THE STUDY OF THE RABBIT BONE MARROW USING DIFFERENT ELECTRON MICROSCOPIC TECHNIQUES

THESIS

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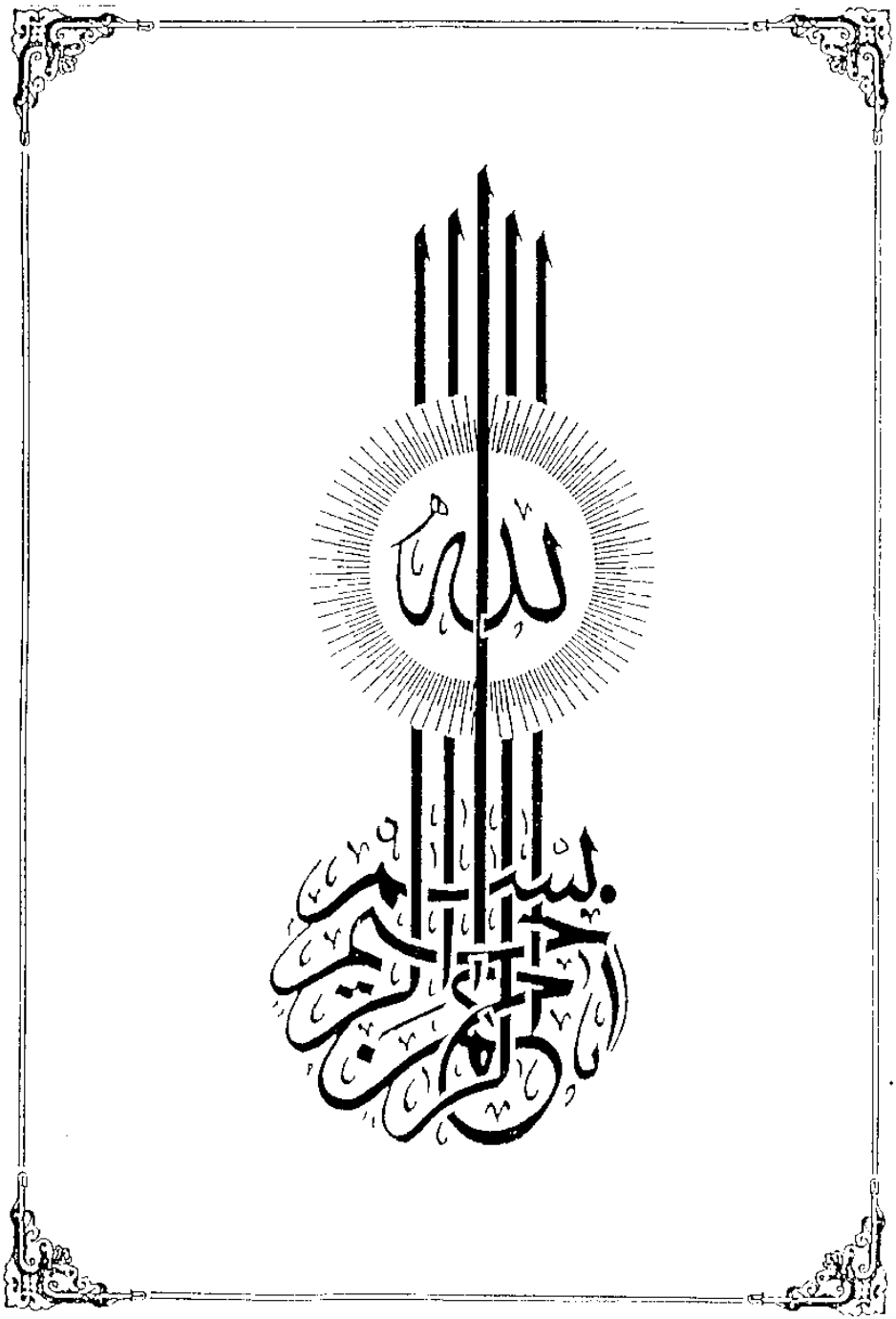

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INTRODUCTION & AIM OF WORK

The preservation of the bone marrow tissue is rather difficult. The main idea of the preservation is to keep and preserve the cell constituents outside the body as it is inside the body. Immersion fixation techniques for minute pieces of tissue for electron microscopy are available ***(Trump and Bulger, 1966)***. However, material for light microscopy was frequently poorly fixed, distorted and findings difficult to interpret. This was due to the large size of the specimens which result in fixation of the outer part while the inner part could not be penetrated by the fixative. In addition, difficulties arised as many pathological changes in tissues were focal. It seems essential, therefore, to develop methods for good fixation of large pieces of tissue for examination with light microscope so that focal localized lesions on these sections could be subsequently studied in the electron microscope ***(Jones, Gallant and Butler, 1977)***.

The ultrastructural effect of various buffer osmolality, and temperature on paraformaldehyde fixation of the blood constituents and bone marrow was studied ***(Carson, Lynn and Martin, 1972)***. They chose these tissues because the fixative was immediately in very intimate contact with the cells.

In this study, different electron microscopic techniques were tried on normal adult rabbit's bone marrow. In addition to the close contact with the fixative, the bone marrow was used because of its increasing importance in clinical practice and for filing of bone marrow patients, for their appropriate follow up.

Review Of Literature

I— FIXATION

Bahr (1955) studied the uptake of osmium tetroxide by different tissues at different times and with the extractability of tissue constituents after osmium fixation. He noticed that, in fat tissue, the outer layers reacted very quickly with osmium tetroxide, thus preventing further penetration of the fixative into the tissue. From other tissues (Liver, muscle, skin, etc...) constant amounts of fat were extractable, independent of the fixation time. He also noticed that osmium fixation, particularly when prolonged, brought about progressive destruction of tissue constituents and resulted in increased extractability with water.

Luft (1959) used acrolin, in a buffered aqueous solution as an effective fixative for animal tissue for light and electron microscopy. He observed that the fixative penetrated rapidly and shrinkage was slight. Cytoplasmic components, particularly mitochondria, were well preserved, however, lipid droplets were not retained. He added that materials fixed in acrolin could be fixed subsequently in osmium tetroxide with good preservation of cellular structure when examined with the electron microscope.

Richardson, Jarett and Finke (1960) dehydrated fixed tissue with tertiary butyl alcohol overnight. On the following day, it was cleared in toluene, infiltrated and embedded in Araldite resin-hardner-accelerator mixture without dibutyl phthelate, and polymerized at 60°C. Authors added that, such method proved more rapid than previous techniques and gave blocks which did not fracture on trimming. Moreover, this method provided sections of 1 µm for phase contrast microscopy as well as ultrathin sections which cut as easily with glass knives as sections of methacrylate.

Holt and Hicks (1961) studied the preservation of fine structure, phospholipids, and the activity of acid phosphatase and esterase in rat liver fixed in various solutions containing 4% formaldehyde. They noticed that veronal-treated or phosphate-buffered formalin gave excellent results if the tonicity of the solution was suitably adjusted by addition of sucrose. Phospholipids were retained almost quantitatively in tissue fixed in formol-calcium. As regarding the morphological and biochemical integrity of the fixed tissues, they suggested that 4% formaldehyde-buffered at pH 7.2 with 0.067 M phosphate, and containing 7.5% sucrose, was the most suitable of the fixatives for the combined cytochemical staining and electron microscopical studies.

Trump and Ericsson (1965) noticed that the composition of the buffer vehicle used with osmium tetroxide exerted a profound effect on the final structures visualized in electron micrographs. The effects observed involved differences in relative density, configuration of internal structure, and distribution of individual cell components. There was also an evidence of change in the rate and degree of heavy metal binding as the staining times with lead and uranyl salts exhibited some variation depending on the buffer used.

Wood and Luft (1965) used rat liver and pancreas for comparison of their microscopic and ultrastructural morphology after fixation in osmium tetroxide buffered with six different solutions. They noticed that the buffer affected fixation significantly. Differences in penetration of the fixative, stainability and cellular appearances by light microscopy and on sectioning properties, acceptance of heavy metal stains and general morphology for electron microscopy were observed. These results were not easily explained on the basis of tonicity differences alone but were interpreted as evidence for specific ion effects.

Hirsch and Fedorko (1968) noticed that neutrophilic polymorphnuclear leukocytes were commonly not well preserved by standard methods of fixation for electron microscopy.

They reported a method for obtaining improved fixation of neutrophil and of other white blood cells. They processed human leukocytes to the stage of dehydration as single cell in suspension or as very small clumps. Initial fixation was accomplished with a freshly made mixture of glutaraldehyde and osmium tetroxide. The cells were postfixed by suspension in uranyl acetate. Simultaneous exposure to glutaraldehyde and osmium tetroxide eliminated many of the drawbacks seen when either of these agents was used alone as the initial fixative.

Carson et al., (1972) studied the ultrastructural effects of various buffers, osmolalities, and temperatures on paraformaldehyde fixation of the formed elements of blood and bone marrow. They noticed that the most critical factor in preservation of cellular ultrastructure was the buffer system. Fixatives containing S-collodine gave the poorest; while those with phosphate gave the best preservation. Little effect on ultrastructure was noticed in varying the tonicity or temperature of the fixative within a given buffer system. They concluded that phosphate-buffered paraformaldehyde solutions, regardless of osmolality or temperature of fixative gave excellent ultrastructural preservation of peripheral blood and bone marrow specimens.

Litman and Barnett (1972) observed that the primary reaction in the staining and fixation of unsaturated tissue components by osmium tetroxide was generally involving the initial formation of cyclic osmium (V1) mono- or diesters. They noticed that most of the osmium tetroxide attached itself by hydrogen bonds to aliphatic side chains and proteins in the tissue.

Carson, Martin and Lynn (1973) found that several common formaldehyde fixative solutions using various buffer systems were evaluated for use with both light and electron microscopy. The fixative, formalin-buffered with monobasic sodium phosphate and sodium hydroxide, had been found to produce consistently high quality ultrastructural results and was of such low cost that it was currently used as routine fixative for all specimens. Use of this fixative had obviated the need for preselection of various tissues to be subsequently examined ultrastructurally.

Collin and Griffith (1974) tried to clarify the mechanism of staining tissue component by osmium tetroxide. They prepared a number of model osmium (V1) esters for studying hydrogen bonding properties of osmium tetroxide. By direct experimental evidence, they noticed that osmium tetroxide had negligible hydrogen-bonding properties to any

proton, either in simple model system or in the far more complex assemblage of lipids, proteins, etc... in the tissue.

Nielson and Griffith (1979) noticed that it was generally believed that fixation of cell membrane by osmium tetroxide involved unsaturated lipids. However, they recognized the importance of osmium tetroxide-protein interaction. They found that osmium tetroxide had a fixative or a cross linking function on tissue protein and that the protein lipid cross-linking might occur. The potentially reactive sites for osmium tetroxide in protein were histidine, lysine and arginine.

II— EMBEDDING

Glauert, Rogers and Galuert (1956) used Araldite as a new embedding medium for electron microscopy. They found that on addition of the hardener, the resin set slowly without shrinkage to form a light-gold block. Also, the viscosity of the mixture and the hardness of the final block could be controlled with a plasticizer, dibutyl phthalate, and the rate of hardening could be varied with an amine accelerator.

Luft (1961) presented an improvement in epoxy resin embedding methods with many advantages. This improvement included the addition of propylene oxide, increasing temperatures, decreasing the anhydrite : epoxy ratio and using two different anhydrite curing agents. Amongst the advantages of this method were rapid embedding, easy sectioning of the embedded tissue, good contrast of the electron microscopy and a wide range of hardeness.

Freeman and Spurlock (1962) developed a new epoxy embedding mixture using Maraglas and Cardolite with benzyldimethylamine as a curing agent. This epoxy mixture permitted cellular preservation, easy preparation of tissue, a wide range of miscibility, low viscosity and easy sectioning on microtome comparable to that obtained with Epon 812. They