INTRACELLULAR LACTATE DEHYDROGENASE CONCENTRATION AS AN INDEX OF CYTOTOXICITY IN RAT HEPATOCYTE PRIMARY CULTURE

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In searching for a reliable index for cytotoxicity testing in rat hepatocyte primary culture, lactate dehydrogenase (LDH) concentrations in lysates of attached hepatocytes and LDH released into the culture medium were compared under conditions of exposure to various dosages of sodium chloride, sodium salicylate, R-warfarin, acetaminophen, phenylbutazone, and furosemide (frusemide). The amount of intracellular LDH was assessed by inducing the cells to release the enzyme with 0.1% Triton X-100. The induced LDH leakage was completed in 1 hr and the LDH activity was stable in storage at 10°C for 2 weeks. We found that intracellular LDH is a direct indicator of the number of viable hepatocytes in contrast to the LDH released, because released LDH does not account for the significant number of cells detached from monolayer but which are not leaky, during the 6-hr test period. Based on IC₅₀ values (50% inhibitory concentration), the relative cytotoxicities are R-warfarin > phenylbutazone > furosemide > acetaminophen > sodium salicylate > sodium chloride.

INTRODUCTION

The fact that maintenance cultures of hepatocytes are metabolically competent in contrast to established cell lines, such as Hela cells and Chang liver cells, is a major advantage for their use in toxicity screening and for studies of toxic mechanisms. Hepatocytes from adult rats, in particular, have proven to be a useful model for

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3. Abbreviations: DMSO, dimethyl sulfoxide; HPC, hepatocyte primary culture; IC₅₀, 50% inhibitory concentration; LDH, lactate dehydrogenase.
studies of xenobiotic biotransformation (Guzelian et al., 1977; Croci and Williams, 1985). While cultured cell lines and hepatocyte primary cultures (HPC) may have comparable sensitivities for general cytotoxicity, HPC are more sensitive than Hela cells towards xenobiotics such as carbon tetrachloride and acetaminophen, which require metabolic activation for the expression of toxic effects (Ekwall and Acosta, 1982).

A convenient end-point for cytotoxicity assessment in hepatocytes is the determination of the leakage of lactate dehydrogenase (LDH) from damaged hepatocytes into the culture medium, generally referred to as LDH release (Mitchell and Acosta, 1981; McQueen and Williams, 1982; Tyson et al., 1983). During a given incubation time, the concentration of a test chemical required to release 50% of the total intracellular LDH (50% inhibitory concentration or IC_{50}) is a useful parameter for comparing the toxicities of drugs and other chemicals (Ekwall and Acosta, 1982).

Most studies with HPC require information on the number of viable hepatocytes and the cytotoxicity, if any, of the compounds administered to the HPC. Direct viable cell counts on monolayer cultures of hepatocytes using trypan blue are not feasible. Indirect viable cell counts by measuring intracellular LDH concentrations appears to be promising (Jauregui et al., 1981). We report here our successful attempt to use the latter in assessing the number of viable hepatocytes in monolayer cultures. The effects of short-term exposure to six test chemicals including four known hepatotoxic drugs are described in support of this claim.

**METHODS**

Amino acids, collagenase Type I, NADH, EGTA, insulin, sodium ascorbate, and pyruvate were purchased from Sigma (St. Louis, MO). R-warfarin was prepared from the racemate as previously reported (Kaminsky et al., 1981; Fasco et al., 1978). MEM vitamin 100X solution was purchased from GIBCO (Grand Island, NY). Vitrogen solution was obtained from Collagen Corp. (Palo Alto, CA). All other chemicals were of either reagent grade or spectral grade, in the case of those used in HPLC analyses. Rat serum was used as a LDH standard and was prepared in this laboratory. Male Sprague-Dawley rats were purchased from Charles River Laboratory (Wilmington, MA). Surgical equipment was purchased from Foster Medical Supply (Guilderland, NY), Hoechst dye 33258 from Poly Sciences (Warrington, PA), and nylon mesh (Nitex Nylon Monofilament bolting cloth) from Tetko Inc. (Elmsford, NY).

**Hepatocyte Primary Culture.** Hepatocytes were prepared from adult male Sprague-Dawley rats (350-450 g) by techniques described by Bissell and Guzelian (1980) with the following modifications. The two-step liver perfusion technique following inferior vena cava cannulation with a 16 ga I.V. catheter involved initial perfusion (15 ml/min) with 150 ml Hanks solution with the addition of EGTA (0.05 mM) and the omission of Ca^{++} and then with 400 ml modified Waymouth MB-752/1 medium containing