ELECTRON MICROSCOPE STUDY ON THE REGENERATIVE PROCESS OF PERIPHERAL NERVES OF MICE*

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With 16 Figures in the Text

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Introduction

The regenerative process taking place in the sectioned peripheral nerves was examined with the electron microscope only in few opportunities. An investigation was made some years ago in this laboratory (ESTABLE, ACOSTA, SOTELO; 1957). The main facts found at that time were: a) the existence in the neuroplasm of the growing segment of the fiber (growing cone) of a high amount of microvesicles of about 500 Å diameter; b) an increase of the mitochondrial content of the fiber and the presence of dark bodies which seemed to derivate from mitochondria; c) the existence of bodies similar to those found in the soma of the neurons (PALAY and PALADE, 1955) in the intestinal epithelium (ZETTERqvist, 1956) and in germinal cells of several species (multivesicular bodies, SOTELO and PORTER, 1959; SOTELO and TRUJILLO-CENVZ, 1957). It was also found during the same research that in many fibers the microvesicles take an elongated shape giving thus place to the formation of tubuli which after a process of thinning would constitute filamentous units (neurofilaments).

Other electron microscopic investigations on this subject have been also reported: GLIMSTEDT and WOHLFART (1960) for instance described the occurrence of microvesicles and found that they are strikingly similar to those described by DE ROBERTIS and BENNETT (1955) in the synaptic junction; VAN BREEMEN, ANDERSSON and REGER (1958) also contend that there is a similarity between the vesicles appearing in the nerve sprouts and the so-called synaptic vesicles. VAN BREEMEN et al. admit that the microvesicles of the regenerating nerves may flow from the trophic center to the growing segment.

The study of nerve regeneration was continued in this laboratory with the immediate aim of investigate which is the earliest response of the nerve fiber to injury and how may lead to nerve regeneration. It was projected to investigate separately, whether the above mentioned morphological reaction also takes place in the fibers of the interganglionic connectives of species belonging to another Phylum (Arthropodes).

The results obtained up to now are reported in two papers. The present one describes the observations made in mice nerve fiber studied from the first 30' after section of the nerve trunk. In another paper, J. MELAMED and O. TRUJILLO-

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Cenóz (in press) report the results obtained studying the connectives of Laplata-
tacris dispar (Orthopterian).

The findings made by J. MELAMED and O. TRUJILLO-CENÓZ being concordant
with ours will be mentioned again in the discussion of this paper.

Material and Techniques

The posterior branch of dorso-spinal nerves were sectioned in mice. Care was taken of
perform a clean cut with a sharp razor edge. The sectioned stumps were left in contact and
the skin incision was closed as soon as possible in order to avoid changes in the nerve fiber
structure due to dessication. In order to compare the effect of section with other type of
lesion some experiments were made by crushing or by ligature of the nerves. Fixation was
performed at varied times after section, namely: 30'; 60'; 80'; 100'; 24 hs.; 48 hs.; 3 days;
4 days; 8 days; 11 days; 20 days and 30 days. The fixative (1% osmium tetroxide solved in
veronal buffer) was dropped “in situ” and the nerve (proximal stump) removed after darken-
ing by osmium reduction; fixation was continued in a bottle containing cold osmium solu-
tion for about 30'. Embedding was carried out in n. butyl methacrylate and polymerization
was performed in small cavities drilled in already polymerized blocs (IZQUIERDO y VIAL, 1962).
This procedure, used in this laboratory for about 4 years, gives very good results in regard
to polymerization damage. It is performed in the following way: a small cavity (2 mm in
width and 2 mm depth) is drilled in the extremity of a polymerized bloc, and a groove is
carved in its lateral face. The pieces are placed in the bloc together with a drop of metha-
crylate as described by IZQUIERDO and VIAL, and a new gelatine capsule is half-filled with
liquid monomer, the bloc is then inserted in the capsule having care of situate the groove
upwards while the capsule is kept at an angle of 45°. The air trapped in the capsule bottom
comes then out via the lateral groove and the bloc is sealed off with liquid methacrylate.
Sections of the tip of the sectioned nerve were made with a Porter-Blum microtome, and were
examined with an E M U — 2 C, R C A electron microscope. The data described below were
obtained from about 50 animals.

Light microscope observations

Light microscope observations on the regenerating process of nerves were
largely done in the past. The information collected by many authors has been
reviewed in several opportunities during the last decades (CAJAL, 1928; HOLMES
and YOUNG, 1942; GUTH, 1956).

The data reported in the following paragraphs correspond to observations made
in thick sections (0,5 μ) of methacrylate-embedded material (Phase contrast
equipment). These observations were done with the purpose of facilitate the
electron microscope examination of each material studied. Due to the relatively
low thickness of sections, the observations are hardly useful for comparison with
the thorough histological studies referred to above, which involved the use of
various techniques of staining and metallic impregnation. Nevertheless it is
considered interesting to make here a short mention of the main facts observed.

Few degenerative changes can be depicted with the light microscope few
hours after section. Those observed should not be estimated as proper degenera-
tive changes but the effect of trauma and bleeding. Firstly noticed changes indi-
cating a reactional state of the fibers consist of a granular appearance which is
visible near the zone where the excision was made. This granular aspect can be
also depicted at later stages and in each case it allows the detection of the growing
sprouts. The reaction of the connective sheath is clearly visible two days after
section. It mainly consists of a layer of cells enveloping the nerve trunk.