metabolic activation may be necessary for phosphatase inhibition. Using horse liver ADH, we isolated and instrumentally characterized two products arising from the enzymatic oxidation of TDG. One, with an Rf of 0.65- 0.70 by thin-layer chromatography (TLC)(silica gel, hexane:ethyl acetate 1:3, vol), gave a color consistent with an aldehyde when sprayed with 0.4% 2,4-dinitrophenyl hydrazine (DNPH) in 2.0 M HCl. The second, with an Rf of 0.74-0.78 in the same TLC system, did not react with DNPH. Electron impact (EI) GC-MS analysis of material isolated by preparative TLC indicated a small molecular ion peak at m/e 120 for the lower Rf compound with a base peak at m/e 46 and additional peaks at m/e 61 and 74. Methane chemical ionization GC-MS, which characteristically gives an unambiguous M+H+ peak, instead gave a peak at m/e 103 with this compound indicating the loss of H2O (120 + H+ - 18), behavior characteristic of an aldehyde. The higher Rf compound gave an EI mass spectrum with a base peak at m/e 46, additional peaks at m/e 59 and 74 and a molecular ion at m/e 118. This is consistent with published mass spectral data for the 1,4-oxathian-2-one. Methane chemical ionization produced an M+H+ at m/e 119. We conclude that the compound with Rf of 0.65-0.70, DNPH (+) and with a molecular ion at m/e 120 is the monoaldehyde of TDG. The compound with the higher Rf, DNPH (-), and a molecular ion at m/e 118 is the 1,4-oxathian-2-one.

233 MECHANISM OF INHIBITION OF LUNG CYP2E1 BY DIALLYL SULFONE.

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We have tested the hypothesis that lung CYP2E1 is inactivated by diallyl sulfone ($DASO_2$) through formation of the metabolite diallyl monoepoxide ($DASO_3$). $DASO_3$ was chemically synthesized and reacted with [^{3}H]glutathione (GSH) to produce the $DASO_3$-GSH standard. HPLC analysis of lung microsomal incubations with $DASO_2$ in the presence of [^{3}H]GSH and NADPH showed the formation of the $DASO_3$-GSH conjugate. This conjugate was not produced in incubations performed in the absence of $DASO_2$ or NADPH. The formation of the $DASO_3$-GSH conjugate in the incubations was time-dependent, peaking at 2 h. Conjugate formation was concentration-dependent; saturation was detected at 0.8 mM of $DASO_2$. Generation of $DASO_3$ in the microsomal incubations, as estimated by formation of a 4-nitrobenzyl pyridine derivative, peaked at 35 min. Levels of $DASO_3$ formed in the incubations were concentration-dependent, and were maximal at 1.0 mM of $DASO_2$. The $DASO_3$-GSH conjugate was also generated in incubations containing CYP2E1-expressed human lymphoblastoid microsomes. Furthermore, the formation of this conjugate was significantly inhibited in lung microsomes preincubated with an inhibitory CYP2E1 antibody. CYP2E1-mediated *p*-nitrophenol hydroxylase activity was significantly inhibited by $DASO_2$ in the microsomal incubations. This inhibitory effect was also observed in microsomes isolated from the lungs of mice treated with $DASO_2$. The loss in catalytic activity coincided with diminution of immunodetectable CYP2E1 protein in both the *in vitro* and *in vivo* systems, as assessed by protein immunoblotting. These results supported the conclusion that $DASO_3$ is responsible for the inactivation of lung CYP2E1. (Supported by NCI Grant RO1 CA73220-01.)

234 EFFECTS OF QUINONE METHIDES ON MOUSE LUNG EPITHELIAL CELL LINES.

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There is substantial evidence that butylated hydroxytoluene (BHT)-mediated mouse lung injury and tumor promotion are consequences of metabolic activation. BHT is oxidized by pulmonary cytochrome P450 forming two quinone methides, BHT-QM and BHTOH-QM. Both of these electrophiles readily form covalent adducts with cellular thiols but the latter is several-fold more reactive. In order to probe the targets of these metabolites possibly related to tumor promotion, cell lines derived from normal alveolar type II pneumocytes (C10 and E10) and the corresponding spontaneous transformants (A5 and E9) were incubated with quinone methides. Compared to the transformed cells, C10 and E10 cells had lower levels of glutathione S-transferase (GST) activity and greater sensitivity to quinone methide-induced toxicity. In all four cell lines, BHTOH-QM was substantially more toxic than BHT-QM in agreement with previous results using isolated bronchiolar Clara cells. The quinone methides irreversibly inhibited GST activity and both toxicity and GST inhibition were enhanced by depleting glutathione (GSH) levels. These results demonstrate that both GSH and GST are intracellular targets of BHT-derived quinone methides and suggest a possible mechanism for tumor promotion involving selective destruction of normal versus transformed cells. (Supported by NIH Grant CA41248.)

235 SUCCINYLACETONE ELICITS AN OXIDATIVE STRESS RESPONSE IN MOUSE HEPATOMA HEPA-1C1C7 CELLS.

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Succinylacetone is a tyrosine catabolite that accumulates in the absence of fumarylacetoacetate hydrolase (FAH), the final enzyme in the tyrosine catabolism pathway. Succinylacetone is a reactive oxygenated metabolite (yet a weak Michael reaction acceptor) found in the tissues, plasma, and urine of *Fah(-/-)* knockout and *14CoS/14CoS* mice, both of which lack a functional FAH enzyme. The presence of urinary succinylacetone is also used to screen patients for hereditary tyrosinemia type 1 (HT-1), an inherited deficiency of FAH. An oxidative stress response, characterized by the induction of stress-inducible genes and changes in glutathione status, has been observed in *Fah(-/-)* and *14CoS/14CoS* mice. We have tested the hypothesis that succinylacetone might cause an oxidative stress response. Northern blot analysis of succinylacetone-treated mouse hepatoma Hepa-1 cells demonstrates significant elevations in mRNA levels of the oxidative stress-responsive genes glutamate-cysteine ligase (catalytic and regulatory subunits, *Gcls* and *Gclr*), NAD(P)H: quinone reductase-1 (*Nqo1*) and heme oxygenase-1 (*Ho1*). Transient transfection of succinylacetone-treated cells with an electrophile response element (EPRE)-driven luciferase (*LUC*) construct generated a 3-fold increase in luciferase activity. Succinylacetone is a potent inhibitor of 5-aminolevulinic acid (ALA) dehydratase, the second enzyme in the heme synthesis pathway. Northern blot analysis and transient transfection of EPRE-*LUC* in ALA-treated cells demonstrate that an oxidative stress response is caused directly by ALA. We hypothesize that FAH deficiency leads to accumulation of succinylacetone, thus inhibiting ALA dehydratase, leading to build-up of ALA and resulting in the oxidative stress response. These findings may have clinical relevance for HT-1 patients as well as porphyria patients, and may partially explain the oxidative stress response observed in untreated *Fah(-/-)* and *14CoS/14CoS* mice. (Supported in part by NIH Grant R01 AG09235.)

236 ANTIOXIDANT BALANCE AND FREE RADICAL GENERATION IN VITAMIN E DEFICIENT MICE AFTER DERMAL EXPOSURE TO CUMENE HYDROPEROXIDE.

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Organic peroxides are widely used in the chemical industry as initiators of oxidation for the production of polymers and fiber-reinforced plastics, in the manufacture of polyester resin coatings, and as antimicrobial agents in pharmaceuticals. Free radical production is considered to be one of the key factors contributing to skin tumor promotion by organic peroxides. *In vitro* experiments have demonstrated metal-catalyzed formation of alkoxyl, alkyl and aryl radicals in keratinocytes incubated with cumene hydroperoxide. The present study investigated *in vivo* free radical generation in lipid extracts of mouse skin exposed to cumene hydroperoxide. The electron spin resonance (ESR) spin-trapping technique was used to detect the formation of *alfa*-phenyl-*N*-*tert*-butylnitrone (PBN) radical adducts, following intradermal injection of 180 mg/kg PBN. It was found that 30 min after topical exposure, cumene hydroperoxide (12mmole/kg) induced free radical generation in the skin of female Balb/C mice (13-14 weeks old) kept for 10 weeks on vitamin E deficient diets. In contrast, no radical adducts were detected when cumene hydroperoxide was applied to the skin of mice fed a vitamin E sufficient diet. Importantly, levels of GSH and vitamin E in the skin of vitamin E deficient mice decreased 30% and 80%, respectively, compared to vitamin E sufficient controls. PBN adducts detected by ESR in vitamin E deficient mice provide direct evidence for *in vivo* free radical generation in the skin after exposure to cumene hydroperoxide.

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