LIGHT MICROSCOPIC STUDIES ON THE MORPHOLOGY OF TISSUES EMBEDDED IN EPOXY RESIN

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The best preservation of tissue structures for a long time has been reached by the embedding of tissues into epoxy resins. The aim of the current research is to show the potential of light microscopic studies on the research of blood vessels of tissues embedded and conservated in epoxide resins. Our results demonstrated that preservation of tissue in epoxide resins proved to be a convenient method for theirs studying under light microscope independently of the age and temperature conditions of the sample storage.

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INTRODUCTION

Making stable thin sections of biological tissues with their subsequent staining and analysis using light microscope helps to analyze animalcular texture of the organs.[1-9]

Recently paraffin, celloidin and gelatin pouring of tissues allow to extract histologic specimen applying different ways of staining and to carry out immunohistochemical investigations. At the same time, the quality of tissue structures' preservation remains moderate.[9] The best preservation of tissue structures is being reached at tissues' epoxy injection followed by preparation of semithin sections. Epoxy resins provide optimal tissue morphology at both the light and the electron microscopic level and therefore enable correlative studies on semithin and thin sections from the same tissue block.[6] In the three-dimensional reconstruction of a neuronal structure, it is imperative that ribbons of semithin or ultrathin sections be obtained. Resin-embedded semithin sections display better structural details than paraffin-embedded sections.

The aim of the current research is to show potential for light optical examination of blood vessels of tissues included in epoxide resins for a long-term.

MATERIALS AND METHODS

Reagents: Powdered paraformaldehyde; OsO_4; Sodium cacodylate trihydrate; 96º ethyl alcohol, acetone, Epon 812, Epon Hardener MNA, Epon Hardener DDSA, Epon accelerator DNP-30, uranyl acetate, citrate Na, nitrate Pb, photo plates, AzurII, sodium borate. All reagent used were of analytical grade and purchased from Sigma Chemical Co. (USA).

Biopsy materials of human being myocardium at mitral valve replacement in patients with rheumatic heart disease and coronary artery disease were received in 1997. Biopsy materials of liver were taken while carrying out an investigation of chronic stress in 2010.

All procedures involved human subject were approved by institutional review board/bioethical committee (Erevan State Medical University, RA) conformed to the Legal Aspects of Research Ethics and Science in European Community directive (2001/20/EC). All procedures involving animals were approved by the Institutional Review Board\ Institutional Animal Care and Use Committee (H. Buniatian Institute of Biochemistry, Yerevan, NAS RA) and Ethics Committee of the National Academy of Sciences, the Republic of Armenia, and they were conformed to the European Communities Council's directives (86\609\EC).

Small pieces of tissue have immediately put in a cold mix of paraformaldehyde in a sodium cacodylate buffer and glutaraldehyde for 12 hours at 4 °C with following post fixation in 1% OsO_4 solution for 2, then dehydration in ascending series of spirits; saturation in a mixture of acetone and Epon resins of different proportions to make gelatinous capsules were performed.

Observation under a light microscope: Semifine (semi-thin) epoxy sections with 1 μm thickness were made using LKB (Swedish) and Reichert (Austria) tools and the obtained semithin epoxy sections stained with Azur 2 and studied under a light microscope supplied with 40 x10 ocular lens.

RESULTS

The research of the biopsy material (right auricle atrial of myocardium) and the experimental material (liver) had been carried out. The material had been collected in different years. So biopsy materials of human myocardium were received in 1997 during mitral valve replacement in patients with rheumatic heart disease and coronary artery disease.

Biopsy material of liver was received in 2010 during the investigation of chronic stress. Sampled material was poured into epoxide resins by standard method for transmission electron microscopy. It must be mentioned that for the last years the collected material had been stored at different temperature conditions as low so high.

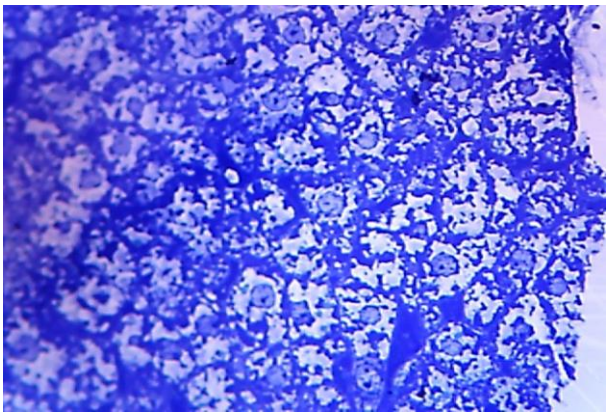

Figure 1. Liver tissue at chronic stress. X 400

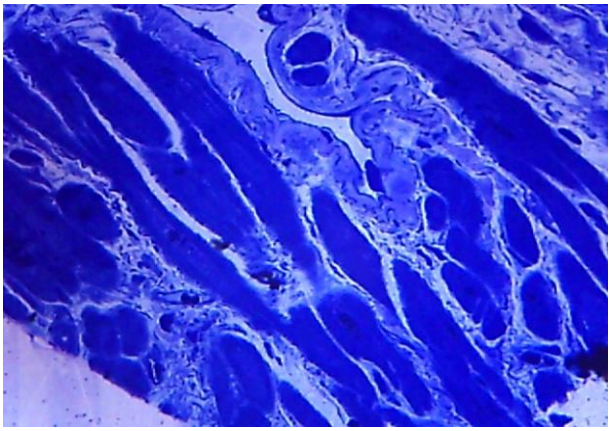

Figure 2. Heart tissue at coronary artery disease. X400

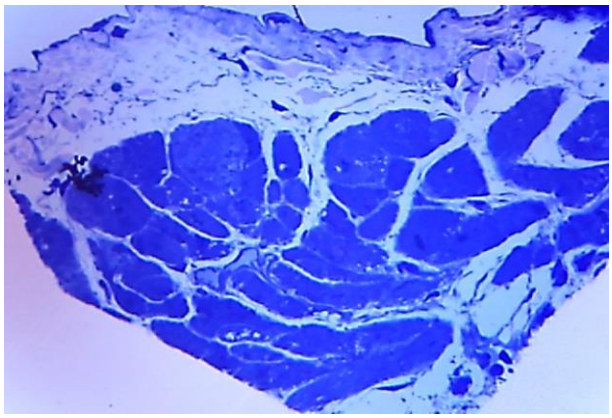

Figure 3. Heart tissue at a rheumatic disease. X 400

The investigation of those materials poured into epoxide resins in different years was carried out. It was necessary to check up how our invention worked[7] on material collected years ago. Nowadays the research on semithin epoxide sections of biological material embedded in epoxide resins provokes more and more interest as it allows seeing thinner morphologic picture under a light microscope. Azur II used by us with sodium borate shows quite interesting results in carrying out current research in diagnosing work on blood vessels in biopsy as it can be seen in Figure 1, 2 and 3.

That pointed out that the current method of investigation allows diagnosing micro circulatory changes as in pathology of a human being so during experimental researches irrespective of the years the material had been collected and the temperature conditions kept at its storing.

DISCUSSION

TEM-examination of ultrathin sections is usually a prerequisite for the researchers working with semi thin epoxide sections of epoxy injected tissue samples.

Semifine epoxide sections are stained in a standard way by Toluidine blue, Methylene blue, AZURE II in different combination. It should be noted that AZURE II almost is not used separately for staining.[2,3] The most common stain used in the electron microscopy lab for thick sections is toluidine blue.[1,4,8,10,11] Unfortunately its general lack of polycromasia makes it unsuitable for photomicrography. Much better results are obtained for general work as well as for photomicrography (especially with the use of filters for the black and white film) with the methylene blue, azure II combinations.[5] They are prepared in advance and most EM-Labs only stained by Toluidine blue because only an approximate morphological information on the area to be sectioned for ultrathin is sufficient.

Toluidine blue is indeed commonly used for stain semithin sections to show the general structure of the cell. Methylene blue-azure is an alternative.[7,9,11,12]

In our research, we used only AZURE II 1% solution prepared on 1% sodium borate by our staining method which turned out to be quite applicable for analyzing blood vessels. Our results show to the well preservation of tissue in epoxide resins for its studying under light microscope irrespective of the time factor and temperature conditions' storage.

CONCLUSION

Collected in different years biological material with shelf life from 18 to 5 years was useful for purposes of light optical research of microvessels on semithin epoxide sections.

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