MORPHOLOGICAL CORRELATES OF INCREASED COUPLING RESISTANCE AT AN ELECTROTONIC SYNAPSE

GEORGE D. PAPPAS, Y. ASADA, and M. V. L. BENNETT

From the Department of Anatomy and the Rose Fitzgerald Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Yeshiva University, New York 10461. Dr. Asada's present address is the Department of Neuropsychiatry, Osaka University Medical School, Asahi-Machi, Abenoku, Osaka, Japan.

ABSTRACT

Close appositions between axonal membranes are present in the septum between adjacent axonal segments of the septate or lateral giant axons of the crayfish *Procambarus*. In sections the closely apposed membranes appear separated by a space or gap. The use of lanthanum indicates that there may be structures connecting the apposed membranes. The apparent gap is actually a network of channels continuous with the extracellular space. Adjacent axonal segments are electrotonically coupled at the septa. The coupling resistance is increased by mechanical injury of an axon, immersion in low Cl^- solutions, and immersion in low Ca^{++} solutions, followed by a return to normal physiological solution. Septa at which coupling resistance had been measured were examined in the electron microscope. The induced increases in coupling resistance are associated with separation of the junctional membranes (with the exception of the moderate increases during immersion in low Ca^{++} solutions). Schwann cell processes are present between the separated axonal membranes. When nerve cords in low Cl^- solutions are returned to normal physiological solution, coupling, i.e., electrotonic synapses. A model of an electrotonic synapse is proposed in which tween axonal membranes are again found. The association between the morphological and physiological findings provides further evidence that the junctions are the sites of electrotonic coupling, i.e., electrotonic synapses. A model of an electrotonic synapse is proposed in which intercytoplasmic channels not open to the extracellular space are interlaced with a hexagonal network of extracellular channels between the apposed junctional membranes.

INTRODUCTION

The preceding paper describes several treatments that increase coupling resistance at septa of the crayfish lateral giant axon and thereby reduce electrotonic spread between axonal segments (1). Mechanical injury of an axonal segment, immersion in certain low Cl^- solutions, and immersion in low Ca^{++} solutions, followed by a return to normal saline all increase the resistance that is measured between cytoplasms of adjacent segments. The present study was undertaken to determine the morphological correlates of these changes, as well as to clarify the normal morphology (21). An important aspect of this study is that a particular septum that has been directly shown to have changed its physiological properties can be examined with the electron microscope. The evidence indicates that the sites of electrotonic coupling are the regions of close apposition of axon membranes that occur scattered over the septum. It was found that the major increases in coupling resistance are associated with separation of these appositions, and that recovery of coupling is as-

sociated with recovery of the normal junctional relationships. Preliminary results of this work have been reported elsewhere (27).

MATERIALS AND METHODS

The ventral nerve cord of the crayfish *Procambarus* was dissected out and placed in crayfish physiological solution at room temperature as described in the preceding paper (1). The morphological correlates of changes in coupling resistance were evaluated from the same septa at which coupling resistance had been measured physiologically. This included five nerve cords in which an axon segment was mechanically injured, ten nerve cords from experiments in which Na propionate was substituted for NaCl, and five preparations from low Ca^{++} experiments. During the physiological measurements the preparations were kept in a perfusion chamber at room temperature for up to 4 hr. At the termination of the electrical measurements, the preparation was removed and placed in a vial containing fixative at room temperature. For control material, the nerve cord was removed from the abdomen and immediately immersed in fixative. Satisfactory preservation was obtained with both procedures.

A variety of fixatives were employed. Usually a 2.5% glutaraldehyde solution in Sorensen's phosphate buffer, pH 7.3, was used as the primary fixative. The tissue was fixed for 2–3 hr, either at room temperature or in the cold (4°C). The tissue was then rinsed 6–8 times in cold phosphate buffer and left in the refrigerator overnight and sometimes for 2–3 days. A cold 1% OsO_4 solution in phosphate buffer (pH 7.3) was then used for postfixation for 1–2 hr. In some experiments osmium tetroxide solution was used at room temperature as the only fixative. Also, 1.8% $KMnO_4$ in phosphate buffer was used, as recommended by Robertson (32). Following fixation, tissue was dehydrated with ethanol, placed in propylene oxide which was changed twice at 5-min intervals, and embedded in Epon. Nerve cords were also fixed in the presence of lanthanum hydroxide and subsequently prepared for electron microscopy by the procedure of Revel and Karnovsky (31). Thin sections were cut with either an LKB or a Sorvall MT-2 microtome, stained with uranyl acetate and lead citrate, and examined in an RCA-EMU-3D or a Philips 200 electron microscope. Care was taken to examine the same septum that was used for the electrical measurements. Usually, several serial sections were examined from each of three to five different sites along the septum. Thicker sections (0.5–2 μ) were stained with toluidine blue and examined with a light microscope.

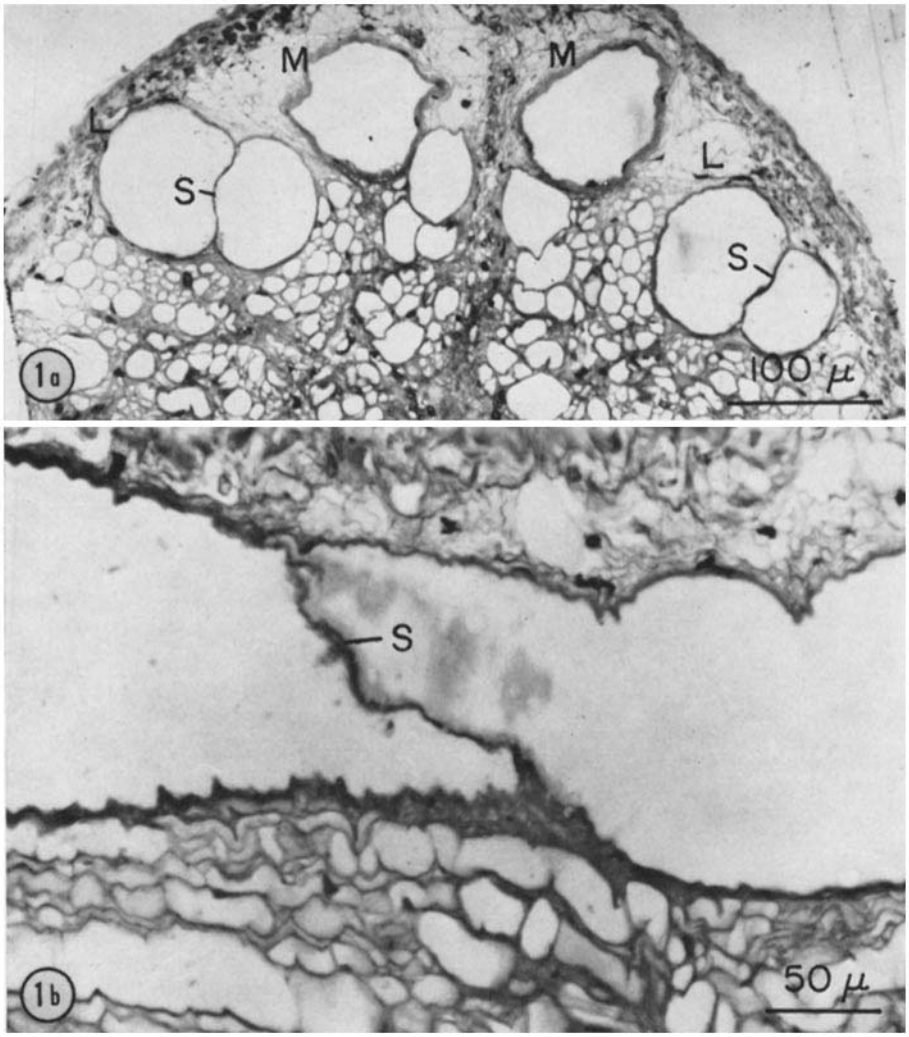
OBSERVATIONS

Normal Morphology

The lateral giant or septate axon is about 100–150 μ in diameter (Fig. 1 *a*). The septum separating adjacent segments runs obliquely and can extend for several hundred microns (Fig. 1 *b*). The septum formed by the two axons is about 2 μ thick over most of its area. In these regions the axons are separated by connective tissue fibrils and Schwann cell processes (Fig. 2 *a*). A central layer of regularly arranged connective tissue fibrils makes up about one-third to one-half of the thickness. Each side of the fibrillar layer is covered with Schwann cell processes that separate it from the axon membrane. Within the Schwann cell cytoplasm, anastomosing tubules are present. Examination of many sections revealed that these elements are connected to the plasma membrane and appear to open to the extracellular space. Holtzman et al. (22) have demonstrated peroxidase reaction product within similar anastomosing tubules in sheaths of peripheral axons from lobster, indicating that the tubules do communicate with the extracellular space.

Irregularly scattered over the septum are regions where both the fibrillar layer and the Schwann cell processes are interrupted (Figs. 2 *a* and 2 *b*) These "windows" are oval to circular in shape and have a diameter of 1–3 μ. A short process from one axon may extend through the "window" to become closely apposed to the other axon (21). Alternatively, a process may extend from each axon to meet the other. As will be considered in the Discussion, it can be concluded that these close appositions are the sites mediating electrotonic coupling between the axons, i.e., they are electrotonic synapses. The number of close appositions in a septum is estimated, from sections 2 μ in thickness, to be about 6–10. In these sections the windows appear as small regions in which the septum is greatly attenuated or is even interrupted.

The fine structural details and thickness of the sites of close apposition (i.e., the electrotonic synapses) depend on the methods of preparation. Furthermore, it is difficult to be sure that the apposing membranes are not viewed at a slight tilt that would increase their apparent thickness. The over-all thickness of the junctions, i.e., the distance from cytoplasm to cytoplasm, falls in the range of 130–200 A (Figs. 2 *c*, 3 *a* and 3 *b*). Fol-



The tissue shown in Figs. 2 *a*, 2 *b*, and 2 *c* was fixed with buffered OsO_4, while the glutaraldehyde-lanthanum technique followed by OsO_4 was used for the preparations in Figs. 3 *b* and 4. All of the other micrographs were prepared from tissue fixed in glutaraldehyde, followed by OsO_4.

FIGURE 1 *a* Light micrograph of a portion of a ganglion in the abdominal nerve cord of the crayfish. Near the dorsal surface are seen the paired medial giant axons (*M*) and the septate or lateral giant axons (*L*). The section passes through the septum (*S*) between axonal segments of the septate axon. × 200.

FIGURE 1 *b* Light micrograph of a longitudinal section through a septate axon. In this section the septum (*S*) runs obliquely and is about 150 μ in length, while the diameter of the axons is about 100 μ. × 300.

lowing $KMnO_4$ fixation, the apposed membranes appear thinner, falling in the lower range of 130–150 A. In OsO_4 fixation, as well as glutaraldehyde followed by OsO_4 postfixation, the thickness falls in the higher range of 150–200 A. In Fig. 2 *c* the over-all thickness of the axo-axonal junction is 150 A, including a clear zone of 35–40 A. These measurements contrast with the 300 A over-all thickness of ordinary Schwann cell-axon appositions where there is a clear zone of 75–120 A. In

very thin sections of glutaraldehyde–OsO_4–fixed tissue, unit membrane structure is evident and a 30 A space can be resolved between the two membranes (Fig. 3 *a*). The nature of this apparent region of separation was further studied by fixation in the presence of lanthanum as described by Revel, and Karnovsky (31). In this procedure lanthanum is a very fine-grained marker for extracellular space that is readily detected with the electron microscope. Lanthanum penetrates into the small space between the close appositions of the septum. In sections perpendicular to the apposing membranes, the space or gap appears to be uniformly filled with the dense lanthanum deposit. However, in sections more or less tangential, it is seen that the lanthanum does not form a uniform layer in the gap region; rather it forms a roughly hexagonal network surrounding electron-lucent regions (Fig. 4, also seen Fig. 3 *b*). The center-to-center spacing of these regions is about 100 A. Thus, it can be concluded that in the appositional region there are structures connecting the apposing membranes. The separation is actually a network of channels continuous with extracellular space, rather than a uniform gap.

In the cytoplasm of both apposing axons subadjacent to the junctional regions, round-to-elongated vesicles are present (21). Some vesicles can be seen to be interconnected and appear to be dilated elements of a smooth, tubular endoplasmic reticulum (Figs. 2 *b* and 2 *c*). The appearance in sections of round or elongated profiles of the vesicles does not vary greatly in osmium tetroxide-, glutaraldehyde-, or permanganate-fixed tissue.

Effects of Mechanical Injury

As described in the preceding paper, a large increase in coupling resistance at the septum follows mechanical injury to one of the axons forming the junctions. Septa at which coupling resistance had been increased by injury were examined and showed none of the close appositions that are found in normal tissue. The former locations of the synapses were identified by interruptions in the fibrillar layer of the septum at which synapses were invariably found in normal tissue. In these regions in the high resistance septa, the axons are separated by distinctive Schwann cell processes containing accumulations of anastomosing tubules (Figs. 5 *a* and 5 *b*). While similar tubules are present in Schwann cells in normal tissue, there is a pronounced increase in their number following injury. Examination of several serial sections from the former synaptic regions showed no direct axonal apposition at these sites. The absence of close appositions was a constant finding in all five of the septa studied at which coupling resistance had increased following injury. The contralateral, uninjured axons of the same nerve cords served as controls in these experiments, and appeared normal.

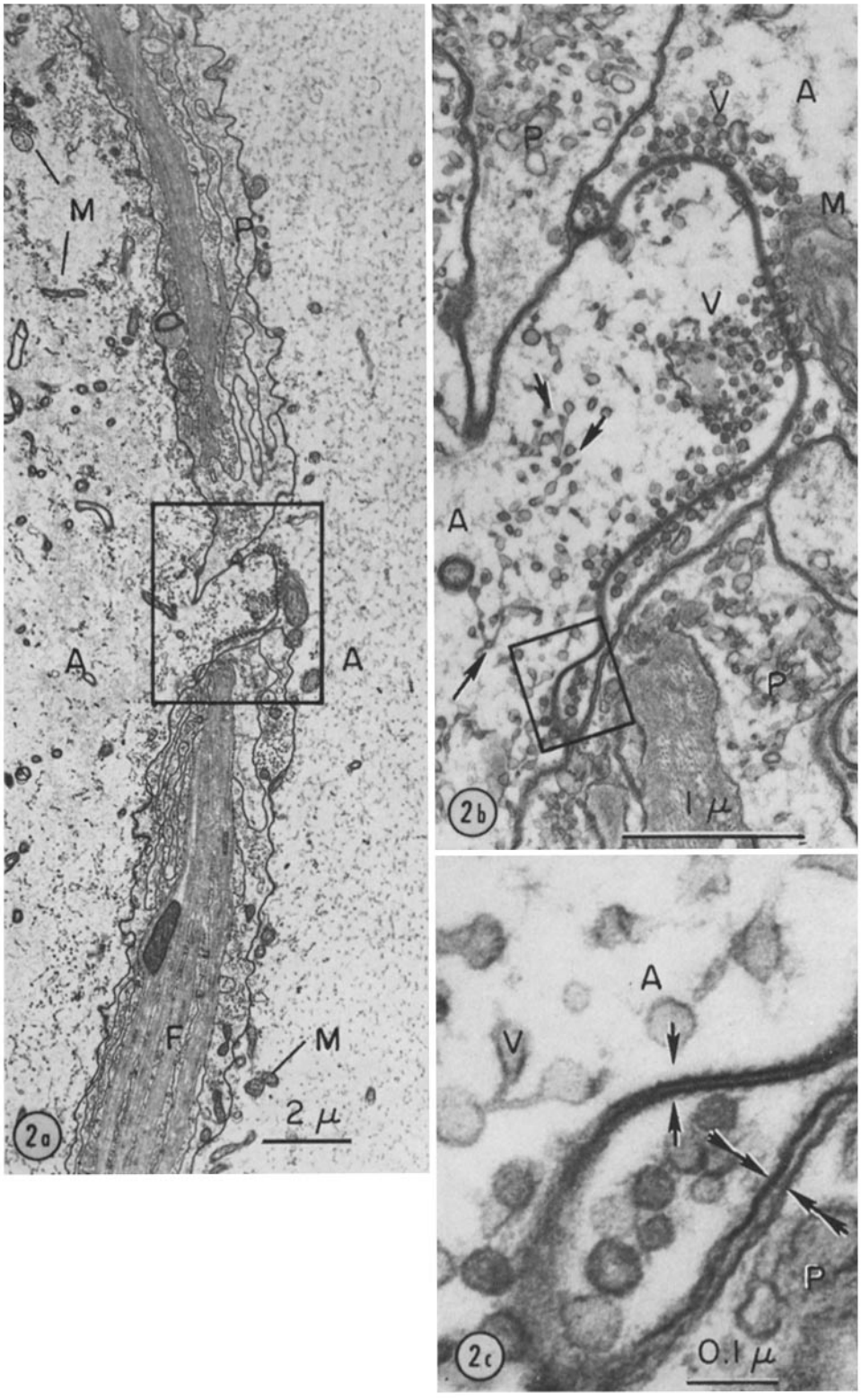
Effects of Propionate Solutions

Immersion in saline in which Cl^- is largely replaced by propionate causes a large increase in coupling resistance at the septa; the coupling resistance recovers its initial value upon return to normal saline as described in the preceding paper.

FIGURE 2 *a* Electron micrograph of a section through a septum of the septate axon. The septum contains a central layer of thin, regularly arranged connective tissue fibrils (*F*) surrounded by Schwann cell processes (*P*). At a synapse, the sheath structures are absent and the membranes of the apposing axonal segments (*A*) come into direct contact (in rectangle). *M*, mitochondria. × 6750.

FIGURE 2 *b* An enlargement of the synapse shown in Fig. 2 *a*. The two apposing axonal membranes are in close proximity. Vesicles (*V*) are present in both axons subadjacent to the junctional membranes. Some vesicles appear to be interconnected (at arrows). A region where the section appears perpendicular to the junctional membranes is outlined (rectangle) and enlarged in Fig. 2 *c*. The rest of the junctional area appears to have been sectioned obliquely. *A*, axon; *M*, mitochondria; *P*, Schwann cell processes. × 27,000.

FIGURE 2 *c* An enlargement of the area outlined in Fig. 2 *b*. The over-all thickness of the apposed axonal membranes is 150 A at the single arrows; a clear space of 35–40 A is present in this region. At the ordinary apposition between Schwann cell (*P*) and axon (double arrows) the over-all thickness is about 300 A and there is a clear space of 75–120 A. *A*, axon; V, vesicles. × 133,000.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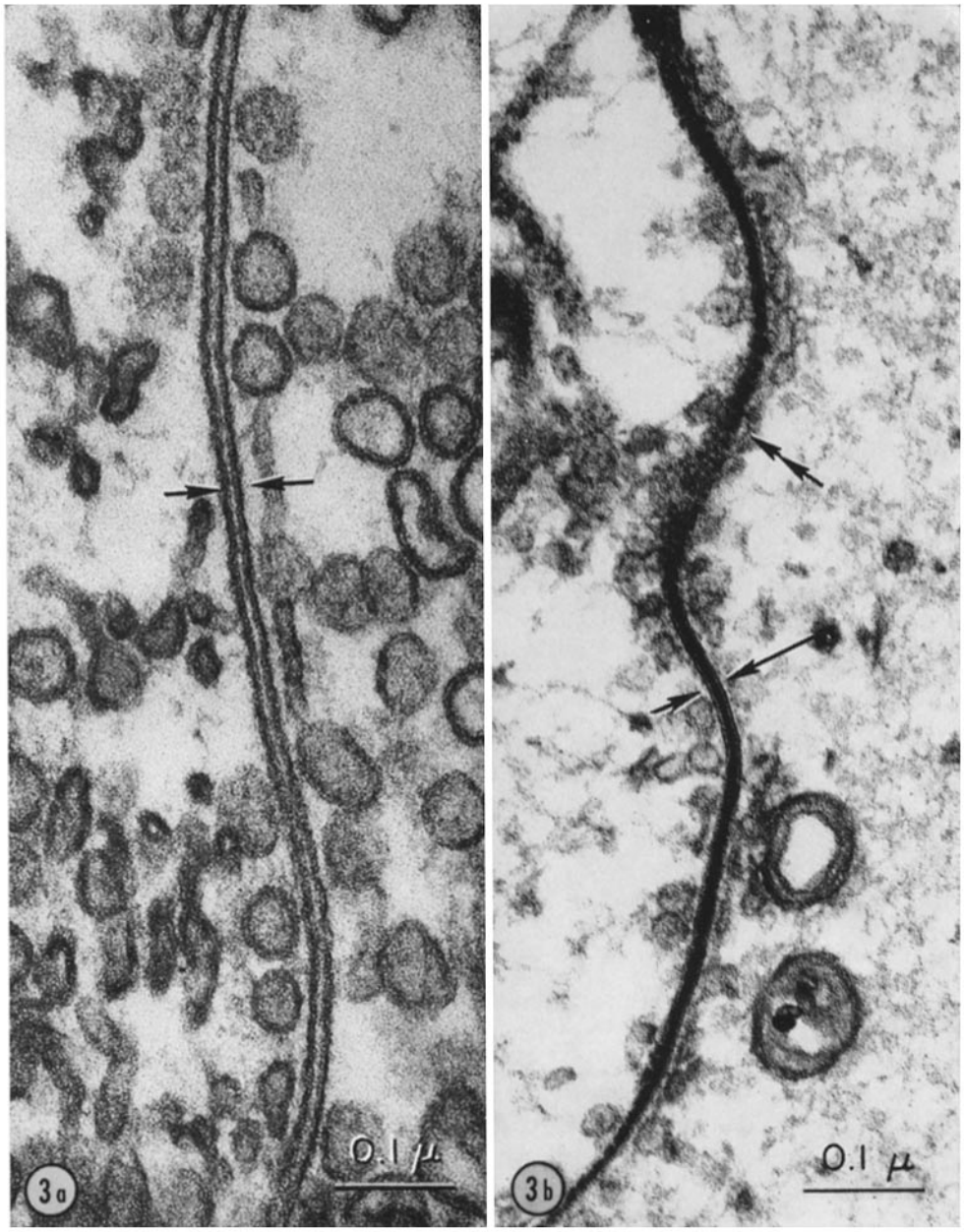


FIGURE 3 *a* High magnification of a very thin section through the junctional region. The over-all thickness of the junction is about 180 A. There is a central light area between the two unit membranes (arrows) which is about 30–40 A wide. × 160,000.

FIGURE 3 *b* Similar to Fig. 3 *a* but lanthanum was used in the fixative. Lanthanum has penetrated between the apposed junctional membranes. At the single arrows the over-all thickness of the junction is 165 A. At the double arrow is a region where the membranes are cut somewhat tangentially; in this region the lanthanum deposit appears not to be a uniform sheet. × 140,000.

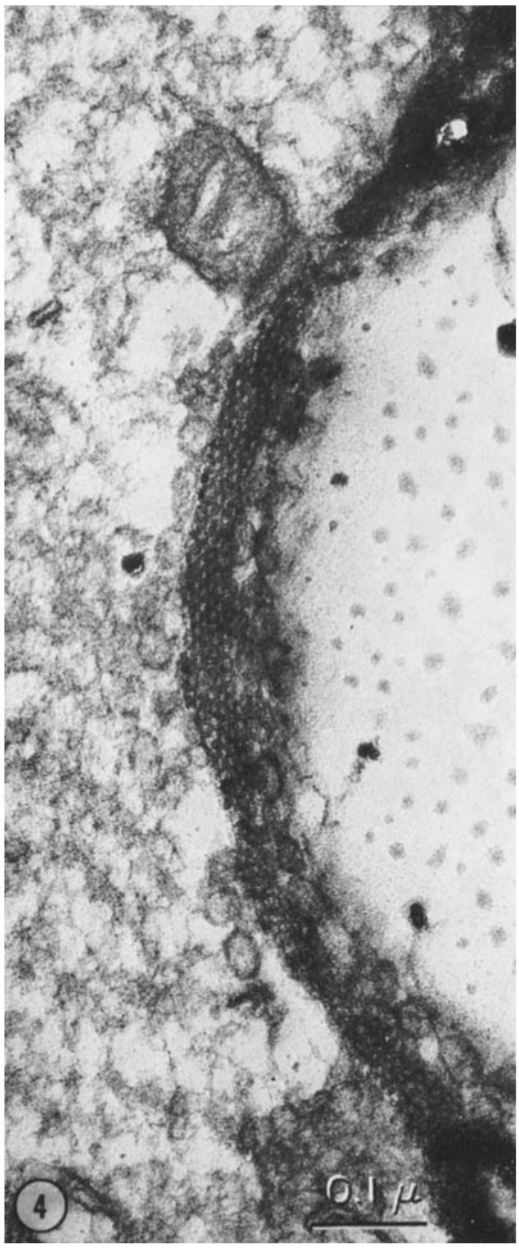


FIGURE 4 An approximately tangential section through the junctional region following fixation in the presence of lanthanum. The lanthanum deposit forms a network outlining a hexagonal array of clear regions. × 150,000.

Septa at which coupling resistance had been measured were fixed for electron microscopy while in the high resistance condition. Other preparations were fixed after recovery.

High resistance septa had many fewer of the close appositions than were found in normal tissue. As in septa at which coupling resistance had been increased by injury, the former sites of synaptic contact were identified by interruptions in the fibrillar layer of the septum. In these regions the axons are separated by distinctive Schwann cell processes that contain large accumulations of anastomosing tubules (Figs. 6 and 7), similar to those found in the injury preparations. At some points tubules are seen open to the extracellular space (at arrows in Fig. 7). Serial sections revealed that Schwann cell processes are interposed between the axons over the entire region in which the fibrillar layer is absent.

In no case was the entire septum sectioned, but in five high resistance septa all of the former synapses that were found had Schwann cell processes interposed between the axons. In only three of the eight high resistance septa examined, a few synapses were found where the apposing axonal membranes remained in close apposition. Since propionate treatment increases septal resistance by a factor of about six on the average, it is to be expected that some close appositions remain even in treated preparations.

Two of the propionate-treated preparations were fixed after they had been returned to physiological saline and the coupling resistance had returned to its normal low value. Close appositions like those of untreated axons were readily found in these septa which appear by and large normal (Fig. 8). However, the Schwann cell processes next to the synapses contain more tubular elements than control preparations, but apparently not as many as those septa in the high resistance condition in propionate (Figs. 6 and 7).

Effects of EDTA

Preparations were treated with 1–2 mM EDTA solutions as discussed in the previous paper. After 30 min in these solutions, two nerve cords were fixed and prepared for electron microscopy. Coupling resistance at the septa had increased about 3-fold prior to fixation. The distribution and appearance of the close appositions in these septa appeared normal (Fig. 9). If the preparations were returned to normal physiological solution after EDTA, the coupling resistance could increase irreversibly. Three preparations were fixed after 1½ hr in normal saline following EDTA treatment and prepared for electron microscopy. In two of

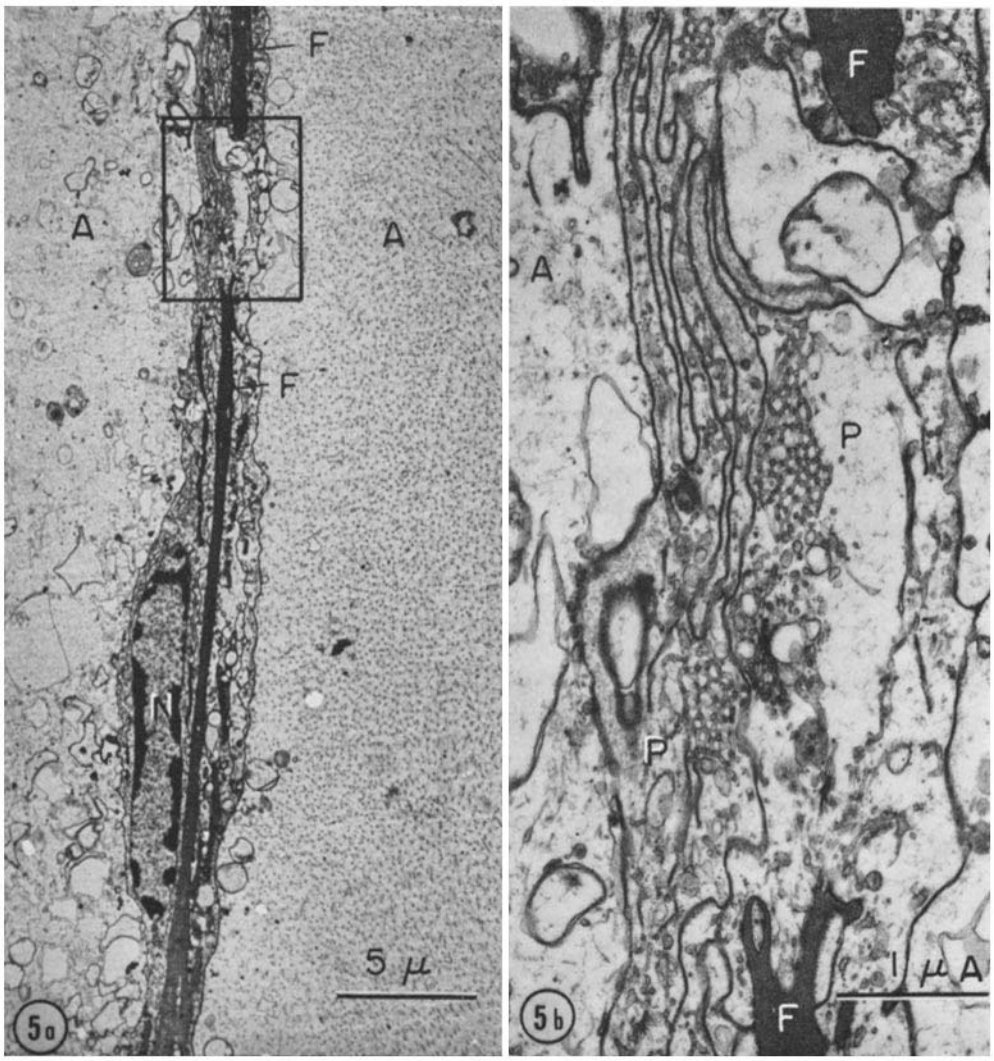


FIGURE 5 *a* Section through a septum following mechanical injury to the rostral axonal segment. The coupling resistance at the septum had greatly increased. The section contains an interruption in the fibrillar layer (*F*) of the septum (outlined in rectangle and enlarged in Fig. 5 *b*). In this region the membranes of the adjacent axons (*A*) are separated by intervening Schwann cell processes. In normal preparations interruptions of the fibrillar layer are always associated with close appositions between axons. *A*, axons; *F*, fibrillar layer; *N*, nucleus of Schwann cell. × 3800.

FIGURE 5 *b* An enlargement of the area outlined in Fig. 5 *a*. The interposed Schwann cell processes (*P*) have anastomosing tubular inclusions. *A*, axons; *F*, fibrillar layer. × 20,000.

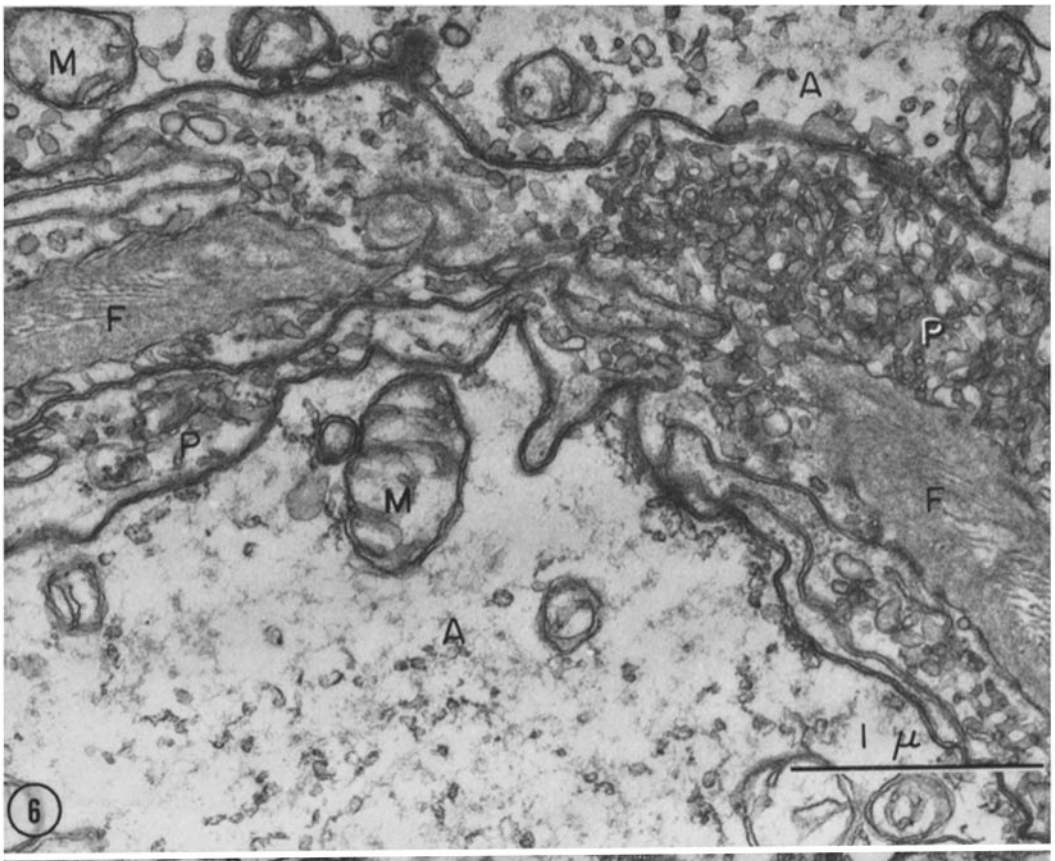
these preparations the coupling resistance had increased 11- and 50-fold; no direct axonal membrane appositions were found in the septa. Schwann cell processes were interposed between the axons at these sites as following other treatments that increased septal resistance (Figs. 10 and 11). In the third preparation the coupling resistance had increased about 3.5-fold over the initial value. Two normal-appearing synapses were found. The axonal membranes were directly apposed without Schwann cell processes separating them.

DISCUSSION

It has been inferred in a number of cases that junctions formed by closely apposing plasma membranes represent the morphological basis of electrotonic coupling between cells (2, 7, 13, 25, 28, 32). Previously, electrotonic junctions were considered to be characterized by fusion of the apposing plasma membranes, completely obliterating the extracellular space between them (13, 28). This criterion has been called into question by recent studies involving a more rigorous examination of very thin sections. A narrow region of extracellular space has been described separating the closely apposed membranes of junctions between a number of electrotonically coupled cells (15, 31). The appearance in sections perpendicular to the membranes is of a uniform 20–30 A gap. This space can be filled with electron-opaque markers for extracellular space such as lanthanum or horseradish peroxidase (15, 31). In tangential sections the electron-opaque deposit of lanthanum is seen to comprise a network outlining a hexagonal array of small, electron-lucent regions with a center-to-center spacing of 90–100 A. Thus, the apparent gap is really not a uniform space in the form of a sheet between the apposed membranes. The hexagonal structure can also be seen following $KMnO_4$ fixation (32). In oblique sections the network of extracellular space can give the appearance of striations crossing the junctional complex with about the same periodicity. Following OsO_4 fixation, similar periodic structures have been seen in junctions in several other preparations where cells are electrotonically coupled (7, 11–13, 25, 28), and these junctions are apparently of the same kind.[1]

[1] These junctions, which have been termed "gap junctions," are to be distinguished from the close appositions at zonulae occludentes where extracellular space is completely occluded between adjacent cells (15). The latter junctions are found in epithelia and serve a barrier function by sealing off extracellular space in continuous bands around cells; they prevent both large molecules and small ions from crossing the epithelia through intercellular clefts (6, 14, 15, 18, 26). There is no evidence of intercytoplasmic channels at these junctions, and no evidence that they electrotonically couple cells. Previously, no distinction was made as to membrane arrangement at the two types of junction, and both were often termed "tight junctions." This latter term has been retained to distinguish the appositions at zonulae occludentes from "gap junctions" (15, 31).

It is evident that the junctions between segments of the septate axon are very similar to those just described as implicated in electrotonic coupling. The question remains as to how these junctions provide low resistance paths between cell cytoplasms. There is the theoretical possibility that low resistance membranes separated by a slight gap can still mediate significant electrotonic coupling between cells, and in most instances the electrical measurements do not exclude the presence of a space between junctional membranes which allows a leak to the surrounding medium (8). However, use of the dye Procion Yellow as a tracer indicates that there is a pathway between cell cytoplasms that does not involve the extracellular space (29). The conclusion is based on two observations: (*a*) Procion Yellow can cross the septum from the cytoplasm of one axonal segment to that of the next; and (*b*) the dye does not enter the axons from the extracellular space, although it stains the sheath tissue of the septum and may even get into the extracellular space within the junctions in the same manner as does lanthanum. The low and fixed resistance of the junctional membranes (33) and their nonspecific permeability to small anions, small cations, fluorescein, neutral red, and even sucrose (9, 10), lead to the conclusion that there are hydrophilic channels between cytoplasms of the coupled cells. The most probable location for these intercytoplasmic channels is in the electron-lucent areas outlined by lanthanum, for these are the only areas in the junction that extracellularly applied lanthanum does not penetrate. From these considerations, the diagrammatic representation of Fig. 12 can be proposed for the junctions. The central placement of the intercytoplasmic channels is supported by the observation of electron-opaque spots in this location at the close appositions of club endings on Mauthner cells following $KMnO_4$ fixation (32); the fixative presumably reacts with the hydrophilic inner walls of the channels. A central dark spot is also found in negatively stained (4) and lanthanum-treated preparations of similar junctions (15, 31). In the latter case, the proposed structure requires that the staining results from some penetration of lanthanum into the cytoplasmic compartment. The amount required does not appear inconsistent with the absence of obvious staining of the general intracellular contents. Freeze-cleaved preparations of similar junctions reveal a hexagonal array of particles (20)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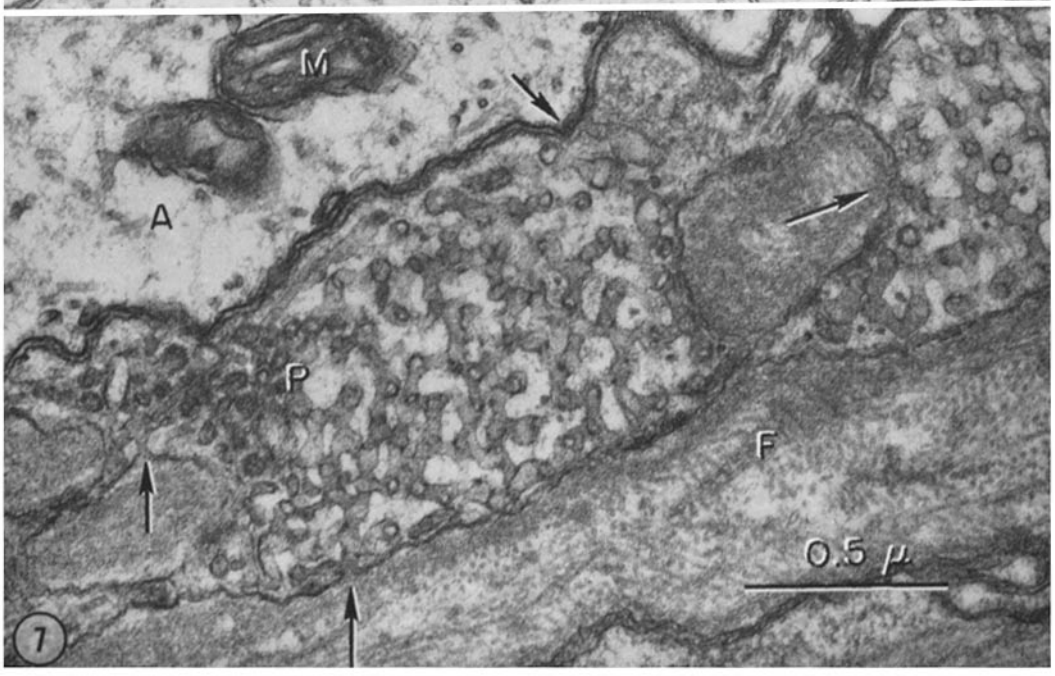
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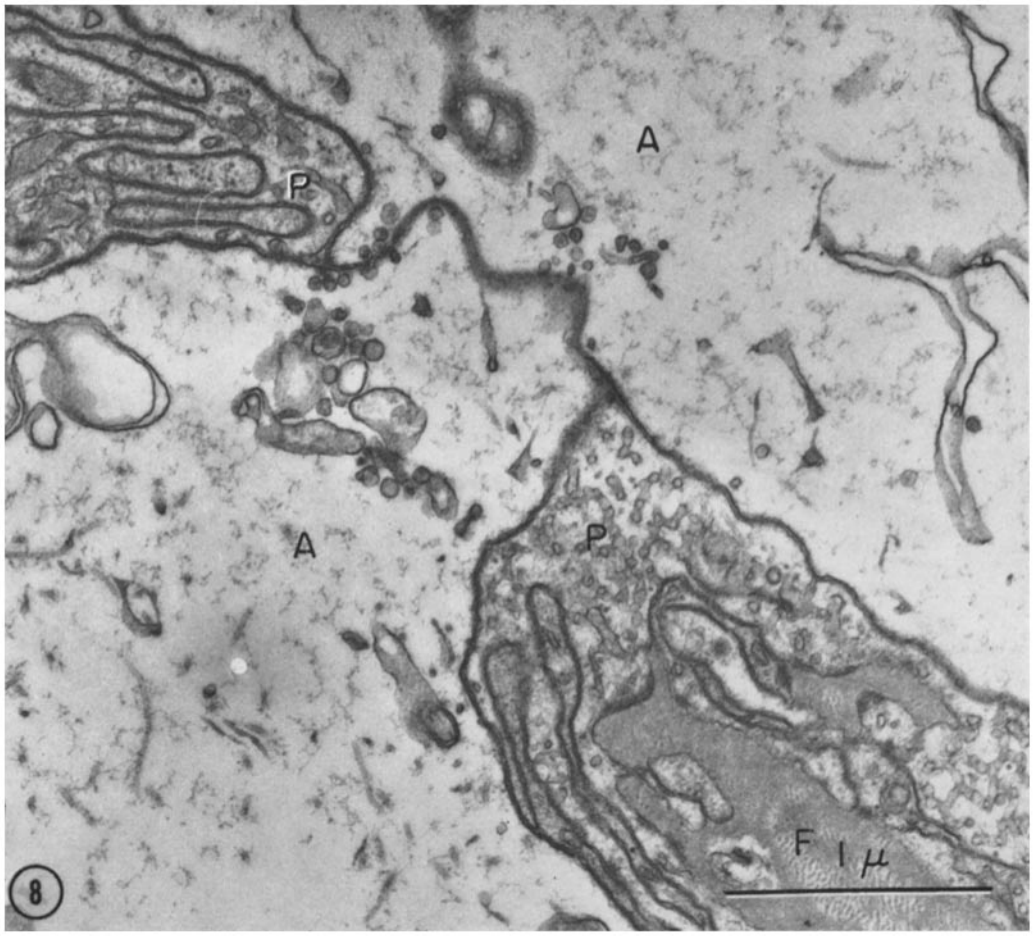


FIGURE 8 Section through a septum from a preparation which had been returned to normal physiological solution after exposure to low Cl^- solution. The coupling resistance at this septum had increased in low Cl^- solution and had recovered its initial value in physiological solution before fixation. As in the normal septum, there are direct appositions of adjacent axons without intervening Schwann cell processes. *A*, axon; *P*, Schwann cell processes; *F*, fibrillar core. × 35,000.

FIGURE 6 Section through a septum from a nerve cord which was fixed while immersed in propionate saline. The coupling resistance at this septum had increased. The fibrillar layer (*F*) is interrupted, but the axonal processes are separated by Schwann cell processes (*P*). The anatomosing tubular inclusions of the Schwann cell processes are more conspicuous than in normal preparations. *A*, axons; *M*, mitochondria in the adjacent axons. × 33,000.

FIGURE 7 Section through a Schwann cell process (*P*) in a septum from a preparation similar to that used for Fig. 6. In the process there is a high density of anastomosing tubular inclusions, a number of which are continuous with the surface membrane (at arrows). *A*, axon; *M*, mitochondrion; *F*, fibrillar layer. × 54,000.

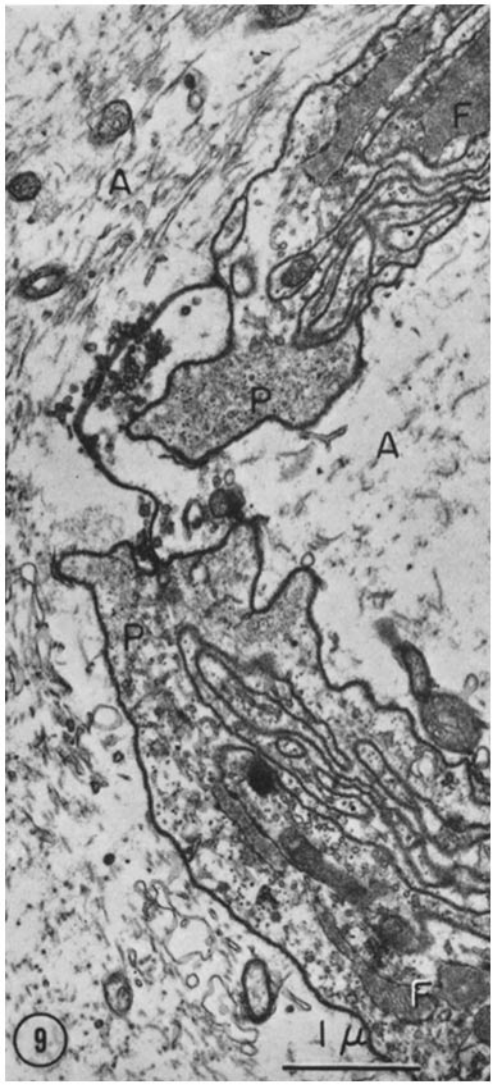


FIGURE 9 Section through a septum from a preparation that was fixed while immersed in EDTA solution. The relation between apposed axonal membranes appears normal. *A*, axon; *P*, Schwann cell processes; *F*, fibrillar layer. × 18,000.

with the 90 A spacing which is appropriate for them to represent the intercytoplasmic channels and their walls. More recently, Chalcroft and Bullivant (17) used a modified freeze-fracture technique which permitted them to study the replicas from both faces of the fracture through the junctions. They concluded that each apposing membrane at the junctions contains hexagonally packed particles, and presumed that these particles function in "cell-to-cell communication."[2]

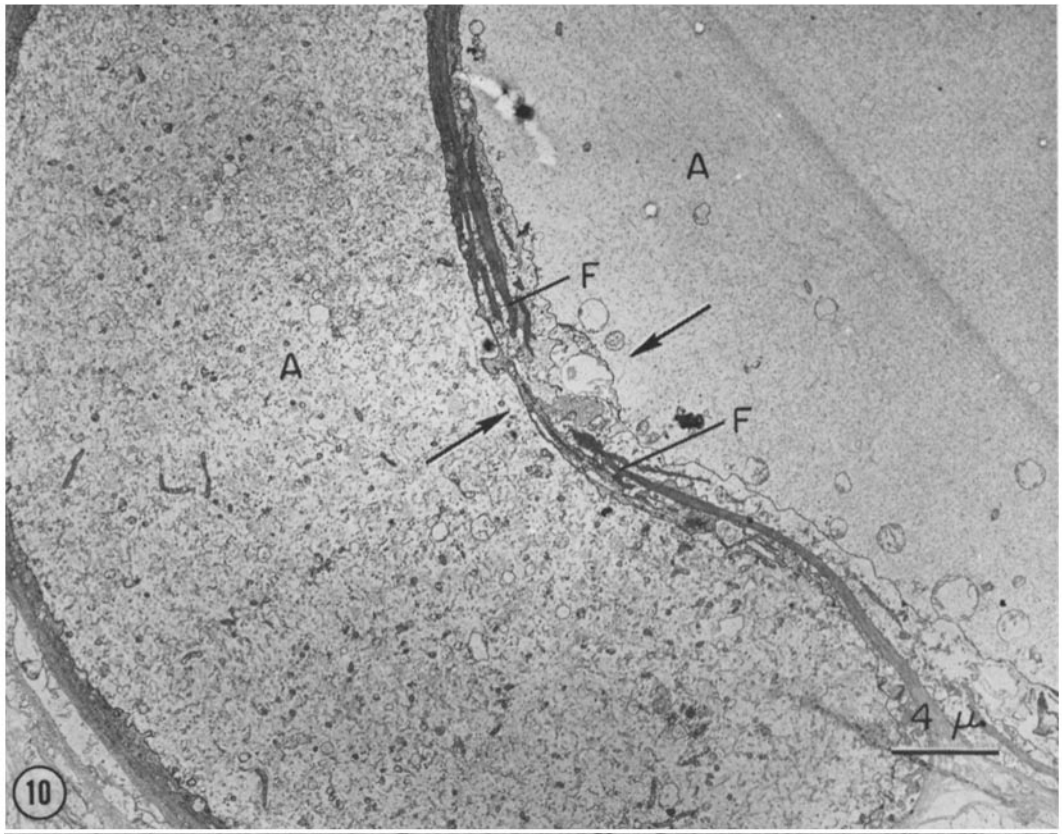
The precise arrangement of protein and lipid at the junctional complex remains to be worked out. The diagram of Fig. 12 requires that within the 100 A repeat period of the array there should be the thickness of the extracellular channel, about 20 A, and the diameter of the intercytoplasmic channel, perhaps 10 A, and twice the thickness of the walls of the intercytoplasmic channel. This allows only 35 A per channel wall in the gap region, which is less than the normal unit membrane thickness of about 75 A but which is conceivable for a bimolecular lipid membrane without protein coats (24). The absence of electron-opaque structures crossing the gap at the junctions indicates that the channel walls are not identical with the neighboring plasma membrane.

The induced increases in coupling resistance are shown here to be associated with separation of the junctional membranes (with the exception of the moderate changes during immersion in low Ca^{++} solutions). The association between the morphological and physiological findings provides

[2] *Note added in proof:* Small depressions are discernable in the center of similar particles seen in freeze-fracture preparations of cardiac muscle (McNutt, N. S., and R. S. Weinstein. 1970. *J. Cell Biol.* 47:666.). These depressions may represent a portion of the intercytoplasmic channels. Recently the dye injection results indicative of intercytoplasmic channels (29) have been confirmed with microperoxidase, a synthetic enzyme of molecular weight 1800 (Reese, T. S., M. V. L. Bennett, and N. Feder. 1971. *Anat. Rec.* Abstr. In press.).

FIGURE 10 Section through a septum fixed after return to normal saline following EDTA treatment Coupling resistance at this septum had markedly increased. There are no close appositions of axonal membranes, and Schwann cell processes are interposed between the axons (*A*) at the interruption in the fibrillar layer (*F*), as indicated at arrows. × 3500.

FIGURE 11 Section through a septum after a return to normal saline following EDTA treatment. Higher magnification than in Fig. 10 to show more clearly the absence of direct axonal appositions. *A*, axon; *F*, fibrillar layer; *P*, Schwann cell process. × 20,000.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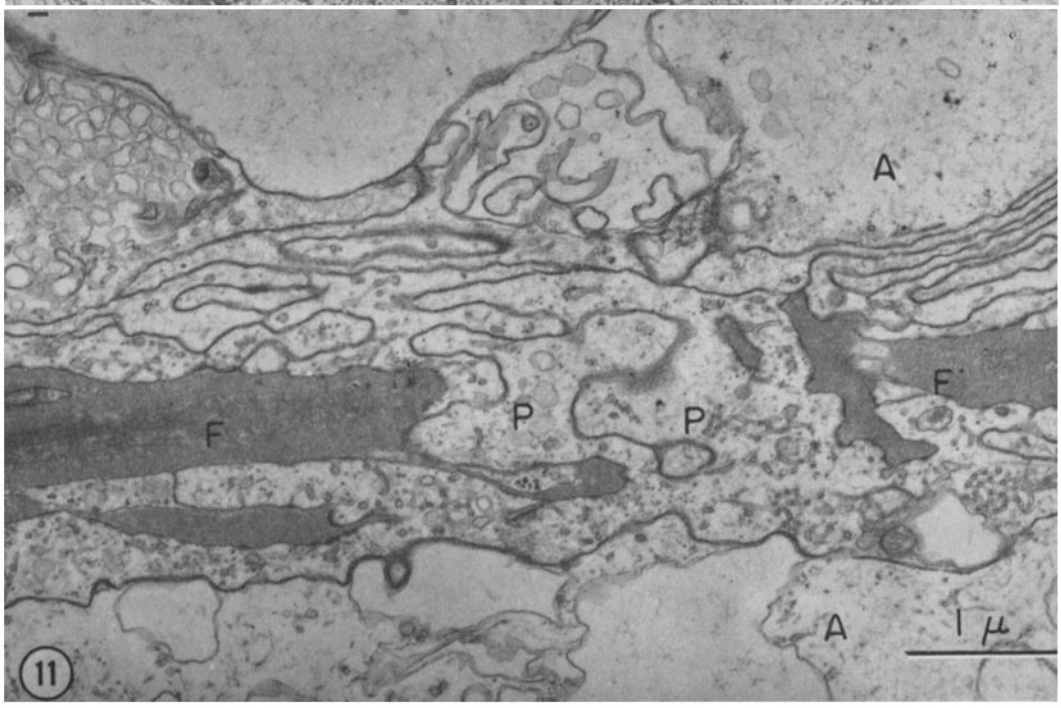
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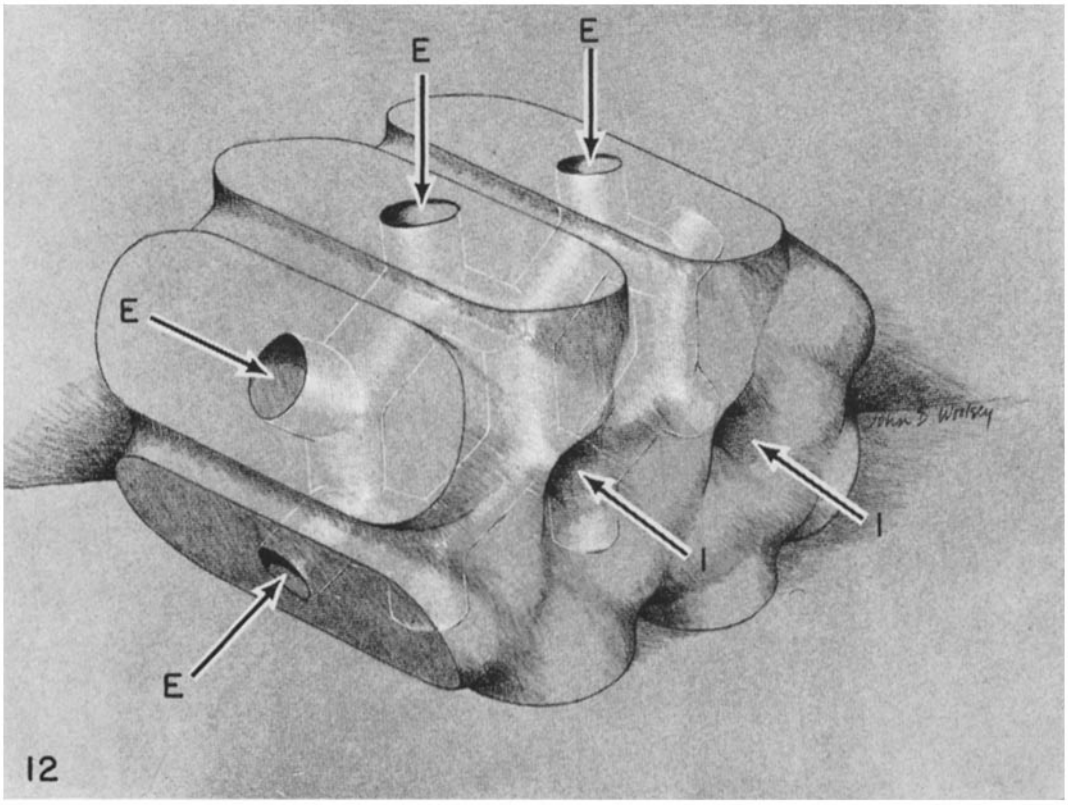

FIGURE 12 Proposed structure of junctions mediating electrotonic coupling. The arrows labeled *I* indicate intercytoplasmic channels. The arrows labeled *E* indicate channels continuous with extracellular space. Two complete *I* channels are shown which pass through two hexagons of *E* channels. Other *I* channels are shown in section. The spacing between *I* channels is about 100 A and the over-all thickness of the junction is about 150 A. The intracytoplasmic channels (*I*) are probably smaller than the extracellular channels (*E*).

further correlational evidence that the junctions are the sites of electrotonic coupling. The correlation is imperfect, however, for no modification of junctional structures was found to be associated with the moderate increases in coupling resistance shat occurred in low Ca^{++} solutions. Furthermore, tomewhat greater increases in coupling resistance occur as a result of cooling, but do not involve any detectable change in junctional structure (30).

An important aspect of our observations is that the resistance of the separated junctional membranes is greatly increased. We return to the question of whether increase in junctional resistance precedes or follows separation of the apposed membranes. It is possible that the structure of the junctional complex is modified during the resistance increase so as to break down the intercytoplasmic channels and reform the conventional bimolecular leaflet structure of the membranes. This change could lead to a loss of adhesion between the membranes and allow the junctions to come apart, and Schwann cell processes could then insinuate themselves between the axonal membranes. The interposition of Schwann cell processes is most likely a secondary phenomenon. Moreover, the reversible changes in coupling observed in cardiac and smooth muscle would appear not to require the participation of satellite cells (2, 3). The small resistance increases in low Ca^{++} solution may be of the same kind, but not involving sufficient reduction in the number of intercytoplasmic channels to allow the membranes to separate. Active movements of the axons or Schwann cells might also be prevented by low

Ca^{++} concentrations, and proximity could result in the maintenance of a significant fraction of the intercytoplasmic channels. At this time we are inclined to ascribe the relatively rapid increases of coupling resistance caused by cooling to a different mechanism, perhaps one that involves increased viscosity in the interior of the intercytoplasmic channels.

It has been suggested that Ca^{++} (or Mg^{++}) must be at a low concentration in the junctional region for coupling resistance to remain low, and that coupling resistance rises when Ca^{++} concentration is increased in the junctional region, either by passing through the cytoplasm, or by coming from the extracellular space following breakdown of a hypothetical perijunctional barrier (23).[3] The existence of a perijunctional barrier is contradicted by evidence for extracellular channels within the junctional complexes discussed here, but our experiments have done little to test for an effect of intracellular Ca^{++} on junctional resistance. The major increases in junctional resistance which we have observed could be interpreted as arising from increased internal Ca^{++} concentration, although this explanation is somewhat tortured in the case of Cl^- substituents. In the absence of direct evidence, it does not seem necessary to speculate along this line at the present time.

Our data demonstrate the lability of a junctional relationship that mediates electrotonic coupling between cells. The experimental treatments that alter coupling resistance are certainly unphysiological, perhaps with the exception of injury, yet they suggest the possibility that changes in electrotonic coupling could be involved in long-term alterations of neural signaling. To be sure, electrotonic coupling has been described primarily in simple reflex systems that exhibit little in the way of "plastic changes," yet this distribution may in part be an artifact of the simplicity of experimental study of these systems (5). Coupling is widespread in embryonic tissues (14, 19), but must decrease during differentiation of at least somatic muscle and nervous tissue; these changes are certainly "normal" responses, although the significance of coupling for development is uncertain. Further physiological and morphological studies of the processes described here should help to elucidate the structure of the junctional complexes and the mechanisms whereby their formation and dissolution are controlled.

[3] It should be noted that most of the evidence implicating Ca^{++} in electrotonic coupling has been obtained from the salivary gland of dipterans (23). The morphological basis of coupling in this tissue was thought to be a septate desmosome which is quite different from the junctions described here (16). Recently evidence has been obtained that there are junctions between salivary gland cells like those described here (B. Rose, personal communication) which may mediate the coupling. Morphological correlates of changes in coupling between these cells (16) require reinvestigation. A role for Ca^{++} in maintaining cell attachments has long been known, but we do not review this literature because our concern has been with the correlation of electrotonic coupling and junctional relations.

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