Toxicology II

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BIOLOGICAL CHANGES IN PRIMATES PRODUCED BY A POLYCHLORINATED BIPHENYL AND TRIPHENYL. J. G. Allen, L. J. Abrahamson* and D. M. Norbeck*. Dept. of Pathology and Primate Res. Ctr., Univ. of Wisconsin, Madison, WI. 53706.

Male rhesus monkeys were fed a diet containing either 300 ppm of a polychlorinated biphenyl (Aroclor 1248) or 5,000 ppm of a polychlorinated triphenyl (Aroclor 5460) for 5 months. In addition to alopecia, subcutaneous edema, conjunctivitis and acne, there was a decided liver hypertrophy. The increase in liver size was attributed to an increase in smooth endoplasmic reticulum and collection of lipid droplets within the hepatic cells. Enzymatically, the enlarged livers had increased activities of their microsomal enzymes. There was also thickening of the stomach caused by edema of the submucosa and marked hyperplasia of the gastric mucosa. The hyperplastic epithelium penetrated the muscularis mucosa and invaded the submucosa. These displaced epithelial cells lined large mucinous cysts, formed acinar structures and assumed a sheet-like appearance. Penetration of the basal lamina and invasion of the adjacent connective tissue by the hyperplastic epithelial cells was a frequent occurrence. Focal necrosis of the surface epithelium and development of ulcers in some of the areas where the muscularis mucosa had been penetrated by mucosal epithelium was observed.
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132 NUTRITION

THE IN VITRO INHIBITION OF LACTIC DEHYDROGENASE BY SELECTED POLYCHLORINATED PESTICIDES. Constance M. Hendrickson* and Joe A. Borden* (SPON: R. E. Allen). Dept. Biochemistry, Louisiana State University, Baton Rouge, La. 70803.

Since little is known about the in vitro effects of pesticides and related compounds on energy metabolism, a series of experiments was devised using rabbit muscle lactic dehydrogenase (IUB 1.1.1.27) (LDH), the terminal enzyme of anaerobic glycolysis, as a model system. Selected compounds Dieldrin (T), Endrin (E), Aldrin (A), DDT isomers, Mirex (M), Lindane, Malathion, Duraban, Methyl parathion, carbamate herbicides and a polychlorinated biphenyl were assayed for inhibitory effects on LDH at 340 nm in 0.1 M phosphate (20X ethanol) at pH 7.5, pyruvate at 6.6×10^{-4} and 1.3×10^{-3}M, and DPNH at 7.0×10^{-5} M. D, A, E, M, o,p'-DDT and p,p'-DDT were found to inhibit LDH in the range 10^{-6}-10^{-2}M. Dixon plots indicate that all are competitive inhibitors (Ki values 1×10^{-5}-6×10^{-5}M). Among dieldrin isomers the position of the epoxide function has no effect (D,E) but its removal (A) lowers the inhibition. Of the DDT isomers o,p'-DDT is more inhibitory; DDT analogue 1, 1-diphenylethane and chlorobenzene have no effects, indicating that intramolecular proximity of chlorines may be a factor. Although M has highest percent chlorine, its Ki remains in the same range. It appears from this data that the polychloro functions common to these compounds play an important part in LDH inhibition; whether this competitive inhibition is specific for the selected group of compounds or is more generalized is not yet known.

133 NUTRITION

METABOLITE LEVELS, REDOX AND PHOSPHORYLATION STATES OF FREEZE-CLAMPED LIVERS OF RATS INGESTING POLYCHLORINATED BIPHENYLS. Ruth A. Mehlman, Biochemical Toxicology Br., Div. of Toxicology, Bureau of Foods, FDA, Washington, D.C. 20204.

Polychlorinated biphenyls (PCB) were administered for 12 days in the diet as Aroclor-1254 to male Osborne-Mendel rats at concentrations of 0, 75, and 400 ppm. PCB effects on the liver cyt. and mito. redox states and cyt. phosphorylation states were calc. from the conc. of intermediary metabolites. Rats receiving 400 ppm of Aroclor-1254 in the diet exhibited decreased body weight gains, food consumption, and adipose tissue weight with respect to controls. Significant differences in the concentrations of intermediary metabolites found in the liver were exhibited by rats fed Aroclor-1254 at both the 75 and 400 ppm levels. The cyt. redox state of the liver was greatly shifted toward a highly oxidized condition, as evidenced by increased [NADP$^+$]/[NADPH] ratios, in rats receiving Aroclor at 400 ppm; this effect was less drastic in rats receiving 75 ppm. A similar increase in [NADP$^+$]/[NADPH] in the cytosol has been observed in livers from thiamin-deficient rats. No significant differences could be observed in the liver cyt. or mito. [NAD$^+$]/[NADH] ratio with respect to control values in either expt. group. The phosphorylation state of liver cyto. was increased in rats receiving 400 ppm of Aroclor, and somewhat less in rats receiving 75 ppm. The data obtained with PCB are similar to that observed in thiamin-deficient animals.

134 PHARMACOLOGY

ALTERATION OF HEPATIC MICROSOMAL HEMOPROTEIN BY POLYCHLORINATED BIPHENYLS. A.P. Alvares, D. Bickers* and A. Kappas. The Rockefeller University, New York, N.Y. 10021.

The CO-difference spectrum of microsomes from rats treated with polychlorinated biphenyls (PCB, 25mg/kg/day/x6) has an absorption maximum at 448nm. With ethyl isocyanide as the ligand for reduced microsomes, PCB treatment causes a shift in the 455nm peak to 453nm, and increases the ratio of the 455:430 peaks from 0.53, obtained with untreated rats, to 1.34. These findings are similar to those seen in rats treated with 3-methylcholanthrene (3-MC) but differ from those which characterize cytochrome P-450 in control or phenobarbital-treated rats. PCB treatment leads to a 3-fold increase in cytochrome P-448 content and an 8-9 fold increase in benzpyrene hydroxylation. However, with hexobarbital the PCB-induced P-448 shows a typical type I difference spectrum whereas P-448 induced by 3-MC shows a modified type II difference spectrum. Also unlike 3-MC, PCB treatment causes a 3-fold enhancement of ethylmorphine N-demethylase. Administration of actinomycin D, an inhibitor of nucleic acid synthesis, prior to PCB treatment blocks the induction of P-448, the shifts in absorption maxima, the change in the 455:430 peak ratio, and the increases in benzpyrene hydroxylation and N-demethylation of ethylmorphine, suggesting that PCB induces synthesis of a spectrally distinct hemoprotein. PCB-induced P-448 may be catalytically different from 3-MC-induced P-448; or the hemoprotein(s) induced by PCB, may be a mixture of P-448 and P-450 exhibiting catalytic properties of both cytochromes.

135 PATHOLOGY

EFFECT OF POLYCHLORINATED BIPHENYLS (PCB) ON GLUCONEOGENIC ENZYME ACTIVITIES OF RATS EXPOSED TO COLD. Beverly Friend*, Myron A. Mehlman and Richard B. Tobin*. (SPON: W. M. Berton). Dept. of Biochem., Univ. of Nebraska, Omaha, Nb. 68105, and Biochemical Toxicology Br., Div. of Toxicology, Bureau of Foods FDA, Washington, D.C. 20204.

Rats were fed diets containing 0 to 400 ppm of Aroclor-1254 for four weeks. After two weeks on the diets, half of these rats were placed in the cold at 3°C for an additional two weeks. PCB effects on body weight gain, food consumption, organ and tissue weight, and enzyme activities were determined. Rats maintained in the cold gained less weight than those at ambient temperature on both control and experimental diets. At both temperatures, there was a two-fold increase in the liver weight and a significant decrease in the activity (umoles/g tissue/min) of hepatic pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase (FDPase) and glucose-6-phosphatase (G-6-Pase) in rats fed PCB. Cold exposure significantly increased PEPCK of both groups. When enzyme activities were expressed as total umoles/100 g body weight/min, there were no significant differences in PC, PEPCK, FDPase, and G-6-Pase of PCB-ingesting rats at 25°C. In rats exposed to cold, the total activities of PC, PEPCK, FDPase, and G-6-Pase were increased in the PCB-fed rats. These studies show that ingestion of PCB (as Aroclor-1254) increases total gluconeogenic capacity of rats exposed to cold, while at room temperature the activities remain unchanged.
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Effects of Chlorinated Hydrocarbons Applied to Skin on Hepatic Cytochrome P-450 and Drug Metabolizing Enzymes. D.R. Bickers*, A. Kappas and A.P. Alvares. N.Y.U. and Rockefeller Univ., New York, N.Y.

DDT and polychlorinated biphenyls (PCB) were applied (75mg/kg/day x6) to rat skin to determine their comparative effects on the hepatic mixed function oxidase system (MFOS) following percutaneous absorption. DDT-treatment produced a less than 2-fold induction of cytochrome P-450 with a CO-difference spectrum peak at 450nm. The ethyl isocyanide difference spectrum showed peaks at 455nm and 430nm with a 455:430 ratio of 0.78. PCB caused a 3-4 fold induction of a cytochrome showing a CO-difference spectrum peak at 448nm. The ethyl isocyanide complex of this cytochrome showed a shift of the 455nm peak to 453nm and an increase of the 455:430 ratio to 1.6; these are similar to the effects induced by skin application of the polycyclic hydrocarbon dimethylbenzanthracene. Application of DDT or PCB to skin led to enhanced activities of ethylmorphine N-demethylation and aniline hydroxylation in liver microsomes. PCB caused an increase in liver weight and microsomal protein while DDT did not. PCB also caused a marked increase in benzpyrene hydroxylase activity in skin and liver. Following a single application of DDT or PCB to skin there were similar though lesser effects on the hepatic MFOS. Peak responses evoked by both compounds were seen at 4 days; however the PCB effects were greater and persisted for 4 weeks whereas those produced by DDT lasted only 8-10 days.

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