

ULTRA-HISTOCHEMICAL STUDY ON LEMMOCYTES IN RABBIT

Ali A.A. Al-Taii¹ *MBCChB MSc PhD*, Fakher S. Al-Ani² *MBCChB MSc PhD*

Abstract

Background: The biosynthesis of myelin in the peripheral nervous system of adult healthy male rabbits was studied utilizing the activity of NSE at EM level.

Materials & Methods: One centimeter length of sciatic nerve from each animal was obtained, fixed and processed for demonstration of NSEs activity using ANA as a substrate. The specimens were stained with uranyl acetate and lead citrate then examined with EM.

Results: There is high reactivity of NSEs in the layers of myelin that wrap the axoplasm and nuclear pore of Schwann cells. No reactivity can be demonstrated at the axoplasm itself or at the microtubules and microfilaments.

Conclusion: These findings hypothesize that NSEs may play a role in the processes of regeneration and regeneration processes.

Key words: Myelin, Non-specific Esterases, Alpha naphthyl Acetate.

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Introduction

Schwann cells or lemmocytes are non-excitabile, supporting cells of the peripheral nervous system, they are associated with all peripheral nerve trunks and they form the capsular or satellite cells of the dorsal root and autonomic ganglia¹.

They are of neural crest origin, morphologically, Schwann cells are large, rounded with rather heterochromatic and ellipsoidal nucleus and of dense cytoplasm. Their cytoplasm is rich in mitochondria, microtubules & microfilaments, in addition to prominent lysosomes and a well-developed rough endoplasmic reticulum. A basement membrane is present at its external surface, except where this lies adjacent to a nerve cell process².

Functionally, Schwann cells are important for supplying energy and metabolites to the nerve cells. In addition embryonic growth of nerve cell processes and re-growth of axon after local injury was found only in the presence of Schwann cells³, so these cells are also responsible for trophic function that maintain and preserve the ensheathed nerve cell processes. Schwann cells are also responsible for myelin production, thickness and internodal lengths, which are related to the axonal caliber that may be determined genetically³. Thus myelin sheath is important for preservation of energy that is utilized during conduction of nerve impulses and

so enhanced conduction along myelinated nerve fibers.

The chemical composition of myelin is a protein lipid complex. The protein forms about thirty percent of adult human myelin; peripheral myelin protein contains a low molecular weight glycoprotein and two basic proteins⁴.

Many enzymes were identified in Schwann cells that accompany myelin metabolism. Some of them like 2:3 Cyclic AMP, and 3-phosphohydrolase, are of unknown role⁵. While other enzymes like Lipoprotein lipase (triacylglycero-protein acylhydrolase, EC (3.1.1.34) which mediated hydrolysis of exogenous triacylglycerol was found to be an important source of free fatty acids for the schwann cell and may play a critical role in myelin biosynthesis in the peripheral nervous system⁶. Moreover, histochemical localization of the activities of other enzymes (like nonspecific cholinesterase, alkaline phosphatases, acid phosphatase, adenosine tri- and diphosphatases, adenylate cyclase, and dipeptidylpeptidase-IV) in the cutaneous sensory nerve formations, mainly sensory corpuscles, has been also reviewed⁷.

In regard to the carboxylic ester hydrolases, there are three commonly designated esterases; these are carboxylesterase (EC 3.1.1.1), arylesterase (EC 3.1.1.2) and acetyesterase (EC 3.1.1.6). The identification of the non-specific esterases has been rested on their ability to hydrolyse synthetic ester substrate; however natural substrates have not been identified for most of the non-specific esterases (NSE), various metabolic roles are

¹Dept. Human Anatomy ²Dept. Clinical Physiology, College of Medicine, Al-Nahrain University.

Address correspondence to Dr. Ali Al-Taii

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attributed to NSE in the nervous system. It seems that the normal role of esterases in metabolism is poorly understood and probably represents a wide variety of functions⁸. This point directed our mind to study the biosynthesis of myelin in the peripheral nervous system utilizing the activity of NSE at EM level

Materials & Methods

Adult male, healthy, New Zealand rabbits weighing 1-1.5 Kg were used in this study. The animal was sacrificed by sectioning of the great vessels in the neck under light chloroform anesthesia. Sciatic nerve was exposed after reflection of gluteus maxims muscle and 1 cm length of sciatic nerve was obtained (1 cm above the knee joint).

This tissue specimen was processed for demonstration of NSEs activity using an incubation medium prepared according to the simultaneous coupling methods of (Nachlas & Seligman, 1949)⁹.

Small cubes of sciatic nerve (0.1 cm³) were obtained with a surgical blade and were placed in a fixative solution for 2 hours at 4°C. The fixative solution is composed of 2.5% glutaraldehyde buffered to pH 6.8 with 0.15M-phosphate buffer. Then the blocks were further minced into smaller pieces and washed (2-3) times with the same buffer prior to incubation.

The substrate (10mg of alpha naphthyl acetate; ANA), was dissolved in 0.5 ml of acetone. The dissolved substrate was added to 8.9 ml of 0.15 M phosphate buffer pH 6.3. Then 0.6 ml of freshly prepared hexazotized pararosaniline (Fluka) was added to the mixture. The minced tissues blocks then were incubated for 6 hours in this medium (Modified after Bozdech & Bainton, 1981)¹⁰. (The hexazotized pararosaniline was prepared by mixing equal volumes, 1ml of 4% pararosaniline in 2N HCl & 4% sodium nitrite newly made, and then the cloudy mixture was filtered with No.1 Whatman filter paper).

Post incubation, another fixation step was performed with 2.5% gluteraldehyde buffered pH 7.2 with phosphate buffer, then the tissue was fixed in 1% osmium tetroxide and dehydrated in ethanol for embedding in araldite.

The semi-thin sections (0.5-1µm) were stained with 1% methylene blue for selecting the most adequate areas for farther processing. Ultra thin

sections were obtained and doubly stained with uranyl acetate and lead citrate and examined by electron microscopy.

Results

Minced tissue block obtained from transverse section shows different diameters of axons (Figure 1).

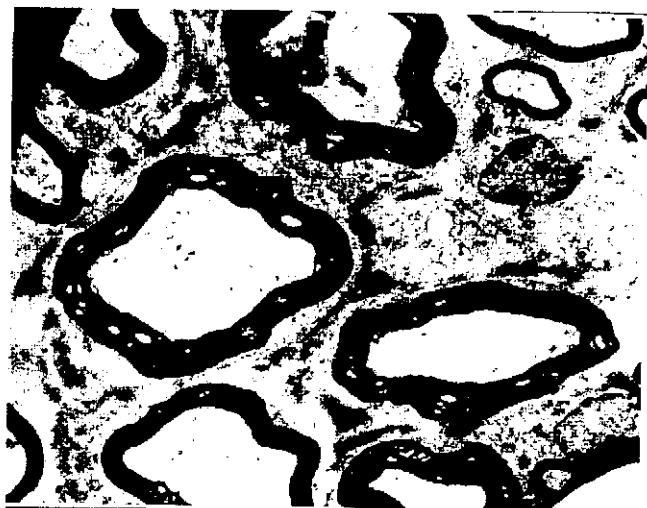


Figure 1: Cross section in the sciatic nerve treated with ANA prior to Osmification x3000

However, this study was focused on the largest diameter one. (A-α skeletomotor) that belongs to the α-motoneurons. Osmifications of the lipid content of the axons was perfect.

The method of processing and fixation and the preincubation fixation with a solution composed of 2.5% glutaraldehyde buffered to pH 6.8 with 0.15M phosphate buffer for 2 hours at 4°C, was found to be very efficient in visualizing esterase enzyme reactivity with the using of ANA as a broad spectrum substrate. It shows perfect reactivity regarding the intensity of FRP (vide infra).

The reactivity was demonstrated in a form of deep stained granules in the axonal wall, in the layers of the myelin that warp the axoplasm. However; no reactivity can be demonstrated in the axoplasm (Figure 2), neither in the microtubules and microfilaments nor in the mitochondria

In the axonal wall two forms of reactivity could be encountered with regarding the intensity of FRP especially in the largest diameter axons (Figure 3).

In the same consequences the nucleus of Schwann cell shows two pattern of reactivity (Figures 4 A & B) precisely in the nuclear

membrane, nucleoleous nuclear pores & nucleoplasm, moreover a homogenized distribution of FRP was encountered within the nucleus (Figure 4 B).

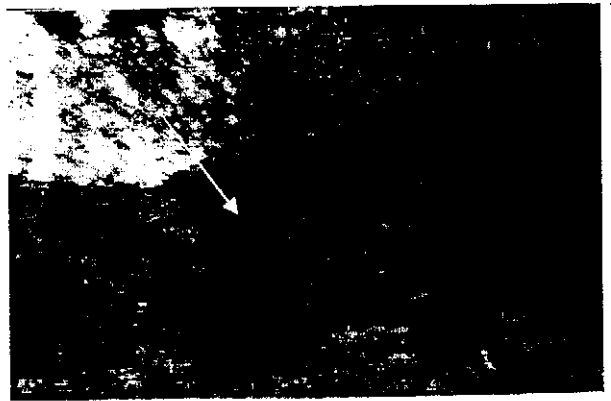


Figure 4: Axonal wall shows intense reactivity of FRP (arrow) x 31000

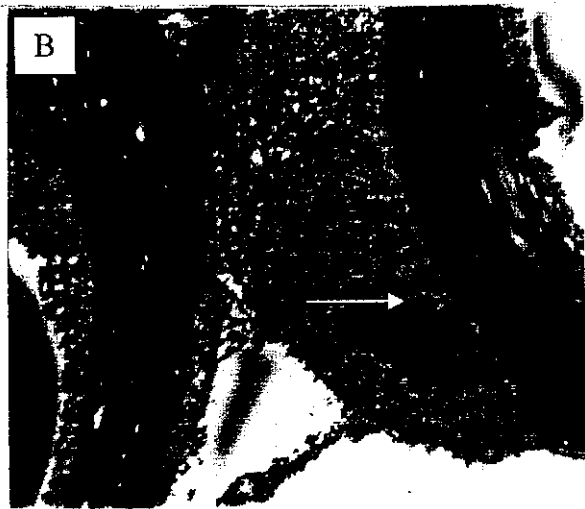


Figure 2: **A:** Schwann cell nucleus (arrow); nuclear pore x 18000 **B:** another form of reactivity in Schwann cell (arrow); nucleolus x 18000

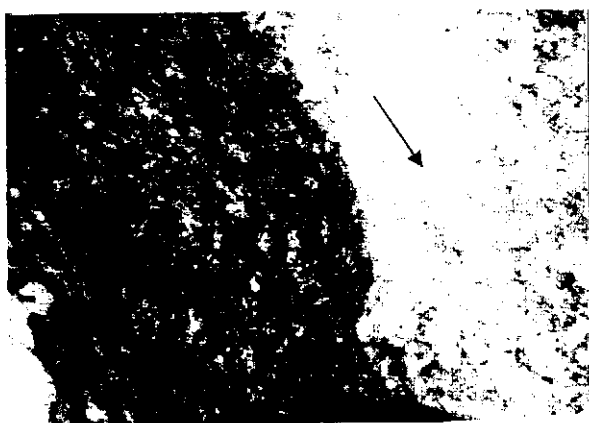


Figure 3: Axonal wall shows discrete granules of FRP. No reactivity could be detected in the axoplasm (arrow) x 31000

Discussion

Reactivity of ANAE was perfectly visualized in a form of granular appearance with the circumstances used, regarding different parameters of fixation, incubation media, and duration of incubation.

In the present study especial attention was paid on the pattern of reactivity in regard to the Schwann cells of the motor& sensory nerves and specifically in the largest diameter nerve fibers. Different reactivity can be encountered within the nucleus of Schwann, nucleoplasm & the myelin sheath.

Esterases have been found to be suitable marker enzymes for certain changes in the physiological state¹¹, as it reflects from their functions in the issue of synthesis and hydrolysis of lipid and lipoprotein¹².

Moreover, the high reactivity of the enzyme at the axoplasmic membrane and nuclear pore that we demonstrate at the nuclear pores of the Schwann hypothesized that they may play a role in intracellular transport^{13,14}.

Myelin is a lipoprotein so the identification of NSE in the Schwann cell may refer to their involvement in lipid metabolism. One of these NSE is A-esterase which is arylesterase (EC 3.1.1.2), that involved in the metabolism of free fatty acids primarily in the transesterification reaction between acids of long carbon chains and acetic acid, or acts as co-factor with lipase in hydrolysis of lipoproteins¹⁵. This confirmed other studies which showed that histochemistry for non-specific esterases were able to demonstrate the age-related development and maturation of myelin at the terminal Schwann cells¹⁶.

Since the activity of non-specific cholinesterases persists in terminal Schwann cells for a long time

after loss of the sensory axons, this combined enzyme useful for experimental studies involving denervation and re-innervation of sensory nerve endings⁷. The finding of this study (Fig. 4 A, & B).shows two varieties of reaction fo the NSE, and this reflects two functional categories of Schwann cells.

Farther studies of histochemistry are needed to confirm the specific role of the esterases iso-enzyme in the Schwann cell at different circumstances, like variation in the activity of enzymes with aging and their relation to degenerative regenerative processes.

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