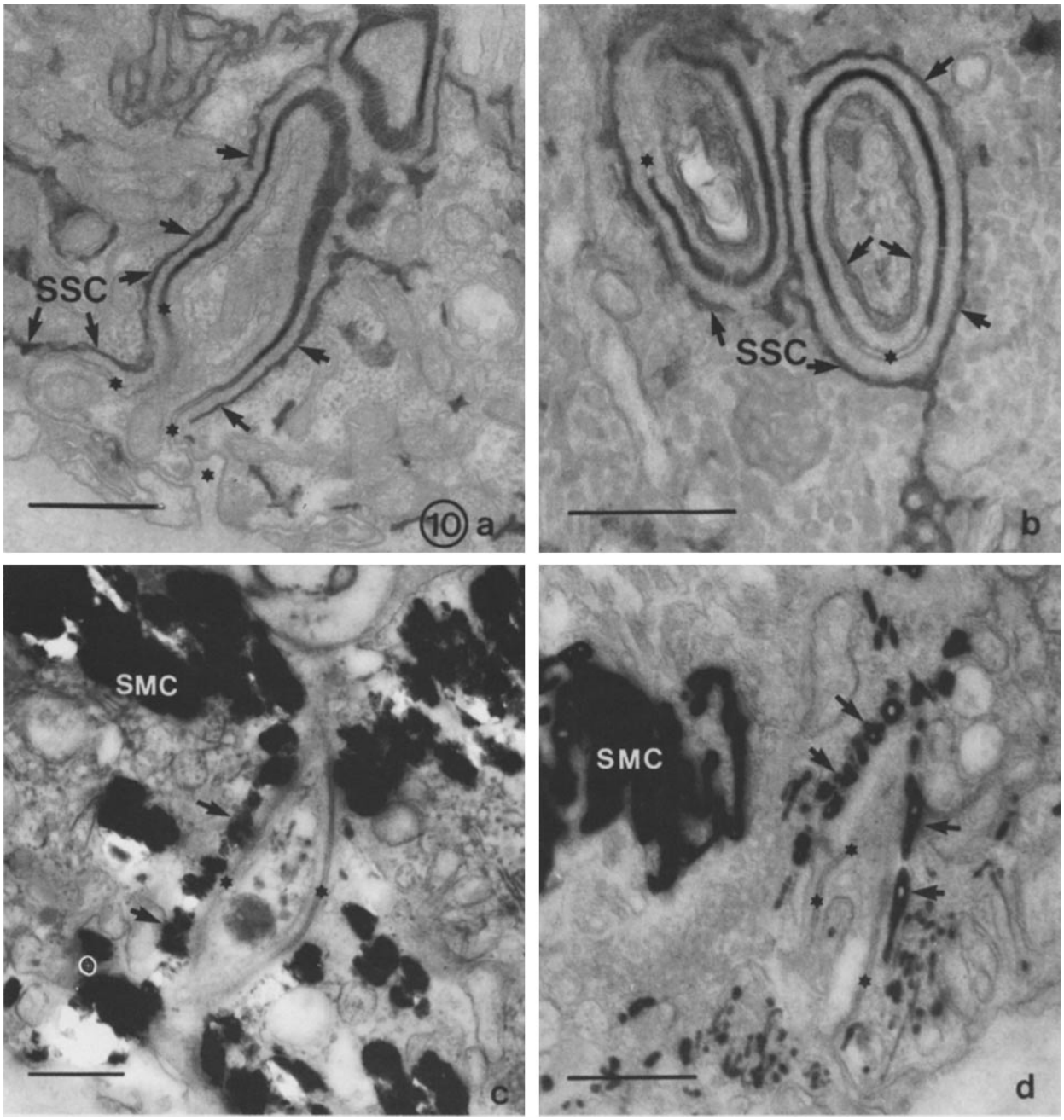


FIGURE 10 Micrographs of that cell region where one of the clefts (stars) connects the "vacuole" to the intercellular space. (*a*) and (*b*) were taken from cells prepared by the OsFeCN method; (*c*) and (*d*) were taken from cells which were permeabilized by saponin and subsequently incubated in a loading medium containing Ca^{2+}, MgATP, and oxalate to promote Ca^{2+} uptake by Ca^{2+}-sequestering ER. The extracellular space of the clefts is labeled with a star in all micrographs. In (*a*) and (*b*) the cleft lumen is positively stained due to block staining with uranyl acetate.

(*a*) and (*b*) show that the lumina of the submicrovillar cisternae (*SMC*) of the SER as well as the subsurface cisternae (*SSC*) associated with the nonreceptive plasma membrane are positively stained and form a morphological continuum (arrows) due to the concentrically arranged *SSC* backing the entire cleft membrane. In (*c*) and (*d*) the *SMC* and the *SSC* (arrows) are heavily loaded with Ca oxalate precipitates indicating that the cisternal elements which are positively stained in (*a*) and the (*b*) are able to actively accumulate Ca^{2+}. Bars, 0.5 μm. (*a*) × 35,000, (*b*) × 51,000, (*c*) × 29,000, and (*d*) × 40,000.

OsFeCN-negative reaction does not result from methodological inconsistencies but might reflect a true functional diversity between these cisternal elements.

Golgi cisternae (Figs. 11, 12) and Golgi-derived vesicles remain unstained. This is remarkable in view of micrographs (see Fig. 12) which show a stained SER cisterna closely juxtaposed to what appears to be the forming face of a dictyosome. Stained vesicles that seem to bud off from the SER to the dictyosome are frequently observed at these sites.

Identification of Ca^{2+}-Sequestering SER Elements

It has been shown previously (41) that the SMC are able to accumulate Ca^{2+} in an ATP-dependent uptake process. Due to the discovery of an interconnected SER network which stains selectively with the OsFeCN procedure and exhibits continuity with the SMC, the question as to whether all these SER components are able to actively accumulate Ca^{2+} is of primary interest in the present study.

When photoreceptor cells of the leech are permeabilized by saponin treatment (41) and subsequently perfused with a loading medium containing $\simeq 1$ μM Ca^{2+} (Ca-EGTA-buffer), MgATP and oxalate, SMC are triggered to take up calcium (Fig. 8 in reference 41, and Figs. 10 *c*, *d*, and 14 in this report). The products of this uptake process are Ca-oxalate precipitates located within the cisternal lumen (41, see also reference 32).

In accumulation experiments of this kind the sections are not stained so as to avoid the loss of Ca-oxalate precipitates. Thus, the low overall contrast of the sections often makes it difficult to prove definitively the presence of a membrane around the precipitates, since the surrounding membrane is mostly masked by the high electron density of the precipitate (compare Fig. 7 with 10 *c*, *d*). Two facts prove that the presence of a Ca-oxalate precipitate per se is indicative of the presence of a Ca^{2+}-sequestering structure, even when its membrane is masked: (*a*) precipitate formation is ATP-dependent (38, 41), (*b*) Spontaneous precipitation of Ca-oxalate does not occur since the concentrations of Ca^{2+}_{free} and $oxalate^{2-}$ in the loading medium do not exceed the Ca-oxalate solubility product.

In spite of this difficulty in interpreting the micrographs, the present study shows that the accumulation experiments lead to Ca-oxalate precipitate formation in the submicrovillar cisternal SER system (reference 41; and Figs. 10 *c*, *d*, and 14) and within the nuclear envelope (Fig. 7). Precipitates located closely juxtaposed and parallel to the nonreceptive plasma membrane (Figs. 9, and 10 *c*, *d*) demonstrate that the subsurface cisternae (SSC) are also able to accumulate Ca^{2+}.

The paracrystalline SER (Figs. 11, 13) never contains Ca-oxalate precipitates (Fig. 14), even if the submicrovillar cisternal system in the immediate vicinity of this structure is heavily loaded with Ca-oxalate.

In no case is there any evidence from the accumulation experiments that the tubular SER cisternae, which do not stain with the OsFeCN method, accumulate Ca^{2+}.

DISCUSSION

Heterogeneity of Smooth Endoplasmic Reticulum

The OsFeCN method turned out to be a valuable tool for the morphological characterization of the various SER elements within the photoreceptor cells of the leech, *Hirudo medicinalis*. This method has been introduced for the specific staining of the sarcotubular network in muscle cells (13, 14) and proved to be useful for the specific staining of SER elements associated with the mitotic apparatus of barley cells (21).

The mechanism that leads to the positive staining behavior of the SER cisternal lumina is poorly understood. The observations (*a*) that prefixation with a fixative containing di- or trivalent cations (Mg^{2+}, Mn^{2+}, Sr^{2+}, Ba^{2+}, or La^{3+}) is a prerequisite for this staining to occur (13), and (*b*) that extraction of calsequestrin from isolated skeletal-muscle sarcoplasmic-reticulum vesicles prevents their staining (42) indicate that the positive staining reaction might be due to the ability of these structures to bind di- and/or trivalent cations. However, the often observed coincidence between OsFeCN-stained and Ca-buffering SER elements (13, 14, 21, 44) seems to be coincidental, because the present study indicates that not all stained SER elements are able to actively accumulate Ca^{2+}.

In photoreceptor cells of the leech the OsFeCN method allows discrimination between two SER cisternal systems (summarized in Fig. 15): the tubular, OsFeCN-negative cisternae beneath and between the SMC, and the continuous OsFeCN-positive cisternal system.

The functions of the tubular SER are unknown. Its OsFeCN-negative staining behavior indicates a functional difference relative to the other SER elements. Neither this nor the previous study (41) provides any evidence that the tubular SER is able to accumulate Ca^{2+}.

Among the OsFeCN-positive SER cisternae (Fig. 15) are the elaborate SMC, the nuclear envelope, subsurface cisternae associated with the nonreceptive plasma membrane areas, SER cisternae associated with the Golgi apparatus, with glycogen areas, and the paracrystalline SER specialization. All these elements belong to a continuous reticular network with confluent lumina.

Some of these SER elements could be functionally characterized in this and the previous study (41) with the help of *in situ* Ca-accumulation experiments (see 41, 32 for discussion on methodological aspects). Not only the elaborate SMC but also subsurface cisternae associated with the nonreceptive plasma membrane regions as well as the nuclear envelope are shown to be able to actively accumulate Ca^{2+} with high affinity (38) (functional implications: see below).

The observed associations between some OsFeCN-positive SER cisternae and glycogen areas have been observed in other cell types and probably indicate a close functional relationship between these structures. In hepatocytes (see reference 8 for review), key enzymes of glycogen synthesis and degradation were demonstrated to be associated with SER preparations (31). Recently, Campbell and Shamoo (7) identified enzymes of glycogen metabolism as being associated with the heavy sarcoplasmic reticulum of skeletal muscle. This and previous results indicate that a certain SER element might perform multiple functions.

The functions of the paracrystalline SER (Fig. 15), which is also found in many other cell types, remain obscure. Although this structure is in continuity with Ca^{2+}-sequestering SER elements and stains positively with OsFeCN, the present study failed to show that it can actively accumulate Ca^{2+}. In summary, these observations show that there is also a functional heterogeneity among the subregions of the OsFeCN-positive SER system.

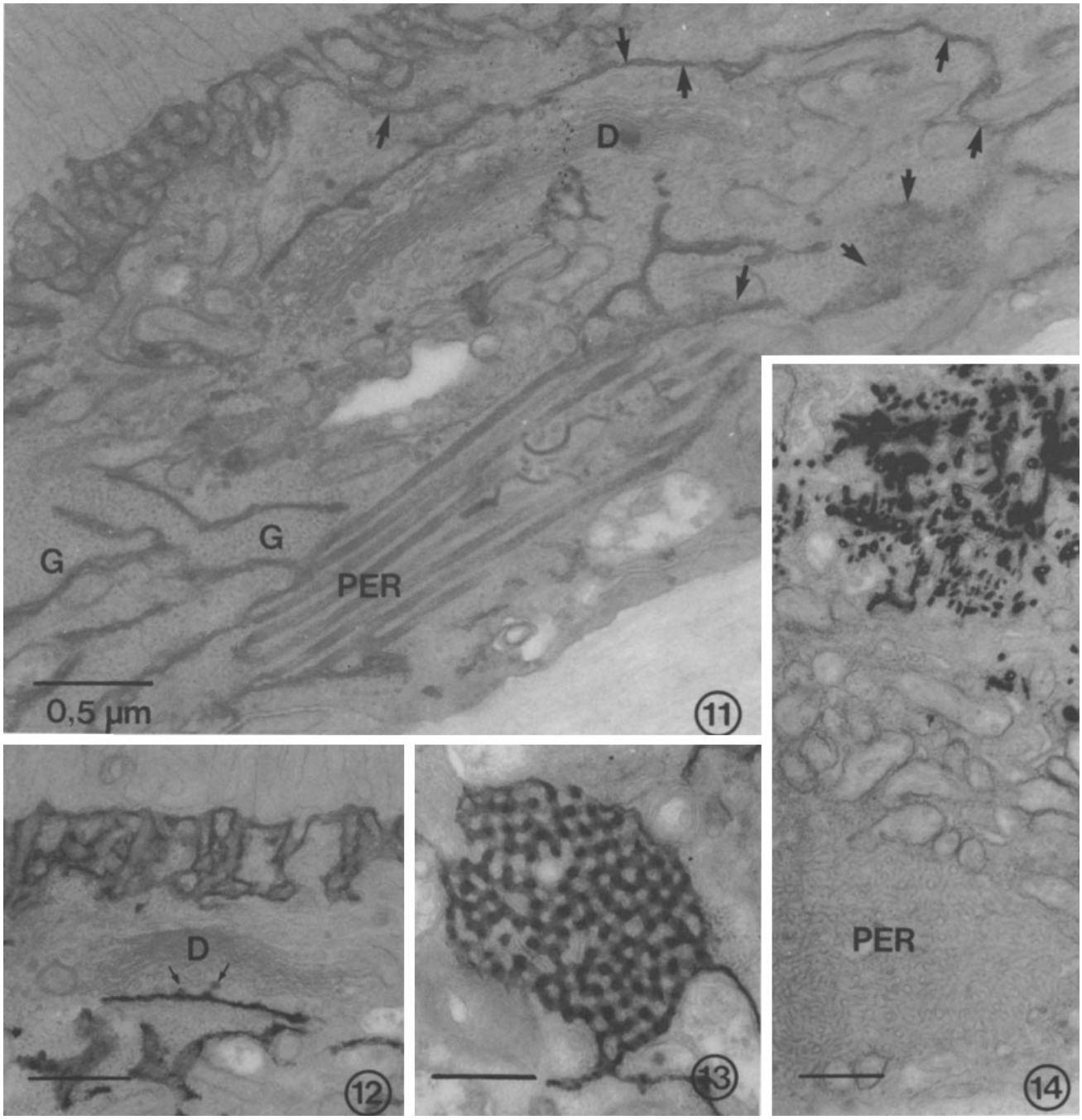


FIGURE 11 The micrograph documents that all ER cisternae that are stained by OsFeCN postfixation form a continuum. The arrows label a cisterna which originates from the submicrovillar smooth ER (top), continues into the cell adjacent to a dictyosome (*D*), and provides direct luminal continuity with the paracrystalline smooth ER (*PER*) and the ER associated with the large glycogen area (*G*). × 39,000.

FIGURE 12 Golgi-associated ER cisterna postively stained by OsFeCN postfixation. While small vesicles which seem to bleb from the Golgi-associated ER membrane are stained (arrows), the cisternae of the dictyosome do not stain with OsFeCN. Bar, 0.5 µm. × 32,000.

FIGURE 13 Paracrystalline smooth ER of a cell that has been postfixed with OsFeCN. Bar, 0.5 µm. × 32,000.

FIGURE 14 Paracrystalline smooth ER (*PER*) of a cell which was permeabilized by saponin and subsequently incubated in a loading medium containing Ca^{2+}, MgATP, and oxalate. While the submicrovillar cisternal system of the ER is heavily loaded with Ca oxalate (top of the picture), the PER does not contain Ca oxalate precipitates, suggesting its inability to actively accumulate Ca^{2+}. Bar, 0.5 µm. × 27,000.

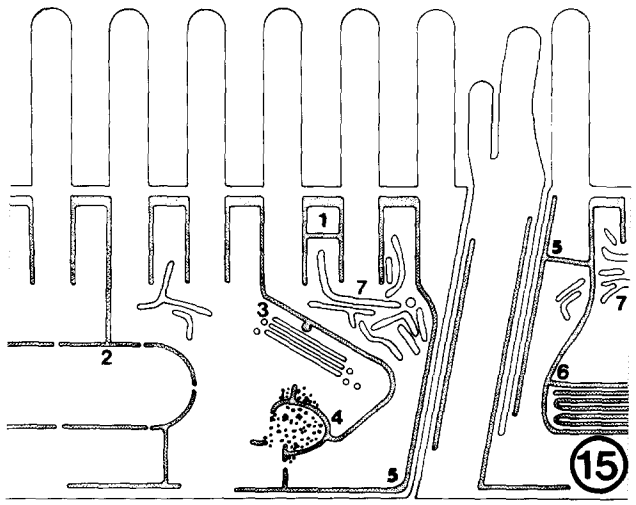


FIGURE 15 Diagrammatic representation of a part of the cytoplasmic cortex of the photoreceptor cell of the leech, *Hirudo medicinalis*, showing the diverse types of the smooth endoplasmic reticulum, and summarizing their structure and interrelation. The ER cisternae which are stained by OsFeCN postfixation (dotted) form a morphological continuum with confluent lumina. The OsFeCN-positive ER cisternae are the submicrovillar cisternae (*1*), the nuclear envelope (*2*), Golgi-associated cisternae (*3*), cisternae associated with glycogen areas (*4*), subsurface cisternae associated with the nonreceptive plasma membrane (*5*), and the paracrystalline cisternae (*6*). The tubular ER cisternae (*7*) do not stain by OsFeCN postfixation. The *in-situ* Ca^{2+}-accumulation experiments reveal that submicrovillar cisternae (*1*), subsurface cisternae (*5*), and the nuclear envelope (*2*) are able to actively accumulate Ca^{2+} with high affinity, while the paracrystalline ER (*6*) and the tubular OsFeCN-negative cisternae (*7*) can not do so.

Functional Implications of the Topography of Ca^{2+}-Sequestering SER

An important result of the present study is the observation that the Ca^{2+}-sequestering ability is not confined to the elaborate SMC but is an intrinsic property of all subsurface cisternae and some intermediate SER elements over which the SSC are in continuity with the submicrovillar cisternal system (see Fig. 15). The following paper (38) shows that the Ca^{2+}-sequestering SER elements in leech photoreceptor cells share important properties with other Ca^{2+}-sequestering SER preparations (e.g. the skeletal muscle sarcoplasmic reticulum and the well-characterized nonmitochondrial SER Ca^{2+} buffer in presynaptic nerve terminals [3]).

The high affinity for Ca^{2+} (38) and the proximity to the receptive and nonreceptive plasma membrane areas thus make the SER an ideal buffer for the localized regulation of Ca^{2+} in the immediate vicinity of the plasma membrane in leech photoreceptor cells. Ca^{2+} buffering close to the plasma membrane is not only important in view of the proposal that a light-induced increase in Ca_{in}^{2+} is one factor controlling light adaptation (see introduction for references) but also a factor modulating Ca^{2+}-dependent potassium conductance in the plasma membrane (see, for example, reference 18) of invertebrate photoreceptors.

Recently, Mullins and Requena (33) have shown that the stimulus-induced changes in Ca_{in}^{2+} in squid giant axons remains confined to the cell periphery. Henkart et al. (19) localized Ca^{2+}-sequestering SER in the periphery of the squid giant axon. Tillotson and Gorman (37) provided evidence that the machinery for short-term buffering of Ca^{2+} in the pacemaker neuron R 15 in *Aplysia* is localized near the inner surface of the plasma membrane. Henkart (20) identified SER cisternae in this preparation as possible intracellular Ca^{2+} stores. The results of the previous (41) and the present studies (this and reference 38) fit into the rapidly emerging scheme of a contribution of SER in the buffering of cytoplasmic Ca^{2+} levels near the plasma membrane of excitable cells.

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