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IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF HUMAN BONE MARROW EXTRACELLULAR MATRIX

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Fragments of human bone marrow were obtained from volunteers ranging between 35 and 60 years old, without presenting any systemic disease. Such fragments, previously fixed in paraformaldehyde and embedded in paraffin have been studied in the light microscope by the use of classical staining methods for connective tissue (L. G. Luna, 1968, ***Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology***, 3rd ed., McGraw-Hill Book Company, New York) and also the immunoperoxidase methods for detecting the presence of type I, III, IV and VI collagens, fibronectin, heparan sulfate, chondroitin sulfate and elastic microfibril (J. M. Polak & S. Van Noorden (eds), 1983, ***Immunocitochemistry, Practical Applications in Pathology and Biology***, Wright-PSG Inc., London).

Five micrometer sections, previously treated with methanol-hydrogen peroxide, were incubated with goat antiserum and then exposed to primary antibodies. After treatment with rabbit or mouse antiserum followed by avidin-biotin-peroxidase complex, the sections were incubated with diaminobenzidine-hydrogen peroxide, washed, stained with hematoxylin, dehydrated and mounted with Canada balsam. Specificity controls included: a) substitution of primary antibody by PBS and b) use of mouse and quail tissues as muscles, cartilages, nervous tissue and embryonic tegument.

Other bone marrow fragments were fixed in a solution containing 3% glutaraldehyde and 0.25% tannic acid in Millonig buffer, 0.1 M, pH = 7.3 for 2 h and then washed in the same buffer solution and postfixed for 1 h in a solution containing 1% osmium tetroxide in Millonig buffer, 0.1 M, pH = 7.3. After this period, the fragments were washed, dehydrated in changes of ethanol and embedded in Epon, and the sections were stained by uranyl acetate and lead citrate prior the observation in the electron microscope (G. Cotta-Pereira et al., 1976, *Stain Technol., 51*: 7-11).

Observing the immunoperoxidase preparations, it was noted an intense reaction with antibodies to type III collagen and chondroitin sulfate, while weaker positivity occurred with other components as type I, IV and VI collagens, fibronectin and heparan sulfate. Positive reaction was also observed with antibodies to elastic microfibrils (monoclonal antibody HB8) which is corroborated by the visualization, at the electron microscopical level, of bundles of tubular microfibrils 10-12 nm in diameter. These microfibrils represent the ultrastructural pattern of oxytalan fibers (G. Cotta-Pereira et al., 1976, *J. Invest. Dermatol., 66*: 143-148). Also many proteoglycan filaments have been observed binding cell membranes and wrapping scarce collagen fibrils.

The presence of the extracellular components above mentioned may be related not only with structural role but also with hematopoiesis control.