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The Role of High-Energy Synchrotron Radiation in Biomedical Trace Element Research

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The Role of High-Energy Synchrotron Radiation in Biomedical Trace Element Research

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1. INTRODUCTION

Trace elements are intimately involved in biological function and dysfunction at all levels of biological organization. At the molecular level, trace elements perform innumerable catalytic and structural roles in macromolecules and other cell constituents. At the cellular level, trace elements are necessary for maintenance and regulation of compartmentation of cell function, stimulus-response coupling, gene regulation, etc. Perturbations in trace element homeostasis and utilization at the molecular and cellular level is manifested in many disease states. Biological systems have evolved elaborate and diverse control mechanisms to provide for trace element homeostasis at the subcellular, cellular, and organismal levels. These critical functions of trace elements have profound influence on human health and disease states. Trace elements are well recognized as contribution factors in modifying development, aging, oncogenesis, and many chronic diseases including cardiovascular disorders.

X-ray fluorescence analysis with high-energy synchrotron radiation has several characteristics, including high sensitivity, multi-elemental capabilities, good spatial resolution, and minimal matrix effects and sample preparation which make it a powerful tool for understanding the role of trace elements in biological systems. The application of the high-energy x-ray microscope is directed at three general areas: first, the role of trace element interactions in normal biochemical and physiological processes; second, the microscopic distribution and metabolism of toxic elements, and third, the microscopic distribution and metabolism of the metal moiety of metal containing drugs. A description of the high-energy x-ray microscope is presented by GORDON *et al.* /1/ and an investigation of the microscopic distribution of gallium, following administration of gallium nitrate, an experimental drug for treating tumor related bone loss is given by BOCKMAN *et al.* /2/.

This paper will present the results of an investigation of the distribution of essential elements in the normal hepatic lobule. The liver is the organ responsible for metabolism and storage of most trace elements. Although parenchymal hepatocytes are rather uniform histologically, morphometry, histochemistry, immunohistochemistry, and microdissection with microchemical investigations have revealed marked heterogeneity on a functional and biochemical level. Hepatocytes from the periportal and perivenous zones of the liver parenchyma differ in oxidative energy metabolism, glucose uptake and output, ureagenesis, biotransformation, bile acid secretion, and plasma protein

synthesis and secretion /3/. Although trace elements are intimately involved in the regulation and maintenance of these functions, little is known regarding the heterogeneity of trace element localization of the liver parenchyma. Histochemical techniques for trace elements generally give high spatial resolution, but lack specificity and stoichiometry. Microdissection has been of marginal usefulness for trace element analyses due to the very small size of the dissected parenchyma. The characteristics of the high-energy x-ray microscope provide an effective approach for elucidating the trace element content of these small biological structures or regions. The preliminary results of this investigation are presented below.

2. METHODS AND MATERIALS

2.1 Specimen preparation

Male, Sprague-Dawley rats of 300 - 350 grams were used for this study. The animals were fasted for 12 hours to deplete liver glycogen stores prior to sacrifice. The animals were deeply anesthetized with ether, and the liver perfused *in situ* for one minute with 300 mM Sucrose / 5 mM HEPES buffer, at pH 7.0 and 4° C, to flush the liver sinusoids of both the cellular and acellular components of blood. This buffer was previously shown to remove unbound extracellular elements while minimizing the loss and exchange of intracellular elements /4/. Five mm thick sections of the caudal lobe were removed, placed in cryomolds with Tissue Tek and frozen in liquid nitrogen. The livers from five rats were sectioned at 20 μm (approximately 1 cell thick) with a freezing microtome, mounted on 7.3 μm Kapton support film, and lyophilized prior to trace element analysis.

2.2 Microprobe analysis

Filtered white synchrotron radiation was used for trace element analyses. The beam was filtered by 100 μm aluminum before irradiating the target. The fluorescence signal was filtered by 50 μm of Kapton placed in front of the Si(Li) detector. Tantalum slits were used to collimate the incident x-ray beam to 50 x 50 μm (approximately 2-3 cells wide). The fluorescence spectra were collected from at least 10 locations within 100 μm of the periportal and perivenous regions of the parenchyma from each rat.

2.3 Data analysis

The peak areas for each measurement were determined using the program HEX as slightly modified for synchrotron radiation. The efficiency for x-ray fluorescence for each element studied was determined from cryosections of gelatin with selected elements added to 10 μg per ml gelatin. The gram atoms of Ca, Cr, Mn, Fe, Cu, Zn, Se, and Br, were normalized to the gram atoms of K for each measurement. The rationale for this normalization is that there may be regional and section-to-section variation in section thickness making normalization to the volume tenuous. Moreover, the liver is highly vascular and each measurement would include a variable volume of blood space which had to be flushed of elements. However, K is distributed uniformly, for the purposes of this study, in the cell water, and is proportional to cell water. Thus the normalization of elements to potassium provided an appropriate and rational approach to normalize without concern for variations in section thickness or the relative amounts

of parenchyma and sinusoidal space irradiated during measurement. The differences in the trace element content of the periportal and perivenous regions of the parenchyma was determined by a paired t-test.

The elemental content of liver parenchyma was determined in tissue volume of approximately 7 x 10^{-4} μl , that is to say, a tissue volume approximately equivalent of 4 to 8 parenchymal cells. The concentrations of K, Ca, Cr, Mn, Fe, Cu, Zn, Se, and Br, averaged 1700, 88, 1.8, 1.4, 35, 1.0, 32, 0.8, and 1.2 μg / cm^3 fresh tissue respectively. These values are consistent with values for fresh liver determined by conventional chemical analysis /5/. A representative spectrum is shown in Figure 1. The relative distribution of these elements, normalized to potassium was not significantly different between the periportal and perivenous regions (Table 1).

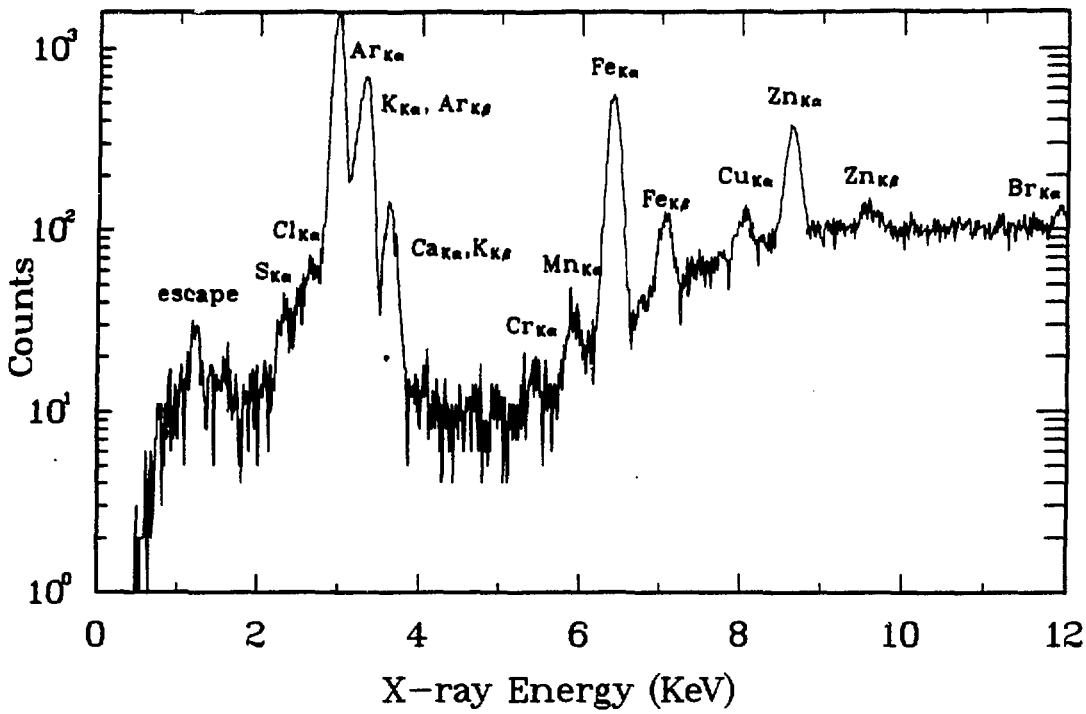

Figure 1. Representative x-ray fluorescence spectrum from periportal liver parenchyma

The current study provides a basis to evaluate the distribution, kinetics, and function of these elements in periportal and perivenous liver parenchyma in situation where the whole liver metabolism is known to be altered. These situations include acute phase reactions, acute and chronic alcohol intoxication, iron and copper over load, and intoxication with non- essential elements. This example illustrates how a high-energy x-ray microscope may be used to gain information about the trace element content of biological structures which are too small or complex in shape to dissect for chemical analyses.

This lack of a difference between regions is somewhat surprising considering the important and multiple roles of Fe in biotransformation, Zn in the dehydrogenases of intermediary metabolism, and Cu and Se in enzymes of biotransformation. Certainly the biochemical form of the trace elements. i.e. the functional trace element complex is the important form. These measurements provide the distribution of these elements in the normal, fasted liver parenchyma and in the current study do not provide information on the dynamic state of the elements.

Table 1. The ration of selected essential elements in periportal to perivenous region of normal liver parenchyma.

Animal	Ca	Cr	Mn	Fe	Cu	Zn	Br
#1	0.83	0.83	1.17	0.94	0.94	0.87	1.15
#2	1.14	1.05	1.04	0.94	1.07	0.97	0.73
#3	1.17	0.86	0.99	0.71	1.43	1.26	1.53
#4	0.95	1.32	0.87	1.11	0.96	1.03	0.54
#5	0.58	0.85	0.91	1.05	0.88	1.07	0.57

The gram atoms of each element in the beam spot were normalized to potassium. The data represent the ratio of the element/K in the periportal region to the element/K in the perivenous region. No statistical differences were detected with a paired t-test.

4. ACKNOWLEDGEMENTS

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