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The histochemical method of revelation of myelin sheaths of the nerve fibers in the central nervous system

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Myelin, named so by Virchow[9], which constitutes sheaths around most axons, is an abundant membrane structure in the vertebrate nervous system. The function of myelin is also clustering of sodium channels at the node of Ranvier during axogenesis essential to fast nerve conduction velocity in myelinated fibers[6], participation in the development and regulation of axonal caliber and maintenance of axons. Myelin also plays an important role in inhibition of axonal growth and regeneration [8].

The study of myeloarchitecture became possible only after the discovery of the staining technique of myelin fibers by Weigert [10]. Later this method was used in histology for the systemic topographic study of the brain. Presently, there are many new methods and various modifications of the classical methods far from being confined [4,5]. A large number of neuro-histological methods have wide application in the experimental-morphological investigations. The progress of morphological technique and the discovery of cellular markers by immunocytochemical technique indicate the notion of multiple functional macroglial subclasses [1]. These investigations have enriched our knowledge about myelin and the oligodendrocytes – as the main myelin forming cells. The major drawback of many classical methods, used for the detection of myelin of nerve fibers is their laboriousness which limits the use in routine investigations. Another major insufficiency lies in the differentiation of the stain, which often hampers the interpretation of the results. The present situation has formed a basis for planning a new approach with the aim of liquidating these limitations. In this relation the method of fractional blocking of metal-organic compounds and selective reaction of Fe^{3+} with myelin has been adopted [2].

Material and Methods

The experiments were conducted on 30 albino rats weighing 220-250 g. The brain was extracted from animals deeply anesthetized by sodium pentobarbital (Nembutal,45 *mg/kg*). Furtherly, the brain was fixed in 10 % neutral formalin solution for a few days to weeks. It has been shown that the long consolidation in the fixative gives better results. Freezed slices of 40–50 *μm* thickness were cut from the brain tissue and placed in freshly prepared 3–5 % ferric chloride solution for a period of 20–30 *min*. Later the slices were washed with special care in several changes of the distilled water during 15 *min*, after which they were transferred to a ex tempore-prepared incubating mixture for histological investigation. This mixture is based on determining the activity of acid phosphatase from the point of view of conformity to the natural laws of concentration interactions [3]. According to this, in the formation of the lead precipitates in the cellular structures, a major significance is attributed to the concentration interactions of the metal ions and the buffer at the given pH. For determining the activity of the ferment we recommend the following incubating mixture: 20 *ml* 0.04 % lead acetate, 7 *ml* 1 *M* acetate buffer pH 5.6, 2.5 *ml* 1 % solution β-glycerophosphate. To this composition distilled water is added up to 100 *ml*.

The incubation period constituted 10–12 *hrs*. Later the sections were washed and placed in 1 % solution of sulfosalicylic acid for 1–2 *sec*. Once again the slices were washed and revealed in sodium sulfide, prepared using physiological solution. After washing the slices were placed in Canada balsam.

Results and their Discussion

The nerve fibers stained intensively are seen on the slides (Fig.1, 3 A). The nerve fiber does not have a similar thickness on its entire length. On the fibers, in regular intervals, thinner regions are arranged at some distance from each other.

Analogously in Fig.1 B,C the myelin sheath is not continuous, it is discontinuous at regular intervals at nodes of Ranvies, which provide the segmental structure for the nerve fiber. In the white matter of the brain, nuclei of

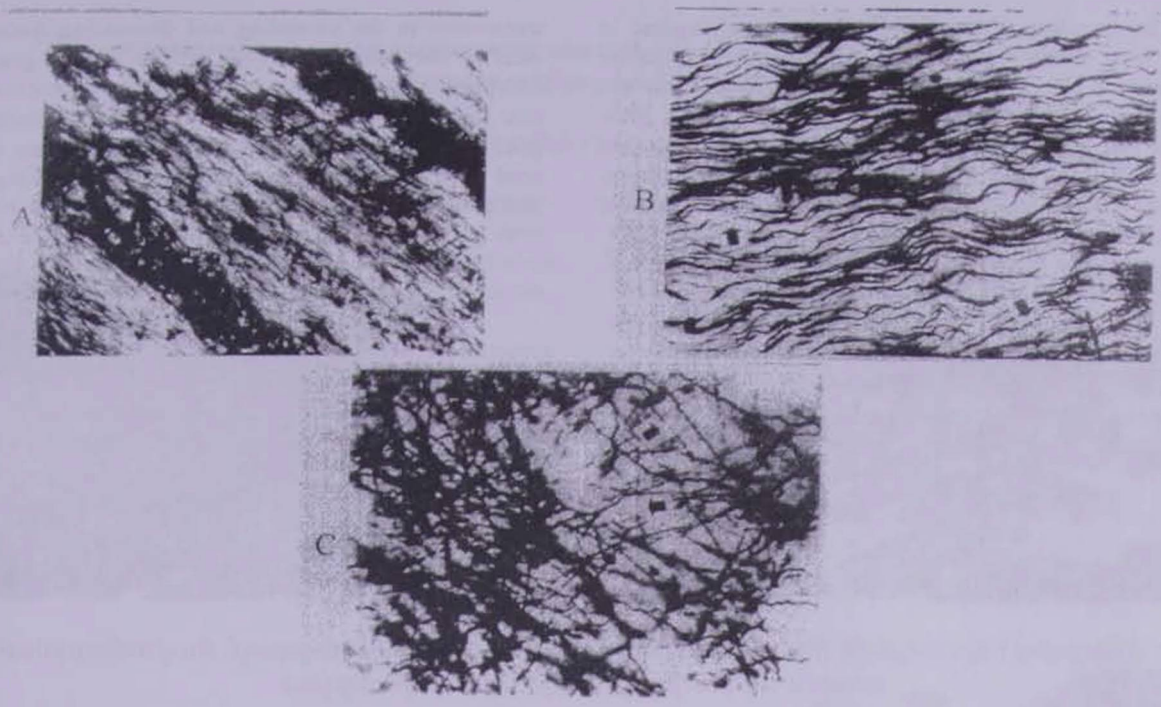


Fig. 1. Microphotographs of the rat medulla oblongata (A, B), frontal sections.
Myelinated nerve fibers, solitary tract. Midbrain (C), frontal sections. Myelinated nerve fibers around aqueductus cerebri (Sylvii). The closed arrowheads indicate nodes of Ranvier.
Scale bars = 30μm (B,C), 90 μm (A)

glial cells are not often revealed among the nerve fibers (Fig.2 B,C). In case of a shorter period of fixation of the brain, a weak reaction of the perikaryon of nerve fibers in the gray matter is observed.

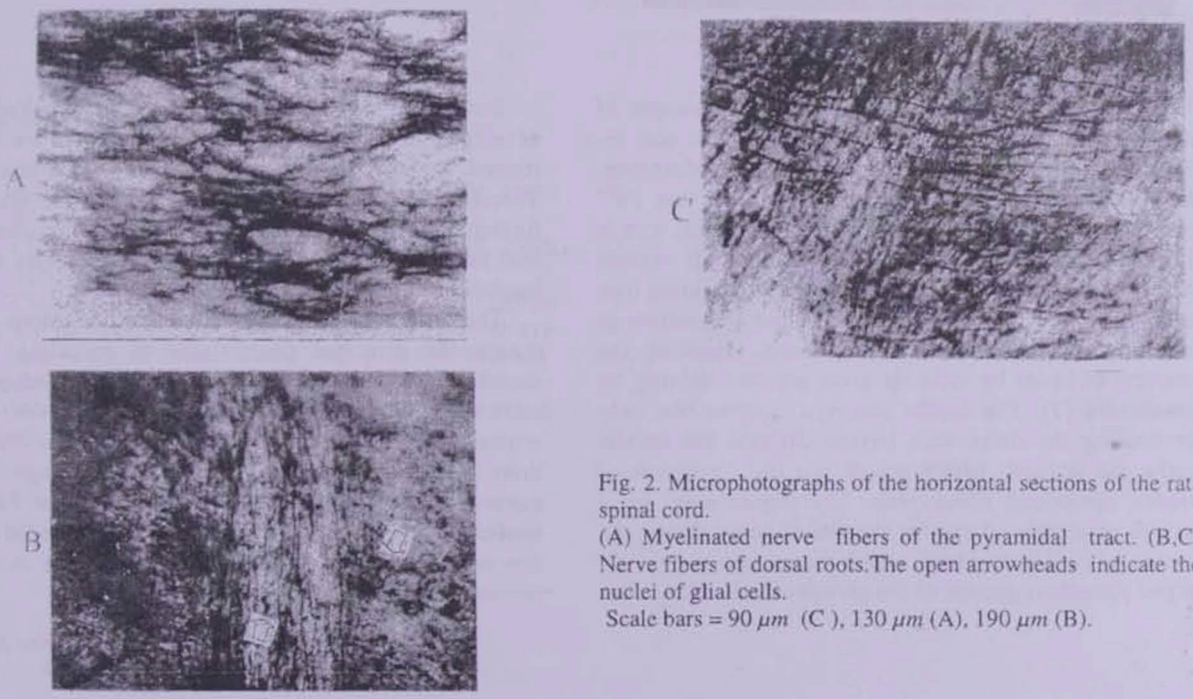
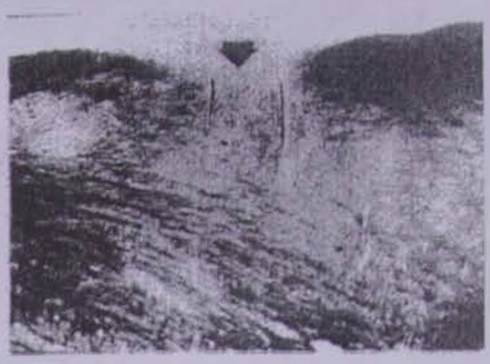


Fig. 2. Microphotographs of the horizontal sections of the rats spinal cord.
(A) Myelinated nerve fibers of the pyramidal tract. (B,C) Nerve fibers of dorsal roots.The open arrowheads indicate the nuclei of glial cells.
Scale bars = 90 μm (C), 130 μm (A), 190 μm (B).

The revealing of myelin sheaths was investigated in adult rats, which had undergone transection of bulbar pyramids. The corresponding region of brain was extracted after the elaboration of conditioned reflexes. Horizontal and sagittal sections of the brain were processed according to the above mentioned method. The degeneration of myelin was observed above and below the point of transection in the ascending and descending tracts. The ends of the transected fibers became thinner gradually. The point of transection was filled with a gliomesodermal scar (Fig.3 B,C), representing an unorganized mixture of glial and connective tissue structures, which are considered to be the major obstacles in the successful regeneration of central nervous system.



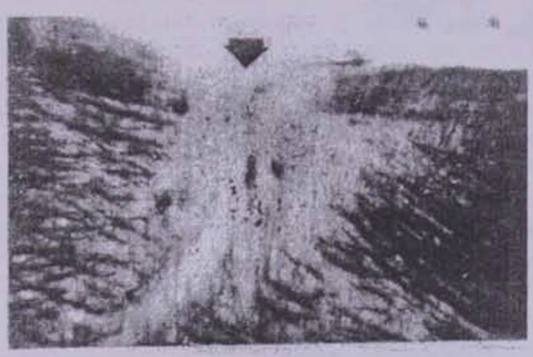


Fig. 3. Microphotographs of the rat medulla oblongata. (A), frontal section. Myelinated nerve fibers. The open arrowhead indicates the transected nerve fibers.(B,C)
Sagittal sections. Degenerating nerve fibers.The closed arrowhead indicates the point of transection.
Scale bars = 90 μm (A), 130 μm (B,C).

The method elaborated is based on the principle of fractional blocking of metal-organic compounds and selective reaction of Fe^{3+} ions with myelin [2]. Moreover, from histochemical practice it is well known that Fe^{3+} forms complex organic compounds and the bound iron is easily detected by various methods. On this basis various methods of staining of cellular structures by prickling iron were executed. This is either ferric ammonium sulfate or ferrous chloride. The present procedure, involving the extraction of lipids by solvents gives a better staining by haematoxilin [7]. The results received confirm that only after treating the slices with ferrous chloride the myelin sheaths are stained, which points out the formation of complex compound iron-myelin. Correspondingly, for a selective revelation of myelin sheaths of nerve fibers, the presence of Fe^{3+} is required, which reacts with negatively charged phosphate groups of the phospholipids of myelin.

But on the slices treated only with ferric chloride and developed in sodium sulfate the nerve fibers are weakly stained. Perhaps, the compound iron-myelin is less stable. Therefore, for intense and stable staining the slices are further transferred into the incubation mixture containing lead ions. The latter, being a heavy metal, forms a stable insoluble compound with phosphate groups.

Thus, we have worked out suitable conditions, which ensure the principal possibilities of revealing myelin sheaths of the nerve fibers on the formalin-fixed material more selectively and precisely. In addition to the peculiarities of the method adopted, the results of our investigations involve the standardization of all the stages of the material processing, which is very important from the histochemical aspect. The simplicity and the rapid execution of the proposed method enable its usage in routine investigations.

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Гистохимический метод выявления миелиновых оболочек нервных волокон в центральной нервной системе

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Разработан новый гистохимический метод выявления миелиновых оболочек нервных волокон в центральной нервной системе.Основой метода является дробное блокирование металло-органических составных и избирательная реакция Fe^{3+} с миелином.

Из фиксированного в формалине мозга готовятся замороженные срезы, которые обрабатываются 20–30 *мин* в свежеприготовленном 3–5% растворе трехвалентного железа. После тщательной промывки в нескольких сменах дистиллированной воды в течение 15 *мин* для усиления интенсивности окрашивания срезы переносятся в свежеприготовленную инкубационную смесь, предназначенную для гистохимических исследований. Эта смесь основана на выявлении активности кислой фосфатазы с точки зрения закономерности концентрационного взаимоотношения. Сроки инкубации – 10–12 *час*. После промывки срезы опускаются на 1–2 *сек* в 1% раствор сульфосалициловой кислоты для просветления, снова промываются, проявляются в растворе сульфида натрия и заключаются в бальзам.

Կենտրոնական նյարդային համակարգում նյարդաթելերի միելինային թաղանթի հայտնաբերման հիստոքիմիական մեթոդ

Ի. Բ. Մելիքսեթյան, Օ. Ա. Նազարյան, Վ.Բ. Ֆանարջյան

Մշակված է նոր մեթոդ, որը նախատեսված է կենտրոնական նյարդային համակարգում նյարդաթելերի միելինային թաղանթի հայտնաբերման համար: Մեթոդի հիմքն է հանդիսանում մետաղաօրգանական միացությունների մասնատված ռադեկապումը և եռվալենտ երկաթի ընտրողական ռեակցիան միելինի հետ: Նոր մեթոդը հասարակ է և ոչ աշխատատար:

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