ULTRASTRUCTURAL ANALYSIS OF PREPARATIONS IMPREGNATED WITH SILVER SALTS

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Tissue sections from the juxtacardial segments of the pulmonary veins and venae cavae of dogs impregnated with silver salts by the Kampos method were studied. An electron-microscopic investigation showed that the reduced silver adsorbed by the tissue of the impregnated preparations had a granular structure, with single granules ranging from 30 to 400 Å in diameter. The largest granules were found in the axoplasm of myelinated and unmyelinated nerve fibers, and the small granules were in various cellular and fibrous components of the tissue substrate. As a rule no silver granules were found in the substance of the myelin sheath. The patterns of adsorption and distribution of the silver granules in the impregnated preparations explain the morphological manifestations of the phenomenon of argentophilia.

KEY WORDS: nerve fiber; impregnation with silver salts; phenomenon of argentophilia.

Methods of silver impregnation occupy a leading place in the arsenal of neurohistological techniques. Their principle of action is based on the special affinity of nerve tissue for silver salts, or argentophilia, as a result of which selective staining of particular nerve structures is obtained: neurons, nerve fibers, receptors, and synapses. Despite careful study of the impregnation process and knowledge of the basic physicochemical and biochemical reactions taking place at the various stages of this process [4, 10, 12, 13], the intimate mechanism of impregnation has not yet been fully explained. This is particularly true of the nature of the tissue substrate of argentophilia. This phenomenon has been shown to be based on the property of certain tissue proteins of forming stable protein–metal complexes during interaction with silver ions [3-5, 9]. However, from the morphological point of view, the structural equivalent of argentophilia has been inadequately studied. Previous investigations in this field have not yielded the required results because of the low resolving power of the optical microscope. Essentially new opportunities for the study of these problems have been provided by electron microscopy. This was shown by the first attempts at ultrastructural analysis of impregnated preparations of the mammalian spinal cord [10] and the frog sciatic nerve [7]. Despite the fragmentary and incomplete nature of the submicroscopic observations contained in the above publications, they nevertheless confirm without reservation the potential value of electron microscopy as a method of studying aspects of the mechanism of silver impregnation and of disclosing the morphological substrate of argentophilia.

The investigation described below was devoted to an ultrastructural analysis of impregnation preparations in order to study the character of adsorption and the patterns of distribution of silver particles in peripheral nerve formations as well as in cellular and fibrous structures of the tissue substrate.

EXPERIMENTAL METHOD

Preparations impregnated with silver salts by the Kampos method and kept for different times (up to several years) mounted in Canada balsam were studied with the electron microscope. Preparations taken

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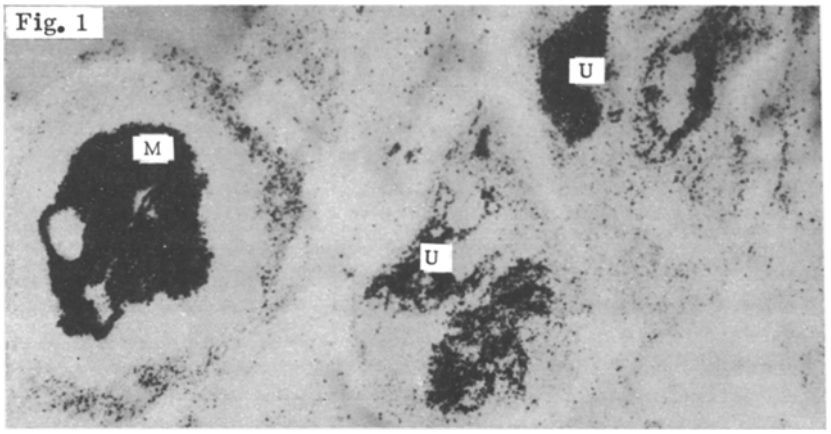

Fig. 1. Axons of myelinated (M) and unmyelinated (U) fibers from juxtacardial portion of the dog pulmonary vein (7000×).

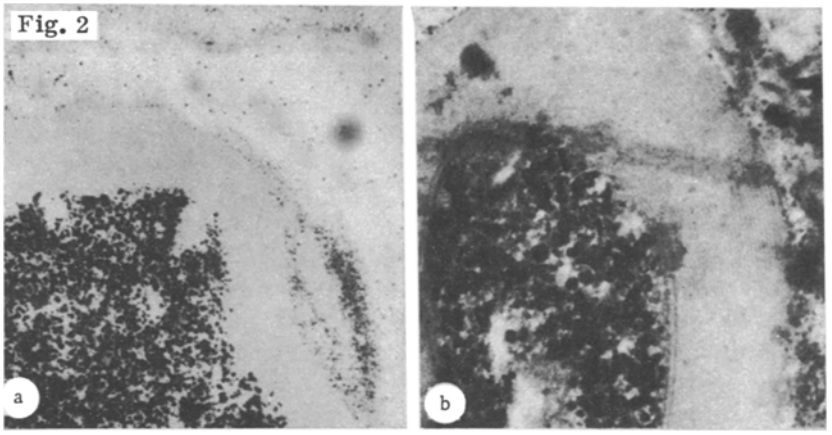

Fig. 2. Axons of myelinated fibers from juxtacardial portion of the dog pulmonary vein (explanation in text): a) 15,000×; b) 25,000×.

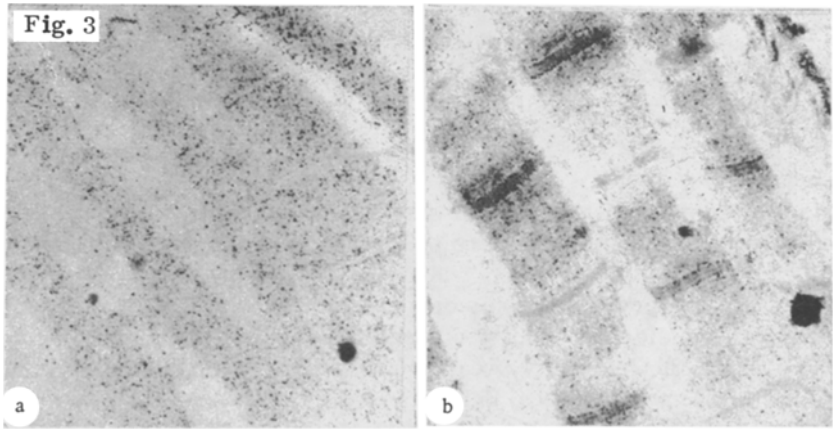

Fig. 3. Myocardial fibers from juxtacardial portion of dog pulmonary vein (explanation in text): a) 15,000×; b) 20,000×.

from the juxtacardial portions of the pulmonary veins and venae cavae of the dogs, in which the various innervation structures are richly represented [1, 2, 6], were chosen as the principal test objects. After examination and photomicrography of appropriate areas of the preparations, the balsam was removed with xylol and the sections taken through absolute alcohol and embedded in Araldite by the sandwiching method (between two thin transparent plastic films). In this form the preparations were re-examined in the light microscope in order to choose areas from which to cut ultrathin sections. Thus it was also possible to verify that the manipulations were not reflected in the general state of the preparation and had not affected the structure of the innervation formation and tissue substrate to be examined. Ultrathin sections (700 Å) were examined and photographed in the electron microscope without further staining or after staining with uranyl acetate.

EXPERIMENTAL RESULTS

Electron-microscopic analysis of the neurohistological preparations impregnated with silver salts reveals the character of distribution of reduced silver particles not only in the various components of nerve fibers (myelinated and unmyelinated), but also in the tissue substrate surrounding them. The first feature to be noted was the abundant deposition of particles in the axoplasm of the nerve fibers. Under low power (5000-7000×) the axons appeared to be almost continuously black; only with magnification of over 10,000× could the granular structure of the silver deposits be distinguished (Figs. 1 and 2). They are composed of granules of different sizes. The smallest granules were hardly 30-60 Å in diameter, the middle-sized 150-200 Å, and the largest 400-500 Å. They were often joined together to form larger particles with an irregular, angular shape.

If the electron-microscopic pictures of impregnation of nerve fibers were compared with those of the neighboring connective-tissue and muscular components, it was easy to see in general no large or medium-sized silver granules were present outside the axons, although small granules were regularly found in the form of a delicate, punctate deposit. The density of the finely granular deposit was usually greatest in the nuclear substance of the Schwann and perineural cells, and also in the nuclei of the macrophages, fibroblasts, etc. The distribution of the granules was less dense in the cytoplasm of those same cells and along the course of the fibrous structures of the connective tissue. The pattern of distribution of the reduced silver granules in the striated muscle fibers of the contractile myocardium was very striking. In some parts of these fibers the uniform deposit was interrupted by light transverse bands (about 600 Å wide), bounded by a distinct row of silver granules (Fig. 3a). Detailed analysis of the specimens showed that the pale bands were nothing more than the sites of the anisotropic disks, and after additional staining of the sections with uranyl acetate they assumed the appearance of dark electron-dense bands (Fig. 3b).

The character of impregnation of the myelinated nerve fibers must be specially mentioned. Besides abundant deposition of silver particles in the axoplasm, their total absence from the myelin sheath was conspicuous. Staining the sections with uranyl acetate showed conclusively that neither the outer nor the inner lamellae of the myelin sheath contained silver granules, regardless of the caliber of the fibers. A regular finely granular deposit could be seen only in the cytoplasmic (Schwann) membrane.

The observations described above agree on the whole with the data of light microscopy and they indicate a high degree of argentophilia of the axons but very low argentophilic properties of the myelin sheath. Meanwhile electron-microscopic analysis introduced important corrections into the existing idea on the nature of the phenomenon of argentophilia of nerve fibers. It is generally considered that axons have a so-called neurofibrillary core and that the argentophilia of nerve fibers is due to its special affinity for silver salts. Yet investigations at the ultrastructural level do not confirm the existence of neurofibrils. These formations have not been found in either myelinated or unmyelinated nerve fibers. It has been suggested that they arise as a result of aggregation of protein-micellar structures (in particular, neurofilaments) during formalin fixation of the material, to be revealed subsequently by silver impregnation [7, 11]. Although this method was used for treating the material in the present investigation, nevertheless no formations with a structure of the neurofibril type, that could be connected with any special concentration of silver particles, could be found in the axons. It is very tempting to suggest that the intra-axonal silver granules may be connected with those specialized and particularly numerous formations of the axoplasm – neurofilaments and neurotubules. In all probability, however, the question of which ultrastructural components of the axoplasm are the actual carriers of the reduced silver granules must be answered in the light of the well-known universality of the argentophilia phenomenon. This means that the mechanism of impregnation must be based on principles of reduction and adsorption of silver salts that are common to nerve and other tissues, and these in turn must be connected with a common ultrastructural (and ultimately molecular) substrate of the corresponding reactions. Biochemical and histochemical investigations show that this substrate is

formed by active argentophilic components of certain tissue proteins. According to some workers, these are amino groups of histidine [8, 9], whereas according to others they are sulfhydryl groups of cystine and cysteine [3]. Regardless of the soundness of these views, the discovery of the nature of the phenomenon of argentophilia as a whole must depend, in the present writers' opinion, not only on a study of the biochemical nature of silver impregnation, but also on a correct understanding of the special features of the adsorption and distribution of silver granules revealed by electron microscopy in impregnation preparations. It is in this light that it becomes clear that the intensity of the argentophilia phenomenon is directly dependent upon the dimensions of the silver granules adsorbed by a particular tissue during impregnation. It is not surprising, therefore, that axons of nerve fibers, which possess the largest granules, are also the most argentophilic. As regards a quantitative index for the adsorbed granules, judging from the electron micrographs accompanying this paper, this is of no decisive importance as a characteristic of the phenomenon of argentophilia.

The facts described above emphasize the exceptional role of ultrastructural analysis in the study of the mechanism of silver impregnation and in the explanation of the phenomenon of argentophilia, so widespread in normal and pathological neuromorphology. In this context it is most important to note that impregnation preparations kept for various and long periods of time can be used for electron microscopy. This accordingly provides essentially unlimited opportunities for their retrospective analysis at the ultrastructural level.

LITERATURE CITED

1. B. A. Dolgo-Saburov, Innervation of Veins [in Russian], Leningrad (1958).
2. V. V. Kupriyanov, The Nervous Apparatus of the Pulmonary Vessels [in Russian], Leningrad (1959).
3. G. V. Orlovskaya and A. A. Tustanovskii, Arkh. Pat., No. 1, 13 (1954).
4. G. I. Roskin, Arkh. Anat., No. 3, 62 (1954).
5. V. A. Florensov and M. V. Demidov, in: Inventions and Rationalization [in Russian], Irkutsk (1961), p. 125.
6. E. B. Khaisman, Arkh. Anat., No. 10, 45 (1961).
7. E. Gray and R. Guillery, J. Physiol. (London), 157, 581 (1961).
8. W. Haarmann and E. Frühaut-Heilmann, Biochem. Z., 309, 13 (1941).
9. A. Peters, Nature, 171, 613 (1953).
10. A. Peters, Quart. J. Micr. Sci., 96, 317 (1955).
11. A. Peters et al., The Fine Structure of the Nervous System, New York (1970).
12. E. Samuel, J. Anat., 87, 278 (1953).
13. M. Wolman, Quart. J. Micr. Sci., 96, 337 (1955).