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A Proposed Mechanism of Benzene Toxicity: Formation of Reactive Intermediates from Polyphenol Metabolites[1]

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A Proposed Mechanism of Benzene Toxicity: Formation of Reactive Intermediates from Polyphenol Metabolites. GREENLEE, W. F., SUN, J. D., AND BUS, J. S. (1981). *Toxicol. Appl. Pharmacol.* **59,** 187–195. Male Fischer-344 rats were given 100 μCi (14 mg/kg) [^{14}C]catechol or [^{14}C]hydroquinone by injection into the lateral tail vein. For a period of at least 24 hr, soluble radioactivity associated with either compound was retained in the bone marrow, but not in the liver or thymus. The amount of covalently bound radioactivity increased with time in all tissues examined and was significantly depressed in liver, white blood cells, and bone marrow in rats pretreated with Aroclor 1254, a regimen which protects against benzene toxicity. Potential enzymatic and nonenzymatic activation pathways for catechol, hydroquinone, and other known benzene metabolites were examined. In air-saturated 50 mM phosphate buffer (pH 7.4) at 37°C, only hydroquinone and 1,2,4-benzenetriol autoxidized. The oxidation product of hydroquinone had an uv absorption maximum (248 nm) identical to that of benzoquinone. With 250 units superoxide dismutase, hydroquinone autoxidation increased fivefold, whereas the oxidation of 1,2,4-benzenetriol was inhibited (4% of control). Epinephrine autoxidation, an indirect measure of superoxide anion generation, was stimulated by 1,2,4-benzenetriol and hydroquinone, but was barely detectable in the presence of catechol. Of the compounds studied, only benzoquinone augmented the oxidation of NADPH by a 3000*g* rat bone marrow supernatant. These data support a mechanism for benzene toxicity in which the formation of potentially cytotoxic metabolites, semiquinone, and quinone oxidation products and superoxide radicals, result from autoxidation of at least two polyphenol metabolites of benzene, hydroquinone, and 1,2,4-benzenetriol.

Chronic exposure to concentrations of benzene ranging from 25 to 1000 ppm results in a progressive degeneration of the hemopoietic system (Carpenter *et al.*, 1944; Hough and Freeman, 1944) and immune dysfunction (Goldwater, 1941; Irons and Moore, 1980). A number of studies indicate that the expression of benzene toxicity requires metabolism of the parent compound to one or more toxic species (for reviews see Snyder and Kocsis, 1975; Laskin and Goldstein, 1977; Cohen *et al.*, 1978). Several metabolites including phenol, catechol, hydroquinone, 1,2,4-benzenetriol, and benzene dihydrodiol have been identified in the urine of rats given [“Clbenzene (Parke and Williams, 1953a,b; Timbrell and Mitchell, 1977). Andrews *et al.* (1977) detected high concentrations of benzene metabolites in the bone marrow of mice given 880 mg/

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kg [^{3}H]benzene. The appearance of these metabolites in the bone marrow correlated temporally with their disappearance from the liver. Rickert *et al.* (1979) reported that hydroquinone and catechol were retained in the bone marrow of rats exposed to 500 ppm benzene for 6 hr and studies on the disposition of ^{14}C-labeled benzene metabolites using whole body autoradiography indicate that radioactivity associated with hydroquinone or catechol, but not phenol, concentrate in the bone marrow and lymphoid organs (Greenlee et *al.*, 1980).

Polyphenol metabolites of benzene such as hydroquinone will spontaneously autoxidize to quinones (Mason, 1979). The formation of semiquinone free radical intermediates, quinones, and superoxide anion ($O_2^{\cdot-}$) during this oxidative process may be critical events leading to cytotoxicity. In this report findings on the disposition and covalent binding of catechol and hydroquinone are presented and discussed with respect to potential enzymatic and nonenzymatic pathways leading to the formation of reactive intermediates.

METHODS

Animals. Male Fischer-344 rats, weighing 250 g, were obtained from Charles River Breeding Laboratories (Wilmington, Mass.). The rats were housed in stainless-steel suspended wire cages, exposed to a day–night light cycle of 12 hr, and given food (Wayne Lab Blox, Allied Mills, Chicago, Ill.) and water *ad libitum*.

Disposition of ^{14}C-labeled hydroquinone and catechol. Five days prior to the administration of radiolabeled substrates, rats were given a single 500 mg/kg ip injection of a 50% solution of Aroclor 1254 (a kind gift from Monsanto Co.) in corn oil or corn oil vehicle alone. [^{14}C]Catechol (100 μCi, 14 mg/kg) or [^{14}C]hydroquinone (100 μCi, 14 mg/kg) were given by a single injection into the lateral tail vein to control and Aroclor-pretreated animals. Both radiolabeled compounds (specific activity, 37.9 mCi/mmol) were purchased from Midwest Research Institute (Kansas City, Mo.). The purity of each radiolabeled metabolite was >98% as assessed by thin-layer chromatography using two solvent systems, benzene:methanol:acetic acid (30:15:1) and ethyl acetate:benzene (1:1), and by high-pressure liquid chromatography (Radial Pak A column containing octadecylasilane) using a 12-min linear gradient (flow rate, 2.4 ml/min) from water to methanol. All solutions were prepared using cold (4°C) phosphate-buffered saline immediately before dosing.

Rats from the control and Aroclor-pretreated groups were sacrificed 2 and 24 hr after dosing with each radiolabeled metabolite. Liver, spleen. and thymus were homogenized in 4 vol 10 mM phosphate buffer (pH 7.0). Bone marrow was aspirated from both femurs using 2 ml of the same buffer and homogenized in a Kontes glass–glass (3 ml) homogenizer. Total radioactivity was determined by solubilizing 100 μl of each homogenate in 1 ml of Protosol overnight at ambient temperature. Each sample was then neutralized with 60 μl of glacial acetic acid and 10 ml of ACS scintillation cocktail (Amersham Corp., Arlington Heights, Ill.) were added. Radioactivity was quantified in a Searle Mark III scintillation. counter. Quenching was corrected by automatic external standardization. ^{14}C efficiency in the various samples ranged from 90 to 95%.

Total radioactivity covalently bound was measured by exhaustive extraction as described by Irons et al. (1980) with the following modifications: to 0.5 ml of each sample an equal volume of 20% TCA was added. The resulting precipitate was then washed sequentially, four times each, with 1 ml of 40, 60, 80, and 100% methanol. After each wash, the precipitate was sedimented by centrifugation and resuspended in fresh extraction solvent. After the final wash with 100% methanol, the precipitate was dissolved in 250 μl 10 N NaOH at ambient temperature for 12 hr. The solubilized mixture was acidified with 200 μl 16 N HNO_3 and radioactivity quantified in 10 ml of ACS. Total protein concentration was measured by the method of Peterson (1977).

Preparation of bone marrow homogenate. Bone marrow was aspirated from each femur of untreated rats with 1 ml of 50 mM phosphate buffer (pH 7.4). Aspirates from 5 to 50 animals were pooled, passed two times through a 16-gauge needle. two times through a 21-gauge needle, and the cells disrupted using a Teflon–glass homogenizer. The homogenate was centrifuged at 3000g for 10 min. The resulting supernatant was diluted 1:5 with phosphate buffer and used as an enzyme source in studies on NADPH oxidation. All procedures were carried out at 4°C.

Measurement of NADPH oxidation. For studies on NADPH oxidation and autoxidation of polyphenols, an Aminco DW-2A spectrophotometer equipped with anaerobic cells with plunger assemblies (American Instrument Co.. Silver Spring, Md.) was used. Incubation mixtures contained 63 mg of protein and an initial concentration of 1.6 mM NADPH in a total volume of 2.5 ml of 50 mM phosphate buffer (pH 7.4). After a 1-min preincubation period, the reaction was started by the addition of 5 μmol (final concentration equivalent to 2 mM) of substrate and incubated for 5 min at

TABLE I

DISPOSITION OF ACID-SOLUBLE RADIOACTIVITY IN CONTROL AND AROCLOR-PRETREATED RATS GIVEN [^{14}C]CATECHOL OR [^{14}C]-HYDROQUINONE

	[^{14}C]Catechol[a]				[^{14}C]Hydroquinone[a]			
	2 hr		24 hr		2 hr		24 hr	
Organ	Control	Aroclor	Control	Aroclor	Control	Aroclor	Control	Aroclor
Liver	279 ± 18	188 ± 11	45 ± 1[b]	49 ± 10[b]	203 ± 31	111 ± 12	27 ± 2[b]	17 ± 1[b]
Thymus	149 ± 35	336 ■34	43 ± 2[b]	44 ± 4[b]	163 ± 27	207 ± 37	38 ± 2[b]	45 ± 1[b]
Bone marrow	335 ± 72	370 ± 9	252 ± 19	196 ± 43	235 ± 17	215 ± 21	156 ± 4[b]	122 ± 23
WBC[c]	—	—	145 ± 3	126 ± 13	—	—	129 ± 7	99 ± 10

[a] Values are expressed as dpm/mg protein and represent the mean ± SE of tissues from three animals.
[b] Value for given treatment group at 24 significantly different ($p < 0.05$) from corresponding group at 2 hr.
[c] White blood cells.

37°C. NADPH oxidation was monitored at AOD 340–400 nm using the split-beam mode. The reference cuvette contained all components except the substrate. For anaerobic measurements N_2 was bubbled into both cuvettes for 3 min and NADPH oxidation was measured under a N_2 atmosphere. Absorbance measurements were converted to concentration units using a value for ϵ of 6200 M^{-1} cm^{-1}.

Autoxidation of polyphenols. The autoxidation of phenol, catechol, 1,2,4-benzenetriol, hydroquinone and benzoquinone was monitored by measuring substrate and product absorption wavelength maxima in the range 200–350 nm at a scan speed of 200 nm/sec. Substrate (final concentration equivalent to 0.10 to 0.45 mM) was added to 2.5 ml of 50 mM phosphate buffer (pH 7.4) using a plunger assembly fitted to an anaerobic cell. The generation of O_2 from the autoxidation of benzene metabolites was monitored by quantitating adrenochrome formation from epinephrine (Misra and Fridovich, 1972) at 480 nm in a Cary 219 spectrophotometer (Palo Alto, Calif.). 1,2,4-Benzenetriol, benzoquinone, and hydroquinone were purchased from Aldrich Chemical Company (Milwaukee, Wisc.). Catechol, epinephrine, superoxide dismutase, and NADPH were obtained from Sigma Chemical Company (St. Louis. Mo.).

Statistical analysis. The data were analyzed via analysis of variance, with difference among means analyzed with Duncan's multiple range test. The level of significance chosen was $p < 0.05$.

RESULTS

Disposition and Covalent Binding of [^{14}C]-Catechol and [^{14}C]Hydroquinone

Male Fischer-344 rats were given 100 μCi (14 mg/kg) [^{14}C]catechol or [^{14}C]hydroquinone by injection into the lateral tail vein. The distribution of soluble radioactivity (not precipitable by TCA, see Methods) and covalently bound radioactivity (measured after exhaustive extraction) was monitored in various tissues from control rats and rats pretreated with Aroclor 1254, a regimen which protects against benzene toxicity (Greenlee *et al.*, 1980). Two hours after dosing with either compound, the amount of soluble radioactivity measured in liver, thymus, and bone marrow ranged from 100 to 300 dpm/mg protein [Table 1). No difference was observed in control versus Aroclor-pretreated animals. After 24 hr, the amount of soluble radioactivity measured in liver and thymus was markedly lower than at 2 hr, whereas in bone marrow no statistically significant difference was found at the two time points, except for control rats given [^{14}C]hydroquinone (Table 1). Even for the latter group, the amount of soluble radioactivity detected at 24 hr was *66%* that detected at 2 hr. These findings indicate that soluble radioactivity associated with [^{14}C]catechol and [^{14}C]hydroquinone is retained in the bone marrow and are in agreement with a previous study by Rickert *et al.* (1979) showing a selective retention of these metabolites in the bone marrow of rats exposed to benzene.

After the administration of [^{14}C]catechol or [^{14}C]hydroquinone to control rats, the

TABLE 2

DISPOSITION OF COVALENTLY BOUND RADIOACTIVITY IN CONTROL AND AROCLOR-PRETREATED RATS GIVEN [^{14}C]CATECHOL OR [^{14}C]HYDROQUINONE

	[^{14}C]Catechol[a]				[^{14}C]Hydroquinone[a]			
	2 hr		24 hr		2 hr		24 hr	
Organ	Control	Aroclor	Control	Aroclor	Control	Aroclor	Control	Aroclor
Liver	210 ± 21	121 ± 49	268 ± 22	82 ± 13[b]	160 ± 14	30 ± 10[c]	239 ± 19[d]	46 ± 5[b]
Thymus	406 ± 42	390 ± 61	347 ± 41	209 ± 4	556 ± 142	262 ± 18	330 ± 17	289 ± 14
Bone marrow	424 ± 68	198 ± 62	715 ± 27[d]	291 ± 73[b]	363 ± 48	296 ± 4	558 ± 10[d]	349 ± 49
WBC[e]	—	—	1059 ± 74	497 ± 21[b]	—	—	726 ± 122	547 ± 15

[a] Values are expressed as dpm/mg protein and represent the mean ± SE of tissues (same as those in Table I) from three animals.
[b] Aroclor value at 24 hr significantly different ($p < 0.05$) from control value at 24 hr.
[c] Aroclor value at 2 hr significantly different ($p < 0.05$) from control value at 2 hr.
[d] Control value at 24 hr significantly different ($p < 0.05$) from control value at 2 hr.
[e] White blood cells.

amount of covalently bound radioactivity measured in the bone marrow after 24 hr was significantly greater than the amount detected after 2 hr (Table 2). Pretreatment with Aroclor resulted in a significant decrease in the amount of covalently bound radioactivity measured at 24 hr in both the bone marrow and peripheral white blood cells. In the liver covalently bound radioactivity associated with [^{14}C]catechol was significantly depressed after 24 hr and with [^{14}C]hydroquinone after both 2 and 24 hr in Aroclor-pretreated rats. No significant differences in the temporal pattern of covalent binding were observed in the thymus.

Autoxidation of Polyphenols

The potential for autoxidation of catechol and hydroquinone as well as other benzene metabolites, phenol, and 1,2,4-benzenetriol was determined at various pH values ranging from 6.5 to 9.5. At physiologic pH (7.4) hydroquinone and 1,2,4-benzenetriol spontaneously autoxidized to products with ultraviolet absorption maxima of 248 and 267 nm, respectively (Table 3). No decomposition was observed anaerobically. The ultraviolet absorption maximum of the hydroquinone autoxidation product was identical to that of benzoquinone (248 nm) and both species decomposed under alkaline conditions. In the presence of 250 units of superoxide dismutase, the rate of autoxidation of hydroquinone was stimulated fivefold, whereas the rate of autoxidation of 1,2,4-benzenetriol was markedly inhibited to a value less than 4% of control (Table 3).

At pH values greater than or equal to 8.5, the autoxidation of both hydroquinone and 1,2,4-benzenetriol was quite rapid and was essentially complete within 1 min after the addition of either compound to the buffer (data not shown). Under the same experimental conditions, phenol and catechol did not spontaneously autoxidize at any of the pH values examined.

The ability of the various metabolites to stimulate the formation of adrenochrome from epinephrine was determined as an indirect measure of the generation of O_2^- (Misra and Fridovich, 1972). As shown in Table 4, adrenochrome formation was stimulated by 1,2,4-benzenetriol (0.298 μmol/min) and hydroquinone (0.0042 μmol/min), but was barely detectable in the presence of catechol (0.0004 μmol/min). Addition of superoxide dismutase inhibited 1,2,4-benzenetriol and catechol stimulated adrenochrome formation, while it resulted in a two-

fold increase in hydroquinone-induced epinephrine autoxidation.

Augmentation of NADPH Oxidation

The effect of benzoquinone, hydroquinone, catechol, and 1,2,4-benzenetriol on **NADPH** oxidation by rat bone marrow was studied. Only benzoquinone augmented the oxidation of **NADPH** by a 3000*g* bone marrow supernatant (Table 4). Anaerobically, the total amount of **NADPH** oxidized (141 nmol) was twice the amount oxidized aerobically (68.5 nmol) (Table 4).

DISCUSSION

It has been suggested that the chronic production **of** small amounts of benzene oxide in bone marrow may result in reactions with critical macromolecules, ultimately leading to bone marrow depression or leukemia (Snyder *et al.*, 1978). Studies on the covalent binding of benzene to liver microsomal protein, however, have indicated that a metabolite of phenol and not benzene oxide was responsible for the observed binding (Tunek *et al.*, 1978). Several recent lines of evidence have indicated that benzene-induced bone marrow toxicity is associated with two polyphenol metabolites, hydroquinone and catechol. In a study of the metabolism of [^{14}C]benzene in isolated rat femurs, it was reported that 3% of the radioactivity was recovered as benzene metabolites. The bone marrow/blood concentration ratios for both hydroquinone and catechol were approximately 400 (Irons *et al.*, 1980). *In vivo*, hydroquinone and catechol were found to be retained at steady-state concentrations for at least 18 hr in the bone marrow of rats following a 6-hr exposure to 500 ppm benzene (Rickert *et al.*, 1979). In studies on the disposition **of** ^{14}C-

TABLE 3

RATES OF FORMATION AND SPECTRAL CHARACTERISTICS OF AUTOXIDATION PRODUCTS OF 1,2,4-BENZENETRIOL AND HYDROQUINONE[a]

Compound (λ_{max}, nm)	Concentration (mM)	Incubation conditions	λ_{max} of product(s)	Rate of product formation[a]
				ΔOD/min[b]
1,2,4-Benzenetriol (290)	0.40	Air	267	0.87 ± 0.04
	0.40	Nitrogen	—	0
	0.10	Air	267	0.55 ± 0.01
	0.10	Air + 62.5 U SOD[c]	267	0.21 ± 0.01
	0.10	Air + 125 U SOD	267	0.08 ± 0.01
	0.10	Air + 250 U SOD	267	0.02 ± 0.01
				nmol/min[d]
Hydroquinone (292)	0.10	Air	248	2.2 ± 0.2
	0.40	Air	248	4.8 ± 0.3
	0.40	Air + 62.5 U SOD	248	13.6 ± 0.9
	0.40	Air + 125 U SOD	248	17.2 ± 0
	0.40	Air + 250 U SOD	248	23.9 ± 1.9
	0.40	Nitrogen	—	0

[a] Autoxidation rates are the mean ± SE of triplicate determination.
[b] ϵ_{max} of autoxidation product not known.
[c] Superoxide dismutase.
[d] Based on a ϵ_{max} value for benzoquinone of 16.3 mM^{-1} cm^{-1} (pH 7.4).

TABLE 4

STIMULATION OF NADPH OXIDATION AND ADRENOCHROME FORMATION BY BENZOQUINONE, 1,2,4-BENZENETRIOL, HYDROQUINONE, AND CATECHOL

Incubation conditions	Compound[a]	Total NADPH oxidized[b] (nmol)	Adrenochrome formation[c] (μmol/min)
Air atmosphere	Benzoquinone	68.5 ± 28.0 (3)	—
	1,2,4-Benzenetriol	0 (3)	0.298 ± 0.007 (4)
	+ SOD[d]	—	0.120 ± 0.002 (4)
	Hydroquinone	0 (3)	0.0042 ± 0.0001 (4)
	+ SOD	—	0.0080 ± 0.0002 (4)
	Catechol	0 (3)	0.0004 ± 0.0000 (4)
	+ SOD	—	nd[e]
Nitrogen atmosphere	Benzoquinone	141 ± 48 (8)	—
	1,2,4-Benzenetriol	0	—
	Hydroquinone	0	—
	Catechol	0	—

[a] Compounds were present at a concentration of 0.1 mM in a total incubation volume of 3.0 ml.

[b] NADPH oxidation (1.6 mM starting concentration) was monitored for 5 min at AOD 340–400 nm in the presence of 3000*g* rat bone marrow supernatant (63 mg total protein). Values shown are the mean ± SE. The number of determinations made are given in parentheses.

[c] Epinephrine present at 0.5 mM. The values shown were calculated using an $\epsilon_{max} = 4.01 \times 10^3\ M^{-1}\ cm^{-1}$ and were corrected for background epinephrine oxidation. All values represent the mean ± SE of four determinations.

[d] Superoxide dismutase; 25 μg protein, 62.5 U per 3-ml incubation.

[e] No adrenochrome formation detected.

labeled benzene metabolites using whole body autoradiography, radioactivity associated with hydroquinone or catechol, but not phenol, was found to concentrate in bone marrow and lymphoid organs. Further, the level of radioactivity in these tissues was reduced in rats pretreated with Aroclor 1254, a regimen which protects against benzene toxicity (Greenlee *et al.*, 1980).

The results in this study further support the hypothesis that polyphenol metabolites of benzene may be important in benzene-induced cytotoxicity. Following administration of a single iv dose of [^{14}C]catechol or [^{14}C]hydroquinone, soluble radioactivity was retained in bone marrow, but not liver or thymus, for at least 24 hr. Covalently bound radioactivity increased with time in all tissues examined, with the largest increase observed in bone marrow. Covalent binding was depressed in liver, white blood cells, and bone marrow by Aroclor pretreatment, which protects against benzene myelotoxicity.

The observations of this study suggested that covalent binding of hydroquinone and/or catechol in the bone marrow may be an important event in the expression of toxicity. Thus, potential pathways for the activation of these compounds and subsequent metabolites such as 1,2,4-benzenetriol were examined. At physiologic pH, hydroquinone and 1,2,4-benzenetriol (a putative metabolite of catechol) spontaneously autoxidized as detected by the appearance of ultraviolet absorption products. A proposed metabolic scheme is presented in Fig. 1. For the triol, the sequence, 9 → 10 → 11, is modeled after studies on the autoxidation of 6-hydroxydopamine (Graham, 1978). The pathway shown for the oxidation of hydro-

quinone (4 → 5 → 6) is based on studies of the autoxidation of durohydroquinone (James and Weissberger, 1938). It is proposed that benzene-induced cytotoxicity may result from binding of highly reactive semiquinone and/or quinone metabolites to cellular macromolecules, which is supported by the observation of binding after *in vivo* treatment with both [^{14}C]hydroquinone and [^{14}C]catechol. Although catechol did not significantly autoxidize at physiologic pH, it is possible that the *in vivo* binding seen with [^{14}C]catechol may originate from the formation of 1,2,4-benzenetriol, which readily autoxidizes (Fig. 1).

Autoxidation may not be the only mechanism whereby polyhydroxy metabolites of benzene can be activated to semiquinone and quinone metabolites. Studies by Dybing et *al*. (1976) have demonstrated that several catechols such as a-methyldopa, dopa, and dopamine are metabolically activated to presumed semiquinone and/or quinone products via O_2^- produced by microsomal cytochrome P-450. A recent study (Hanson *et al*., 1978) has shown that rabbit bone marrow microsomes are capable of generating O_2^-, as determined by adrenochrome formation. Thus, the presence of endogenously generated O_2^- might also be expected to activate benzene metabolites and would provide, for example, a direct mechanism for activation of catechol to radical or quinone products.

It cannot be excluded, however, that O_2^- generated by autoxidation of benzene metabolites may also be important component of benzene-induced cytotoxicity. The stimulation of epinephrine oxidation by 1,2,4-benzenetriol and catechol, and its inhibition by superoxide dismutase, suggests O_2^- as a product of metabolite autoxidation (Fridovich, 1975). In contrast, the autoxidation of hydroquinone and subsequent stimulation of epinephrine oxidation were increased by superoxide dismutase, which may be accounted for in the reaction described below in which removal of O_2^-

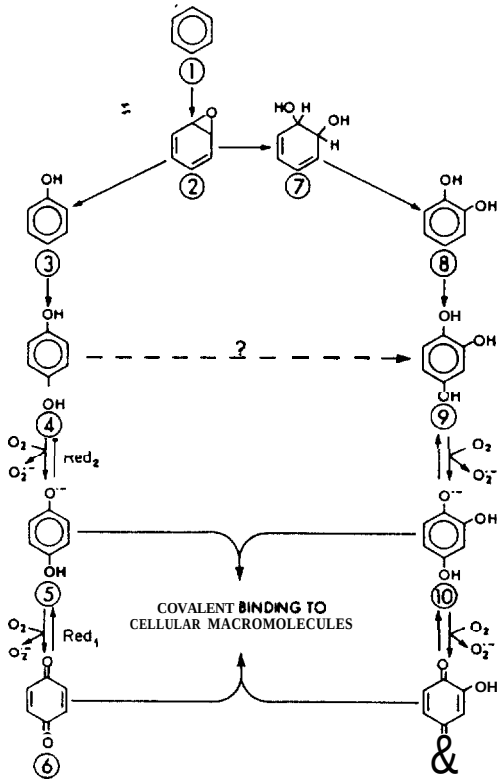

FIG. 1. Proposed oxidative pathways for various benzene metabolites. 1, benzene; 2, benzene oxide; 3, phenol; 4, hydroquinone; 5, semiquinone; 6, benzoquinone; 7, benzene dihydrodiol; 8, catechol; 9, 1,2,4-benzenetriol; 10, semiquinone of benzenetriol; 11, 2-hydroxybenzoquinone.

would displace the equilibrium to the right:

$$\text{hydroquinone} + 2O_2 \rightleftarrows \text{benzoquinone} + 2O_2^- + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

The enhanced cooxidation of epinephrine induced by superoxide dismutase can not clearly be attributed to an increased flux of O_2^-, however, in that semiquinone and/or quinone autoxidation products may also be capable of oxidizing epinephrine directly.

Superoxide anion is known to be cytotoxic, presumably mediated via formation of hydrogen peroxide and hydroxyl radicals (Fridovich, 1978). In rabbit bone marrow

the activity of superoxide dismutase, an enzyme which functions to detoxify O_2^- (Fridovich, 1978), is less than 10% of the activity in liver tissue (Hansen *et al.*, 1978). Thus, bone marrow may be particularly sensitive to O_2^--induced tissue injury.

It should be noted that the mechanism of toxicity of several other chemicals may be similar to that proposed here for benzene. The neurotoxic agent 6-hydroxydopamine is thought to destroy dopaminergic neurons via O_2^- and/or semiquinone formation (Sachs and Jonsson, 1975; Graham *et al.*, 1978). Likewise, the diabetogenic agent alloxan appears to be reduced to dialuric acid in the β cell of the pancreas, with subsequent autoxidation producing O_2^-, H_2O_2, and OH (Cohen and Heikkila, 1974). Finally, the herbicide paraquat may produce pulmonary damage by O_2^- generated from a cyclic single-electron reduction–oxidation of the parent molecule (Bus et *al.*, 1976). It is particularly interesting that the specific target organ toxicity of these agents correlates with a selective uptake and/or retention of the agent in the target tissue (Cohen et al., 1976; Hammarstrom and Ullberg, 1966; Rose et *al.*, 1974), which is analogous to the retention of the proposed cytotoxic metabolites of benzene in bone marrow.

A second source of semiquinone and O_2^- can result from a one-electron transfer from NADPH to benzoquinone (6 → **5**, Fig. 1). The reduction of benzoquinone was found to be catalyzed by a 3000*g* rat bone marrow supernatant (Table 4). Under aerobic conditions, the one-electron reduction product would be reoxidized to benzoquinone with formation of O_2^-. Anaerobically, the total amount of NADPH oxidized was twice the amount oxidized aerobically (Table 4), suggesting the two-electron reduction sequence 6 → **5** → 4 (Fig. 1).

In summary, the data in this report support a mechanism for benzene toxicity in which the formation of potentially cytotoxic products result from the autoxidation of at least two of the reported in vivo polyphenol metabolites of benzene, hydroquinone, and 1,2,4-benzenetriol.

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