ARGENTIC IMPREGNATION METHOD DERIVED FROM SILVER NITRATE PROCESS
APPLIED TO THE NERVOUS TISSUE

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The mechanisms and difficulties met in reaching final diagnoses in cases of peripheral nervous pathologies
worry both our neurologists and their colleagues worldwide. In the Molecular Structure Laboratory (LEM)
of the IVIC, we were offered the possibility of contributing to neurological diagnosis research in the aim of
providing neuropathologists with new techniques to obtain data from biopsies of sural nerves, in addition to
those already existing X-rays scattering analysis - (Luzatti - Mateu). The numerous difficulties met in
analyzing the texture of nervous tissue in normal or pathological state in the histological cuts generally
reside in the use of inappropriate micrographic processes. The paraffin embedding method reduces relative
usefulness of the samples when applied to nervous tissue experimentation. The main artifact generated are
characterized by an accentuated retracting of the tissue, and even worse by a total extraction of the lipid
component of the myelin because of the solvents used, if it could not be insoluble previously. That’s why
we used another method to analyze the structure of peripheral and central nerves. The microtome, i.e.
freezing of fixed material, represents a more effective method than that of the paraffin in order to study
nervous tissue. This method preserves the integrity of the cellular and fibrous components, thus found free
from the artifacts generated during the paraffin embedding. The common and almost unique artifact met in
this process is tears happening in thin cuts of nerves with rare connected supports, due to the manipulation
of samples during coloration. The plastic resins used both in light and electronic microscopy as a way of
embedding widely surpasses the method using paraffin. Obtaining very thin sections preserving the cells,
maintaining the individual relation of irremovable anatomical structures in their natural location, and
manipulating the cuts safely constitute some of these advantages. With embedding in Epon 812, artifacts
derived from paraffin embedding are eliminated and the results obtained are similar to those reached with
the freezing method. However, the embedding in Epon 812 is a little slower than the other processes in its
carrying out. When the thin nervous cuts are embedded in plastic and get saturated with metallic salts, the
results obtained reach a degree of perfection in terms of exploitation, surpassing historologically those
obtained with conventional methods. These considerations led us to use Epon 812 in the analysis of the
nerves objects of morphometric research through image analysis for the clinic diagnosis that is taking place
in the Engineering Laboratory of Automated Systems (LISA). In the figures (1a), (2b), (1c), (1d), (1e), and
(1f) the results of the argentic embedding process (reduces silver nitrate and gold nitrate) are illustrated on
various pathologies, which permits to differentiate the individual state, distribution and disposition of the
components of the nerve that serve as anatomic support for the morphometric study, which integrated to the
histological alterations will bring in more information to establish a reliable diagnosis. The results obtained
with the argentic saturation method, derived from the reduced silver nitrate of CAJAL (Lisson) and gold
saturation (Parthe) allowed us to differentiate the nerves, clearly contrasted in sober an indelible colors, the
myelin, the axon, the intercellular space, and myelin remainders produced during the degeneration and
degradation of the myelin, as well as the remaining microscopic structures of the peripheral nerves in
normal or pathological states. The preparation and saturation used is more effective than the paraffin
method to study the nervous tissue, since this new method preserves the integrity of cellular and fibrous
components. What is more, the remaining material in the resin blocks can be used to do cuts to be observed
for transmission of electronic microscopy.
References:
- Parthe, Valentin. Personal Communication.

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Figure 1. Micrographs show different pathologies: a) Neuropathy, segmental demyelination, mag.20X, b,c) Fascicules, mag. 40X, d) Chronic Axonal Polyneuropathy, mag. 20X, and d,f) Fasicule and detail of the previous, mag. 40X, 60X.