on tumor yield or on the number of mice bearing papillomas. Mice receiving either diethyl ether or CuDIPS and acetone in lieu of TPA developed no tumors, indicating that CuDIPS has no tumor-promoting properties itself.

That the antipromoter effects of CuDIPS in mouse epidermis can be ascribed to its existence as a chelate in vivo with SOD-like chemical reactivity rests on the observed failure of cupric acetate or 3,5-diisopropylsalicylic acid to appreciably inhibit either phorbol ester–induced ODC activity or tumor promotion. Cupric acetate, used as a control for the copper component of CuDIPS, does have the capability of scavenging O_2^-; however, in biological systems, ionizable forms of copper frequently lose their SOD-like activity because they form complexes with protein (*14*). 3,5-Diisopropylsalicylic acid alone has no O_2^- scavenging activity (*11*). This ligand presumably confers a high degree of lipophilicity to copper allowing for the preferential subcellular localization of the copper complex at sites of O_2^- production, accumulation, or reactivity, such as membrane matrices and membrane-cytoplasmic interfaces. By contrast, exogenously applied bovine erythrocyte SOD is excluded from such sites (*10*) because of its size and lack of lipophilic character and as such is an ineffective antagonist of phorbol ester action (*15*).

The finding that a low molecular weight, lipophilic, copper chelate with SOD-mimetic activity can inhibit certain phorbol ester–induced biochemical and biological responses strengthens the arguments for an essential role of oxygen radicals in the promotion stage of carcinogenesis and of SOD or SOD-like compounds in the homeostatic prevention of carcinogenesis. The action of oxygen radicals in the stimulation of cell proliferation and progression by tumor promoters may involve a direct or indirect modification of genomic integrity (*3*) or modulation of biochemical processes such as the arachidonate cascade and cyclic nucleotide metabolism (*5*). Although O_2^- is clearly a component of the phorbol ester–induced response, the identification of the pertinent activated oxygen species and their macromolecular targets in the tumor promotion process is an important goal.

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Metabolism of Polycyclic Aromatic Hydrocarbon Derivatives to Ultimate Carcinogens During Lipid Peroxidation

Abstract. *Lipid peroxidation triggered by ascorbate or reduced nicotinamide adenine dinucleotide in rat liver microsomes can initiate the epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo*[a]*pyrene. The stereochemistry of epoxidation is indicative of a peroxide-dependent free radical process. Since the epoxides formed may be the most carcinogenic derivatives of benzo*[a]*pyrene yet identified, lipid peroxidation can effect the metabolic activation of proximate carcinogens to ultimate carcinogens.*

Oxidation is a critical step in the metabolic activation of many xenobiotics to toxic, mutagenic, and carcinogenic derivatives (*1*). Benzo[*a*]pyrene (BP), a widespread environmental pollutant, is converted to a number of derivatives by the combined action of oxygenases and conjugating enzymes (*2*). One of the metabolites, 7,8-dihydroxy-7,8-dihydrobenzo-[*a*]pyrene (BP-7,8-diol) (Fig. 1), is further oxidized to dihydrodiol epoxides, which represent ultimate carcinogenic

Table 1. NADPH- and ascorbate-dependent oxidation of BP-7,8-diol. Microsomes were suspended (0.5 mg of protein per milliliter) in 0.1*M* tris buffer (*p*H 7.5) at 37°C. Incubations contained 40 μ*M* BP-7,8-diol and various combinations of the following cofactors: NADPH (1.0 m*M*), ADP (4.0 m*M*), Fe^{3+} (15 μ*M*), EDTA (100 μ*M*), Fe^{2+} (110 μ*M*), and ascorbate (1.0 m*M*). Reactions were initiated by the addition of NADPH or ascorbate, and the incubation period was 20 minutes. Values are means ± standard errors.

Cofactors	TBA-reactive material*	Diol metabolism†	Tetraol formation‡	*Anti/syn* ratio
NADPH	0.30 ± 0.07	6.0 ± 1.9	0.6 ± 0.2	1.1 ± 0.3
NADPH + ADP-Fe^{3+} + EDTA-Fe^{2+}	14.0 ± 1.3	27.0 ± 4.3	19.0 ± 4.4	2.5 ± 0.4
NADPH + ADP-Fe^{3+} + EDTA-Fe^{2+} + EDTA§	0.4 ± 0.1	4.0 ± 0.8	0.4 ± 0.1	0.5 ± 0.1
Ascorbate + ADP-Fe^{3+}	23.0	31	14	2.6
Hematin-ROOH (*8*)				2.5

*Nanomoles of malondialdehyde formed per milligram of protein. †Percentage of total radioactivity eluting from high-pressure liquid chromatography in zones not cochromatographing with the authentic standard of BP-7,8-diol. ‡Percentage of total radioactivity eluting in zone cochromatographing with authentic tetraol standards. §Includes 1 m*M* excess EDTA, which completely inhibits lipid peroxidation under these conditions (*25*).

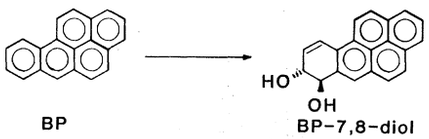


Fig. 1. Formation of BP-7,8-diol, a key step in the metabolic activation of BP.

forms of BP (*3*). The oxidation of BP and BP-7,8-diol can be catalyzed by mixed-function oxidases (*4*), prostaglandin H (PGH) synthase (*5*), and an uncharacterized oxygenase from *Cunninghamella elegans* (*6*).

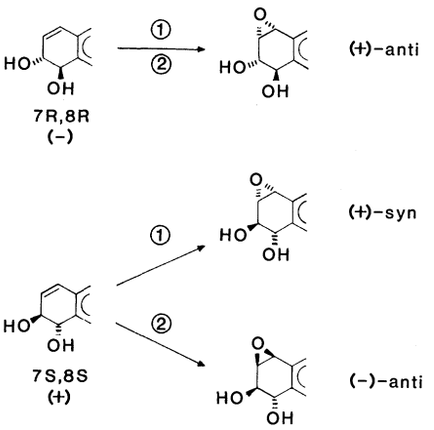
Oxygenations by PGH synthase are hydroperoxide-dependent oxidations catalyzed by a heme-containing peroxidase activity that is a functional component of the enzyme (*7*). The oxidations of BP and BP-7,8-diol occur by free radical mechanisms and are potently inhibited by antioxidants (*7*). We recently reported that hematin and unsaturated fatty acid hydroperoxides epoxidize BP-7,8-diol in a reaction that appears mechanistically related to the PGH synthase–catalyzed reaction (*8*). This suggests that other systems that generate and metabolize unsaturated fatty acid hydroperoxides oxygenate BP-7,8-diol to diol epoxides.

A well-known method for the generation and metabolism of unsaturated fatty acid hydroperoxides is lipid peroxidation (*9*). Chain autoxidation of unsaturated fatty acyl groups and decomposition of the product hydroperoxides can be triggered by the addition of ascorbate or reduced nicotinamide adenine dinucleotide (NADPH) and metal ions to microsomal preparations (*10*). In fact, Morgenstern *et al.* (*11*) reported that BP is oxidized to quinones during ascorbate-dependent lipid peroxidation in rat liver microsomes (*11*). The quinone products are identical to those generated by the free radical oxidation of BP by PGH synthase (*12*). We now report that the epoxidation of BP-7,8-diol—the terminal activation step in BP carcinogenesis—occurs during both ascorbate- and NADPH-dependent lipid peroxidation.

Liver microsomes were prepared from Long-Evans rats (*13*). Ascorbate-dependent lipid peroxidation was induced in the presence of Fe^{3+} and adenosine diphosphate conjugate (Fe^{3+}-ADP) (*14*), NADPH-dependent lipid peroxidation was initiated by the addition of Fe^{3+}-ADP and Fe^{2+} and EDTA complex (Fe^{2+}-EDTA) (*15*), and lipid peroxidation was determined by the thiobarbituric acid (TBA) assay (*16*). Metabolism of BP-7,8-diol to diol epoxides was quantitated by analysis of the *cis* and *trans* tetraol hydrolysis products after solvent extraction and separation on a high-pressure liquid chromatography column (Zorbax reversed-phase) with methanol and water gradients. Epoxide formation was also estimated by determining the amount of radioactivity bound to microsomal protein after termination of the reaction (*17*).

Figure 2 shows TBA-reactive material generation, tetraol formation, and protein binding after the addition of ascorbate and Fe^{3+}-ADP to microsomes. Parallel increases are seen in the generation of TBA-reactive material and tetraols. A slight lag is seen in protein binding. Omission of Fe^{3+}-ADP eliminates the increase in lipid peroxidation and BP-7,8-diol epoxidation. These results clearly indicate that ascorbate-dependent lipid peroxidation triggers the epoxidation of BP-7,8-diol.

Demonstration of BP-7,8-diol epoxidation during NADPH-dependent lipid peroxidation is complicated by the mixed-function oxidase–catalyzed epoxidation that occurs simultaneously. We have used the stereochemistry of BP-7,8-diol epoxidation to distinguish between these two competing pathways. Thakker *et al.* (*18*) reported that the 7R,8R enantiomer of BP-7,8-diol is metabolized by cytochrome P-450c or methylcholanthrene-induced microsomes predominantly to the (+) enantiomer of the *anti*-diol epoxide, whereas the 7S,8S enantiomer is metabolized primarily to the (+) enantiomer of the *syn*-diol epoxide (*18*). Similar results were reported by Deutsch *et al.* (*19*). We have confirmed these findings and have shown that the same stereochemical pattern is exhibited by the mixed-function oxidases in microsomes from untreated animals (*20*). In contrast, fatty acid hydroperoxide–dependent epoxidation of either enantiomer of BP-7,8-diol by PGH synthase or hematin yields predominantly the (+) or (−) enantiomers of the *anti*-diol epoxide; little, if any, *syn*-diol epoxide is formed (*7, 8*) (Fig. 3). Therefore, the ratio of *anti*-diol epoxide–derived products to *syn*-diol epoxide–derived products (the *anti*/*syn* ratio) is a qualitative indicator of the pathway by which BP-7,8-diol is oxidized. A ratio of 2.5 is diagnostic of peroxide-dependent metabolism, whereas a ratio of 1.0 is indicative of mixed-function oxidase–dependent metabolism.



① Mixed-function oxidase-dependent
② Peroxide-metal-dependent

Fig. 3. Epoxidation of BP-7,8-diol enantiomers by mixed-function oxidase and peroxidative pathways. The difference in the orientation of the introduced oxygen provides a stereochemical basis for discriminating between the two pathways.

Table 1 summarizes our experiments designed to probe for BP-7,8-diol epoxidation during NADPH-dependent lipid peroxidation. In the absence of metal complexes, minimal amounts of TBA-reactive material were generated and 0.6 percent of the added BP-7,8-diol was converted to tetraols. The *anti*/*syn* ratio of 1.1 is consistent with epoxidation by mixed-function oxidases. The addition of Fe^{3+}-ADP and Fe^{2+}-EDTA dramatically increased the amount of TBA-reactive material and the yield of tetraols (more than 30-fold). In addition, the *anti*/*syn* ratio was elevated to 2.5. Inclusion of an excess of EDTA in the incubations to

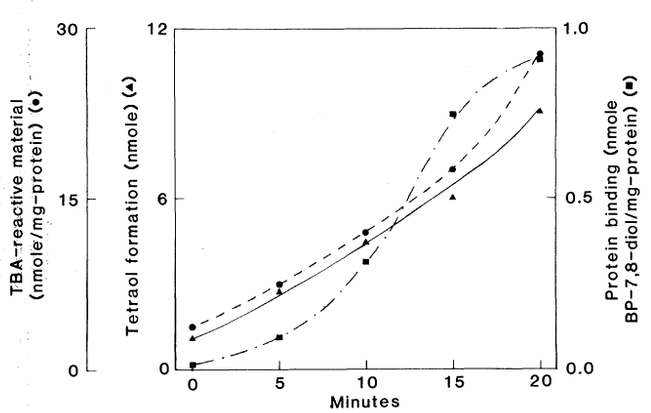


Fig. 2. Ascorbic acid–dependent BP-7,8-diol epoxidation. Rat liver microsomes were suspended (0.5 mg of protein per milliliter) in 0.1*M* tris buffer (*p*H 7.5) at 37°C. Incubations contained 40 μ*M* BP-7,8-diol, 4.0 m*M* ADP, and 15 μ*M* Fe^{3+} and were initiated by the addition of 1.0 m*M* ascorbic acid. Experiments were performed in duplicate.

inhibit lipid peroxidation abolished the increase in both TBA-reactive material and tetraols and lowered the *anti/syn* ratio to 0.5. The addition of EDTA also appeared to partially inhibit the low level of epoxidation by the mixed-function oxidases. The data from a typical ascorbate-dependent reaction are included in Table 1. Also included is the *anti/syn* ratio of 2.5 that we previously measured for the epoxidation of BP-7,8-diol by hematin and unsaturated fatty acid hydroperoxide (ROOH) (*8*). The data provide strong evidence that BP-7,8-diol is epoxidized during NADPH- and ascorbate-dependent lipid peroxidation in rat liver microsomes.

The results in Table 1 were obtained with racemic BP-7,8-diol. We carried out similar experiments with the resolved (+) enantiomer of BP-7,8-diol. Since mixed-function oxidases convert this enantiomer to only the (+)-*syn*-diol epoxide, tetraols derived from the (+)-*anti*-diol epoxide should be formed only by lipid peroxidation. Incubation of 40 μM (+)-BP-7,8-diol with NADPH and liver microsomes yields 0.4 μM tetraols derived from the *syn*-diol epoxide and 0.1 μM from the *anti*-diol epoxide. Inclusion of Fe^{3+}-ADP, and Fe^{2+}-EDTA generates 1.2 μM *syn*-diol epoxide–derived and 4.0 μM *anti*-diol epoxide–derived tetraols. The *anti/syn* ratio is 0.3 in the absence of metal complexes and 3.5 in their presence. The dramatic increase in metabolism and in the *anti/syn* ratio in these experiments forces the conclusion that BP-7,8-diol is epoxidized as a result of NADPH-dependent lipid peroxidation.

Lipid peroxidation is a consequence of the evolution of organisms to oxygen utilization. Some of the products of unsaturated fatty acid degradation are toxic and mutagenic (*21*), and the disruption of membrane integrity can lead to cell lysis (*22*). The generation of potent oxidizing agents during lipid peroxidation can cause the selective inactivation of heme-containing proteins such as cytochrome P-450 (*23*). This has been implicated as a principal factor in the alteration of hepatic xenobiotic metabolism after glutathione depletion in rats (*24*). Our report indicates that oxidizing agents generated during lipid peroxidation can epoxidize xenobiotics and can, in the case of polycyclic aromatic hydrocarbons, trigger the formation of their ultimate carcinogenic forms.

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Calicivirus Isolation and Persistence in a Pygmy Chimpanzee (*Pan paniscus*)

Abstract. *What may be the first calicivirus isolate from any primate species, including man, was recovered from a herpesvirus-like lip lesion on a pygmy chimpanzee and then, 6 months later, from the throat of the same animal. The infected individual and its cage mates had circulating antibodies that were type-specific for this calicivirus. The agent was antigenically different from 30 other calicivirus serotypes and is tentatively designated primate calicivirus* Pan paniscus *type 1 (PCV-Pan 1).*

We have isolated from a rare and endangered primate, the pygmy chimpanzee (*Pan paniscus*), a new calicivirus tentatively designated primate calicivirus *Pan paniscus* type 1 (PCV-Pan 1). In the naturally infected chimpanzee this agent persists and is shed for up to 6 months. The virus was first isolated from a vesicular lip lesion (Fig. 1). Although the pathogenesis in pygmy chimpanzees has not been established, inferences from infections in other species suggest an array of possible disease effects. One group of caliciviruses or calicivirus-like agents that has not yet been isolated in vitro causes enteritis in calves, piglets, and humans (*1–3*). This group includes the Norwalk agent and other closely related agents that have been associated with outbreaks of gastroenteritis among children and adults on several continents (*4–7*). Other caliciviruses isolated from swine, cats, and pinnipeds cause systemic disease resulting in pneumonia, abortion, myocarditis, and encephalitis. Most notably, however, they cause oral vesicular lesions or epidermal erosions (*8–11*).

A young adult pygmy chimpanzee (Loretta) showing signs of upper respiratory disease had a small lip lesion like those induced by Herpes hominis type 1. The lesion was scraped and swabbed, and the sample was placed in 2 ml of tissue culture medium with 10 percent