a 50% loss in hematocrit. Survival of radiolabeled red cells in vivo was also reduced following in vitro exposure to divicine (1.0 mM). Prior depletion of red cell GSH with diethyl maleate (5 mM) exacerbated divicine hemotoxicity by about 10-fold, whereas preincubation with the G6PD inhibitor, epiandrosterone (200 μM), induced only a two-fold enhancement of divicine toxicity. These data indicate that favism can be reproduced in the rat, and substantiate the value of the rat as a model for human favism. In addition, the data suggest that the GSH levels may be the determining factor, rather than loss of G6PD activity, in susceptibility to favism. Supported by NIH grant HL-30038.

1497 MITOCHONDRIAL TOXICITY OF BHTOOH, THE HYDROPEROXYL METABOLITE OF BUTYLATED HYDROXYTOLUENE

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The food additive butylated hydroxytoluene can be oxidized to form a hydroperoxyl metabolite BHTOOH. The cytotoxicity of BHTOOH and its tumor promoting activity in skin may be mediated by further metabolism to its electrophilic quinone methide (BHT-QM), which occurs during incubation with cytosol or hematin. Previous investigations indicated that the earliest signs of BHTOOH toxicity in keratinocytes occurred in the mitochondria. The present research investigates the covalent binding of BHT-QM to subcellular fractions and effects on mitochondrial respiration and oxidative phosphorylation following BHTOOH. A plurality of radioactivity was found in the mitochondria following 30 or 120 min exposure of murine keratinocytes (PA cells) to [^{14}C]-BHTOOH. To examine further the toxicity of BHTOOH on mitochondria, oxygen consumption of isolated rat liver mitochondria was measured using an oxygen electrode, while oxidative phosphorylation was measured using luciferase-coupled bioluminescence. Incubation with micromolar doses of BHTOOH alone caused a decrease in mitochondrial respiration and a decrease in ATP production, while incubation of BHTOOH with hematin resulted in a greatly enhanced respiration and a decrease in ATP production. These data indicate that BHTOOH is toxic to mitochondria and that BHT-QM alkylates mitochondrial proteins which may result in uncoupling of oxidative phosphorylation. (Supported by NIH grants PO1 CA 44530 and ES 03819).

1498 BENZOYL PEROXIDE MEDIATED FORMATION OF 8-HYDROXY-2′-DEOXYGUANOSINE IN MURINE KERATINOCYTES

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Benzoyl Peroxide (BzPO) is a free radical generating compound that acts as a tumor promoter and progressor in mouse skin. BzPO is cleaved in the presence of Cu^{+1} to produce benzoyloxyl and phenyl radicals. Treatment of mutation reporter plasmids with BzPO and copper yields predominantly single strand breaks and G→T transversions. To explore the role of base modifications in the possible mutagenicity of BzPO, the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) within the DNA of cultured murine keratinocytes was investigated. Treatment with 10 μM BzPO produced a maximum 3 fold increase in levels of 8-OHdG versus vehicle controls within 1–2 hrs with significant levels of 8-OHdG persisting 6 hours after initial BzPO exposure. The copper chelator, bathocuproinedisulfonic acid, reduced the levels of 8-OHdG generated by BzPO to near background. However, treatment with the iron chelator, desferal, did not. The metabolic product of BzPO, benzoic acid, was not effective in producing 8-OHdG. These results suggest that the free radical intermediate species of BzPO are responsible for the formation of promutagenic 8-OHdG in the DNA of keratinocyte cells. Supported by PO1 CA 44530 and Training Grant ES 07141.

1499 COPPER-REDOX-DEPENDENT ACTIVATION OF 2-*tert*-BUTYL(1,4)HYDROQUINONE (TBHQ)

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The metabolism of 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), a carcinogenic food antioxidant, includes *o*-demethylation to TBHQ and its subsequent oxidation to 2-*tert*-butyl(1,4)paraquinone (TBQ). In this study, we have examined the capacity of copper, a nuclei- and DNA base-associated transition metal, to mediate the oxidation of TBHQ. In phosphate buffered saline, TBHQ underwent a slight autoxidation to TBQ, while the presence of Cu(II) strongly catalyzed the oxidation of TBHQ to TBQ. Oxidation of TBHQ by Cu(II) was also accompanied by the reduction of Cu(II) to Cu(I), the utilization of O_2 and the concomitant generation of H_2O_2. Using EPR spectroscopy, it was observed that copper mediated the one electron oxidation of TBHQ to semiquinone anion radicals ($SQ^{\bullet}$). The formation of $SQ^{\bullet}$, the utilization of O_2 and the generation of H_2O_2 and TBQ could be completely blocked by bathocuproinedisulfonic acid (BCS), a Cu(I) chelator. 4-Pyridyl-1-oxide-*N*-*tert*-butylnitrone (POBN)-spin trapping experiments showed that the reaction of TBHQ with Cu(II) resulted in the generation of POBN-CH_3 and POBN-CH(OH)CH_3 adducts in the presence of dimethyl sulfoxide (DMSO) and ethanol, respectively, suggesting the formation of hydroxyl radical or a similarly reactive intermediate. The formation of a POBN-CH_3 adduct from the TBHQ/Cu(II) plus DMSO system could be completely inhibited by catalase or BCS, indicating the reactive oxygen species is generated from the interaction of H_2O_2 with Cu(I). Incubation of supercoiled ϕX-174 plasmid DNA with TBHQ/Cu(II) resulted in extensive DNA strand breaks, which could also be prevented by catalase or BCS. The above results indicate that the activation of TBHQ by a copper-redox mechanism results in the formation TBQ, $SQ^{\bullet}$ and reactive oxygen species, and that the reactive oxygen formed may participate in DNA strand breakage. These reactions may contribute to the observed carcinogenic effects of BHA.

1500 REDUCTION OF GLUTATHIONE DISULFIDE IN OXIDATIVELY STRESSED RESPIRING RAT LIVER MITOCHONDRIA

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Factors affecting the reduction of glutathione disulfide (GSSG) by mitochondria after a *t*-butyl hydroperoxide-induced (t-BOOH) oxidative stress were studied. The amounts of ADP and mitochondrial protein were adjusted to consume less than 50% of the available oxygen during the 8 min experimental period using glutamate/malate (20 mM/2 mM), succinate (30 mM), or ascorbate/N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) (30 mM/0.3 mM) as substrates. A four min treatment of mitochondria (25 mg protein/ml) with 60 μM t-BOOH oxidized 91% of total glutathione (6.3 ± 0.4 mmol/mg protein). In the presence of glutamate/malate, succinate or ascorbate/TMPD (state 4 respiration), 84, 84 and 28% of the GSSG formed during t-BOOH treatment was reduced after 4 min, respectively. A similar extent of reduction was seen during state 3 respiration (1.5 mM ADP) with glutamate/malate, but no reduction occurred during state 3 respiration with either succinate or ascorbate/TMPD. The succinate-supported reduction of GSSG was completely blocked by rotenone (0.1 μM), antimycin A (7 μM), carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) (0.4 μM), or 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) (0.1 mM). In contrast, oligomycin (5 μM) potentiated GSSG reduction using either glutamate/malate or succinate as substrates. Rotenone partially blocked glutamate/malate-supported GSSG reduction. Levels of individual pyridine nucleotides are being determined. The current data indicate that the reduction of GSSG in oxidatively-stressed isolated rat liver mitochondria occurs mainly during state 4 respiration and is independent of ATP synthesis. Mitochondrial NADPH is critical since the inhibition of glutathione reductase (an NADPH specific enzyme) by BCNU prevents the reduction of GSSG. Both the availability of mitochondrial NADH and the transmembrane proton gradient appear to be important in NADPH regeneration and consequent GSSG reduction. (Supported by HL51005.)

1501 IMMUNOCHEMICAL DETECTION OF OXIDIZED PROTEINS FORMED BY RADIOLYSIS OF FRACTIONS OF RAT RENAL AND HEPATIC TISSUE

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We have developed an immunochemical assay to detect carbonyl moieties that result from oxidative damage to proteins. Homogenates, microsomes and cytosol prepared from rat renal and hepatic tissue were exposed to hydroxyl radicals generated by a radiolysis mechanism. The resulting protein-derived carbonyls were reacted with 2,4-dinitrophenyl-hydrazine giving the corresponding hydrazones, which were detected by Western blot using anti-dinitrophenyl antisera. Analysis of the immunoblots using a densitometer indicated linear relationships between carbonyl group formation and increasing treatment from radiolysis in all fractions studied. In addition, distinct proteins stained more intensely in the immunoblots, particularly in the liver microsomes and cytosol, which demonstrated increased susceptibility of these proteins to oxidative damage. The characterization of susceptible proteins will increase our understanding of the mechanisms of oxidative toxicity, and may prove useful as early indicators of oxidative protein damage. (This study was supported in part by grant OH03061 from NIOSH and by grant R820970 from the U.S. EPA).

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