 0030-0950/79/0006-0271/$1.00/0

BRIEF NOTE

ELEMENTAL ANALYSIS OF BIOLOGICAL MATERIAL IN THE FRESH-FROZEN STATE[1]

WAYNE B. PARRISH, Department of Zoology
DONALD G. BRONN, JAMES R. WRIGHT JR. and JOHN P. MINTON, Department of Surgery, The Ohio State University, Columbus, OH 43210

OHIO J. SCI. 79(6): 271, 1979

The acceptance of x-ray probe analysis of biological materials depends upon the methods used for the preparation of specimens. Fixation and embedding create a problem since many embedding media contain high levels of electrolytes (Mizuhira 1976). Other fixatives such as glutaraldehyde urea resin, which do not contain electrolytes, contribute to the loss of labile elements. In an effort to prevent this loss during the dehydration, embedding, and sectioning process, additives such as oxalates have been incorporated into the fixative (Poddsky *et al* 1970), but Coleman (1976) has reported that this method causes redistribution of elements into the intercellular spaces. In order to more accurately detect the elemental distribution in biological materials by x-ray probe analysis, a number of methods for tissue preparation have been explored. Critical point drying offers superior preservation of surface structure and morphology for electron microscopy, but this technique promotes the redistribution of the normal intracellular elements and therefore is not useful for x-ray probe studies (Warner and Coleman 1975). Cryogenic techniques have been utilized for the preparation of specimens for x-ray probe analysis. Rapid freezing not only preserves morphological structure but also the normal distribution

[1]Note received 28 July 1978 and in revised form 4 January 1979 (#78-42).

of mobile elements such as sodium and potassium (Coleman 1976). Ingram and coworkers (1976) showed that freeze-drying of rapidly frozen biological material results in a 20% decrease in volume. This shrinkage causes the elements to concentrate in a manner which may not reflect the natural state. Furthermore, the dehydrated tissue is usually exposed to osmium vapor for fixation and contrast, but fixation may change tissue elemental distributions. The fresh-frozen state is therefore a better method of preparation of biological materials for x-ray probe analysis than techniques such as critical point or freeze-drying.

Tissue specimens were obtained at slaughter and were transported on ice to the electron microscopy facilities. Specimens were sectioned to a size of approximately 1 cm^3 and were placed on a stub for examination with the scanning electron microscope (Cambridge S4–10). The stub, which was made of aluminum, was modified to accept a thin beryllium wafer (Ernest Fullam Company, Schenectady), and contact was maintained by electron-conducting paste. The beryllium wafer prevents the appearance of an artifactual aluminum peak in the spectrum. A conducting interface was obtained between the beryllium and the fresh specimen when it was frozen by the stub's contact with the coldstage (Cambridge Hot/Cold Module), which had been previously cooled with liquid nitrogen. By freezing the specimen in the cold stage within the scanning electron microscope (SEM), tissue frosting was maintained at a minimum. The thermocouple, which was 10 mm from the stub, indicated that the specimen was maintained at −170 to −180 °C. A vacuum was then drawn in the SEM, (200 μm aperture), and the specimen was located by imaging. Various sites for probe analyses were chosen, and the dimensions of the x-ray probe were set by determining the magnification of the SEM. Assuming an average cell diameter of 10 μm/cell, the electron beam was of the diameter of approximately 20 cells. The homogeneity of the tissue specimen was confirmed by the SEM image. Specimens were analyzed by an energy dispersive x-ray detector with an amplifier and display system (Ortec 6200 Multichannel Analyzer). The distance from the probe to the specimen was maintained at 4.5 cm. An accelerating voltage of 20 KV, beam current of 170 m amps, count rate of 2000 counts per second for 100 seconds, and a take-off angle of 40° were utilized.

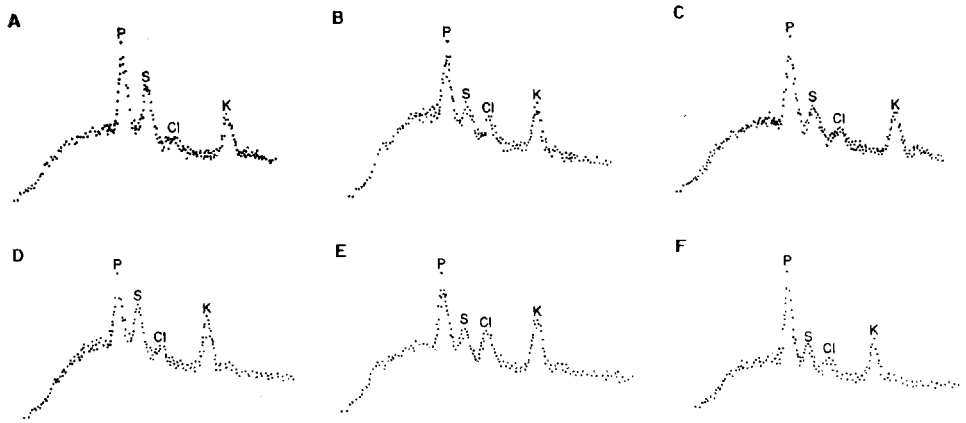

FIGURE 1. X-ray spectra of tissue specimens from cattle and sheep: (A) Bovine liver (B) Bovine brain (C) Bovine pancreas (D) Sheep liver (E) Sheep brain (F) Sheep pancreas. Spectra A and D demonstrate the high sulfur and muted chlorine peaks characteristic of liver samples. Brain samples, spectra B and E, are characterized by low sulfur and high chlorine peaks, while analysis of pancreas samples, spectra C and F, yields both low sulfur and low chlorine peaks.

The four elemental peaks most evident for all tissue samples examined were phosphorous, sulfur, chlorine, and potas-

sium. Comparative examinations of bovine liver, brain (cerebrum), and pancreas revealed a consistent difference in elemental patterns which distinguished each of these organ tissues. The elemental pattern of liver (fig. 1A) was characterized by a comparatively high sulfur peak and a muted or very low chlorine peak. This elemental pattern remained consistent for liver samples obtained from cattle and sheep (figs. 1A and 1D). Elemental patterns of bovine brain (fig. 1B) were characterized by a relatively lower sulfur peak and an almost equally prominent chlorine peak. The elemental patterns of bovine pancreas (fig. 1C) were characterized by a low sulfur peak, but the chlorine peak was consistently lower than the sulfur peak. The differentiating characteristics for bovine brain and pancreas (figs. 1B and 1C) were the same for sheep brain (fig. 1E) and sheep pancreas (fig. 1F). Bovine brain could not be distinguished from sheep brain, nor bovine pancreas from sheep pancreas. Twenty-four repeat analyses were consistent with the original findings and did not vary with different animal donors.

The elemental patterns we found were similar for homologous tissues in sheep and cattle, but tissues from various organs within one animal had different elemental patterns for each of the different organ tissues. Thus, the elemental patterns were tissue-specific but not species-specific (fig. 1).

Our observations suggest that elemental analysis may be used to differentiate organ tissues by differences in their elemental patterns. When the tissue is maintained in the frozen-hydrated state, the integrity of the specimen is not compromised by preparative methods such as fixation, critical point drying, or freeze-drying.

Acknowledgment. This study was supported by funds from the Elsa U. Pardee Foundation. Tissue specimens were obtained through the courtesy of The Ohio State University Department of Animal Science.

LITERATURE CITED

Coleman, J. R. 1976 Electron probe studies of calcium transport. Proc. 11th Ann. Conf. Microbeam Anal. Soc. paper 58. Aug. 9–13, Miami Beach. pp. 58A–M.

Ingram, F. D., M. J. Ingram and A. R. Spurr 1976 Volume changes in freeze dried tissue. Proc. 11th Ann. Conf. Microbeam Anal. Soc. paper 63. Aug. 9–13, Miami Beach. pp. 63A–B.

Mizuhira, V. 1976 A review of the various usages of energy dispersive x ray analysis in biology. Proc. 11th Ann. Conf. Microbeam Anal. Soc. paper 5. Aug. 9–13, Miami Beach. pp. 5A–D.

Poddsky, R. J., T. Hall and S. L. Hatchett 1970 Identification of oxalate precipitates in striated fibers muscle. J. Cell. Biol. 44: 699–702.

Warner, R. R. and J. R. Coleman 1975 Electron probe analysis of calcium transport by small intestine. J. Cell. Biol. 64: 54–74.