SUBSURFACE CISTERNS AND THEIR RELATIONSHIP TO THE NEURONAL PLASMA MEMBRANE

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ABSTRACT

Subsurface cisterns (SSC's) are large, flattened, membrane-limited vesicles which are very closely apposed to the inner aspect of the plasma membranes of nerve cell bodies and the proximal parts of their processes. They occur in a variety of vertebrate and invertebrate neurons of both the peripheral and central nervous systems, but not in the surrounding supporting cells. SSC's are sheet-like in configuration, having a luminal depth which may be less than 100 A and a breadth which may be as much as several microns. They are separated from the plasmalemma by a light zone of $\sim$ 50 to 80 A which sometimes contains a faint intermediate line. Flattened, agranular cisterns resembling SSC's, but structurally distinct from both typical granular endoplasmic reticulum (ER) and from Golgi membranes, also occur deep in the cytoplasm of neurons. It is suggested that membranes which are closely apposed may interact, resulting in alterations in their respective properties. The patches of neuronal plasmalemma associated with subsurface cisterns may, therefore, have special properties because of this association, resulting in a non-uniform neuronal surface. The possible significance of SSC's in relation to neuronal electrophysiology and metabolism is discussed.

INTRODUCTION

Physiological studies of neurons have focused attention on the cell surface as the probable site of the electrical responses of these cells (6). It is generally assumed that the neuronal surface is represented morphologically by the plasma membrane—a trilaminate structure, which has an over-all thickness of $\sim$75 A (38).

In the course of recent morphological studies of vertebrate peripheral ganglia (39, 41, 42) attention was drawn to another structure, whose location and distribution suggested that it too may be a functional component of the neuronal surface. This structure, which was designated "subsurface cistern" (SSC), consists of a broad, flat, membrane-limited vesicle, which occurs within the neuronal cytoplasm in direct *apposition* to the plasma membrane. Although examples of SSC's were encountered frequently in neurons, none were observed in the Schwann cells, or in the endothelial cells and fibroblasts of these ganglia.

The intimate association of subsurface cisterns with the neuronal plasmalemma and their apparent absence in neighboring, non-neuronal cells suggested that their presence might be correlated with some of the distinctive physiological properties of the neuronal surface. This view was supported by reports that comparable structures, although not present in most cells, do

occur in certain sensory cells (8, 43) and muscle cells (18, 33, 44), which resemble neurons in that they generate or conduct electrical potential changes as part of their normal function.

On the basis of these considerations, an effort was made to determine whether subsurface cisterns occur in nerve cells generally, or whether they are limited to neurons of the vertebrate peripheral nervous system. Specimens of rat cerebral cortex and spinal cord were, therefore, examined, and SCC's were identified in the neurons of these areas, but not in the glial cells.[1] In addition, they have also been seen recently in granule cells of the rat cerebellar cortex (25), and in nerve cells of a snail (40). Although there are some differences among SSC's in these different cell types, they all share certain basic characteristics.

The purpose of this paper is to give a detailed description of the structure and distribution of subsurface cisterns as they occur in the acoustic ganglia, cerebral cortex, and spinal cord of the rat, and to discuss their variations, their association with other intraneuronal and extraneuronal structures, and their possible significance in relation to nerve cell physiology.

MATERIALS AND METHODS

Specimens were obtained from adult male Osborne-Mendel rats weighing approximately 150 to 300 gm. The animals were anesthetized with chloral hydrate (0.4 mg/gm) (3), and fixed by perfusion through the aorta (27). The fixative consisted of 1 per cent osmium tetroxide with Veronal acetate buffer (pH 7.4 to 7.5) and either $CaCl_2$ (5.4 mg/ml) or $MgCl_2 \cdot 6H_2O$ (9.9 mg/ml). Blocks of tissue were taken from the cochlear ganglia, the frontal and parietal cerebral cortex, and the spinal cord. No attempt was made to select specific layers or regions within these areas. The specimens were dehydrated in methanol and embedded in either Epon 812 or Araldite (21). Blocks were sectioned with a Porter-Blum microtome and sections were mounted on grids coated with thin films of carbon and Formvar or carbon alone, or on grids with no supporting substrate. They were stained with saturated aqueous uranyl acetate (45) or lead hydroxide (46), and examined with an RCA EMU-3E electron microscope or a Siemens Elmiskop I at initial magnifications of 5000 to 30,000. Preliminary examination of thick sections was performed with a phase contrast microscope, in order to select for thin sectioning those areas which contained a high proportion of neuronal cell bodies.

[1] Comparable structures have been noted in neurons of the cerebral cortex in two recent publications (14, 28).

OBSERVATIONS

Subsurface cisterns are somewhat variable in configuration, but all possess three defining characteristics: they are bounded by a single membrane; they are flattened; and they are very closely applied to the plasmalemma of the cell containing them, with no ribosomes or other organelles intervening. A typical example, illustrating these features, is presented in Fig. 1 which shows a cochlear ganglion cell covered by a sheath consisting of loose and semicompact myelin (39). Just beneath the neuronal plasmalemma (*p*) is a subsurface cistern consisting of an elongated (1.5 μ), flattened, membrane-limited vesicle. The interval between the SSC and the plasmalemma is distinctly narrower than the cleft between the neuron and its sheath. The lumen of the SSC is also very narrow, except at its bulbous lateral edges where the deep and superficial surfaces are continuous with each other. The SSC follows the undulating course of the plasmalemma closely, separated from it by a constant interval (*cf.* Figs. 3 to 5). At the right side of the figure the edge of the cistern dips slightly downwards into the neuronal cytoplasm, and the overlying plasmalemma follows it for a short length, forming a small well in the perineuronal space (*cf.* Fig. 5). Two cisterns of granular endoplasmic reticulum (ER) are lined up parallel to the subsurface cistern.

In Fig. 1 the subsurface cistern and the plasmalemma are sectioned normally throughout most of their course so that the relationships of these structures with one another are clear. Often, however, all or part of an SSC passes obliquely through a section, producing a broad blur in the vicinity of the cell membrane. That portion which is normal to the plane of the section can usually be identified by its characteristic spacing relationships, however. For example, in Fig. 2 the two lines labeled SSC are separated by a light zone which is much too narrow to be confused with the perineuronal space (between *s* and *p*).

The feature of subsurface cisterns which is of most interest is their proximity to the neuronal plasmalemma. Precise determination of the distance between these two structures is difficult to make, however, because their external limits are not clearly defined; in addition, the technical procedures employed in preparing the tissue for

electron microscopic examination may change the spatial relationships present in the fresh tissue. Thus the measurements which are given must be considered approximations.

At high magnification, the SSC and plasmalemma appear to be separated by a light zone which is ~50 to 80 A in width (range, 40 to 100 A). The width of this light zone is usually narrower in spinal cord neurons than in acoustic ganglion cells or cortical neurons. Occasionally, a faint, discontinuous intermediate line bisects this zone longitudinally (Figs. 2 and 5). However, cross-bridges, such as have been described in "septate desmosomes" of *Hydra* (48), and in "attachment areas" of earthworm nerve sheaths (17), have not been observed between the SSC and plasmalemma. Nor are multiple intermediate lines present in this location, such as those occurring in some desmosomes (42). Although the separation between the SSC and plasmalemma is often indistinct (probably due to sectioning artifacts, or obliquity of the membranes), frank continuity between the limiting membrane of an SSC and a plasmalemma has not been observed. In this respect, SSC's differ from pinocytic vesicles, whose lumina can often be seen to communicate with the extracellular space (23).

The profiles of subsurface cisterns seen in sections are broad and shallow, and are generally uninterrupted by fenestrations. The largest SSC's that have been observed occur in spinal cord neurons, where their breadth is usually ~2 μ, and in some instances extends up to 4 μ. These are also usually the flattest cisterns, having a luminal depth of only ~40 to 50 A (Figs. 3 to 5). In contrast, those in neurons of the cerebral cortex tend to be deeper (~100 to 600 A) and not so broad (~1 μ) (Figs. 6, 7, and 11). The dimensions of SSC's tend to cluster in a range which is characteristic of the cell type under observation.

It may be inferred from these observations that in three dimensions subsurface cisterns are sheet-like or discoid in configuration. If they were tubular, it is very unlikely that the long dimension would remain in the plane of a 0.1 μ section for 1 or more microns, as it in fact does.

Based on the assumption of a disc-shaped configuration, the surface area of the neuronal plasmalemma overlying a single SSC would be ~4 μ^2 in spinal cord neurons, and ~1 μ^2 in neurons of the cerebral cortex. In any individual neuron, the total surface area which is associated with SSC's would be difficult to assess, but it probably amounts to no more than a small percentage of the whole neuronal surface.

Associated Intraneuronal Structures

Mitochondria occur in the immediate vicinity of subsurface cisterns, and in some cases one mitochondrion may be very closely apposed to the deep surface of an SSC (Fig. 4). The light zone which separates the mitochondrion from the SSC is of the same order of magnitude as that between the SSC and plasmalemma.

Connections between the subsurface cisterns and the endoplasmic reticulum are prominent, particularly in the cerebral cortex. In Fig. 6, for example, the SSC at the upper left extends down into the neuronal cytoplasm and is continuous with the network of granular endoplasmic reticulum. Deep connections of this kind occur only from one or both lateral edges of an SSC but not from the central portion. When these connections occur, they are always with the granular ER, and not with Golgi membranes. The deep surface of an SSC may be studded with ribonucleoprotein (RNP) granules in regions where the luminal depth of the SSC is greater than ~100 A. In the spinal cord, where SSC's are typically highly flattened, ribosomes are generally absent from their deep surfaces (Figs. 3 to 5), except at their bulbous lateral edges where the luminal size increases. In the cerebral cortex, by contrast, SSC's are often deeper over their entire extent, and here, ribosomes may occur anywhere along their deep surfaces (Figs. 7 and 11).

Association of subsurface cisterns with the deeper ER also occurs without apparent connections between their respective lumina. It is common to find several cisterns of the ER stacked up parallel to an SSC and immediately subjacent to it. In the spinal cord, the members of such a stack are usually typical cisterns of granular ER studded with RNP granules (Fig. 3). In the cerebral cortex, in contrast, the members of the stack are often highly flattened, unfenestrated cisterns, spaced at a regular interval of ~250 to 300 A and not associated with ribosomes (Figs. 9 and 10). Diffuse granular material of intermediate density intervenes between them. Like the SSC, these flat, agranular cisterns may connect with typical granular ER but only at their lateral edges. Stacks of this kind resemble "spine appara-

tus" (13, and Fig. 14) in the spacing of the component cisterns. However, even though an intermediate line of moderate density may occasionally be seen between successive cisterns in such a stack, it never attains the thickness or density of the intermediate line which occurs regularly in the spine apparatus.

Instead of a stack, only one or two cisterns may underlie the SSC (Figs. 1 and 8). The one immediately adjacent to the subsurface cistern is usually atypical in that no ribosomes are present along its surface facing the SSC or in the ~250 to 350 A zone separating it from the SSC. It is usually not very highly flattened, however, and RNP granules may stud its deep surface.

Stacks of unfenestrated, agranular cisterns regularly spaced at ~250 to 300 A also occur *deep* in the cytoplasm of cortical neurons (Fig. 12), and in acoustic ganglion cells as well (Fig. 13). These appear to be identical to the stacks occurring in association with SSC's, differing only in that they are removed from the vicinity of the neuronal surface. The configuration of these stacks distinguishes them from Golgi membranes, as can be seen at a glance in Fig. 12. The component cisterns of the latter are more closely apposed to each other; they are fenestrated; they are associated with vesicles; and their lumina are larger.

Associated Extraneuronal Structures

Subsurface cisterns apposed to the neuronal plasmalemma inevitably appear to be related to extraneuronal structures on the other side of the cell membrane. Whether the association of SSC's with extraneuronal structures is entirely fortuitous and lacking in functional significance, or whether the association is, in some cases, more specific, is open to question. In the case of spinal cord neurons (Figs. 3 to 5), nearly the entire surface of the soma and dendrites is covered by synaptic boutons. SSC's in these cells could, therefore, be regarded as being subsynaptic in position, implying that they are part of the synaptic complex. A specific association between SSC's and overlying synaptic boutons is suggested by the fact that the two structures are sometimes lined up one over the other, each covering about the same length of the neuronal plasmalemma. Since some synaptic endings are associated with SSC's in this way and

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FIGURES 1 AND 2

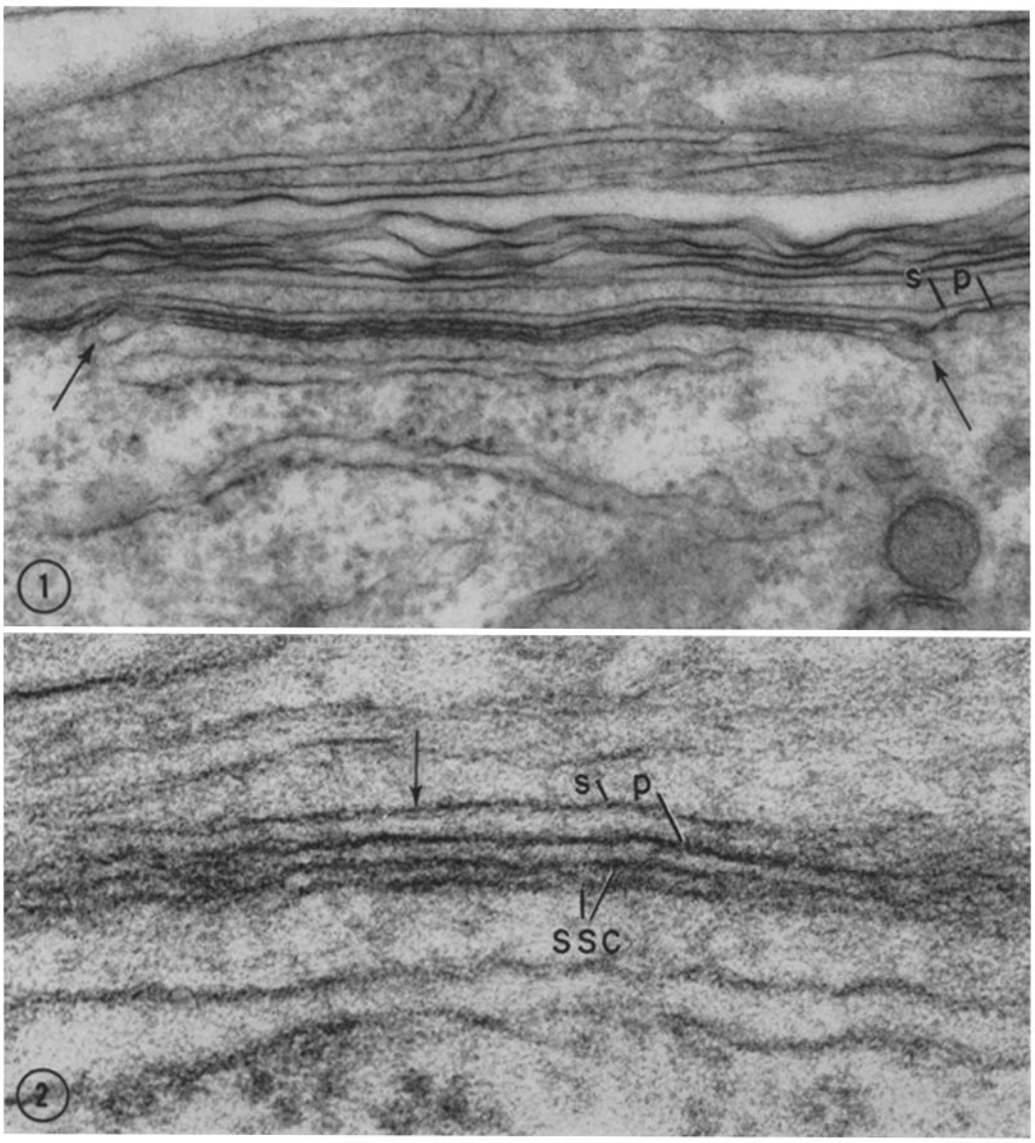
Subsurface cisterns in cochlear ganglion cells.

FIGURE 1

The neuron, which occupies the lower half of the figure, is limited by its plasma membrane (*p*). The adjacent Schwann cell membrane (*s*) is separated from the neuron by a light zone of ~130 A. Arrows indicate the bulbous lateral edges of a subsurface cistern which extends for ~1.5 μ in the plane of the section. The depth of its lumen is ~60 A, and the cleft between it and the neuronal plasmalemma is ~75 A wide. Two cisterns of the endoplasmic reticulum underlie the SSC. The first is separated from it by a distance of ~350 A with no RNP granules intervening between them. The second cistern has RNP granules associated with both its deep and superficial surfaces. × 70,000.

FIGURE 2

A subsurface cistern (*SSC*) lies beneath the neuronal plasmalemma (*p*) separated from it by a light zone of ~90 A, which contains a faint, discontinuous intermediate line. The lumen of the SSC is only ~40 A wide. A cistern of the endoplasmic reticulum parallels the SSC at the bottom of the figure. At the level of the arrow, the Schwann cell membrane (*s*), situated directly above the neuronal plasmalemma, exhibits a triple-layered structure (over-all width ~70 A). The neuronal plasmalemma is represented only by its innermost component which is ~40 A wide and is denser than that of the Schwann cell membrane. (In general, only the innermost portion of the plasma membrane is visible in osmium-fixed specimens.) × 240,000.


S
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1
S
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SSC
2

others are not related to SSC's, it would be possible to make a distinction between two morphological types of synapses on these cells.

On the other hand, a subsurface cistern may underlie only a small part of a synaptic bouton, or extend for a considerable distance beyond the edge of a bouton. Although the breadth of an SSC may coincide with that of an overlying synaptic bouton, the lateral edges of the two structures may not be exactly lined up with each other, so that the SSC extends beyond the bouton on one side, and falls short of its extent on the other (Fig. 4).

Furthermore, in cortical neurons, which are not so heavily invested with synaptic boutons, subsurface cisterns have been observed to underlie dendrites (Fig. 10), glial cells (Fig. 7), and myelinated axons, in addition to synaptic endings. It is probably also significant that the characteristic thickening of the apposed membranes at a synapse (24) is usually completely absent where an SSC is present under a bouton (although that part of the synapse not associated with the SSC may exhibit thickening of the membranes). Similarly, the aggregation of synaptic vesicles against the presynaptic membrane is also usually not seen where an SSC is present. Sometimes, although a bouton is directly apposed to the neuronal plasmalemma over an SSC, the bouton clearly forms a synapse with a dendrite on its other side. In Fig. 11, for example, such an axodendritic synapse occurs. The postsynaptic, dendritic membrane is thickened; there is an intermediate line in the synaptic cleft; vesicles are clustered against the presynaptic membrane; and spine apparatus (sectioned tangentially) is present in the postsynaptic cytoplasm. None of these observations applies to the juxtaposition between this same axon and the neuronal cell body.

It is, therefore, not clear whether the apparent associations between synaptic boutons (or other extraneuronal structures) and subsurface cisterns is significant, or whether the distribution of SSC's is entirely random with respect to extraneuronal structures. In sensory ganglia there are, of course, no synaptic boutons, so that in all cases, SSC's in these neurons underlie sheath cells or the channels of extracellular space which lie between adjacent sheath cells.

Distribution

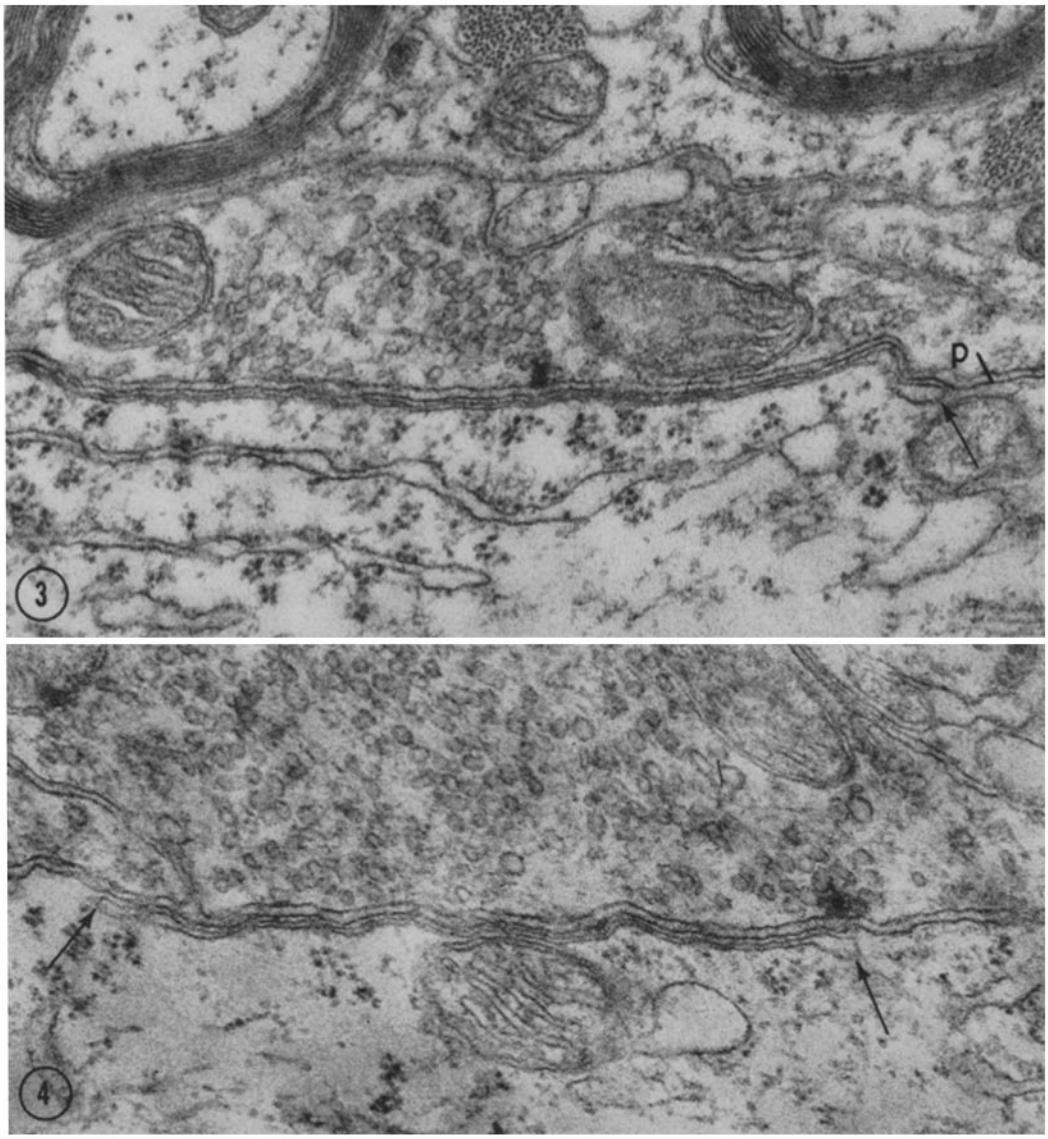
Subsurface cisterns characteristically occur in the perikarya of neurons and in the proximal, large-caliber portions of the processes which issue from them. In peripheral sensory ganglia they have been identified in cell bodies and in the initial segments of their axons, where the investing sheath is still characteristic of that over the perikaryon. In acoustic ganglia, in which the cell bodies are myelinated, there is a preferential association of SSC's with areas covered by loose myelin as opposed to compact myelin (39). With rare exceptions, SSC's have not been seen in C fibers, or in myelinated axons beyond the node of Ranvier adjacent to the cell body.

Similarly, in the central nervous system SSC's occur in neuronal perikarya and in the large-caliber dendritic trunks near the cell bodies. Only rarely have they been encountered in the smaller, more distal extensions of these processes. It is not possible to say whether or not SSC's also occur in the initial segments of axons in the central nervous system, because of difficulty in identifying these processes in electron micrographs.

DISCUSSION

On morphological grounds, the subsurface cisterns and the stacks of flattened, agranular cisterns, which are their counterparts deeper in the cytoplasm, appear to belong to the network of endoplasmic reticulum which is abundant in neurons (26) and which has been shown to occur almost universally in cells (22, 31). They most closely resemble the granular ER, or ergastoplasm, and indeed they are sometimes continuous with it. However, they differ in that they may be entirely devoid of ribosomes (Figs. 1, 2 and 8) and flattened to a much greater degree than are typical cisterns of granular ER. They also differ in configuration from typical Golgi membranes. Thus, SSC's and their counterparts cannot be readily classified as members of either of the two categories of the endoplasmic reticulum which occur most commonly, but may be more akin to special types of endoplasmic reticulum such as the sarcoplasmic reticulum of striated muscle cells (12, 33, 36), or the agranular reticulum in myeloid bodies of retinal pigment epithelial cells (34). They also bear some resemblance to the whorled membranes of "ergastoplasmic nebenkerns" (16), and to the flattened agranular membranes recently described in HeLa cells (9).

The static image of subsurface cisterns seen in electron micrographs may represent a dynamic one in the living system, and consequently the association of an SSC with a particular area of the



FIGURES 3 AND 4

Subsurface cisterns in spinal cord neurons.

FIGURE 3

A synaptic bouton containing mitochondria and vesicles lies against the neuronal plasmalemma (*p*). A markedly flattened subsurface cistern begins at the arrow and extends to the left. It is separated from the plasmalemma by a light zone of ~50 A. Two cisterns of the granular endoplasmic reticulum underlie the SSC and are surrounded by RNP granules. Parts of two myelinated nerve fibers, and a glial cell containing transversely cut filaments, can be seen above the bouton. × 70,000.

FIGURE 4

A mitochondrion is apposed to the deep surface of an SSC, whose lateral edges are indicated by the arrows. A light zone of ~55 A intervenes between the SSC and plasmalemma. The overlying synaptic bouton (containing vesicles) extends beyond the limit of the SSC at the right, but terminates short of it at the left. Part of a second bouton is present at the extreme left. × 70,000.

neuronal plasmalemma may be transient rather than permanent. It would be interesting to know whether physiological or metabolic activity of the neuron has any effect on the sites at which SSC's appose the cell membrane or on the duration of the apposition.

Whatever the dynamics of this association are, it is appropriate to explore some of the effects which SSC's might have on the plasmalemma, and how they might therefore be involved in the normal functioning of the neurons.

1. *Modification of Cell Membrane Properties*

The fact that in electron micrographs each subsurface cistern is invariably separated from the overlying plasmalemma by a narrow light zone does not mean that the two structures are entirely separate from each other, or that this light zone is devoid of structure. In other locations there is reason to believe that narrow light zones in electron micrographs are highly structured regions, but are of low electron opacity. For example, the light bands in compact myelin sheaths are, undoubtedly, the site of radially arranged lipid molecules which are closely packed together (38). It is, therefore, possible that all or part of the light zone which separates each SSC from the plasmalemma is structured, or is occupied by a "cementing material" (43), and that there is, in fact, very little "space" between them. In addition, minute bridges may traverse this light zone, but are not seen because their dimensions are much smaller than the thickness of the sections used for electron microscopy, and their electron opacity is not great enough to delineate them from the relatively great thickness of surrounding embedding plastic. Whether or not the SSC and plasmalemma are structurally connected, they are closely enough apposed so that attractive and repulsive forces of an electrical and chemical nature undoubtedly operate between them (30).

The constancy in the width of the light zone between an SSC and its adjacent plasmalemma, and the faithfulness with which the two structures follow each other as they undulate, support the belief that SSC's are either structurally joined to the overlying plasmalemmas, or are bound to them by forces which operate across distances of ~50 to 80 A.

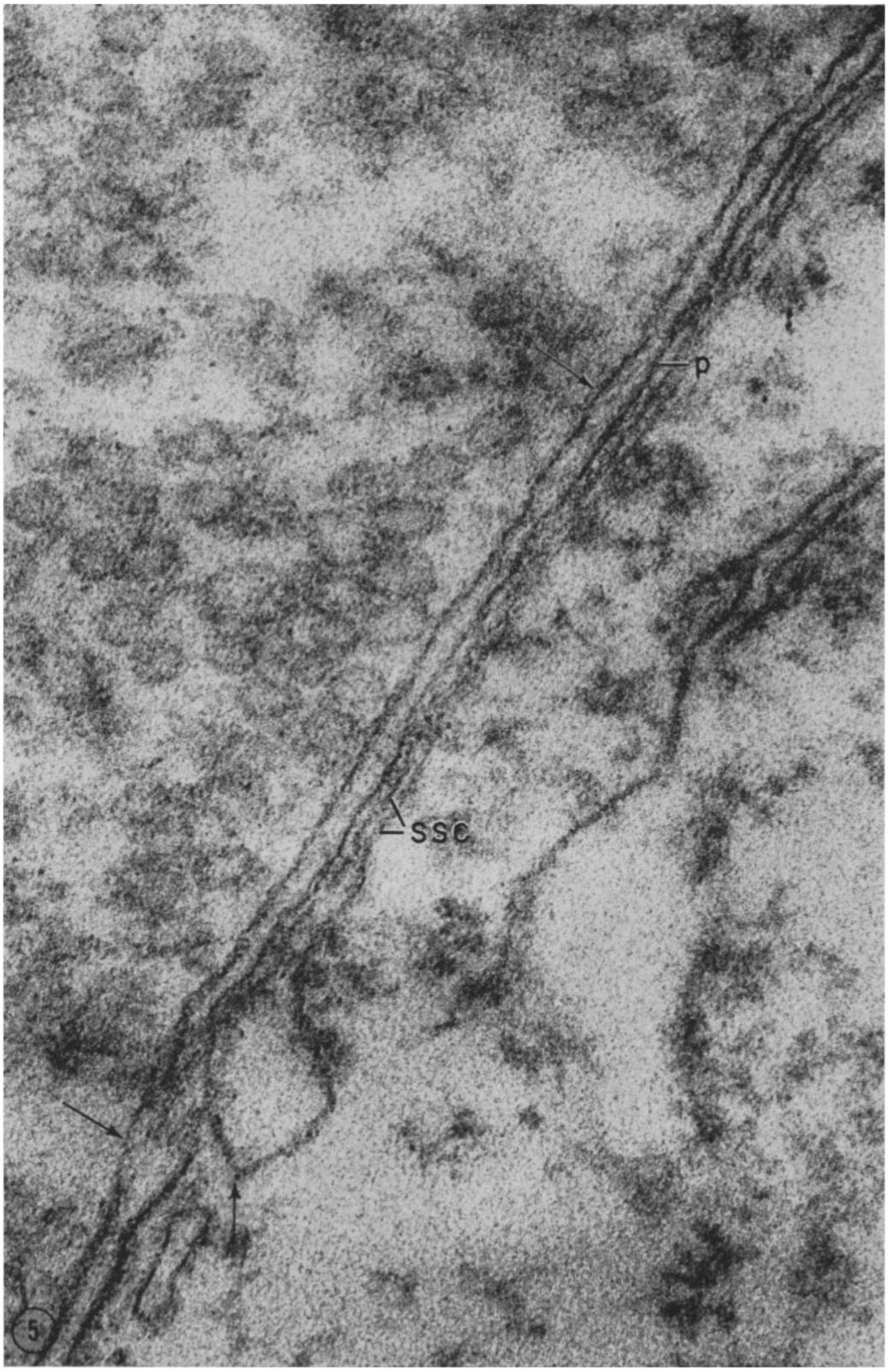
It would be reasonable to expect that in the areas where subsurface cisterns occur the properties of the neuronal surface are a function not of the plasmalemma alone, but of the composite consisting of the plasmalemma and associated SSC. The neuronal surface may, therefore, be thought of as containing patches occupying a small percentage of the total surface area, which may differ functionally as well as structurally from the remainder of the neuronal surface. Although nerve cell membranes are usually assumed to be uniform with respect to certain electrical properties (4, 35), there is some precedent, based on physiological observations, for considering neuronal membranes to be non-uniform with respect to excitability (2, 15), threshold (6), and resistance (11).

The effect of an extra set of membranes underlying the plasmalemma would depend on the extent to which they interact with the plasmalemma. Forces acting between closely apposed membranes may serve to deform or to decrease stability in the structures of the respective membranes, or to otherwise modify the chemical bonds within each of them. The properties of a membrane may depend not only on its structure and composition, but also on its interaction with neighboring, closely apposed membranes. Such an interaction could serve to bring about profound alterations in the properties of the neuronal surface overlying SSC's, even though the basic structure and the chemical composition of the plasma membrane *per se* were no different in these areas than in adjacent portions of the plasmalemma.

The degree of interaction between the SSC and the plasmalemma could also be variable depending

FIGURE 5

Subsurface cistern in a spinal cord neuron. The vertical arrow indicates the bulbous lateral edge of the SSC, which, just above this region, has a few RNP granules associated with its deep surface. Diagonal arrows indicate the limiting membrane of an overlying synaptic bouton containing innumerable vesicles. At the level of the upper diagonal arrow there is a faint intermediate line in the ~60 A cleft between the SSC and the neuronal plasmalemma (p). At the level of the lower diagonal arrow the plasmalemma of the neuron appears to be pulled away from that of the bouton. × 205,000.


p
ssc
5

upon external factors such as the potential across the plasmalemma, or its ionic milieu. The properties of the patch overlying the SSC might then vary with alterations in these parameters.

If there were no interaction between an SSC and the associated plasmalemma, it would be expected that their properties would be additive. The SSC would then constitute simply an additional barrier to the passage of ions and other materials across certain areas of the neuronal surface (perhaps strategically located), so that permeability in these regions would be decreased.

Although the specific properties of the membranes of subsurface cisterns (and of the endoplasmic reticulum in general) are not known, it is possible that, like plasma membranes, they too exhibit selective permeability, and that differences in ionic concentrations and in potential exist between the inside and the outside of these intracellular, membrane-limited compartments. A separation of charges by the limiting membranes of an SSC would lead to capacitive binding of ions at its surfaces. There may also be selective adsorption of specific ions at the surfaces of these intracellular membranes. (It has been suggested (32) that components of the sarcoplasmic reticulum may play a role in the binding of calcium ions.) In either case the effect of such a membrane in the subsurface position would be to alter the mobility and effective concentration of ions at the inner surface of the plasma membrane, and this might secondarily affect the electrophysiological behavior of the neuronal surface in this region.

These various possible effects of subsurface cisterns on the properties of the neuronal surface could have important neurophysiological consequences. For example, if the patches of neuronal surface associated with SSC's are sites of low transneuronal resistance, it would be expected that current flow produced by synaptic depolarization elsewhere on the cell would be channeled selectively through these areas. It would also follow that specific membrane resistance in the perikaryon as a whole would be lower than in the distal portions of the dendrites, since the latter do not exhibit SSC's. It is not fruitful to explore these possibilities in detail until more information is available concerning the effects of close apposition of charged membranes on their permeability, stability, and electrical properties. Data of this kind may be more readily obtainable from studies of physical models rather than biological systems. At the present time, it does seem reasonable to make the suggestion that patches of the neuronal surface may have special electrophysiological properties because of their association with SSC's.

2. *Metabolism of the Neuron*

The cytoplasmic volume of many neurons (including their axons and dendrites) is enormous, and undoubtedly the quantity of nutrients and

FIGURES 6 AND 7

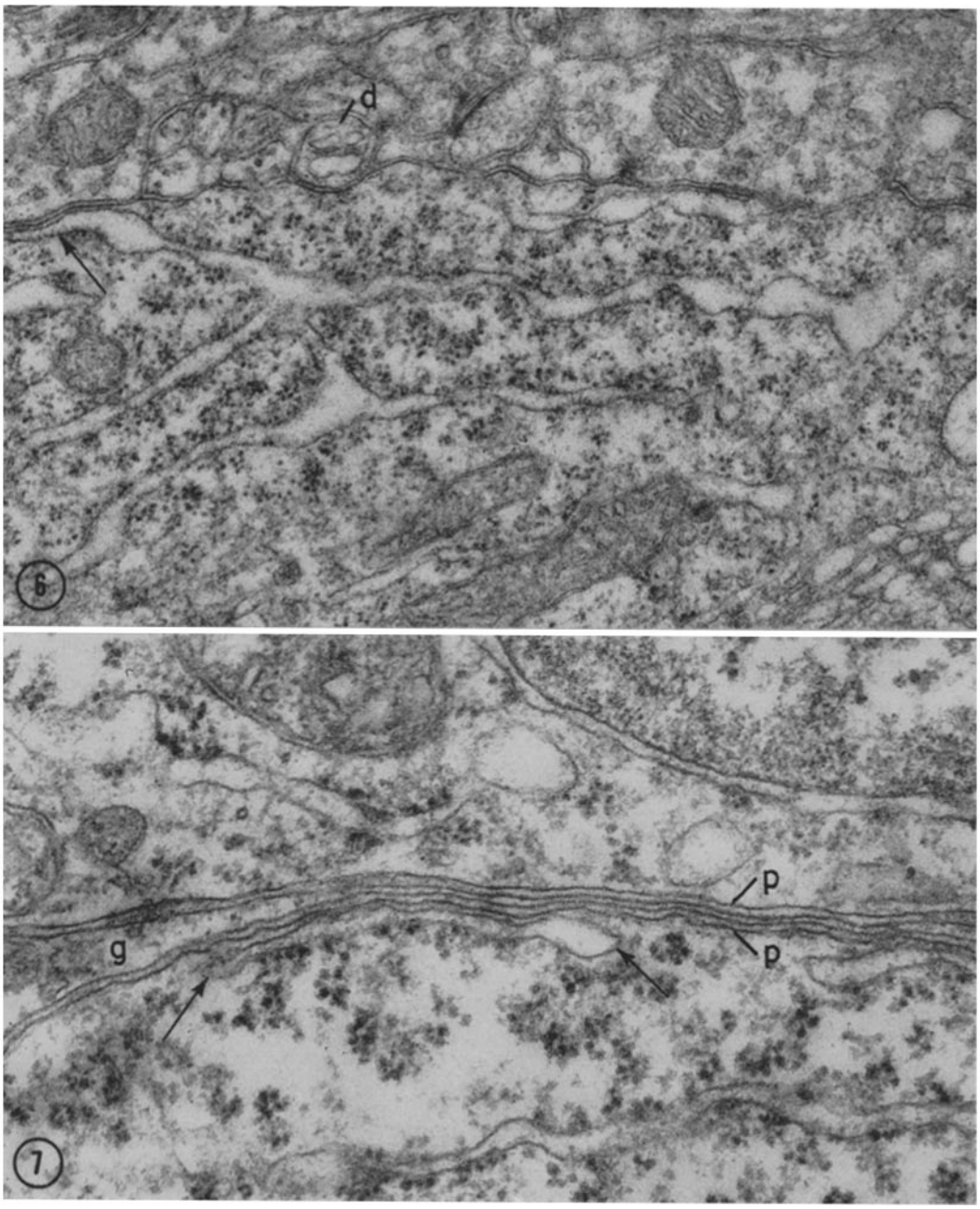
Subsurface cisterns in neurons of the cerebral cortex.

FIGURE 6

The neuron occupies the lower two-thirds of the figure. At the extreme left a subsurface cistern (arrow) lies under a synaptic bouton and is continuous at its right edge with a cistern of granular endoplasmic reticulum, which branches several times. A second SSC is present under a synaptic bouton near the right side of the figure. The luminal size of both SSC's is comparable to that of the deeper-lying granular ER. *d* indicates a dendritic spine containing spine apparatus. × 43,000.

FIGURE 7

Two adjacent neurons separated by an attenuated glial process (*g*). The lower neuron contains a subsurface cistern whose lateral edges are indicated by arrows. Its deep surface is studded by RNP granules. The edge of a second SSC appears at the right. *p* indicates the two neuronal plasmalemmas in a region where the four parallel membranes present could easily be mistaken for two *directly* apposed neuronal plasmalemmas plus a subsurface cistern. This illustrates the importance of identifying at least one of the lateral edges of an SSC in neurons of the central nervous system. × 64,000.


d
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g
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7

metabolites transferred across the plasma membranes of these cells is correspondingly high. A large proportion of this flux probably takes place in the region of the perikaryon since this is where most of the cell's metabolic machinery is concentrated. Subsurface cisterns may therefore represent a surface specialization concerned with the flux of materials which are involved in the general metabolism of the cell. One possibility is that materials entering the neuron in the region of SSC's could be channeled directly into the granular ER by way of its connections with SSC's.

In addition, the neuronal plasmalemma is involved in other metabolic activities which are prominent in excitable cells, *i.e.* the extrusion of sodium ions, and the restoration of membrane integrity, following excitation or subthreshold synaptic stimulation. Both the subsurface cisterns and the stacks of flattened, agranular cisterns deeper in the cytoplasm may serve as repositories of membrane material, which are utilized when the cell is active. This function has been proposed for Golgi membranes in other locations (10).

Subsurface cisterns may also serve to bring specific materials into contact with the deep surface of the neuronal plasmalemma. Enzymes, metabolites, or ions associated with membrane activity or reconstitution, or with sodium pumping, may be synthesized, stored, or concentrated by the endoplasmic reticulum and then conveyed through its lumen to their site of utilization at the cell surface. The close association which sometimes can be seen between SSC's and mitochondria also suggests a metabolic function for the SSC's. It is possible that those areas in which there is close contact between SSC's and mitochondria may be

FIGURE 8

Neuron from the cerebral cortex showing a highly flattened SSC whose lateral edges are indicated by arrows. The cistern of the endoplasmic reticulum (*ER*) lying ~250 to 300 A beneath the SSC is studded with RNP granules on its deep surface only. The process overlying the neuronal plasmalemma (*p*) is probably glial. It contains a large mitochondrion (*m*) running across the top of the figure. A capillary (not shown) is situated above this process. × 102,000.

FIGURE 9

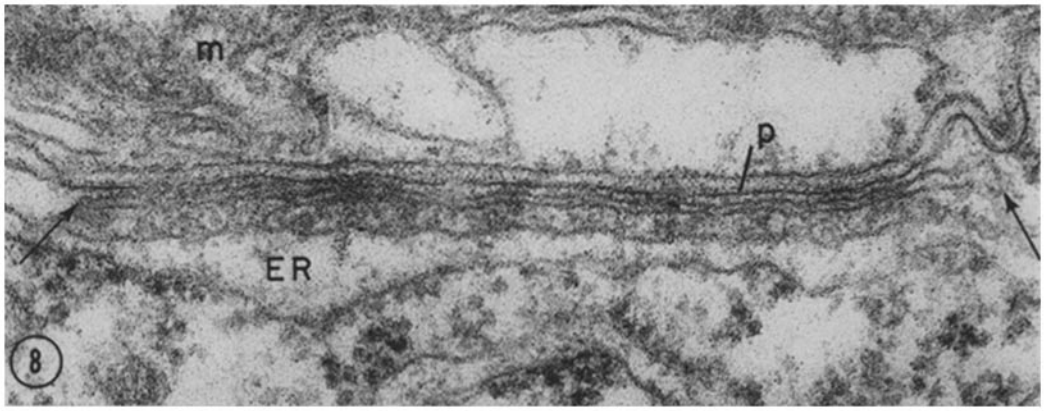
Neuron from the cerebral cortex showing a stack of flattened cisterns in the subsurface position. The arrow indicates the plasma membrane of a glial process, beneath which lies the neuronal plasmalemma. Four highly flattened cisterns, and a fifth one which is slightly more dilated, underlie the neuronal membrane in this region. Some of these cisterns connect with the granular endoplasmic reticulum at their lateral edges. A large mitochondrion is associated with this complex. × 35,000.

FIGURE 10

Neuron from the cerebral cortex showing another example of a stack of flattened cisterns in the subsurface position. The vertical arrow indicates the limiting membrane of a dendritic process directly apposed to the neuronal plasmalemma (*p*). Three highly flattened cisterns, and one which is more dilated, lie subjacent to the nerve cell membrane. The lateral edges of the upper two cisterns are indicated by horizontal arrows. Granular material, finer than RNP, intervenes between the successive cisterns, but not between the SSC and the plasmalemma. × 95,000.

FIGURE 11

A synaptic bouton (*b*) apposed to a neuron (*n*) in the cerebral cortex. An SSC, whose right edge is indicated by the arrow, underlies most of the extent of the synaptic bouton. It is obvious, however, that the bouton is making synaptic contact with a dendritic spine (*d*) lying above it. Vesicles cluster against the axodendritic junction and an intermediate line is present in the synaptic cleft. Spine apparatus (sectioned tangentially) is present at the top of the dendritic spine. × 49,000.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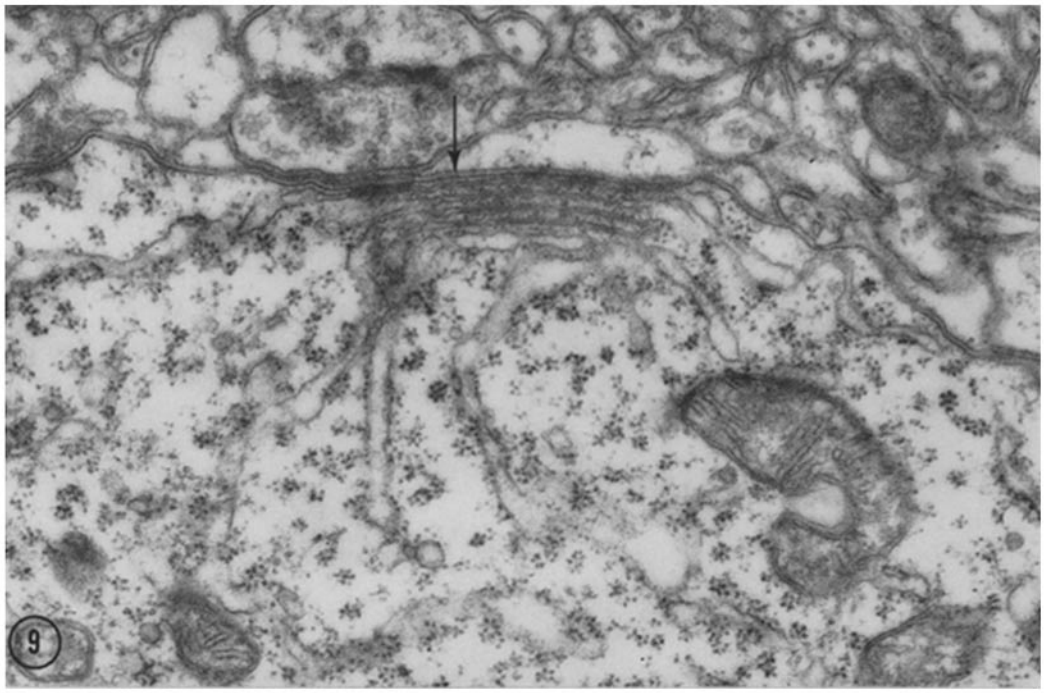
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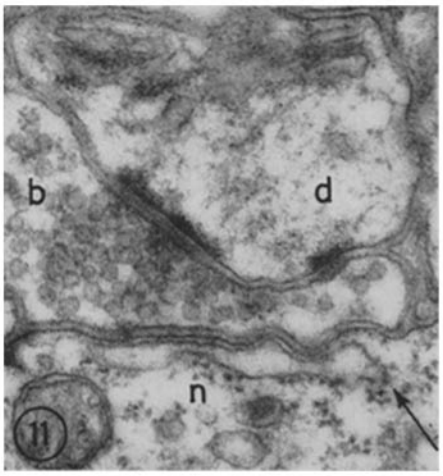
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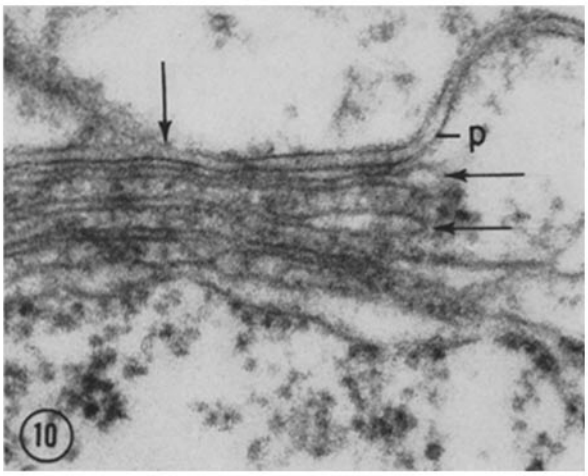
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regions of altered permeability of their respective membranes and that transfer of materials is facilitated in this location.

Counterparts of Subsurface Cisterns in Other Cells

The first description of a structure which is apparently identical to the subsurface cisterns described here was made by Engström (8). He observed that at the bases of outer hair cells of the organ of Corti an extra pair of membranes occurred within the hair cell just under the plasmalemma opposite to an axon terminal. Although he did not describe the pair of membranes as a closed sac, this observation was later made by Smith and Sjöstrand (43), who performed serial sectioning. They noted also that the "membrane pair" occupies approximately the same area of the hair cell plasmalemma as does the associated axon terminal.

Similar structures also occur in certain muscle cells of vertebrates and invertebrates. Porter and Palade (33) in their study of the sarcoplasmic reticulum of vertebrate striated muscle cells found examples of large membranous cisterns in close association with specific regions of the sarcolemma. Bennett (1) has suggested that these structures may underlie "sensitive spots" on the sarcolemma through which stimuli are able to enter the cell to produce localized contraction of sarcomeres (20). The concept of non-uniformity of the sarcolemma was also proposed by Edwards *et al.* (7), who suggested that a kind of saltatory conduction might occur along the surface of muscle cells, with active areas over the Z lines. Large vesicular structures also occur just under the cell membranes of oyster adductor muscle fibers, and these, too, have been implicated in the transmission of impulses into the depths of the cells (18).

A parallel may also be drawn between subsurface cisterns and the lateral elements of the "triads" of sarcoplasmic reticulum, which are prominent in certain muscle cells (12, 36). Although the triads occur deep in the sarcoplasm, it has been suggested that the middle component, or intermediary vesicle, of the triad may be continuous with the sarcolemma (32). In certain insect muscles "dyads" rather than triads occur, and here it is clear that one of the components of the dyad is indeed a deep infolding of the sarcolemma (44). The second component consists of a flattened vesicle of ER closely apposed to it. The resemblance between the configuration of the dyad and that of the SSC and associated neuronal plasmalemma is striking.

A role in the intracellular transmission of impulses has been ascribed to these highly organized elements of the sarcoplasmic reticulum (29) in order to account for the uniform contraction of myofibrils across the diameter of large muscle fibers (19). It has also been suggested that the sarcoplasmic reticulum may be concerned with the synthesis or distribution of metabolites (12). The morphologic similarity of the triads and dyads of muscle to the SSC's and associated plasma membrane of nerve suggests comparable functions in the two instances. A review of the history and present status of the sarcoplasmic reticulum in relation to muscle physiology is presented in a recent article by Porter (32).

Instances of subsurface cisterns have also been found in pineal gland cells (47), whose function is not known. Yamada (49) has published an illustration of "tubular endoplasmic reticulum closely applied to the surface of the inner segment" of cone cells from the turtle retina. It is suggested by Yamada that a transfer of metabolites may take place between the cone and the adjacent glial cell in this location. Structures resembling SSC's have also been observed recently at the surfaces of parenchymal cells of the liver (5). They are, however, separated from the plasmalemma by a light zone which appears to be wider than that between SSC's and the neuronal plasma membrane. Structures comparable to SSC's have also been noted in smooth muscle cells in close association with axon terminals (37).

Subsurface cisterns may be a structural feature common to many cell types but well developed in only certain ones. So far they appear to be most prominent in cells which are engaged in the generation or conduction of electrical potential changes. However, they may ultimately turn out to be more specifically correlated with ion transport or binding, rapid membrane turnover, or metabolic peculiarities which also characterize these cells. Information about the occurrence, size, and distribution of SSC's in other cells which are not primarily engaged in electrogenesis or conduction would help to clarify the functional significance of this structure.

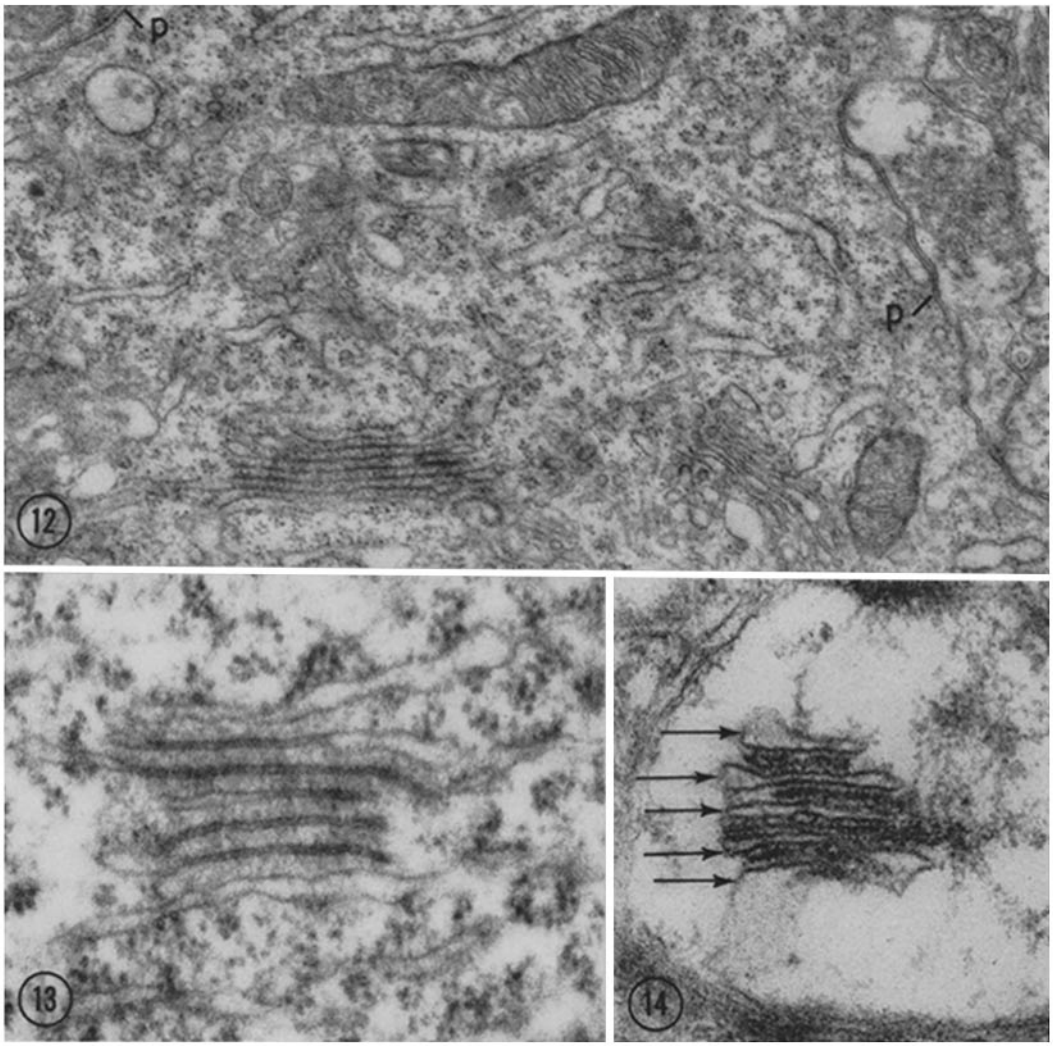

FIGURE 12

A stack of flattened cisterns lying deep in the cytoplasm of a neuron from the cerebral cortex. Their lumina are so narrow as to be almost indistinguishable at this magnification. These cisterns are not fenestrated or branched, and are separated by a constant interval of ~250 to 300 A. The configuration of this complex is distinctly different from that of the Golgi membranes, at the lower right, and of the granular endoplasmic reticulum scattered throughout the cytoplasm. *p*, neuronal plasmalemma. × 32,000.

FIGURE 13

A stack of flattened cisterns deep in the cytoplasm of a vestibular ganglion cell. Their lumina are ~30 to 40 A in depth, and they are spaced at ~250 to 300 A intervals. Some of these cisterns are continuous with granular ER at their lateral edges. × 75,000.

FIGURE 14

Spine apparatus from a dendritic spine in the cerebral cortex. The complex is composed of five flattened agranular cisterns whose left lateral edges are indicated by arrows. Each pair of cisterns is separated by a light zone of ~250 to 300 A, containing a dense intermediate line which is ~100 A thick. There appear to be many fine cross-bridges between the cisterns and the intermediate lines. × 82,000.

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REFERENCES

1. BENNETT, H. S., The structure of striated muscle as seen by the electron microscope, *in* The Structure and Function of Muscle, (G. H. Bourne, editor), New York, Academic Press, Inc., 1960, **1**, 137.
2. BENNETT, M. V. L., CRAIN, S. M., and GRUNDFEST, H., Electrophysiology of supramedullary neurons in *Spheroides maculatus*. II. Properties of the electrically excitable membrane, *J. Gen. Physiol.*, 1959, **43**, 189.
3. BRIGHTMAN, M., personal communication.
4. COOMES, J. S., CURTIS, D. R., and ECCLES, J. C., The electrical constants of the motoneurone membrane, *J. Physiol.*, 1959, **145**, 505.
5. DAVID, H., Zur Morphologie der Leberzellmembran, *Z. Zellforsch. u. mikr. Anat.*, 1961, **55**, 220.
6. ECCLES, J. C., The Physiology of Nerve Cells, Baltimore, The Johns Hopkins Press, 1957.
7. EDWARDS, G. A., RUSKA, H., SANTOS, P. DE S., and VALLEJO-FREIRE, A., Comparative cytophysiology of striated muscle with special reference to the role of the endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 143.
8. ENGSTRÖM, H., On the double innervation of the sensory epithelia of the inner ear, *Acta Otolaryng.*, 1958, **49**, 109.
9. EPSTEIN, M. A., Some unusual features of fine structure observed in HeLa cells, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 153.
10. FARQUHAR, M., WISSIG, S. L., and PALADE, G. E., Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall, *J. Exp. Med.*, 1961, **113**, 47.
11. FATT, P., Sequence of events in synaptic activation of a motoneurone, *J. Neurophys.*, 1957, **20**, 61.
12. FAWCETT, D. W., and REVEL, J. P., The sarcoplasmic reticulum of a fast-acting fish muscle, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, No. 4, suppl., 89.
13. GRAY, E. G., Axo-somatic and axo-dendritic synapses of the cerebral cortex, *J. Anat.*, 1959, **93**, 420.
14. GRAY, E. G., Ultra-structure of synapses of the cerebral cortex and of certain specialisations of neuroglial membranes, *in* Electron Microscopy in Anatomy, London, Edward Arnold Ltd., 1961, 54.
15. GRUNDFEST, H., Electrical inexcitability of synapses and some consequences in the central nervous system, *Physiol. Rev.*, 1957, **37**, 337.
16. HAGUENAU, F., The ergastoplasm: its history, ultrastructure and biochemistry *in* International Review of Cytology, (Bourne, G. H. and Danielli, J. F., editors), New York, Academic Press, Inc., 1958, **7**, 425.
17. HAMA, K., Some observations on the fine structure of the giant nerve fibers of the earthworm, *Eisenia foetida*, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 61.
18. HANSON, J., and LOWY, J., The structure of the muscle fibres in the translucent part of the adductor of the oyster *Crassostrea angulata*, *Proc. Roy. Soc. London, Series B*, 1961, **154**, 173.
19. HILL, A. V., The abrupt transition from rest to activity in muscle, *Proc. Roy. Soc. London, Series B*, 1949, **136**, 399.
20. HUXLEY, A. F., and TAYLOR, R. E., Local activation of striated muscle fibres, *J. Physiol.*, 1958, **144**, 426.
21. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
22. PALADE, G. E., The endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 85.
23. PALADE, G. E., Blood capillaries of the heart and other organs, *Circulation*, 1961, **24**, 368.
24. PALAY, S. L., Synapses in the central nervous system, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 193.
25. PALAY, S. L., unpublished data.
26. PALAY, S. L., and PALADE, G. E., The fine structure of neurons, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 69.
27. PALAY, S. L., MCGEE-RUSSELL, S. M., GORDON, S. JR., and GRILLO, M. A., Fixation of neural tissues for electron microscopy by perfusion with solutions of osmium textroxide, *J. Cell Biol.*, 1962, **12**, 385.
28. PAPPAS, G. D., AND PURPURA, D. P., Fine structure of dendrites in the superficial neocortical neuropil, *Exp. Neurol.*, 1961, **4**, 507.
29. PEACHEY, L. D., and PORTER, K. R., Intracellular impulse conduction in muscle cells, *Science*, 1959, **129**, 721.
30. PETHICA, B. A., The physical chemistry of cell adhesion, *Exp. Cell Research*, 1961, suppl. **8**, 123.
31. PORTER, K. R., The submicroscopic morphology

of protoplasm, *in* The Harvey Lectures, New York, Academic Press, Inc., 1957, 175.

32. PORTER, K. R., The sarcoplasmic reticulum. Its recent history and present status, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, No. 4, suppl., 219.
33. PORTER, K. R., and PALADE, G. E., Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.
34. PORTER, K. R., and YAMADA, E., Studies on the endoplasmic reticulum. V. Its form and differentiation in pigment epithelial cells of the frog retina, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 181.
35. RALL, W., Branching dendritic trees and motoneuron membrane resistivity, *Exp. Neurol.*, 1959, **1**, 491.
36. REGER, J. F., The fine structure of neuromuscular junctions and the sarcoplasmic reticulum of extrinsic eye muscles of *Fundulus heteroclitus*, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, No. 4, suppl., 111.
37. RICHARDSON, K. C., personal communication.
38. ROBERTSON, J. D., The ultrastructure of cell membranes and their derivatives, *Biochem. Soc. Symp.*, 1959, **16**, 3.
39. ROSENBLUTH, J., The fine structure of acoustic ganglia in the rat, *J. Cell Biol.*, 1962, **12**, 329.
40. ROSENBLUTH, J., unpublished data.
41. ROSENBLUTH, J., and PALAY, S. L., Electron microscopic observations on the interface between neurons and capsular cells in dorsal root ganglia of the rat, *Anat. Rec.*, 1960, **136**, 268.
42. ROSENBLUTH, J., and PALAY, S. L., The fine structure of nerve cell bodies and their myelin sheaths in the eighth nerve ganglion of the goldfish, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 853.
43. SMITH, C. A., and SJÖSTRAND, F. S., Structure of the nerve endings on the external hair cells of the guinea pig cochlea as studied by serial sections, *J. Ultrastructure Research*, 1961, **5**, 523.
44. SMITH, D. S., The structure of insect fibrillar flight muscle, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, No. 4, suppl., 123.
45. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.
46. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.
47. WOLFE, D. E., unpublished data.
48. WOOD, R. L., Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 343.
49. YAMADA, E., Observations on the fine structure of photoreceptive elements in the vertebrate eye, *J. Electronmicroscopy*, 1960, **9**, 1.