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The Effect of Decalcification on the Microspectrophotometric Determination of DNA

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Conventional methods of decalcification using acids depolymerize nucleic acids. This is a serious handicap for microspectrophotometric studies on the DNA content of calcified tissues. In the investigation reported here we have evaluated different decalcifying agents for this purpose. Liver, spleen, and maxillae of adult Wistar rats were exposed to the action of nitric acid, picric acid, and EDTA.

Tissues were subjected to the action of 7% nitric acid for 9, 12, and 24 hours, and for 3 days. In picric acid (Bouin fixative) the exposure lasted 3

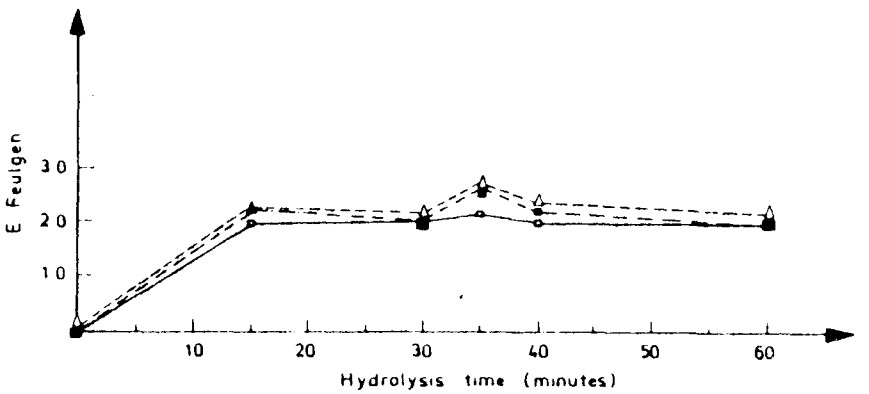

Fig. 1. Feulgen hydrolysis curve for spleen lymphocytes. Control □——□; EDTA-tris pH 7 treated spleen lymphocytes ■- - -■; EDTA-tris pH 7 decalcified bone cells △- - - - -△

days, and in EDTA-tris pH 7 (EDTA 10% in 0.1 M tris, adjusted to pH 7.0 with KOH pellets [Fullmer and Link 1964]), 15 days. The Feulgen reaction was carried out using 5N HCl at room temperature and hydrolysis periods of different duration (De Cosse 1966, Deitch 1967). Microspectrophotometric readings were obtained using a Zeiss Cytoscan with automatic stage under conditions described in a previous report (Giménez 1977).

Tissues treated with nitric acid and picric acid showed severe losses of nuclear DNA. In most cases the Feulgen reaction gave a completely negative response. Material exposed to the action of EDTA-Tris pH 7 yielded hydrolysis curves similar to the controls (Fig. 1); the ploidy relationship of the studied tissues was not affected (Table 1).

Our results obtained in the cells of different tissues apparently indicate that neither the amount of DNA in tissues nor the stoichiometry of the Feulgen reaction is modified by treatment with EDTA-tris at pH 7. Consequently, we believe that tissue decalcification achieved by this method can be

TABLE I DNA AMOUNTS IN CONTROL AND EDTA TRIS TREATED TISSUES

Cell	*n*	N	Control DNA	N	EDTA Tris 15 days DNA
Hepatocytes	2	50	2.61 ± 0.20	50	2.65 ± 0.29
	4	40	5.59 ± 0.34	40	5.63 ± 0.44
Lymphocytes	2	50	2.52 ± 0.17	60	2.66 ± 0.26
Connective Tissue Cells	2	50	2.59 ± 0.19	40	2.74 ± 0.30
Osteocytes	2	–	–	60	2.75 ± 0.28
Epithelial Cells	2	–	–	30	2.83 ± 0.32
Odontoblasts	2	–	–	30	2.81 ± 0.29

DNA values are given in arbitrary units ± S.D. N: number of cells measured; *n*: ploidy class

used as a routine technique preceding microspectrophotometric studies of DNA in calcified tissues.

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