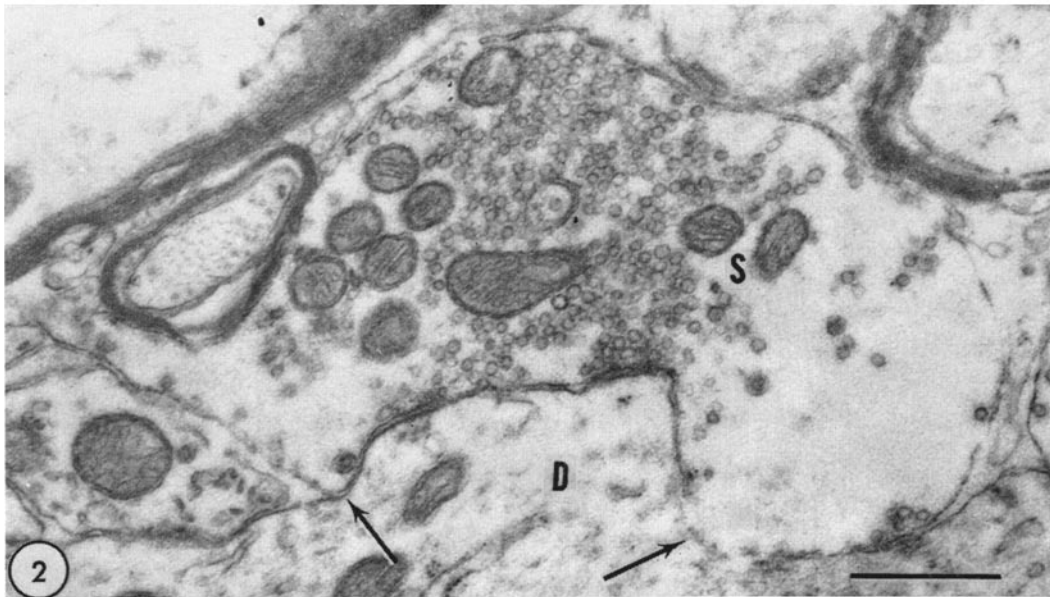
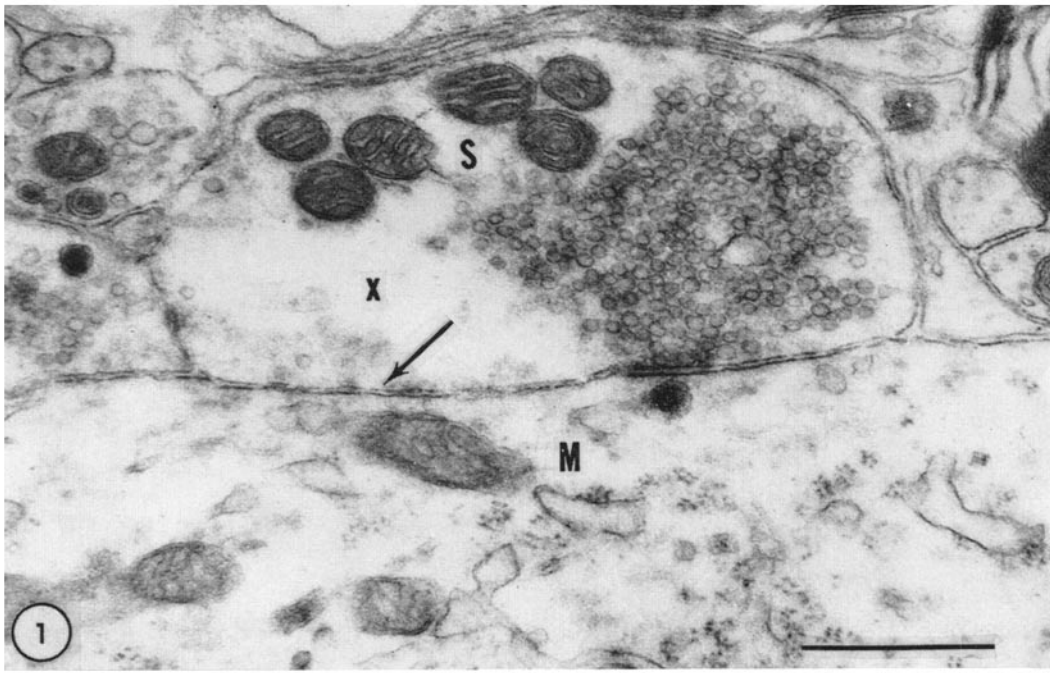


## PRESYNAPTIC ORGANELLES AND JUNCTIONAL INTEGRITY

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The identification of synaptic bulbs at the electron microscope level and the description of their major cytological characteristics (mitochondria, synaptic vesicles, and junctional densities) were soon accompanied by attempts to study their fate after the interruption of parent fibers (1). The degenerative changes in organelles of synaptic bulbs were well described by De Robertis, but several years



FIGURES 1-3. From the same animal (I66). Fixation was by perfusion with 2.5% glutaraldehyde in sodium cacodylate buffer, followed by hardening in osmium tetroxide. Scale markers indicate  $0.5 \mu$ . The macaque had a hemisection of the spinal cord at T10 on the left side, seven days before perfusion. Changes shown at L6 were found only on the left side below the lesion.

FIGURE 1 Synaptic bulb (S) on motoneuron soma (M), and in early stage of degeneration after interruption of parent axon. X marks zone of severe local depletion of synaptic vesicles. Those remaining in this area are ghostlike. Interface membranes in this region (arrow) are less dense than elsewhere, and possibly deteriorating.  $\times 51,000$ .

FIGURE 2 Synaptic bulb (S) in more advanced stage of degeneration of junctional structures. Interface deterioration at right and left margins (arrows) accompanied by invagination into postsynaptic dendrite (D).  $\times 38,000$ .

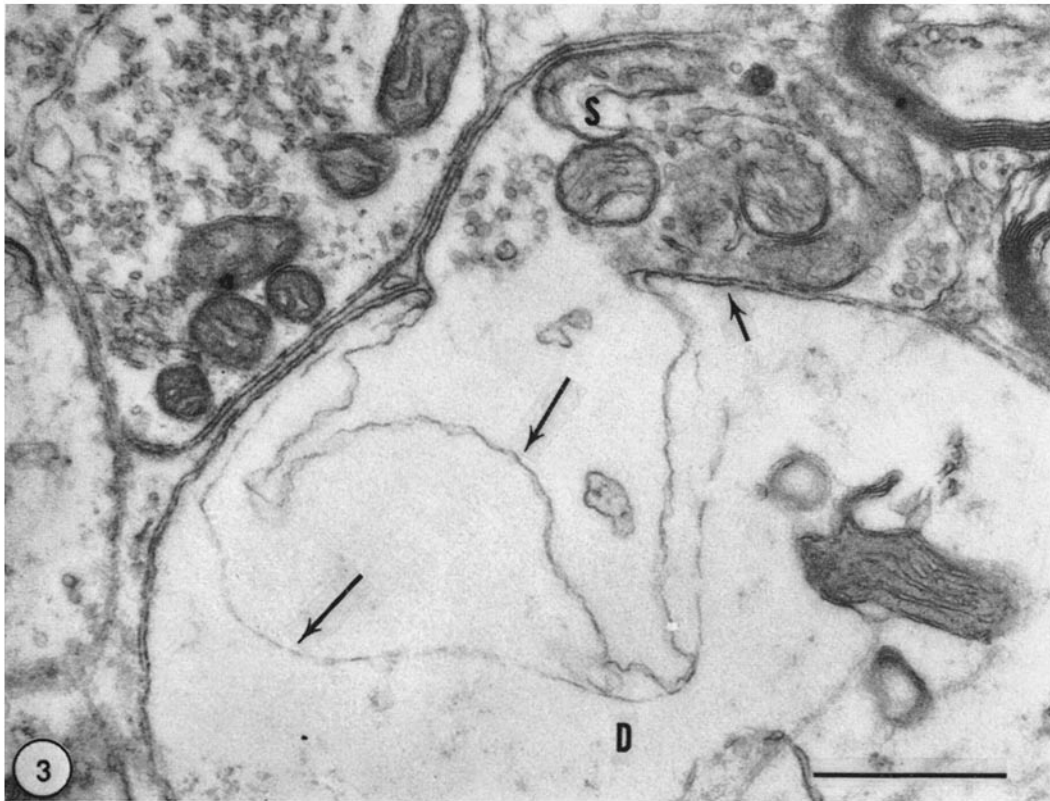


FIGURE 3 Synaptic bulb (*S*) in advanced stage of separation and extrusion of interface membranes on left (arrows), while junction remains rigid on right side (short arrow). *D*, postsynaptic dendrite.  $\times 51,000$ .

elapsed before improved techniques resulted in a large number of reports dealing with phenomena of synaptic bulb degeneration. Most of these reports, beginning with that of Alksne et al. (2), have dealt with the exploitation of synaptic degeneration for purposes of identification of the type of synaptic bulb and its location after interruption of a specified neuron pathway. This neuroanatomical goal has directed attention almost exclusively to organelle changes in the synaptic bulbs, such as swelling and decrease in numbers of synaptic vesicles, and to the appearance of unusual amounts of components such as neurofilaments, glycogen, or matrix density.

In previous studies the assumption has been implicit that presynaptic degeneration would of course affect the integrity of at least the presynaptic member of the junctional membrane pair, the adherence of which led us to refer to the adherent zone as the synaptolemma (3). An effect

on the postsynaptic member was also implied by the finding of Walberg (4) that postsynaptic dendrites appear to engulf the debris of degenerated synaptic bulbs.

In the course of a study of the origin of specific synaptic types in the spinal motoneuron neuropil of macaques, we have been impressed by evidence linking the integrity of the junction, not only to the state of the presynaptic bulb as a whole, but to the integrity of mitochondria and synaptic vesicles in the immediate vicinity of a specified portion of the synaptolemma. Dissolution of synaptic vesicles may occur locally (Fig. 1), or dense clustering of synaptic vesicles may result in the absence or near absence of vesicles in an adjacent region. In either event, the junctional densities, whether presynaptic or postsynaptic, may no longer be seen in regions of vesicle depletion, although junctional density and membrane adherence and rigidity may be present in an adjacent zone where vesicles

are clustered (Figs. 1 and 2). In addition to the loss of junctional density where synaptic vesicle depletion occurs, considerable separation of presynaptic and postsynaptic membranes often occurs. This suggests deterioration of the adherence factors in the membrane, and is invariably accompanied by mild or severe invagination of both membranes into the interior of the postsynaptic dendrite (Figs. 2 and 3). Subsequent extension of this process, as seen in our material, clearly leads to the engulfment by the postsynaptic dendrite described by Walberg (4).

The phenomena of local synaptic vesicle depletion and of adjacent junctional decay were noted in mild form as early as 3 days after the interruption of parent axons (motor cortex ablation or spinal cord hemisection). The rather more severe changes, shown in Figs. 2 and 3, for example, were noted in the spinal cord at L6, 7 days after spinal hemisection at T10. Synaptic bulbs of the S type (spheroid synaptic vesicles) and those of F type (flattened synaptic vesicles) showed similar changes. The "darkening" reaction of synaptic bulbs, most commonly referred to as a criterion of synaptic bulb degeneration, was almost never seen in our experiments under the conditions described above. The eight experimental macaques and four controls will be described elsewhere in connection with an analysis of the origin of specific synaptic types in the spinal motoneuron neuropil. Tissues of all animals were fixed by vascular perfusion with 2.5% glutaraldehyde in cacodylate buffer and hardened with osmium tetroxide as described in a previous report (5). The described phenomena appear not to be due to fixation artifact since they were not present in control animals, and were found in experimental animals in proportion to the expected degree of terminal degeneration.

## DISCUSSION

There can hardly be any doubt that the rigid control of spacing between presynaptic and postsynaptic membrane components of the synaptotemal junctions, whether chemical or electrical, is of crucial importance in synaptic functioning. The rigidity and adherence of the membrane pair at the junction are certainly in contrast with the lack of adherence and more irregular spacing of apposed membranes away from the junctional areas. Although Akert and colleagues have shown a striking relation between synaptic vesicles and

the presynaptic membrane densities of certain synapses (6), their findings have been interpreted as possibly being related to the mechanism of secretion of synaptic transmitter. Our findings seem to open up the additional possibility that vesicles may contain, presumably as part of their membranes, the synthetic enzymes responsible for production of substances which determine the spacing and adherence of synaptic junctions. These substances may include the junctional and cleft densities seen in various forms in synapses of different types and attributed to mucopolysaccharides or glycoproteins. The localized nature of even spectacular instances of detachment of the membrane pair in intermediate stages of synaptic degeneration (Fig. 3) suggests that the so-called "synaptic solder" is the product of activity of local presynaptic organelles rather than the aggregation of material transported from the perikaryon by way of the axon.

The excellent state of mitochondrial structure as late as 7 days after axon interruption is in sharp contrast with the swelling of synaptic vesicles as early as 24 hr (7), followed by blurring of the vesicle membranes in localized areas (Fig. 1). It seems likely that one or more basic components of vesicle structure are dependent upon axonal transport from the neuronal perikaryon.

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