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OPTIMIZATION OF CRYOPRESERVATION PROCEDURES FOR RAT AND HUMAN HEPATOCYTES. L J Loretz, A P Li, M W Flye and A G E Wilson. Monsanto Environmental Health Laboratory and Washington Univ Med School, St. Louis, MO.

Rat hepatocytes were cryopreserved using a number of procedures and the viability, attachment, and metabolic activity of the cryopreserved cells were compared. Several cryopreservation agents (DMSO, glycerol, PVP, dextrans) and combinations of these agents were tested. Other variables tested included the freezing rate and the concentration of serum in the freezing medium. The greatest recovery of viable attached cells was obtained using DMSO at concentrations of 10% or higher, and a freezing rate of  $\sim 1^\circ\text{C}/\text{minute}$ . Varying serum concentration in the freezing medium did not affect cryopreservation results. Using this procedure, the recovery of viable hepatocytes was 70%. Metabolic endpoints used to evaluate cryopreserved cells included activation of pro-mutagens in the CHO/HGPRT gene mutation assay, ethoxycoumarin-O-deethylase activity, p-chloromethylaniline demethylase activity, and urea synthesis. Each of these endpoints remained unchanged following cryopreservation. In addition, peroxisome proliferation at a level similar to that found in freshly isolated hepatocytes was observed in cryopreserved hepatocytes following treatment with Wyeth 14,643. Similar results were obtained with hepatocytes isolated from two human livers.

ROLE OF THE 4S BINDING PROTEIN IN THE INDUCTION OF ARYL HYDROCARBON HYDROXYLASE IN THE RAT. M Harris, C Kamps and S Safe, Departments of Veterinary Physiology and Pharmacology and Biochemistry and Biophysics, Texas A&M University, College Station, TX

A survey of several rat strains demonstrated that the levels of the hepatic cytosolic 4S binding protein (using [ $^3\text{H}$ ]-benzo[a]pyrene as the radioligand) were highly variable. The concentrations of this binding protein in Sprague Dawley (Harlan, -4S), Sprague Dawley (Sasco, +4S), Fischer 344, Wistar, and Lewis rat hepatic cytosol were  $0$ ,  $208 \pm 57$ ,  $0$ ,  $244 \pm 21$ , and  $216 \pm 40$  fmol/mg cytosolic protein, respectively. Dose-response induction of hepatic microsomal aryl hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3-methylcholanthrene (MC) in Sprague Dawley (+4S) and Sprague Dawley (-4S) rat strains gave comparable  $\text{ED}_{50}$  values for AHH induction. Studies with these inducers and other polynuclear aromatic hydrocarbons with affinity for the 4S binding protein suggest that this protein plays a minimal or antagonist role in the regulation of AHH induction in the rat. (Supported by the Texas Agricultural Experiment Station and the National Institutes of Health.)

258 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) ANTAGONISTS PROTECT AGAINST TCDD-MEDIATED PORPHYRIA. C Yao and S Safe, Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine and Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX

Administration of 2,3,7,8-TCDD (75 ug/kg) to C57BL/6 mice causes significant hepatic accumulation of octa and hepta carboxy-substituted porphyrins within 3 weeks after treatment. In contrast, treatment of the animals with either Aroclor 1254 (250 umol/kg) or 6-methyl-1,3,8-trichlorodibenzofuran (MCDF, 750 umol/kg) did not cause hepatic porphyria. Cotreatment of the mice with 2,3,7,8-TCDD (75 ug/kg) plus either Aroclor 1254 (250, 75 and 25 umol/kg) or MCDF (750, 250 and 75 umol/kg) completely inhibited the accumulation of hepatic hepta and octa carboxy-substituted porphyrins. The inhibition of hepatic microsomal aryl hydrocarbon hydroxylase by MCDF or Aroclor 1254 was not observed. Using a staggered treatment protocol for administration of the antagonist, significant protection from the porphyrinogenicity of 2,3,7,8-TCDD was observed when the antagonist was administered 2 weeks after the toxin. The mechanistic implications of these and other observations will be discussed. (Supported by the National Institutes of Health.)

259 EFFECTS OF NITROUS OXIDE AND BODY WEIGHT ON THE GUINEA PIG MODEL OF HALOTHANE HEPATOTOXICITY. RC Lind and AJ GandoIfi, Department of Anesthesiology, University of Arizona, Tucson, AZ.

Halothane (H), is often administered concurrently with  $\text{N}_2\text{O}$ . Since  $\text{N}_2\text{O}$  can exacerbate liver injury, this combination of anesthetics was evaluated in a guinea pig model of halothane hepatotoxicity. Male and female strain 13 guinea pigs (300-1000 g) were exposed to 1% H, 40%  $\text{O}_2$  for 4 hr with or without 60%  $\text{N}_2\text{O}$ . The addition of  $\text{N}_2\text{O}$  affected neither plasma concentration of H metabolites nor the degree of hepatic injury (ALT and histopathology). Animal weight was a factor with larger (572-970 g) animals of both sexes demonstrating significantly greater elevations in 48 hr ALT and incidences of centrilobular necrosis vs smaller (318-565 g) animals (ALT=134 + 74 units/ml vs 27 + 7; necrosis=17/24 vs 0/18, respectively). There were no significant differences between large and small animals in levels of plasma metabolites of H. Following 0.1 ml/kg  $\text{CCl}_4$  (ip), larger guinea pigs also developed significantly greater elevations in ALT over those in smaller animals. Further studies will be required to elucidate factors involved in this variation in hepatotoxic response in guinea pigs of different sizes. (NIH AM16715).