1222 1/1/91 Ref

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ABSENCE OF PAPS-DEPENDENT BIOACTIVATION OF 7-HYDROXY-METHYL-12-METHYLBENZANTHRACENE (7-OH-DMBA) AND N-HYDROXY-2-ACETYLAMINO-FLUORENE (N-OH-2AAF) IN HUMAN LIVER CYTOSOL. Sharon J. Hirshey, Thomas B. Dowling, and Charles N. Falany (SPON: Patricia M. Hinkle). Department of Pharmacology, University of Rochester, Rochester, NY 14642.

Cytosolic steroid and phenolic sulfotransferase (ST) activity has been implicated in the metabolic activation of various polycyclic aromatic hydrocarbons to toxic, reactive intermediates, capable of binding to cellular nucleic acids. The formation of these DNA adducts is believed to be the initiation step in the process of chemical carcinogenesis. The 3'-phosphoadenosine 5'-phosphosulfate (PAPS)-dependent activation of two prototypical carcinogens, 7-OH-DMBA and N-OH-2AAF, to reactive metabolites capable of forming DNA adducts has been examined in human and rat liver cytosol. Female Sprague-Dawley rat liver cytosol displays approximately twenty-five fold higher levels of 7-OH-DMBA DNA adduct formation than male rat liver. However, this sex difference is reversed when N-OH-2AAF is used as substrate; male rat liver cytosol possesses approximately sixteen-fold higher levels of DNA adduct formation than female rat liver. In contrast to the bioactivation observed in rat liver cytosol, no PAPS-dependent DNA adduct formation was observed in human liver cytosol with these two carcinogens. The phenol and steroid STs in rat liver cytosol are a heterogeneous group of enzymes, with the levels of many isozymes showing marked differences between the sexes. In comparison, only two forms of phenol and one form of steroid ST, which do not display a sex difference in activity, have been detected in human liver. These results suggest that the forms of cytosolic ST in human liver are not homologous to the forms involved with the bioactivation of 7-OH-DMBA and N-OH-2AAF in rat liver cytosol. Supported by NIH grants GM/CA 40440 and GM 38953.

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SUICIDE INACTIVATION OF RAT LIVER ARYL SULFOTRANSFERASE IV (AST IV) BY THE SULFURIC ACID ESTER OF N-HYDROXY-2-ACETYL-AMINOFLUORENE (NOH-AAF). D.P. Ringer, T.R. Norton and R.R. Self. The Noble Foundation, Box 2180, Ardmore, OK 73402.

Rat liver NOH-AAF sulfotransferase activity is mediated by AST IV and causes the bioactivation of NOH-AAF to a highly reactive, mutagenic sulfuric acid ester form which putatively has a role in inducing liver cancer. Unexpectedly, AAF has been found to decrease liver NOH-AAF sulfotransferase activity in dietary protocols used to induce hepatocarcinogenesis. We have thus examined reaction-product, suicide inactivation of AST IV as a possible mechanism for the loss in sulfotransferase activity. In initial experiments, purified AST IV was found to undergo a PAPS-dependent binding with [^{14}C]-NOH-AAF. Alkaline hydrolysis and C18-HPLC analysis of the AST IV:AAF conjugates revealed that linkage primarily involved cysteine and methionine residues of AST IV. Experiments testing the effect of pretreatment of AST IV with NOH-AAF upon subsequent assay of sulfotransferase activity, showed that there was a NOH-AAF and PAPS dependent loss in AST IV sulfotransferase activity. These results demonstrate that the highly reactive, sulfuric acid ester of NOH-AAF can covalently link with AST IV causing suicide inactivation of the enzyme, and suggests that it deserves consideration as an in vivo mechanism for loss of NOH-AAF sulfotransferase activity.

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BENZO(A)PYRENE (BaP) TREATMENT RESULTS IN COMPLETE INFERTILITY IN FEMALE PIGEONS. J.L. Hough, D. Darrow, J. Eaton, and M.B. Baird (SPON: H. Hassia). Masonic Medical Research Laboratory, Utica, NY, 13501.

BaP is a carcinogenic polycyclic aromatic hydrocarbon (PAH) and a common environmental pollutant. Show Racer and White Carneau female pigeons injected weekly with BaP (10mg/kg body weight, 12.5mg/ml corn oil) for 3 or 5 months were completely infertile, with ovaries appearing necrotic or oxidized. Fertility in benzo(e)pyrene (BeP, a noncarcinogenic PAH) treated birds was the same as for corn oil treated controls, as was embryo development. Thus, infertility in BaP treated birds appears to be related to its structure-carcinogenic potential. There was no readily apparent affect of BaP treatment on testes from male birds. In order to determine whether BaP metabolites covalently bind to DNA in the ovaries of these birds, pigeons were injected with BaP or BeP (50mg/kg body weight, 12.5mg/ml corn oil), controls were injected with corn oil. Animals were sacrificed 24h later, the ovaries or testes removed, and the DNA isolated and analyzed for PAH-DNA adducts by ^{32}P-post labeling assay. One major and one minor PAH-DNA adduct was found in ovaries and testes from BaP treated birds. However, no PAH adducts were found in BeP treated or control animals. Thus, problems with fertility may arise because of the alteration in DNA by BaP metabolite binding in ovaries where rapid cell growth occurs during egg production.

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PURIFIED RAT LIVER DT-DIAPHORASE POTENTIATES THE MUTAGENICITY OF 1,3-, 1,6- AND 1,8-DINITROPYRENE. A. K. D. Haios and G. W. Winston Department of Biochemistry, Louisiana State University, Baton Rouge, LA 70803, USA

The effect of highly purified rat liver cytosolic DT-diaphorase [E.C. 1.6.99.2] on the mutagenicity of 1,3-, 1,6- and 1,8-dinitropyrene (DNP) was studied in the Ames *Salmonella typhimurium* mutagenicity assay. DT-diaphorase over the range of 0 - 0.78 µg/plate increased the mutagenicity of all three DNP in *S. typhimurium* TA 98 to a maximum of 3-fold. In the nitroreductase-deficient strain, TA98NR, 1,6- and 1,8-DNP were activated to a lesser extent in the presence of equal amounts of DT-diaphorase as compared to TA98. The mutagenicity of 1,3-DNP was markedly lowered in TA98NR and DT-diaphorase did not increase the mutagenicity of this toxicant except at the highest level utilized i.e., 0.78 µg/plate. DT-diaphorase was found to activate 1,6-, but not 1,3-DNP to mutagenic intermediates in TA98 18DNP$_6$, a strain deficient in O-acetyltransferase activity. The results suggest that DT-diaphorase not only catalyzes reduction of the parent DNP but also that of partially reduced metabolites generated from that DNP. This may result in increased formation of the penultimate mutagenic species. Thus, while DT-diaphorase is commonly viewed as having antimutagenic properties with certain compounds the opposite appears to be true for DNP.
Supported by NIAAA grant AA065758-04 to GWW.

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T7 DNA POLYMERASE INCORPORATES dT OPPOSITE O^2-ETHYLDEOXYTHYMIDINE *IN VITRO*. P.C. Grevatt and G.S. Bhanot (SPON: W. Troll). New York University Medical Center, New York, NY 10016 USA.

The significance of alkylation at the O^2 position of thymidine was ascertained by studying in vitro DNA replication properties of O^2-ethyldeoxythymidine (O^2-Et-dT) present at a single site in a 36-nucleotide template. The template corresponds to a DNA sequence in gene G of bacteriophage φX174 and was used to direct synthesis by T7 DNA polymerase (T7Pol). At 10 µM dNTP and 5 mM Mg^{++}, the progress of T7Pol was interrupted by O^2-Et-dT: 80% 3' to O^2-Et-dT and 14% after incorporating a nucleotide opposite O^2-Et-dT. Postlesion synthesis was low (6%). Postlesion synthesis was enhanced by increasing the dNTP concentration, reaching 30% at 200 µM. It was further increased to 45% by inhibiting the 3'→5'-exonuclease activity of T7Pol by addition of 10 mM dAMP to the polymerization reaction. DNA sequencing revealed that both dA and dT were incorporated opposite O^2-Et-dT. Incorporation of dA opposite O^2-Et-dT impedes the progress of DNA synthesis and may contribute to the cytotoxicity of ethylating agents. dT incorporation opposite O^2-Et-dT is efficiently extended, leading to an A•T→T•A transversion mutation. These studies suggest that formation of the persistent O^2-Et-dT lesion in DNA is biologically significant and may contribute to mutagenic and carcinogenic processes long after the original exposure has occurred. (Supported by NSF Grant No. DMB 8607536 and NIEHS Grant No. ES 00260

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CHEMOTHERAPEUTIC EVALUATION OF THE OPIATE ANTAGONIST, NALTREXONE, IN THE RAT MAMMARY TUMOR MODEL. H. Abou-Issa and G. A. Tejwani. Departments of Surgery, Human Nutrition, Pharmacology and Anesthesiology, The Ohio State University, Columbus, Ohio 43210

Previous studies (Adv. Biosc. 75, 615, 1989) showed that naltrexone (NAX) inhibited the induction of rat mammary tumors by 7,12-dimethybenz(a)anthracene (DMBA) and stress. These studies were extended to evaluate NAX for its effectiveness as an anti-tumor agent. For this evaluation, we tested the effect of NAX given daily in the diet on the growth of established DMBA-induced mammary tumors. Tumors continued to grow actively in rats fed chow diet only (control group). In contrast, the NAX-supplemented diet (75 mg/Kg diet) significantly decreased the size of the established mammary tumors in rats over the 25 day observation period, resulting in an average decrease in tumor volume by approximately 23% compared with their sizes at the beginning of the treatment. Tumor regression occurred in 70% of the rats. This growth suppression was rapidly reversed upon transfer of the rats from NAX treatment to the control diet. The inhibitory effect of NAX appears to [illegible] responsive mammary tumor[illegible] has an anti-tumor activi[illegible] (Supported by OSU Devaol[illegible] grant).