LOW RESISTANCE JUNCTIONS IN CRAYFISH

I. Two Arrays of Globules in Junctional Membranes

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ABSTRACT

The ultrastructure of low resistance junctions between segments of lateral giant fibers in crayfish is studied in sections from specimens fixed either by conventional methods or by glutaraldehyde-H_2O_2 or by glutaraldehyde-lanthanum. Cross sections through junctions fixed by conventional glutaraldehyde display the usual trilaminar profile of two parallel membranes separated by a narrow gap. Most of the junctional regions appear covered by 500–800 Å vesicles which lie on both sides of the junction in rows adjacent to the membranes. Cross sections through junctions fixed by glutaraldehyde-H_2O_2 display, in regions containing vesicles, membranes with a beaded profile. The beads correspond to globules ~125 Å in width and ~170 Å in height arranged in a hexagonal pattern with a unit cell of ~200 Å. The globules of one membrane match precisely with those of the adjacent membrane, and opposite globules seem to come in contact with each other at the center of the junction. The membrane of the vesicles also contains globules. Occasionally the globules of the vesicles seem to join with those of the junctional membranes, apparently forming intracellular junctions. In junctions negatively stained by lanthanum the globules are seen organized into two arrangements. Areas containing globules in a hexagonal array with a unit cell of ~200 Å (swollen pattern) are seen adjacent to areas in which the globules are more closely and disorderly packed (close packing), the minimum center-to-center distance between adjacent globules being ~125 Å. At higher magnification each globule appears composed of six subunits arranged in a circle around a central region occupied by lanthanum (possibly a pit).

INTRODUCTION

The occurrence of low resistance junctions between cells has been reported in a variety of vertebrate and invertebrate systems. In most cases, morphological studies have demonstrated the existence of specialized areas of cell junction characterized by a close apposition between two surface membranes. Early works (4, 8, 9, 12, 29, 33, etc.), based mainly on osmium tetroxide- or permanganate-fixed preparations, described these junctions as areas in which fusion occurs between outer layers of the surface membranes, apparently leaving no extracellular space between the two cells. More recently, however, the occurrence of an electron-transparent layer (20–30 Å thick) has been described between the two joined membranes and interpreted as a gap occupied by extracellular material (28). This interpretation has been confirmed in specimens treated with colloidal lanthanum as an extracellular tracer (28). Thus these findings suggested that "gap junctions" (27) be an appropriate designation for these cell contacts.

In recent studies, fairly regular hexagonal arrays have been seen by various techniques in face

views of gap junctions. First reported in permanganate-fixed goldfish brain at the club ending-Mauthner cell junctions (30), hexagonal arrays were also seen in lanthanum preparations (5, 28, etc.), in freeze-cleaved specimens (7, 10, 13, 15, 35, 36, etc.), and in isolated junctions negatively stained with Na-phosphotungstate (PTA) (3, 10). In most cases the hexagonal array appeared as a close packing of globular particles with a unit cell of ~100 Å.

The existence of low resistance nonpolarized junctions between segments of crayfish lateral giant axons is well established. First suggested (39) by the brief delay in the transmission of electrical impulses at the regions (septa) where two axonal segments meet, the existence of ephaptic (electrotonic) and bidirectional transmission at the septa has been confirmed by means of intracellular stimulation and recording (38).

Early morphological works described, in the region of the septum, special areas in which the surface membranes of two joined axon segments come in close apposition (11). A fairly regular hexagonal array with an ~100 Å periodicity has recently been described in these areas, as a result of lanthanum treatment, allowing the classification of these junctions as gap junctions (18, 20). This hexagonal array has been interpreted as a network of extracellular channels intercalated with intercytoplasmic channels (18, 20).

Recently the membranes of these junctions, fixed by glutaraldehyde-H_2O_2 (24), were seen in certain regions displaying a beaded appearance, due to the presence of electron-opaque particles projecting from the dense layers of each membrane (21, 22, 25). The same membranes seen in face view displayed the particles organized in a hexagonal array with a unit cell of ~200 Å. The discrepancy between the periodicity of this array (~200 Å) and that of other hexagonal arrays (~100 Å) previously reported in the same and in other gap junctions suggested the need for further work on this subject.

In a preliminary note (21) I have reported, in the membranes of septal junctions treated by lanthanum, the occurrence of particles organized into two hexagonal arrays different in periodicity, one being a close packing, the other a larger arrangement. The present paper and its companion (23) describe in detail the structure and aggregation of the particles (globules) forming the two arrays, as seen in specimens prepared by glutaraldehyde-H_2O_2, glutaraldehyde-lanthanum, freeze-fracture, and PTA negative staining. Further morphological evidence for the existence and location of the presumed intercellular channels in the junctional membranes as well as information regarding the subunit composition of each globule were obtained, together with suggestions of possible dynamic properties of the membrane structure.

METHODS

Crayfish (*Cambarus clarkii*) about 10 cm in length were kept in well-oxygenated aquaria at about 20°C. The animals were sacrificed by decapitation. Fixative was injected into the abdomen and after a few minutes the abdominal nerve cord was dissected out and the fixation was carried out in a vial. Glutaraldehyde-H_2O_2 fixative (24) was used. 30% hydrogen peroxide (Fisher Scientific Company, Pittsburgh, Pa.) was added to a 3–6% glutaraldehyde solution buffered to pH 7.4 with 0.1 M (Na, K) phosphate in the amount of 3 drops per ml of 50% glutaraldehyde (Fisher Scientific Company). The fixation was carried out for 1–2 h at room temperature. The postfixation was carried out in 2% OsO_4 buffered to pH 7.4 with phosphate at room temperature. Dehydration was carried out in graded alcohols, and embedding in Epon.

Experiments were performed using lanthanum hydroxide as an extracellular stain (28). 25 ml of a 4% solution of lanthanum nitrate were prepared. 0.01 N NaOH was added to the solution within 3–4 min with vigorous stirring to reach pH 8.1. Usually 20–25 ml of NaOH solution were necessary. The final solution was crystal clear. NaCl was added to reach the final osmolarity of ~436 mosM, the osmolarity of crayfish blood (37). Unfixed nerve cords were dissected from decapitated crayfish and the sheaths enveloping the ganglia were cut open in the dorsal or ventral region of each ganglion under a dissecting microscope with dark-field illumination. In some experiments the nerve cords were immersed in the lanthanum-NaCl solution for 5 min to 1 h. They were fixed for 2 h with 3% glutaraldehyde (pH 7.4) containing 1% lanthanum followed by 2 h of immersion in 2% OsO_4 (pH 7.4) containing 1% lanthanum or directly with OsO_4-lanthanum for 2 h. In other experiments the lanthanum-NaCl treatment was omitted and the dissected cords were immersed directly in glutaraldehyde-lanthanum followed by OsO_4-lanthanum. The penetration of the tracer always seemed better in specimens treated with lanthanum solutions before fixation. Dehydration and embedding were carried out as previously described. Lanthanum solutions were not mixed with glutaraldehyde-H_2O_2 solutions because the mixture becomes a gel in the first

hour. This phenomenon seems to depend on the presence of H_2O_2 in the final solution, since the addition of H_2O_2 to a lanthanum solution also produces a gel.

Transverse sections through the ganglia, about 2 μm thick, were cut serially with an LKB Ultrotome microtome and observed by phase-contrast microscopy to localize areas of the septum, in the lateral giant fibers, rich in synapses. From these areas thin sections were cut and collected on uncoated 400-mesh grids. The sections from lanthanum-treated specimens were not stained while the sections from the other specimens were stained by immersion for 15 min in a saturated solution of uranyl acetate in 50% ethanol, followed by a 3 min immersion in a 3% solution of lead salts (32).

All the specimens were examined with an AEI EM801 electron microscope. The microscope magnification was standardized before each photographic exposure. All the magnifications were previously standardized with a carbon grating replica (✻1002, Ernest F. Fullam, Inc., Schenectady, N. Y.).

OBSERVATIONS

Lateral giant axons of crayfish are composed of a series of axonal segments, each 2–3 mm long, joined together at each ganglion by oblique structures called septa. Each septum consists of a sheet (~1 μm thick) of fibrillar connective tissue which is covered on both sides by Schwann cells. The sheetlike Schwann cells' cytoplasmic processes overlap each other either in close contact or separated by thin layers of connective tissue. In certain regions of the septum, large discontinuities in the combined Schwann cell-connective layer allow the surface membranes of the two axons to come in close apposition, forming specialized junctions. The junctions, in most cases, occur between one of the two axons and finger-like processes of the other axon which find their way through the septum in a tortuous manner.

It has been assumed that these junctions represent regions where electrical coupling between the two axons occurs since they are the only areas in which surface membranes of the two axons come in close apposition. This hypothesis is strengthened by the structural similarities between these junctions and gap junctions reported between cells electrically coupled (18, 20).

Cross sections through the junctions display, at low magnification (Fig. 1), the profiles of two axon surface membranes which lie in parallel planes separated by a narrow electron-transparent gap. Vesicles, 500–800 A in diameter, lie on both sides of the junction. At higher magnification, in conventional glutaraldehyde-fixed specimens (Figs. 2, 3), the membranes display a typical trilaminar profile with an overall thickness of ~80 A. The electron-transparent space between the two membranes is 30–40 A in thickness. In glutaraldehyde-H_2O_2-fixed specimens, on the contrary, the same membranes, transversely sectioned, display mostly a beaded profile, apparently due to the presence of electron-opaque globules which lie in the plane of the membrane and protrude from the membrane surfaces (Figs. 1, 4, 5). The globules measure ~125 A on their axis parallel to the membrane surface and ~170 Å on their axis perpendicular to the membrane surface, and repeat with a periodicity of ~200 Å. The globules appear to protrude slightly more from the cytoplasmic than from the outer membrane surface and seem to correspond exactly to those of the adjoining membrane in such a way that two opposite globules give the impression of coming in contact with each other at the center of the junction, apparently forming images of electron-opaque bridges across the gap. The thickness of each membrane is ~170 Å at the globules and ~80 Å between globules. The overall thickness of the junction is ~340 Å at the globules and 200 Å between globules. The gap between the two membranes measures, in areas between globules, 40–50 Å.

In junctions tangentially cut (Fig. 6) the globules are visible as electron-opaque spots ~125 Å in size, organized in a hexagonal array in which the center-to-center distance between adjacent globules is ~200 Å. The vesicles (500–800 Å in diameter) lie close to the junction, most often in a single layer. Occasionally the membranes of the vesicles display areas containing electron-opaque globules similar in size and shape to those seen in junctional membranes, and frequently the globules of the vesicles join precisely with the globules of the junctional membranes, apparently forming small intracellular junctions (Figs. 4, 5).

Although the apposed membranes display, in most of the junction, a globular profile, there are regions in which the globules are not seen (Fig. 1). Here, cross- and face views of the membranes appear similar to those seen in junctions fixed by conventional glutaraldehyde (Figs. 2, 3). Usually, in these regions, the membranes are not covered by vesicles although occasionally cisterns of endoplasmic reticulum may be seen (Fig. 1).

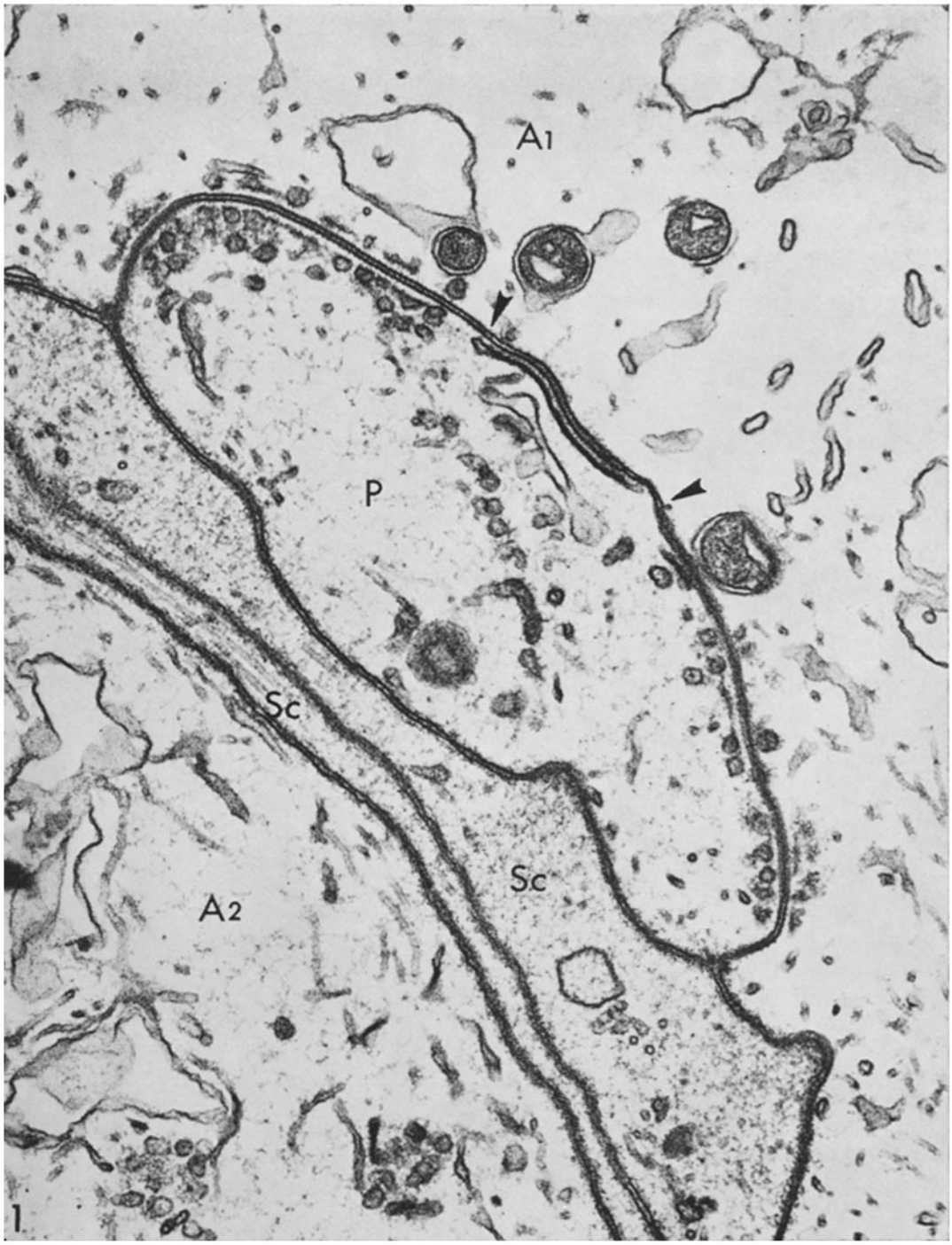


FIGURE 1 Junction between two segments of a lateral giant fiber at the septum (glutaraldehyde-H_2O_2, OsO_4 fixation). The junction is formed by the close apposition between the surface membrane of an axon (A_1) and that of a small process (P) from the other axon (A_2). The continuity between the axon (A_2) and its process (P) is not seen in this section. The junctional membranes display a beaded appearance in most of their profile. The beads (globules) are not seen in the region between the arrowheads. Vesicles 500–800 Å in diameter lie close to the junction preferentially near membrane regions where globules are seen. Occasionally adjacent vesicles are bridged by fuzzy filamentous material. *Sc*, Schwann cell. × 66,000.

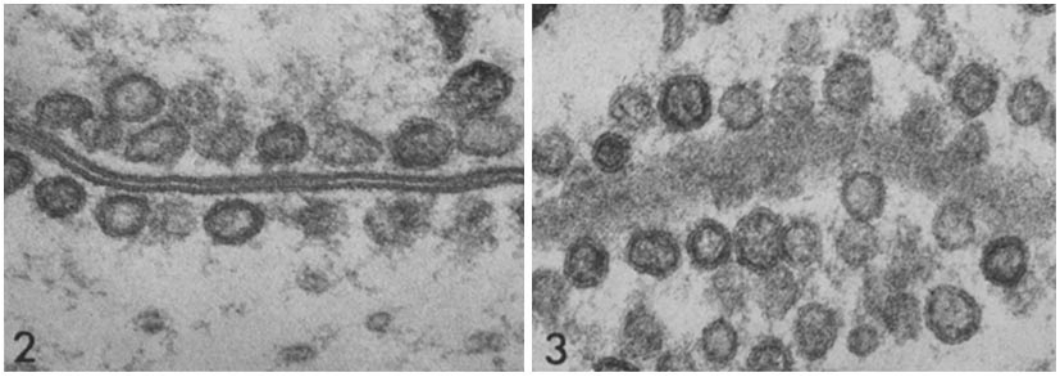


FIGURE 2 Junction in cross section (conventional glutaraldehyde, OsO_4 fixation). The junctional membranes display the conventional unit membrane profile (~80 Å thick). The gap between the two membranes measures 30–40 Å. Notice that vesicles lie adjacent to the junctional membranes. These membranes, in similar regions of junctions fixed by glutaraldehyde-H_2O_2, display periodically repeated globules (Figs. 1, 4, 5). × 100,000.

FIGURE 3 Junction in face view (conventional glutaraldehyde, OsO_4 fixation). The face view of the junctional membranes displays a homogeneous electron opacity. Vesicles lie adjacent to the junctional membranes. Similar regions of junctions fixed by glutaraldehyde-H_2O_2 display a hexagonal array of electron-opaque globules (see Fig. 6). × 100,000.

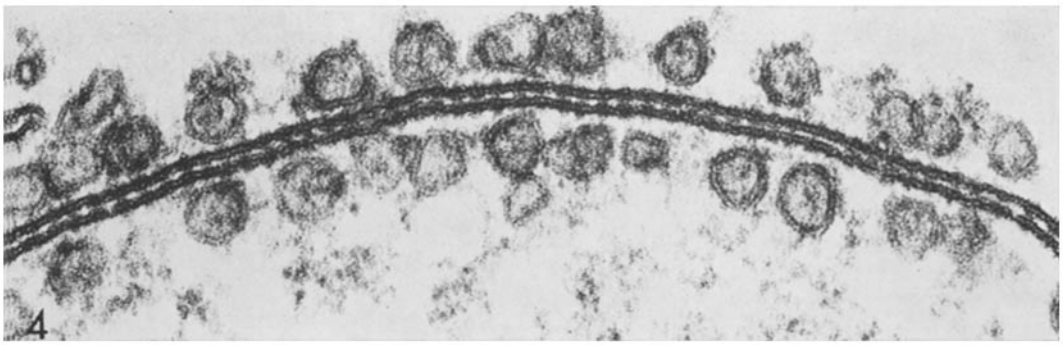
In lanthanum-treated specimens, junctions transversely cut display a very electron-opaque central layer which represents the gap filled with tracer (Fig. 7, inset *a*). In most areas this layer appears interrupted by moderately electron-opaque bridges which repeat with a periodicity of ~200 Å. As previously described, these bridges are formed presumably by two opposite globules projecting from each membrane and coming in contact at the center of the gap. In oblique (Fig. 7) or tangential (Fig. 7, inset *b*) sections, the junctions display regular arrays of electron-transparent spots surrounded by an electron-opaque network. The spots represent the negative images of the globules which project into the gap. In these preparations, the globules, seen at high magnification, are frequently resolved into electron-transparent rings with a central electron-opaque dot ~25 Å in diameter (Fig. 7, inset *c*). Occasionally the rings seem to be composed of six main subunits arranged in a circle (Fig. 7, inset *c*). The number of subunits is confirmed using the multiple rotation exposure method according to Markham (14). Only a six-step rotation, in fact, gives a clear image of the subunits (Fig. 7, inset *e*).

The pattern organization of the globules varies in different areas (Fig. 9, 10). In some areas the globules are displayed in a quite regular hexagonal array with a unit cell of ~200 Å (swollen arrangement) (Figs. 7, 9, 10). In other areas the globules

FIGURE 4 Junction in cross section (glutaraldehyde-H_2O_2, OsO_4 fixation). The junctional membranes contain globules repeating with a periodicity of ~200 Å. Each globule measures roughly 125 × 170 Å and is oriented with the long diameter perpendicular to the plane of the membrane. Opposite globules appear to join precisely at the center of the junction. × 150,000.

FIGURE 5 Junction in cross section (glutaraldehyde-H_2O_2, OsO_4 fixation). Similar to Fig. 4. In addition the membrane of certain vesicles (arrow) display globules similar to those seen in the junctional membranes. The globules of the vesicle are occasionally seen apparently joining precisely with the globules of the junctional membranes (*inset*). × 145,000; *inset*, × 245,000.

FIGURE 6 Junction in face view (glutaraldehyde-H_2O_2, OsO_4 fixation). In junctional membranes tangentially cut, the globules are seen as electron-opaque spots organized in a hexagonal array with a unit cell of ~200 Å. The membrane of the vesicles frequently contains globules (arrowheads). × 123,000.



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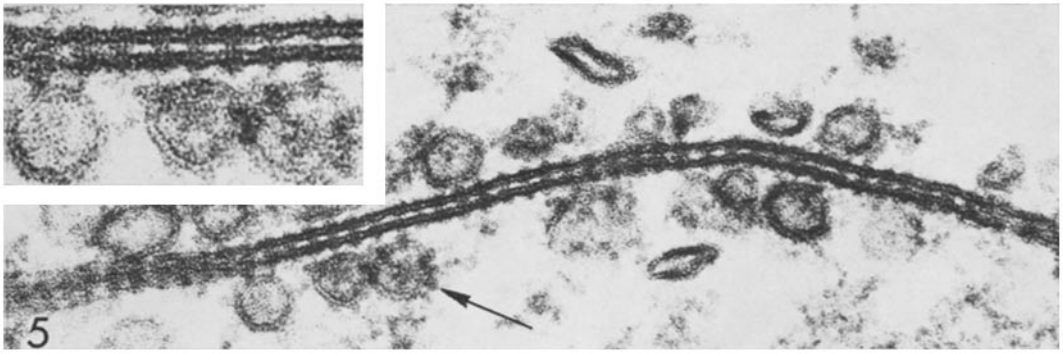

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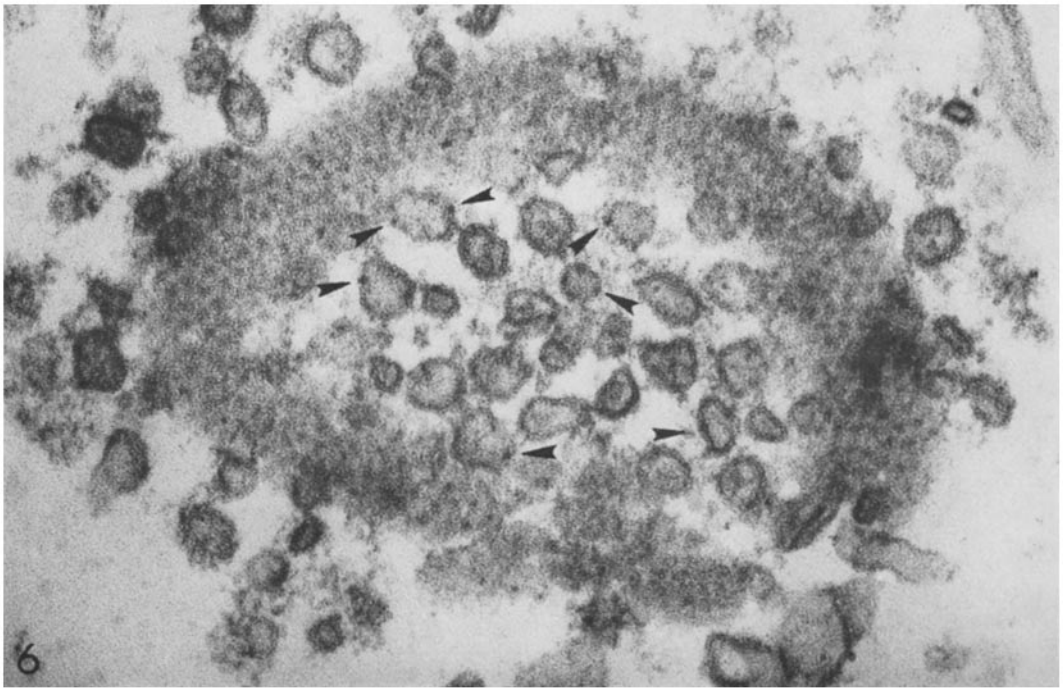

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are more closely and disorderly packed, the minimum center-to-center distance between adjacent globules being ~125 Å (close packing) (Figs. 8, 9, 10). Areas containing one type of arrangement are usually seen switching abruptly into areas containing the other type (Figs. 9, 10), the limit between the two areas being almost a straight line. The 500–800 Å vesicles seem to be

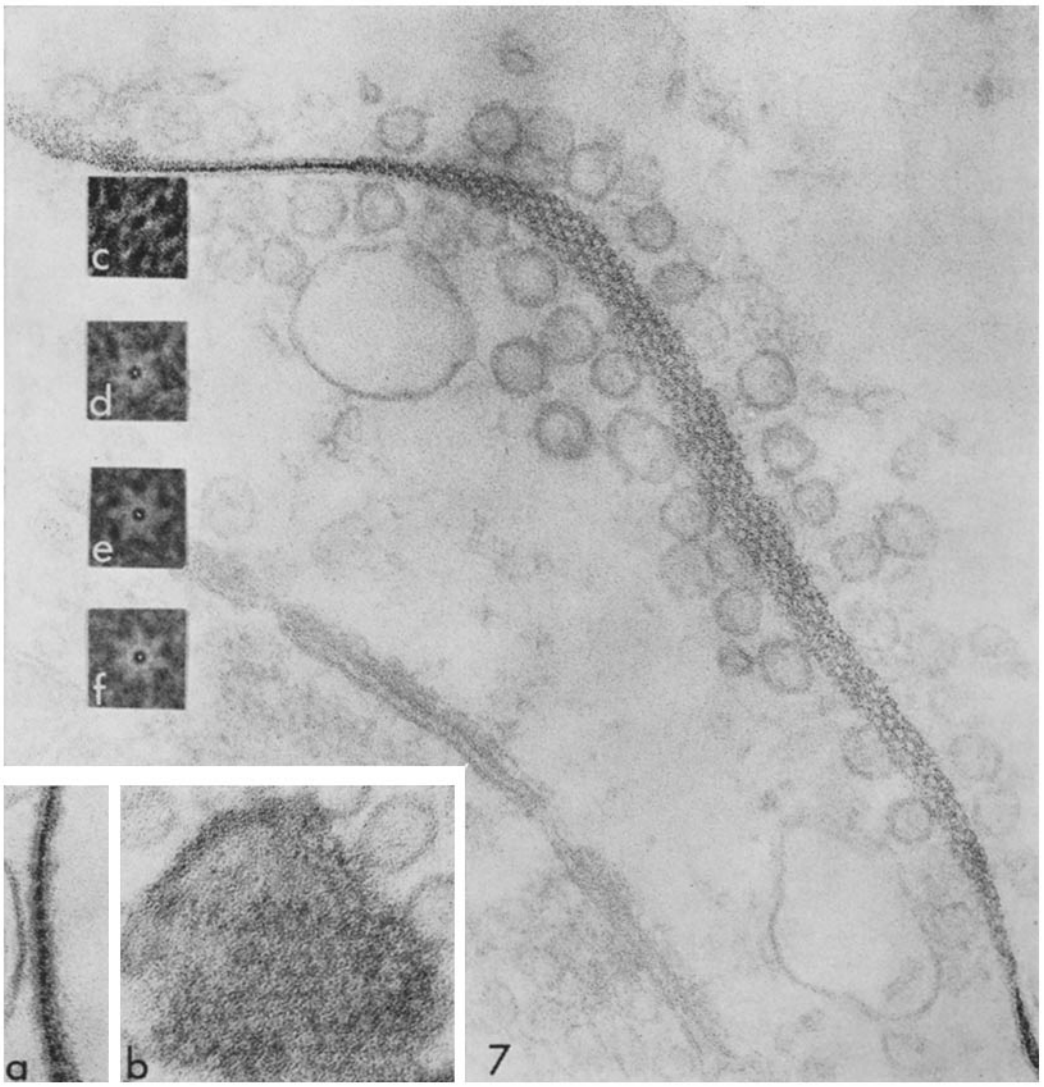


FIGURE 7 Junctions treated with colloidal lanthanum (conventional glutaraldehyde, OsO_4 fixation). In tangential section the globules are seen here as electron-transparent spots organized in a hexogonal array with a periodicity of ~200 Å. Occasionally certain globules (*inset c*) are seen composed of six subunits surrounding a central electron-opaque region. The number of the subunits is confirmed by the photographic rotation method according to Markham (14). Here, only a six-step rotation (*inset e*) gives a clear image of the subunits. A five-step rotation and a seven-step rotation are shown in *insets d* and *f*, respectively. In junctions cross-sectioned (*inset a*) the layer of lanthanum in the gap is periodically interrupted by moderately electron-opaque bridges. Each bridge represents the protrusions of two opposite globules apparently joined at the center of the junction. *Inset b* shows a larger face view of the junctions. × 100,000; *insets: a*, × 145,000; *b*, × 153,000; *c–f*, × 700,000.

located preferentially close to membrane regions containing the swollen arrangement. Both arrangements are also seen with the same characteristics in specimens fixed with OsO_4 alone.

Cross sections through membranes containing globules arranged in close packing display, with lanthanum, the typical, central electron-opaque layer (Figs. 11, 12) which represents the gap occupied by tracer. As previously seen in areas containing the swollen pattern (Fig. 7, inset *a*), occasionally the layer of lanthanum displays a beaded appearance also in areas of close packing. However, in the latter case the electron-opaque beads, which represent the narrow spaces between adjacent globules, repeat, as expected, with a periodicity of ~125 Å. The beads frequently display a square shape (Fig. 12). Occasionally a moderately electron-opaque line, perpendicular to the membranes, is seen crossing the electron-transparent region between adjacent beads (Fig. 12). This line corresponds to the center of the globules, and most likely represents the side view of the electron-opaque dot seen at the center of the globules in membranes tangentially sectioned (Fig.7, inset *c*).

DISCUSSION

The observations reported here suggest that the membranes forming low resistance junctions in crayfish lateral giant fibers contain globular structures organized in two arrangements. The globules are visible in positively stained sections only after glutaraldehyde-H_2O_2 fixation, and in lanthanum preparations seem to be composed of six main subunits arranged in a circle.

An interesting finding is represented by the appearance of two different arrangements of globules in different areas of the junctions. First of all, it should be discussed whether or not one of the two arrangements represents an artifact. One of the two arrays could be, for instance, a moiré pattern. Moiré patterns are artifactual arrays formed by the overposition of two periodic structures. Therefore junctions, resulting from the overposition of two membranes each containing a regular array, could easily produce moiré patterns. However, none of the two arrays observed here is likely to be a moiré pattern, since each one is seen in profile in cross-sectioned junctions with the right periodicity. In fact, the swollen pattern appears in both positively and negatively stained cross-sectioned junctions with a periodicity of ~200 A, and the close packing is visible in negatively stained, cross-sectioned junctions with a periodicity of ~125 Å. The possibility that either one of the two arrays results from a fixation artifact is less likely, since both patterns are seen in junctions prepared with different fixatives such as glutaraldehyde-OsO_4 or OsO_4 alone. Moreover, both arrays were seen in fragments of unfixed, isolated junctions (23). The previous considerations, therefore, may suggest that the two arrays are unlikely to result from some sort of an artifact, although the possibility that, for instance, the regions of closer packing simply represent areas of disorder in the aggregation of the globules due to some unknown factors cannot yet be ruled out.

Several ultrastructural characteristics of the junctions should be discussed. As described in Observations, the globules can be clearly seen in positively stained sections only after glutaraldehyde-H_2O_2 fixation. Obviously this does not mean that glutaraldehyde alone does not preserve the globules, since in these preparations stained with lanthanum they are seen organized in either one of the two arrangements. It seems more likely that in glutaraldehyde-H_2O_2 the globules are visible simply because they are more strongly stained by osmium, uranium, and lead. Areas of cross-sectioned, positively stained membranes in which globules are not seen in glutaraldehyde-H_2O_2-fixed specimens are likely to correspond, in lanthanum preparations, to areas in which the globules are arranged in the small pattern. This interpretation is also suggested by the observation that in both cases these areas are usually poor in vesicles.

Previous studies on the structure of gap junctions could not determine whether the regularly arranged particles seen by lanthanum, PTA, and freeze-fracture techniques are on the external surface of the membranes, penetrate to a certain extent into the membrane, or occupy the entire thickness of the membrane. The observations reported here and in the companion paper (23) suggest that at least in these membranes the globules are likely to be continuous structures which occupy the entire thickness of the membrane, protruding from both the cytoplasmic and the extracellular membrane surface. This suggestion is supported to a certain extent by the appearance of the globules in cross-sectioned, positively stained junctions. Here, in fact, it seems clear that opposite protrusions of the same globule are lo-

cated on the same axis perpendicular to the membrane surface. Although this does not necessarily prove that the constituents of the globules are the same throughout the thickness of the membrane, it implies, at least, that some sort of a linkage must occur across the membrane to hold in place the opposite globular protrusions. Moreover, further and more convincing support for this view is offered by the observations on freeze-fractured junctions which are reported and discussed in the companion paper (23).

Assuming that the two globular arrangements correspond to structures existing in vivo, it could be discussed whether each one of the patterns is a steady and irreversible structure or represents a form of organization which may convert into the other form under peculiar circumstances. The latter hypothesis is not unreasonable since the globules seem to be of the same size in both patterns, and one pattern is seen in direct continuity with the other without interruptions or limiting structures of any sort. It could be, therefore, that changes in the arrangements of the globules from the small to the large pattern are paralleled by changes in the junctional membrane permeability from high to low coupling resistance or vice versa (22). This mechanism could be activated by various modifications in the intracellular environment and could function in the regulation of the intercellular permeability.

A similar suggestion has recently been proposed for gap junctions between epithelial cells of rat intestine (36). Also, in this case, the gap junctions were seen containing particles organized into two hexagonal arrays in close proximity to each other, one array being formed by particles 80–90 Å in diameter, with a center-to-center spacing of 90–100 A, the other containing particles 100–110 Å in diameter with a spacing of 190–200 Å (36).

This hypothesis could easily be tested by studying lanthanum preparations of septal junctions in specimens treated by methods that modify the junctional coupling resistance. Several cases have been reported in the literature in which the intercellular resistance of coupled cells could be modified by various methods up to a complete uncoupling of the joined cells (1, 2, 16, 17, 19, 26, 31, 34). Only rarely, however, have morphological studies been performed in an attempt to establish possible correlations between increase in resistance and structural changes at the junctions (6, 18, 19). On some occasions, uncoupling was paralleled by a disappearance of the junctions themselves as a result of a separation of the joined membranes

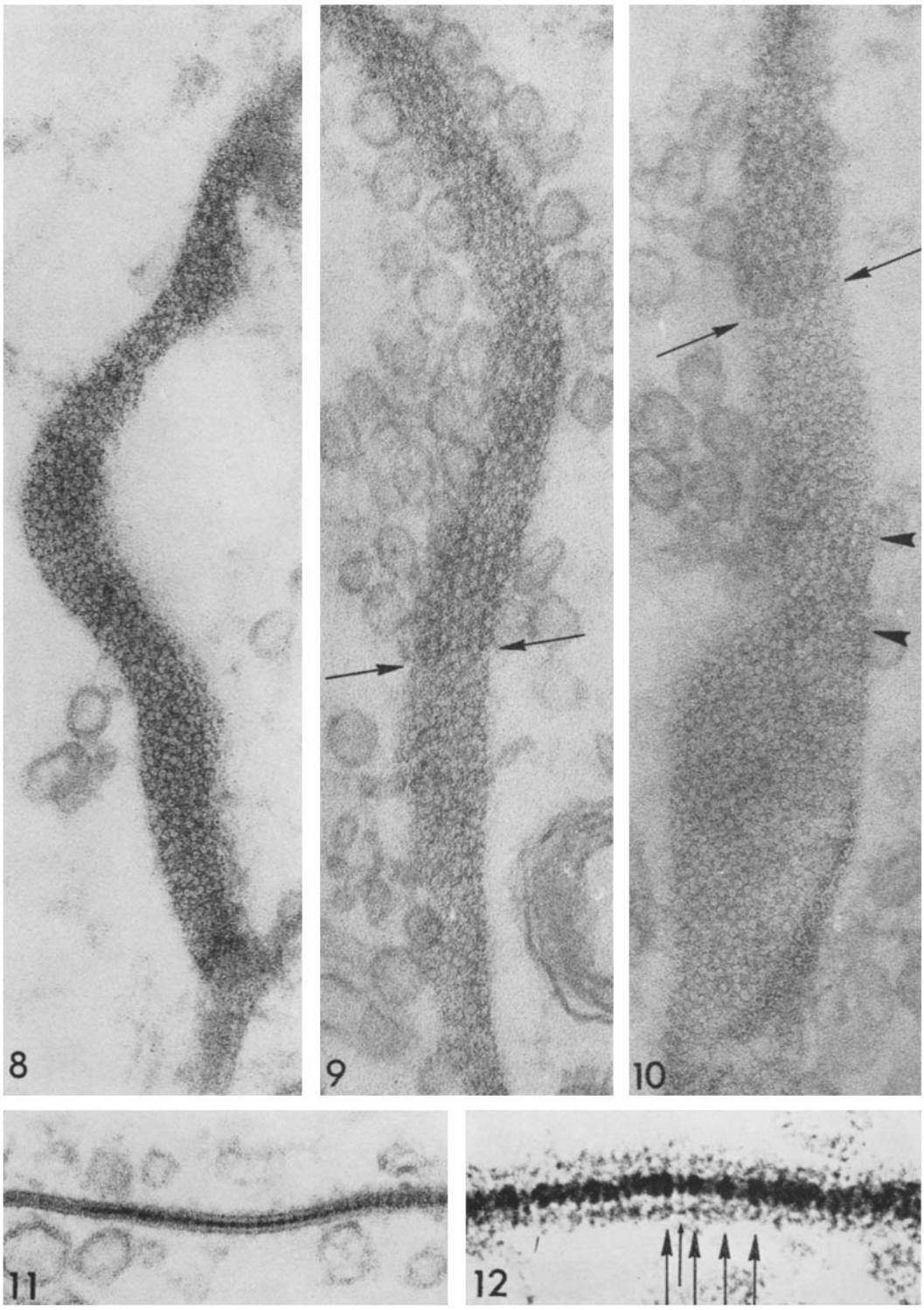
FIGURE 8 Face view of a junction treated with colloidal lanthanum (conventional glutaraldehyde, OsO_4 fixation). In certain junctions the globules are organized in a close packing, the minimum center-to-center distance between adjacent globules being ~125 Å. × 123,600.

FIGURE 9 Face view of junction treated with colloidal lanthanum (conventional glutaraldehyde, OsO_4 fixation). The globules are seen organized in two different arrangements. The two arrows indicate the region where one pattern suddenly switches to the other. The pattern below the line is an irregular close packing in which the minimum center-to-center distance between adjacent globules is ~125 Å. The pattern above the line is a fairly regular hexagonal array with a unit cell of ~200 Å (swollen pattern). × 123,600.

FIGURE 10 Face view of junction treated with lanthanum (conventional glutaraldehyde, OsO_4 fixation). Features are similar to those of Fig. 11. The two arrows indicate the region where one pattern switches to the other. The two arrowheads show a region of swelling in the close packing arrangement. × 123,000.

FIGURE 11 Cross-sectioned junction treated with lanthanum (OsO_4 fixation). The globules here repeat with a periodicity of ~125 Å, as suggested by the periodicity of the electron-opaque beads formed by the lanthanum in the gap. The beads represent the spaces between adjacent globules, occupied by the tracer. × 152,000.

FIGURE 12 Cross-sectioned junction treated with lanthanum (conventional glutaraldehyde, OsO_4 fixation). As in Fig. 11 the globules repeat here with a periodicity of ~125 Å. The electron-opaque beads, representing the spaces between adjacent globules occupied by lanthanum, frequently appear square in shape (large arrows). Occasionally a moderately electron-opaque line (small arrow) is seen crossing the electron-transparent areas between adjacent beads. This line is produced by a certain amount of tracer located at the center of two closely joined globules. × 390,000.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(18). On others, on the contrary, significant increase in coupling resistance could not be correlated with obvious modifications in the ultrastructure. This occurred in septal junctions of crayfish lateral giant fibers kept at low temperature (5°C) (19) or treated by low Ca^{++} media (18). In these cases, however, whether or not fine changes in the membrane organization occur could not be established, because the specimens were not prepared by methods that allow one to study the globular arrays.

As an alternative hypothesis for the function of the two arrays, it could be proposed that each one of them belongs to a junctional region possessing a special function. Areas of junctions containing the close-packing arrangement could function as intercellular pathways for small molecules located in the matrix of the axoplasm. On the other hand, junctional areas which display the swollen globular array could be part of an intercellular structure that allows exchange of molecules between closed compartments of the two adjacent cells. This is suggested by the observation that the vesicles lie preferentially adjacent to junctional regions in which the swollen pattern is present. In fact, although the function of the vesicles is not known, the observation that vesicles have been seen containing globules and occasionally have been seen apparently forming small intracellular junctions with the surface membranes, seems to suggest that communications could be established between the interior of the vesicles lying on one side of the junction and the interior of vesicles lying on the other side. In this case, compounds that must be excluded from the cytoplasmic matrix could be transferred into closed compartments of the other cell by means of three coupled junctions, two intracellular and one intercellular.

In conclusion, this study led to new data regarding the globular structures forming the membranes of crayfish low resistance junctions. Of certain interest is the finding that the globules apparently organize themselves into two arrangements. The two arrangements may represent two forms of aggregation of the globules possibly endowed with specific functional meanings; however, the possibility, although unlikely, that one of the two patterns simply represents an artifact cannot be completely discarded yet.

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BIBLIOGRAPHY

1. Asada, Y., and M. V. L. Bennett. 1971. *J. Cell Biol.* **49**:159.
2. Asada, Y., G. D. Pappas, and M. V. L. Bennett. 1967. *Fed. Proc.* **26**:330.
3. Benedetti, E. L., and P. Emmelot. 1968. *J. Cell Biol.* **38**:15.
4. Bennett, M. V. L., E. Aljure, Y. Nakajima, and G. D. Pappas. 1963. *Science (Wash. D. C.).* **141**:262.
5. Brightman, M. W., and T. S. Reese. 1969. *J. Cell Biol.* **40**:648.
6. Bullivant, S., and W. R. Loewenstein. 1968. *J. Cell Biol.* **37**:621.
7. Chalcroft, J. P., and S. Bullivant. 1970. *J. Cell Biol.* **47**:49.
8. Dewey, M. M., and L. Barr. 1962. *Science (Wash. D. C.).* **137**:670.
9. Farquhar, M. G., and G. E. Palade. 1963. *J. Cell Biol.* **17**:375.
10. Goodenough, D. A., and J. P. Revel. 1970. *J. Cell Biol.* **45**:272.
11. Hama, K. 1961. *Anat. Rec.* **141**:275.
12. Karrer, H. E. 1960. *J. Biophys. Biochem. Cytol.* **8**:135.
13. Kreutziger, G, O. 1968. Proceedings 26th Meeting of the Electron Microscope Society of America. Claitor's Publishing Division, Baton Rouge, La. 234.
14. Markham, R., S. Frey, and G. J. Hills. 1963. *Virology.* **20**:88.
15. McNutt, N. S., and R. S. Weinstein. 1970. *J. Cell Biol.* **47**:666.
16. Nakas, N., S. Higashimo, and W. R. Loewenstein. 1966. *Science (Wash. D. C.).* **151**:89.
17. Oliveira-Castro, G. H., and W. R. Loewenstein. 1971. *J. Membrane Biol.* **5**:51.
18. Pappas, G. D., Y. Asada, and M. V. L. Bennett. 1971. *J. Cell Biol.* **49**:173.
19. Payton, D. W., M. V. L. Bennett, and G. D. Pappas. 1969. *Science (Wash. D. C.).* **165**:594.
20. Payton, B. W., M. V. L. Bennett, and G. D. Pappas. 1969. *Science (Wash. D. C.).* **166**:1641.
21. Peracchia, C. 1971. Abstracts of Papers Presented at the 11th Annual Meeting of the American Society for Cell Biology, New Orleans. 221.
22. Peracchia, C. 1972. *J. Cell Biol.* **55**(2, Pt. 2): 202 a. (Abstr.)
23. Peracchia, C. 1973. *J. Cell Biol.* **57**:66.

24. PERACCHIA, C., and B. S. MITTLER. 1972. *J. Cell Biol.* **53**:234.
25. PERACCHIA, C., and J. D. ROBERTSON. 1971. *J. Cell Biol.* **51**:223.
26. POLITOFF, A. L., S. Y. SOCOLAR, and W. R. LOEWENSTEIN. 1969. *J. Gen. Physiol.* **53**:498.
27. REVEL, J. P. 1968. Proceedings 26th Meeting of the Electron Microscope Society of America. Claitor's Publishing Division, Baton Rouge, La. 40.
28. REVEL, J. P., and M. J. KARNOVSKY. 1967. *J. Cell Biol.* **33**:C7.
29. ROBERTSON, J. D. 1960. *Progr. Biophys. Biophys. Chem.* **10**:343.
30. ROBERTSON, J. D. 1963. *J. Cell Biol.* **19**:201.
31. ROSE, B., and W. R. LOEWENSTEIN. 1971. *J. Membrane Biol.* **5**:20.
32. SATO, T. 1968. *J. Electron Microsc.* **17**:158.
33. SJÖSTRAND, F. S., E. ANDERSSON-CEDERGREN, and M. M. DEWEY. 1958. *J. Ultrastruct. Res.* **1**:271.
34. SOCOLAR, S. J., and A. L. POLITOFF. 1971. *Science (Wash. D. C.).* **172**:492.
35. SOMMER, J. R., R. L. STEERE, E. A. JOHNSON, and P. H. JEWETT. 1972. *In* Hibernation and Hypothermia, Perspectives and Challenges. Elsevier Publishing Co., New York. 291.
36. STAEHELIN. L. A. 1972. *Proc. Natl. Acad. Sci. U.S.A.* **69**:1318.
37. VAN HARREVELD, A. 1936. *Proc. Soc. Exp. Biol. Med.* **34**:428.
38. WATANABE, A., and H. GRUNDFEST. 1961. *J. Gen. Physiol.* **45**:261.
39. WIERSMA, C. A. G. 1947. *J. Neurophysiol.* **10**:23.