CONTRAST BETWEEN OSMIUM-FIXED AND PERMANGANATE-FIXED TOAD SPINAL GANGLIA

JACK ROSENBLUTH, M.D.

From the Department of Anatomy, University of California Medical Center, San Francisco. Dr. Rosenbluth's present address is Department of Anatomy, Harvard Medical School, Boston

ABSTRACT

Chains of vesicles are prominent near the plasma membranes of both the neurons and satellite cells of osmium-fixed toad spinal ganglia. In permanganate-fixed specimens, however, such vesicles are absent, and in their place are continuous invaginations of the plasma membranes of these cells. The discrepancy suggests that the serried vesicles seen in osmium-fixed preparations arise through disintegration of plasma membrane invaginations, and do not represent active pinocytosis, as has been suggested previously. A second difference between ganglia fixed by these two methods is that rows of small, disconnected cytoplasmic globules occur in the sheaths of permanganate-fixed ganglia, but not in osmium-fixed samples. It is suggested that these globules arise from the breakdown of thin sheets of satellite cell cytoplasm which occur as continuous lamellae in osmium-fixed specimens. Possible mechanisms of these membrane reorganizations, and the relevance of these findings to other tissues, are discussed.

INTRODUCTION

The extent to which fixation, dehydration, and and embedding introduce alterations in the morphology of cells and tissues can be checked in some instances by a direct comparison of fixed specimens with their living counterparts. Through the use of phase contrast microscopy together with specialized viewing chambers, such as the Rose chamber (20), structural details can be observed in living cells and indeed their gyrations can be followed in time-lapse cinematographs.

There is, as yet, no equivalent check available for ultrastructural details, however. Although gross distortions in electron micrographs can be recognized as artifacts, verification of the fine structure seen in fixed preparations is in general not possible, except in special instances. (The period of compact myelin, for example, has been determined both by x-ray diffraction (24) and by electron microscopy (6)).

Another approach to this problem is to compare electron micrographs of specimens fixed and embedded by a variety of different techniques. Admittedly, all such processing probably introduces morphological changes, but at least some insight into the nature of these changes is possible when clear cut differences appear between specimens prepared by different methods.

This report focuses attention on the vesicles which are so prominent in satellite cells of peripheral nerve ganglia and which also occur near the surfaces of dorsal root ganglion cells (23). Such vesicles have been noted in satellite cells of mammals (23), reptiles (30), and amphibia (4, 21). In size and configuration they resemble the pino-

cytic vesicles of capillaries (14), and indeed it has been suggested that they are engaged in the transfer of materials across the satellite cells to and from the neurons (4).

These vesicles differ from pinocytic vesicles in several respects, however. They are not distributed uniformly throughout the thickness of the satellite cell, but are generally absent from the outermost portion of the cell. Only rarely can they be found budding from the plasma membrane. It has been noticed, furthermore, that they tend to be serried (30). In this last respect, they resemble the chains of vesicles which occur near the surfaces of macrophages (9, 13) and certain epithelial cells (25, 26). To a lesser degree they are reminiscent of the chains of vesicles which, in some preparations, comprise the intermediary component of the triad of the sarcoplasmic reticulum in vertebrate striated muscle cells (5).

Several investigators (3, 5, 7) have suggested that intracellular vesicles may, in some instances, arise from the breakdown of pre-existing tubular components of the endoplasmic reticulum, and that this transformation may, in fact, occur after the membranes have been fixed.

When serried vesicles were noted in rat spinal ganglia (23), it was suspected that they might represent continuous invaginations of the plasma membrane which had disintegrated at some time during the fixation or embedding of the tissues. A change from methacrylate to epoxy resin as an embedding medium did not, however, affect the occurrence of serried vesicles in these cells.

In the present study, a comparison was made between osmium-fixed and permanganate-fixed toad spinal ganglia. Several modifications of each fixative were used in order to determine whether the ionic composition of the fixative had any significant effect on the occurrence of chains of vesicles. It was found that in all the osmium-fixed specimens serried vesicles commonly appeared in both neurons and satellite cells, while in all the permanganate-fixed ganglia they were entirely absent. Other differences appeared as well, indicating that paired plasma membranes may be subject to considerable morphological reorganization depending on the method of fixation.

MATERIALS AND METHODS

Specimens were obtained from adult toads (*Bufo marinus*), most of which were maintained in the laboratory on a diet of raw liver for several months prior to use. Lumbar spinal ganglia were removed from pithed, living animals and plunged immediately into chilled fixative. (The fixatives used are listed below.) Parallel fixation in permanganate and osmium tetroxide solutions was carried out with ganglia from the same animal. However, since only three or four ganglia could be obtained from each toad, it was not possible to perform parallel fixation, in all five of the solutions listed, with ganglia from the same animal. At the end of 1 to 2 hours at 5°C the ganglia were removed from the fixative and cut into halves or quarters. The ganglia were blackened through and through at this stage. After being rinsed in isotonic saline, the blocks were dehydrated in a graded series of methanol or ethanol solutions and embedded in Epon 812 (11). Sections were cut with a Porter-Blum microtome and mounted on grids coated with a film of carbon and Formvar, or a film of carbon alone. Osmium–fixed specimens were stained with lead salts (8, 31), and examined in a Siemens Elmiskop I at initial magnifications of 5,000 to 30,000. (Accelerating voltage, 80 kv.)

The following fixing solutions were used:

1. 1.3 per cent osmium tetroxide in acetate-veronal (A-V) buffer (pH 7.5) plus the following salts:

	mg/ml
NaCl	3.98
KCl	0.224
$CaCl_2$	0.211
$MgCl_2 \cdot 6H_2O$	0.162
$NaH_2PO_4 \cdot H_2O$	0.124

2. 2 per cent osmium tetroxide in A-V buffer (pH 7.5) plus 5.2 mg/ml of $CaCl_2$.
3. 3 per cent aqueous, unbuffered $KMnO_4$.
4. 1.65 per cent $NaMnO_4 \cdot 3H_2O$ in A-V buffer (pH 7.5). The resulting solution is diluted with water to 75 per cent of its initial concentration in order to make the final fixing solution.[1]
5. 0.83 per cent $NaMnO_4 \cdot 3H_2O$ in A-V buffer (pH 7.5) plus the following salts:

	mg/ml
NaCl	2.8
KCl	0.40
$CaCl_2$	0.20
$MgCl_2 \cdot 6H_2O$	0.20
$NaH_2PO_4 \cdot H_2O$	0.16

The resulting solution is diluted with water to 75 per cent of its initial concentration in order to make the final fixing solution.[1]

[1] The undiluted solution was designed as a fixative for mammalian tissues.

The original observations on permanganate-fixed material were made on specimens fixed in potassium permanganate (10) containing no buffer or added salts (solution No. 3). The potassium content of this fixative is, however, about sixty times that in osmium fixative No. 1. Because of the possibility that this and other gross ionic differences between osmium and permanganate fixatives could account for some of the observed differences in cell morphology, two other permanganate fixatives were subsequently introduced. In No. 4, sodium permanganate (32) was substituted for potassium permanganate, and A-V buffer was added. In solution No. 5 the ionic composition was adjusted to correspond approximately to that in osmium fixative No. 1. In solutions Nos. 1 and 5 the ions Na, K, Ca, Mg, and PO_4 (including the contributions from the buffer and fixative as well as the added salts) constitute an amphibian balanced salt solution. Solution No. 2, which contains approximately twenty-five times as much calcium as solution No. 1, was employed on the basis of a report (16) that discontinuities in the plasma membranes of rat pancreatic exocrine cells could be prevented by increasing the calcium chloride content of the fixative (osmium tetroxide) from 0.13 to 5.40 mg/ml. Although discontinuities in plasma membranes seem to be qualitatively different from the membrane reorganizations considered in the present paper, it was suspected that both phenomena might have a common basis.

OBSERVATIONS

The satellite cells of toads, like those of rats (23), form a sheath which completely surrounds each neuron (21). In some regions the sheath consists of a single satellite cell layer (Fig. 8), and in others of several overlapping layers (Fig. 10). The *outer* or *external* surface of the sheath (facing the pericapillary space) is coated by a basement membrane (Fig. 12); this surface is relatively straight and free of infoldings or protrusions. The *inner* surface of the sheath (adjoining the neuron) is not coated by a basement membrane, and its contour is much more complex. This surface exhibits many deep invaginations of the plasma membrane (Figs. 1, 5) and it also extends slender, villous projections, some of which invaginate the neuronal surface. The inner surface of the sheath is further complicated in that many short processes from the neuron extend into the sheath and interdigitate with satellite cell processes (not shown). In short, the sheath is quite asymmetric; its outer surface is relatively simple, while its inner surface is highly complicated. The neuronal surface exhibits two forms of irregularity, one consisting of evaginations which extend into the sheath, and the second of invaginations of its plasma membrane (Figs. 8, 9, 15). The foregoing observations apply to ganglia fixed in either osmium tetroxide or permanganate solutions.

The principal *differences* between ganglia fixed in these two ways can best be appreciated by direct comparison of Fig. 1 (osmium-fixed) with Fig. 2 (permanganate-fixed). At the bottom of Fig. 1 a slender process (*s*) extends into an invagination in the thick, outermost layer of the sheath, and then ends. The invagination continues for a short length beyond this point, and then it too ends (arrow). Note that a line of vesicles proceeds from the end of the invagination all the way to the top of the figure, paralleling another thin satellite cell process just to its right.

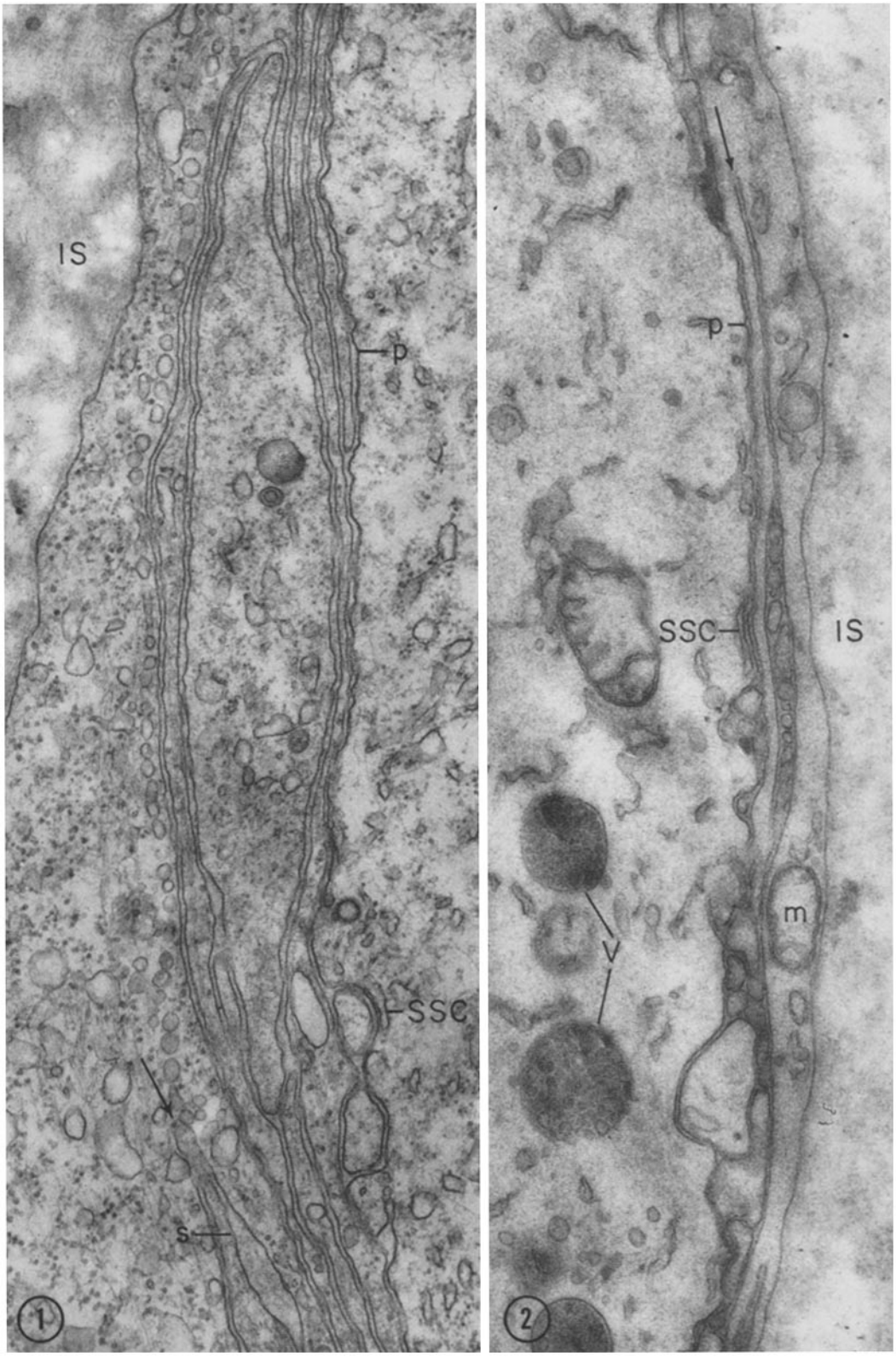
In Fig. 2 there is again an invagination in the outermost layer of the sheath, but in this case it is very long, extending all the way from the level of a mitochondrion (*m*) to the arrow at the top of the figure. No vesicles extend from its point of termination. Within this invagination there is a string of small, disconnected circular profiles whose width is about the same as that of the cytoplasmic process (*s*) at the bottom of Fig. 1.

Extracellular circular profiles such as those in Fig. 2 are not present in osmium-fixed specimens, and intracellular serried vesicles such as those in Fig. 1 do not occur in permanganate-fixed specimens. In addition to these differences, there is no visualization of ribosomes or neurofilaments in permanganate-fixed tissues.

Satellite Cell Vesicles and Invaginations

In satellite cells fixed with osmium tetroxide, chains of vesicles and short tubules are usually very conspicuous. These serried vesicles are absent from the outermost portion of the satellite cell cytoplasm (Figs. 1, 3, 12). They are approximately the same in size as pinocytic vesicles and, like them, are free of attached ribosomes, but only rarely are their limiting membranes continuous with the satellite cell plasma membrane. They are distinct from the cisterns of granular endoplasmic reticulum which occur in the outermost portion of the sheath (Fig. 3). The latter are not serried; they are larger; and they are studded with RNP granules.

The serried vesicles are sometimes connected with one another by narrow bridges, so that continuity is established between the lumina of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adjacent vesicles (Fig. 3). Such a pair of vesicles presents the appearance of a figure eight, the long axis of which is parallel to the long axis of the satellite cell. Serried vesicles are just as prominent in satellite cells fixed in the presence of excess calcium (Fig. 12) as they are in cells fixed in the presence of a balanced ionic environment (Fig. 3).

When the specimens are fixed in permanganate solutions, serried vesicles are absent from the satellite cells, while continuous invaginations of the satellite cell plasma membrane are prominent (Figs. 2, 5, 6, 7). Some of these invaginations end blindly and, at least in the plane of the section, do not extend all the way across the sheath. Most commonly the invaginations arise from the satellite cell plasmalemma immediately adjacent to the neuron (Fig. 5), but they may also originate from the external surface membrane (Fig. 6), or from layers in the middle of the sheath (Fig. 7). Large, irregularly shaped vesicles, resembling in size and configuration the granular endoplasmic reticulum, are present in the outermost part of the permanganate-fixed sheath (Figs. 5, 10), but they are never lined up as are the serried vesicles in the osmium-fixed specimens, and they are never so numerous. The appearance of the permanganate-fixed satellite cell, with respect to plasma membrane invaginations and cytoplasmic vesicles, is independent of the ionic composition or pH of the fixative solution within the limits used.

Neuronal Vesicles and Invaginations

Examination of osmium-fixed neurons also reveals the presence of chains of vesicles near the neuronal surface (Figs. 4, 13, 14, 16). These differ only slightly in their configuration from the serried vesicles of satellite cells. Characteristically, each chain originates from a slight inpocketing of the neuronal plasmalemma, usually at the base of a broad projecting peg of neuronal cytoplasm, where the neuronal plasma membrane is angulated (Fig. 4). The path of the chain may be oblique with respect to the neuronal surface (Figs. 4, 13), or nearly perpendicular to it (Fig. 14). Sometimes, the chain extends as far as another inpocketing of the neuronal surface and ends there. As in the case of satellite cell vesicles, these too are sometimes elongated (Figs. 14, 16), or there may be narrow bridges between adjacent vesicles. Occasionally, continuous invaginations of the neuronal surface can be seen in osmium-fixed specimens (Figs. 4, 15). Neither these invaginations nor the chains of vesicles occurring in this location ever have ribosomes attached to them. Flattened vesicles resembling subsurface cisterns (22) are sometimes closely apposed to the deep surface of a chain of vesicles extending from the neuronal surface (Fig. 16). As in the case of satellite cells, osmium-fixed neurons exhibit chains of vesicles in this location regardless of the presence of excess calcium in the fixative.

In the vicinity of chains of vesicles or invaginations of the neuronal surface, the surrounding cytoplasm sometimes contains a fibrillar material which has the appearance of a terminal web (Fig. 14). Single vesicles which are somewhat larger than the serried vesicles, and which appear to be coated with fibrillar material like that of the terminal web, also occur in this region (Figs. 4, 14, 15). The last vesicle in a chain is sometimes of this type (Fig. 14).

In a study of the surface membranes and cytoplasmic vesicles of certain epithelial cells, Wissig

FIGURE 1

Neuron and sheath fixed in osmium tetroxide. The outermost layer of the sheath is invaginated by a tongue of cytoplasm (*s*) at the bottom of the figure. A chain of vesicles extends from the end of the invagination (*arrow*) up to the top of the figure. *p*, neuronal plasmalemma. *IS*, interstitial space. *SSC*, subsurface cistern. × 28,000.

FIGURE 2

Neuron and sheath fixed in sodium permanganate. An invagination into the outermost layer of the sheath begins at the level of a mitochondrion (*m*) and extends upwards to its termination at the arrow. A row of disconnected circular profiles lies within this invagination. A second blind termination appears in this sheath at the bottom of the figure. *SSC*, subsurface cistern. *V*, multivesicular body. *p*, neuronal plasmalemma. × 30,000.

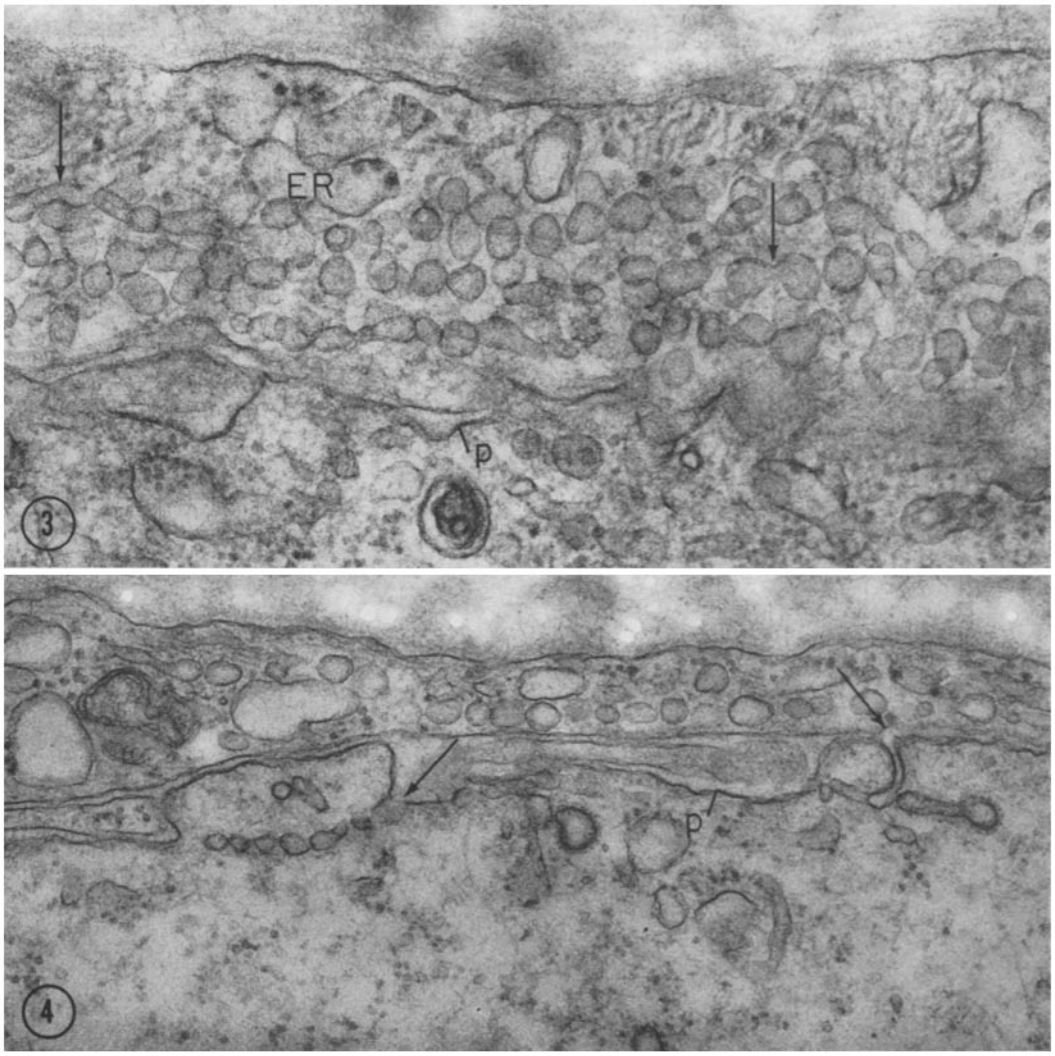


FIGURE 3

Satellite cell containing serried vesicles. The outer fourth of the satellite cell (top) contains fibrillar and granular material as well as several cisterns of granular endoplasmic reticulum. One of these (*ER*) displays superimposition of its face and edge, with the result that some of the associated ribosomes seem to be inside the cistern. The inner three-fourths of the satellite cell is almost entirely filled with chains of vesicles, some of which are elongated horizontally, or joined from side to side (*arrows*). Neuronal plasmalemma, *p*. × 57,000.

FIGURE 4

Neuron showing invaginations (*arrows*) of its plasma membrane (*p*). An arcuate chain of vesicles extends from the invagination at the left towards an angulation in the neuronal surface. A club-shaped tubule is apposed to the invagination at the right. The swollen end of this tubule and another vesicle in the center of the figure are both coated with fibrillar material. × 40,000.

(33) divides the vesicles into three groups on the basis of the structure of their limiting membranes and the presence or absence of an external coat. The vesicles coated with fibrillar material, which occur in osmium-fixed spinal ganglion cells, appear identical to the third group of vesicles in his classification. Neither vesicles of this type nor the terminal web are visualized in permanganate-fixed spinal ganglia.

In permanganate-fixed ganglion cells the neuronal surface shows no short chains of vesicles projecting obliquely into the ectoplasm. Instead, invaginations of the plasma membrane, following approximately the same course as the chains of vesicles in osmium-fixed samples, project into the neuron and end blindly (Figs. 8, 9). Sometimes subsurface cisterns are closely apposed to these invaginations (Fig. 8), producing a configuration strikingly like that of a dyad in an insect flight muscle (29). Invaginations of the plasma membrane have not been described in neurons of the central nervous system.

Thin Satellite Cell Layers and Serried Globules

Another characteristic of permanganate-fixed samples of spinal ganglia is that rows of disconnected circular profiles may be seen in locations where greatly attenuated sheets of satellite cell cytoplasm occur in osmium-fixed specimens. Although these circular profiles bear some resemblance to the intracellular serried vesicles present after osmium fixation, they are clearly *extracellular* as can be seen in Fig. 2. Each of them is limited by a membrane which, in favorable sections, can be resolved into a "unit membrane" (19) having the same over-all thickness as the satellite cell plasma membrane (Fig. 11). These rows of circular profiles can in some cases be seen to extend from the ends of thin satellite cell layers (Fig. 11). They may occur directly against the neuronal plasmalemma (Figs. 6, 8), or directly adjacent to the interstitial space (Fig. 6), or between layers in the sheath (Figs. 2, 10, 11).

Two three-dimensional interpretations of these circular profiles are possible. They could represent cross-sections through villous outpouchings of satellite cell layers which lie outside of the plane of the section. Or they could represent sections through isolated spherules of cytoplasm, each completely enclosed in a plasma membrane. In the first case, they would correspond to cross-sections through intestinal epithelial microvilli, which also appear as disconnected circular profiles (15). However, in longitudinal sections through intestinal epithelial cells it is obvious that the microvilli are in fact perpendicular evaginations of the epithelial cell surface. Such perpendicular evaginations are never seen to arise from satellite cell layers regardless of the plane of section. Consequently, it seems likely that the circular profiles seen in sheaths of permanganate-fixed spinal ganglia are sections through disconnected cytoplasmic globules. It should again be emphasized that no such structures appear in osmium-fixed sheaths.

Other Membranes

Because of the disparity in the observed morphology between the osmium-fixed and permanganate-fixed specimens, some attention was given to other structures which also show vesiculation in fixed preparations. Golgi membranes were found to possess lamellar and vesicular components in about the same proportion in both osmium- and permanganate-fixed specimens. The granular endoplasmic reticulum also showed both cisternal and vesicular components in both cases, but there appeared to be some increase in the vesicular component in the osmium-fixed samples. A cursory examination of permanganate-fixed striated muscle showed clear evidence of pinocytic vesicles along the surfaces of the muscle cells and in the capillary endothelium. Other investigators have shown previously that certain vesicular structures, including Golgi vesicles (10, 12) and synaptic vesicles (10), are preserved with this method of preparation. Thus it is with respect to only certain membranous and vesicular structures that a sharp distinction can be made between osmium-fixed and permanganate-fixed specimens.

DISCUSSION

The principal point to be made in this paper is that spinal ganglia taken from the same animal and handled in the same manner (except for the mode of fixation) appear consistently different with respect to vesiculation and invagination of plasma membranes, depending on whether they are fixed with permanganate solutions or with osmium tetroxide. Which, if either, of the two pictures accurately represents the morphologic aspect of the living cells is impossible to deter-

mine from the information available. It is clear, however, that both pictures cannot be correct and that a systematic artifact has been introduced by at least one of these standard fixatives.

Two interpretations of the data immediately come to mind. The first is that paired plasma membranes may break down to form either serried intracytoplasmic vesicles or rows of extracellular globules, depending on whether the membranes are apposed along their external or internal surfaces. The former process occurs in osmium-fixed samples and affects infoldings of the plasma membrane; the latter occurs in permanganate-fixed material and involves attenuated cytoplasmic sheets. The second interpretation is the converse of this, *i.e.*, that the serried vesicles and isolated cytoplasmic globules fuse to form continuous membrane pairs.

Although a conclusive argument cannot be made to support either of these views, it will be assumed here that it is the continuous membranous structures which are verifactual and that the smaller disconnected units are derived from them. It does not seem likely that isolated cytoplasmic globules having no connection with a nucleus or with other cytoplasmic organelles would survive in life. Nor would serried vesicles be expected to remain neatly lined up in rows in living cells which undoubtedly display cytoplasmic streaming and ruffling of their surfaces.

In a paper on the endoplasmic reticulum of gastric parietal cells Ito (7) also favored the view that continuous tubules were probably verifactual and the vesicles artifactual on the ground that more energy was required to maintain the former state. Sedar (27), studying the endoplasmic reticulum of oxyntic cells in the frog stomach, recently reported that a greater percentage of elongated profiles and relatively few vesicles were evident after permanganate fixation.

Mechanism of Membrane Reorganizations

Membrane recombinations may be regarded as events which are induced in the living or moribund cells by the fixatives, or as biochemical or surface interactions between membranes after they have been "fixed." Both osmium tetroxide and permanganate solutions may be imperfect fixatives in the sense that reactive groups persist in plasma membranes even after the membranes have been exposed to these solutions. Membrane recombinations could then be brought about by interactions between such groups in adjacent membranes at several stages in the processing of the tissues. During dehydration, extraction of alcohol-soluble coatings might uncover these groups. During embedding, components of the unpolymerized plastic mixture might serve to catalyze interactions between these groups. Perhaps the mere increase in temperature to 60°C during embedding is sufficient to bring about such reactions. Another possibility is that both osmium tetroxide and permanganate create physical instability in these membranes by altering their surface properties.

Figures 5 to 7. Sodium permanganate-fixed specimens which show invaginations of the satellite cell plasmalemma.

FIGURE 5

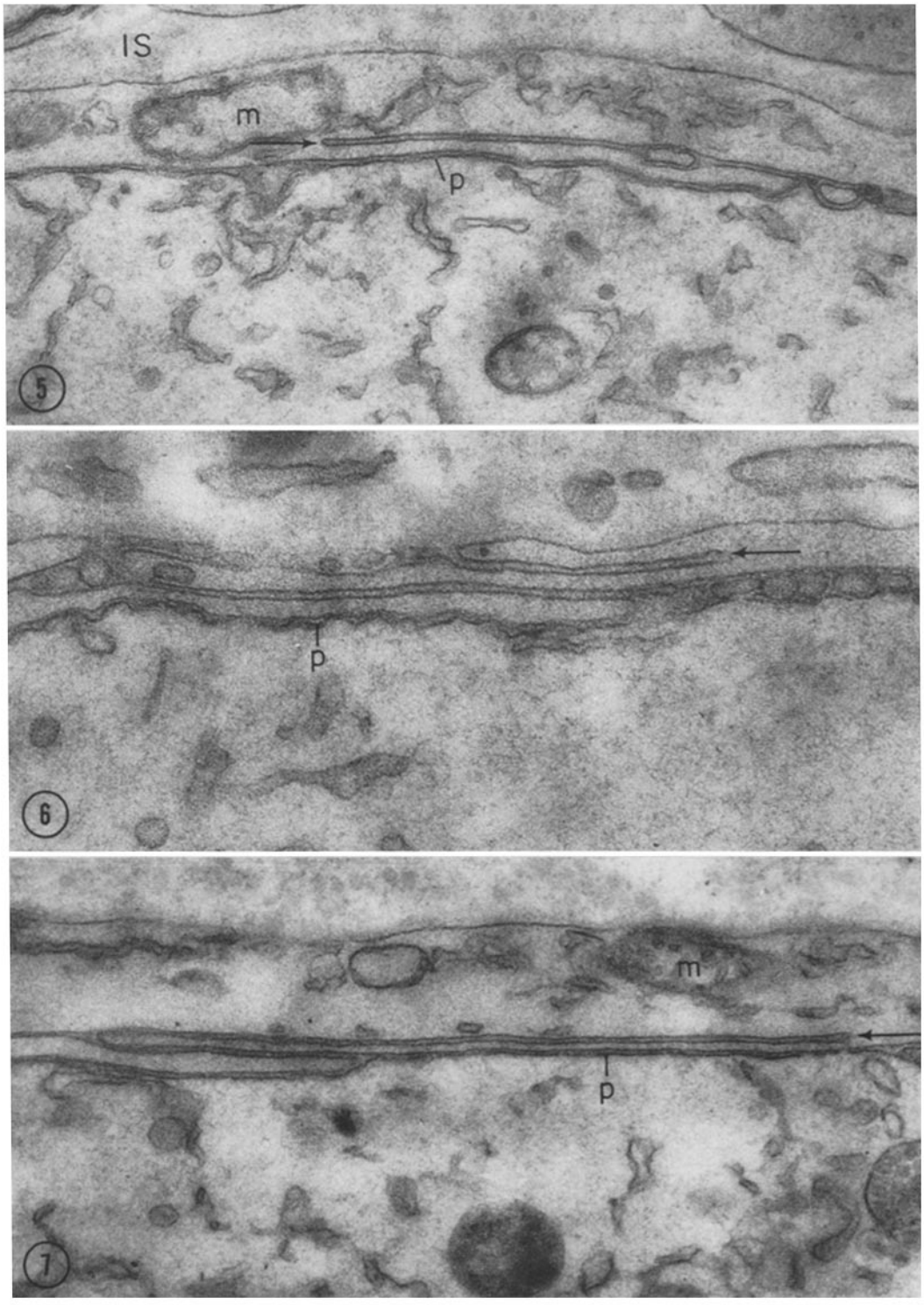
Invagination originating from the inner surface of a satellite cell (at the right) and extending to the arrow where it ends as a *cul-de-sac*. The invagination encloses a short satellite cell process. The outer portion of the sheath contains irregular profiles of the endoplasmic reticulum, and a mitochondrion (*m*). Interstitial space, (*IS*). Neuronal plasmalemma, *p*. × 30,000.

FIGURE 6

Invagination which begins at the outer surface of a satellite cell and ends at the arrow. Below and to the left of this invagination, a channel consisting of a pair of plasma membranes extends all the way across the sheath. This channel probably represents the junction between two adjacent satellite cells. Neuronal plasmalemma, *p*. × 47,000.

FIGURE 7

Invagination beginning between two layers in a sheath. The invagination extends for 2.7 μ in the plane of the section before it ends (*arrow*). The outer layer of the sheath contains irregular profiles of the endoplasmic reticulum, and a mitochondrion (*m*). Neuronal plasmalemma *p*. × 42,000.


IS
m
p
5
p
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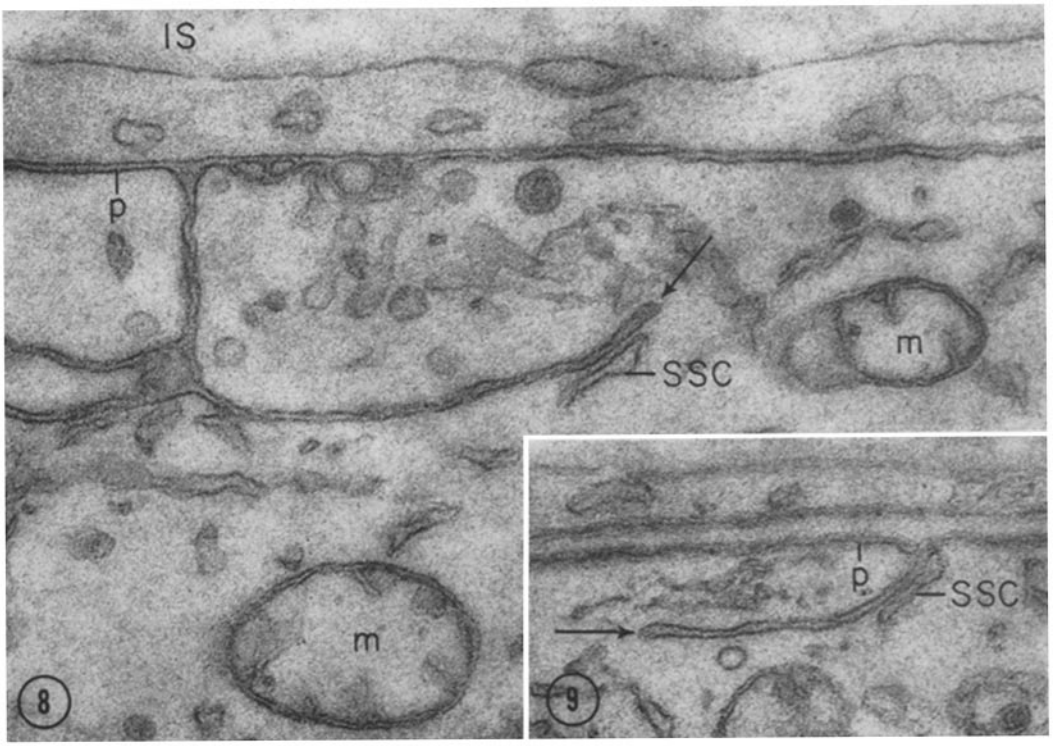


Figures 8 and 9. Specimens fixed in sodium permanganate showing infoldings of the neuronal plasmalemma (*p*).

FIGURE 8

The invagination at the left side of the figure extends perpendicularly into the neuron and then breaks up into three branches, one of which terminates at the arrow. A subsurface cistern (*SSC*) is apposed to the invagination near this termination. *m*, mitochondrion. *IS*, interstitial space. × 46,000.

FIGURE 9

An invagination extends obliquely into the neuronal cytoplasm and terminates at the arrow. A subsurface cistern (*SSC*) occurs near the origin of the invagination. × 44,000.

This could result from addition of the fixative to the membrane surfaces, or from denaturation of surface proteins. A comparable mechanism has been proposed by Brandt (2) to account for the induction of pinocytosis in amebae by charged molecules.

Robertson has demonstrated that of the three layers of a plasma membrane only the innermost one can in general be visualized in osmium-fixed preparations (19) even after lead staining. This may indicate that the two external layers of the membrane are not stabilized well enough by the osmium tetroxide to prevent some degree of extraction by alcohols or unpolymerized plastic. It is of interest that in osmium-fixed tissues it is precisely those plasma membranes that are apposed along their external surfaces that may break down into chains of vesicles.

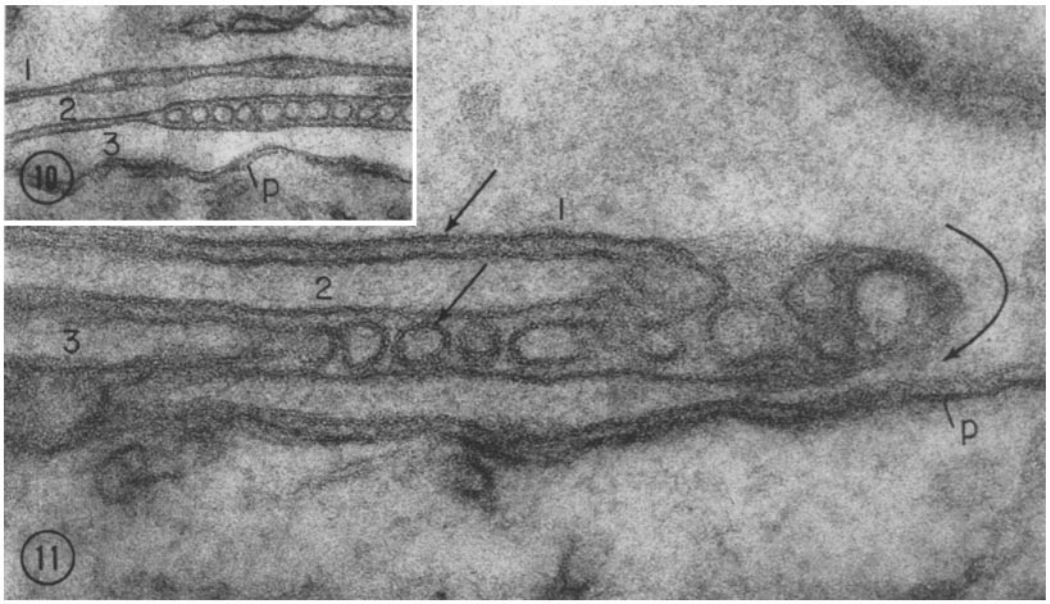
One difficulty in ascribing membrane recombinations to purely physical factors, such as surface tension changes, is that not all paired plasma membranes exhibit this phenomenon. The neuron-satellite cell interface also consists of a pair of plasma membranes apposed along

their external surfaces; yet in the material examined no clear cut examples were seen in which this pair of membranes had broken down into a string of vesicles. Similarly, the mesaxon of unmyelinated fibers is not known to break down into vesicles under any conditions of fixation.

pinocytosis in the living cell. In the latter case also, the milieu of the cell has considerable effect on membrane vesiculation (2).

Applications

The significance of these findings with respect to the mode by which materials are transferred to



Figures 10 and 11. Specimens fixed in sodium permanganate showing disconnected units of satellite cell cytoplasm.

FIGURE 10

Three satellite cell layers are shown (*1, 2, and 3*). The space between layers 2 and 3 widens to enclose a row of small, membrane-limited, circular profiles. Layer 1 contains some endoplasmic reticulum. Neuronal plasmalemma, *p.* × 43,000.

FIGURE 11

Three satellite cell layers are shown (*1, 2, and 3*). Layer 1 folds under layers 2 and 3 (*curved arrow*). A row of disconnected circular profiles extends to the right from the end of layer 3. The limiting membranes of these units, and the satellite cell membranes, are in some places resolved into trilaminate "unit membranes" (*straight arrows*). Neuronal plasmalemma, *p.* × 114,000.

Evidently, there is some specificity to this phenomenon, as if predisposing factors must be present in order for it to occur. In toad spinal ganglia, it appears to occur only where surface infoldings end as blind pouches. Perhaps the blind ending serves as a focal point from which vesiculation proceeds.

Although fixative-dependent vesiculation of plasma membranes can hardly be considered a naturally occurring phenomenon, its mechanism may not be very different from the mechanism of

and from the neuron through the investing layer of satellite cells will be treated separately. Aside from this specific application, it is probably of more general interest to consider that chains of discontinuous vesicles may in many locations represent pre-existing continuous channels.

It has been noted, for example, that the intermediary vesicle of the sarcoplasmic reticulum of striated muscle is visualized in some preparations as an elongated tubule, and in others as a chain of closely apposed vesicles. It was suggested that

the latter configuration may arise from the breakdown of the former (5). Similarly, the connections between the intermediary vesicles (or T system) and the sarcolemma are usually seen as chains of vesicles. This has led to the suggestion that there is no continuity between intermediary vesicles and the plasma membrane (1), or that continuity of these structures is intermittent (17). In vertebrate cardiac muscle deep invaginations of the plasma membrane have been reported (28), and in some invertebrate muscles it is clear that one of the components of the "dyad" is indeed a deep infolding of the plasma membrane (29). It would be interesting to know whether permanganate fixation of vertebrate skeletal muscle would regularly demonstrate tubular intermediary vesicles which are clearly continuous with the sarcolemma.

CONCLUSION

Although the mechanism of the membrane reorganizations described here and the stage at which they occur are still obscure, it is at least important to recognize that these phenomena may occur with the standard preparative procedures now in use. Serried vesicles occurring near cell surfaces in osmium-fixed specimens, and cytoplasmic globules occurring in rows in permanganate-fixed material, should both be suspected of representing paired plasma membranes that have disintegrated under the influence of the fixative. The observation of smooth surfaced ectoplasmic vesicles in electron micrographs need not always indicate that pinocytosis is occurring in the living cell.

In general terms, it appears that even in tissues which look well fixed, and in which there are no obvious architectural disruptions, there may nevertheless be systematic artifacts which can be identified as such only through comparison with specimens fixed by alternative methods.

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Figures 12 to 16. Specimens fixed in osmium tetroxide in the presence of excess calcium.

FIGURE 12

Satellite cell containing rows of vesicles, some of which are elongated horizontally. The outermost region of the satellite cell is free of these vesicles, but exhibits a number of dense glycogen granules (18). Interstitial space, *IS*. Neuronal plasmalemma, *p*. × 40,000.

FIGURE 13

Neuron containing two curved rows of vesicles. The shorter row appears to arise (*arrow*) from a shallow invagination of the neuronal plasmalemma, (*p*). Dense glycogen granules are present in the neuron (18). × 56,000.

FIGURE 14

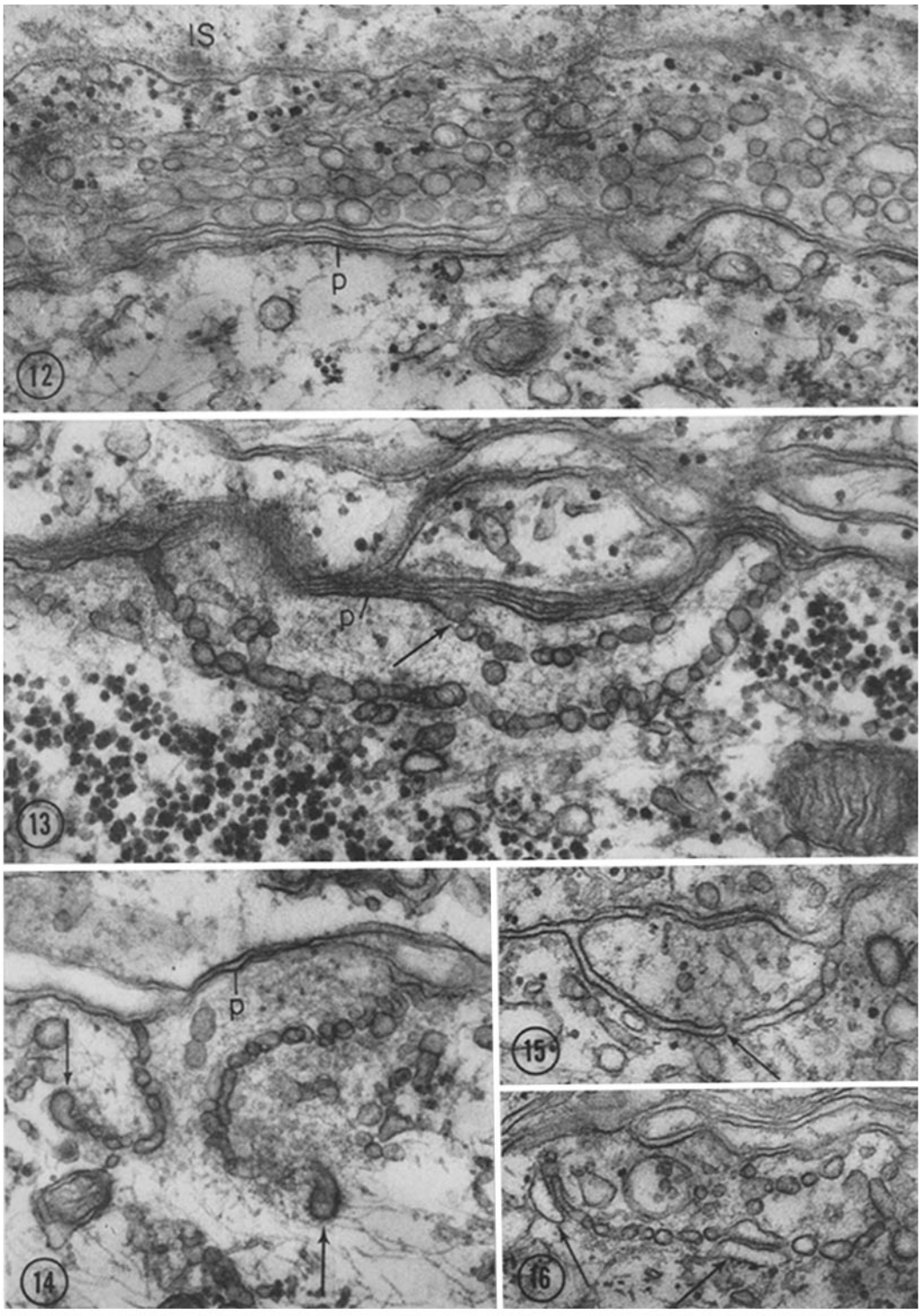
Neuron containing two rows of vesicles. The last vesicle in each row (*arrow*) is larger than the others and is coated with fibrillar material. The neuronal cytoplasm has the appearance of a terminal web. × 48,000.

FIGURE 15

Invagination of the neuronal plasmalemma. The invagination terminates at the arrow. Two disconnected tubules continue from this point upwards towards a depression in the neuronal surface. × 42,000.

FIGURE 16

Chain of vesicles and short tubules in the neuronal cytoplasm. Two flattened vesicles (*arrows*) resembling subsurface cisterns are closely apposed to this chain. × 39,000.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