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Ultrastructure of Two Invertebrate Synapses.* (20071)

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The structure of the synapse has been the subject of numerous light microscope studies since the days of Cajal. These investigations have revealed many significant facts with regard to the grosser microscopic structural elements. However, the capriciousness with which synaptic structures appear in histological preparations has generally necessitated the use of one of the silver technics for their demonstration. These technics, while exhibiting quite well the grosser structures, appear to be subject to many severe technical limitations. They could hardly be expected to yield many significant facts about the finer structural elements intervening between pre- and postsynaptic axoplasm. Indeed, the resolution of the light microscope itself limits such studies. However, some speculation has appeared about the possible intervention of mitochondria, connective tissue, granules, or even about axoplasmic or neurofibrillar continuity(1). The high resolution obtained by the electron microscope (EM) gives promise of a more fruitful approach. Accordingly, an investigation with this instrument has been instituted. The desire to conduct correlated physiological studies and the technical limitations thereby imposed led to the choice of an invertebrate synapse for the initial work. This paper constitutes a preliminary report of these studies.

The structure of several invertebrate synapses has been the subject of light microscope investigations(2-5) and related physiological studies(6-8) have rendered plausible their division into two general types. One type is physiologically polarized, introduces an appreciable conduction delay, fatigues and responds to drugs reversibly(6). A second type is physiologically unpolarized and seems to consist of a transverse septum in certain giant fibers(6-7). Structurally both types can be shown to be variants of the same general plan of side-to-side apposition of the axons of unipolar neurones.

Materials and methods. The physiologically polarized synapse between the median giant fibers and the third motor root giant fibers of the abdominal segmental ganglia of the crayfish (*Cambarus clarkii* and *Cambarus virilus*)(6) has been the primary subject of these studies. The polarized synapse between the second order giant fiber and the third

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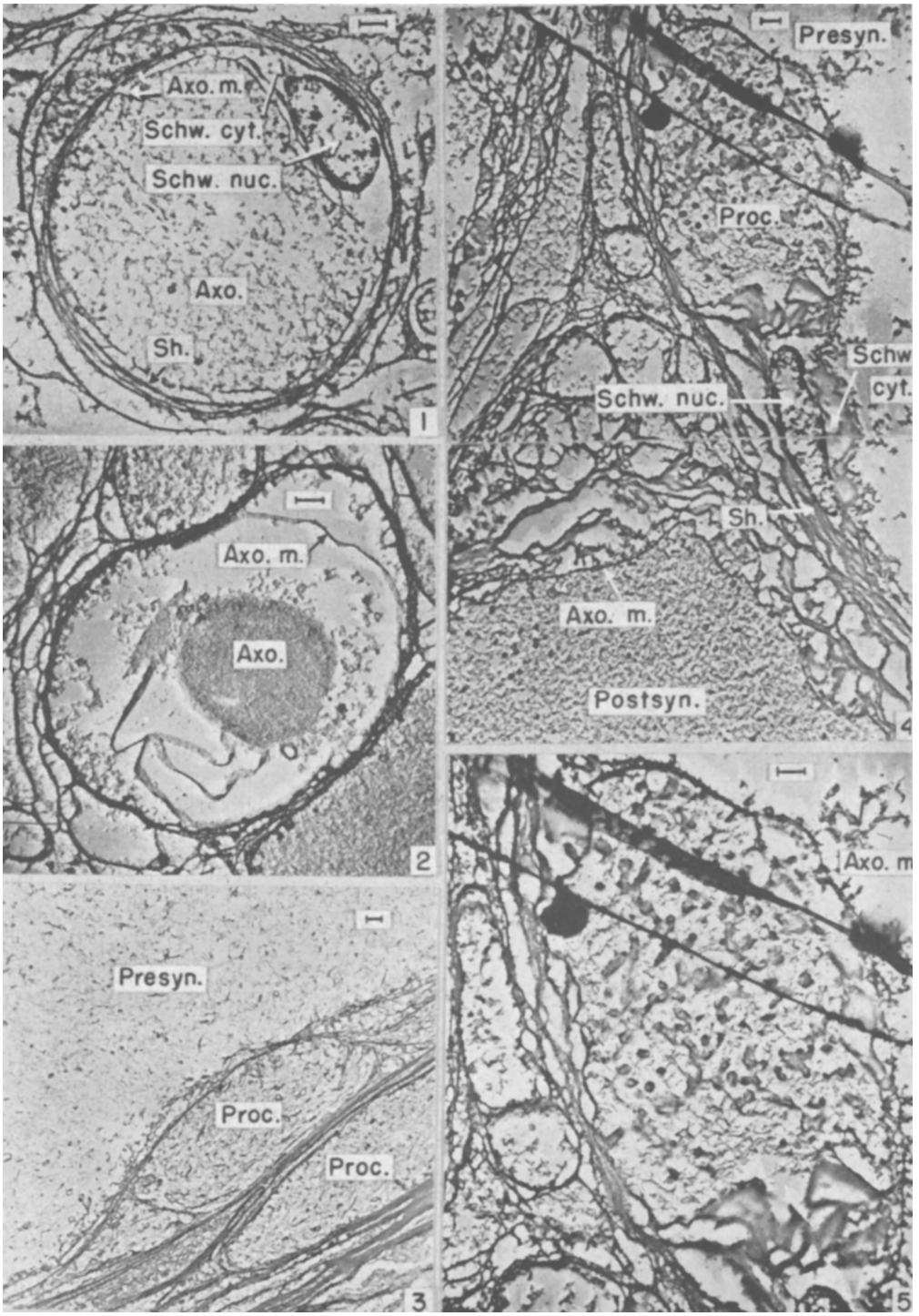

FIG. 1. Electron micrograph of a small crayfish fiber. The axoplasm (axo.), Schwann cytoplasm (Schw. cyt.), Schwann nucleus (Schw. nuc.) and the sheath (sh.) are shown. (The nucleus lying external to the sheath is of uncertain significance.) The axolemmal membrane (axo. m.) is seen separating axoplasm from Schwann cytoplasm. Osmic fixation. 4000 ×.

FIG. 2. Electron micrograph of a cross section of a small fiber in the ganglion showing the shrunken axoplasm (axo.) and the partially detached axolemmal membrane (axo. m.). Formalin-osmic fixation. 4000 ×.

FIG. 3. Electron micrograph of a cross section of the wall of the presynaptic second order giant fiber in the squid. Two synaptic processes are shown penetrating the sheath. Note the distinct difference in the concentration of filaments in the pre- and postsynaptic axoplasm. Osmic acid fixation. 2000 ×.

FIG. 4. Electron micrograph of a cross section of a portion of the crayfish synapse showing the presynaptic median giant fiber (presyn.) to the upper right, postsynaptic motor fiber (postsyn.) below, and the combined sheaths (sh.). The presynaptic Schwann cell cytoplasm (Sch. cyto.) containing a Schwann nucleus (Sch. nuc.) and a synaptic process (proc.) can be distinguished to the upper right. The axolemmal membranes (axo. m.) can be seen separating axoplasm from Schwann cell cytoplasm. Osmic acid fixation. 2700 ×.

FIG. 5. Higher magnification micrograph of the synaptic process shown in Fig. 4. 4000 ×.

order median giant fiber of the squid (*Loligo pealii*) stellate ganglion has also been studied. Fixation was accomplished by perfusion of the ganglia with 10% formalin and/or 1% osmic acid in the appropriate physiological solution (Van Harreveld's solution(9) or sea water). After thorough washing, dehydration was carried out with alcohol or dioxane according to histological methods which will be reported in detail elsewhere. The tissue was then embedded in n-butyl methacrylate. The synapse was located by the observation of serial 10-20 μ sections with the light microscope. The area of the block containing the synapse was trimmed to about 1 mm^2 and thin (circa 0.1 μ) sections were cut with the Minot International Rotary microtome(10) and a glass knife(11). The sections were mounted on 25 mesh nickel grids and the plastic was removed with toluene. After shadowing lightly with chromium the specimens were examined with an R.C.A. model EMU electron microscope.

Observations. Preliminary light microscope studies of the crayfish synapse demonstrated numerous processes of the postsynaptic fiber measuring about 0.5-5 μ in diameter extending toward the presynaptic fiber. These processes penetrate the combined sheaths of the two fibers, attain a larger diameter, up to 20 μ, and end in close apposition with the axoplasm of the presynaptic fiber in essentially the same manner as J. Z. Young had previously described for the larger (5-40 μ) processes in the squid synapse(4). In addition, a marked increase in osmiophilia of the postsynaptic axoplasm over that of the presynaptic axoplasm was noted.

The preliminary light microscope studies of the squid synapse confirmed the findings of J. Z. Young(4). The increased basophilia of the postsynaptic fibers described by Young was related to an increased osmiophilia in the crayfish.

The electron microscope studies of both the crayfish and the squid first permitted a more precise delineation of the structure of the fibers involved in the synapse. The fibers consist of a central axoplasmic core surrounded by Schwann cell cytoplasm and sheath (Fig. 1). An interfacial film between the axoplasm and Schwann cell cytoplasm, measuring about 300 Å in thickness, was observed to invest the axoplasm of the fibers. This film is considered an organized structure since at times it has been seen as a clearly defined entity stripped away from both axoplasm and Schwann cell cytoplasm (Fig. 2). It seems to be structurally related to a similar membrane first observed by Geren in myelinated vertebrate nerve(12) and to the "myelin lamellae" isolated from frozen vertebrate nerve by Sjostrand(13) and Fernandez-Moran(14). It is designated the axolemmal membrane.

The synaptic processes (Fig. 3-5) could be much more clearly differentiated with the electron microscope than with the light microscope. They are pseudopodium-like extensions of postsynaptic axoplasm and consist of densely packed axoplasmic filaments apparently completely surrounded by the axolemmal membrane of the postsynaptic fiber. They penetrate the combined sheaths of the synapsing fibers and enter the Schwann cell cytoplasm of the pre-synaptic fiber where they turn approximately at right angles and run roughly parallel to the long axis of the giant fibers (Fig. 3-6). The axolemmal membrane of the processes and the axolemmal membrane of the presynaptic fiber become closely ap-

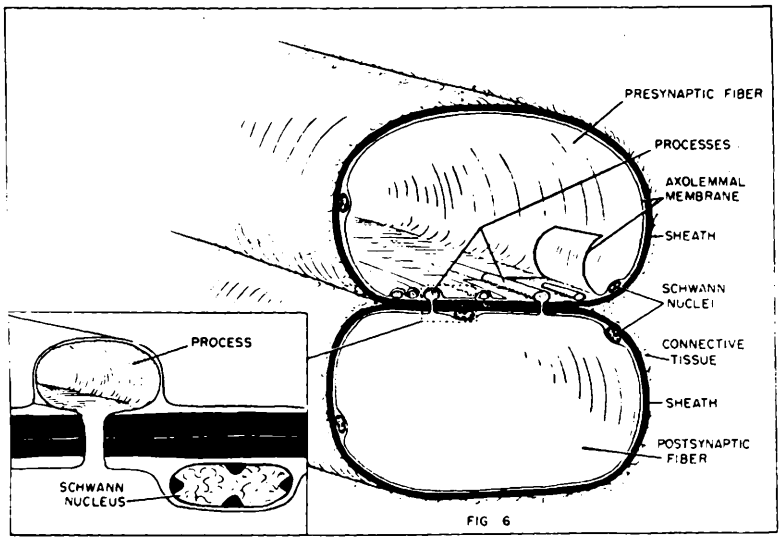

FIG. 6. A diagram of the crayfish synapse showing the presynaptic fiber above and the postsynaptic fiber below. A flap of the presynaptic axolemmal membranes is reflected to show two synaptic processes in presynaptic Schwann cell cytoplasm. The inset is an enlargement of the region designated by the dotted lines. A process of postsynaptic axoplasm surrounded by the postsynaptic axolemmal membrane is shown in presynaptic Schwann cytoplasm. The close apposition of pre- and postsynaptic axolemmal membranes is indicated.

plied to one another (Fig. 4-5). In many regions it appears that these apposed membranes fuse to form a single membrane of double thickness. However, in other regions, probably as a result of alterations associated with fixation and preparation, these closely applied membranes are separated from one another by a distance which may reach several hundred Å units. The intervening space when it was observed contained no definite structures. It is impossible to state at present with any degree of certainty whether or not such a space separates these membranes in the living synapse. But it may be stated that if such a space does exist it must be of an order of magnitude of no more than a few hundred Å units. Indeed, it is deduced from the examination of many electron micrographs that pre- and postsynaptic axoplasm are separated in the crayfish synapse by a distance of the order of magnitude of 600 Å, *i.e.*, merely by the thickness of the two apposed membranes. It is hoped that studies now in progress will lead to a more exact determination of this synaptic thickness.

The synaptic processes have consistently been observed to contain the filaments characteristic of axoplasm(15) and these filaments have provided one fairly reliable means of identifying them. Postsynaptic axoplasm, whether in the giant fiber or its synaptic processes, has been found to contain much more densely packed axoplasmic filaments than presynaptic axoplasm (Fig. 3). This increased number of axoplasmic filaments per unit volume may account for the increased osmiophilia of the postsynaptic axoplasm.

The extension of postsynaptic axoplasm toward presynaptic axoplasm and the more dense packing of the axoplasmic filaments of postsynaptic axoplasm at the synapse provide a demonstration of two types of morphological polarization which may be related in some way to the physiological polarization of these synapses.

Summary. 1. Electron microscope studies of a type of physiologically polarized synapse in the crayfish and the squid have shown that the synapse consists of a region characterized by processes of postsynaptic axoplasm lying in presynaptic Schwann cell cytoplasm. 2. The axolemmal membrane of the postsynaptic fiber completely envelops the synaptic processes and is closely applied to the correspond-

ing axolemmal membrane of the presynaptic fiber in a manner such that a single membrane of double thickness is sometimes produced. 3. Pre- and postsynaptic axolemmal membranes are structurally similar and are about 300 Å in thickness. 4. The axoplasm of the postsynaptic fiber and of the synaptic processes contains a distinctly increased number of axoplasmic filaments per unit volume over that of presynaptic axoplasm.

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Hepatic Glycogen in Acute Radiation Death.* (20072)

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In radiobiological research involving acute x-radiations, interest is directed toward discovering sensitive indicators of irradiation damage. Liver glycogen may prove to be such an indicator. It might be hoped that glycogen depletion has a quantitative relationship to irradiation dosage.

Materials and methods. Hamsters (*Cricetus auratus*), mice (Swiss albino), and salamanders (*Triturus pyrrhogaster*) were exposed to x-radiation until they were moribund. The animals were then sacrificed by decapitation, and their livers were immediately removed and fixed in cold Rossman's solution. These tissues were studied for glycogen by the histochemical technic of Hotchkiss and McManus (1,2) which involves an oxidative treatment of the sections with periodic acid and subsequent treatment with Schiff's leuco-fuchsin followed by sodium bisulfite. The specificity of the reaction is controlled by treating alternate sections with saliva or diastase before staining. As an additional check for the presence of glycogen in the hamster livers, the microchemical method of Boettiger(3) was used.

The irradiation conditions were as follows: The hamsters were killed under the 2 million volt x-ray machine made available through the courtesy of Dr. Vincent Collins of the Delafield Memorial Hospital. The dose rate was 534 r/minute at a distance of 61 cm, and the animals were moribund at 110,000 r in 3 hours and 26 minutes. The mice and salamanders were exposed between parallel tube sources at the Radiological Research Laboratory, stabilized at 110 KV and 30 MA, without filtration, with a distance of 30 cm from the source to the center of the body of the animals. The dose rate was 2200 r/minute. In all cases the animals were enclosed in plastic boxes. The salamander bodies were kept moist with cotton dampened with tap water.

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