# BENZENE METABOLISM

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## *IN VIVO* BENZENE METABOLISM

The metabolism of benzene in humans and animals shows many parallel pathways. It has been the subject of a recent review by Snyder and Kocsis (886). The major site of conversion appears to be the liver and its primary oxidative products include phenol, catechol, and quinol. Further oxidation may produce 1,2,4-trihydroxybenzene (hydroxyquinol). Subsequently, these oxidative products are transformed to phenylsulfuric and phenylglucuronic acids, which are excreted as their alkaline salts. It must be recognized that these represent classical conversions of organic materials in the liver. The major toxic action, however, occurs in the bone marrow, and the specific metabolic products *or* intermediates occurring in this tissue have not been well characterized.

Historically, the basic understanding of benzene metabolism was obtained via animal experimentation. The conversion of benzene to phenol in animals was first observed in 1867 (363). Subsequently, the presence of quinol and catechol was detected in the urine of animals previously exposed to benzene (343). Other minor metabolites isolated from urine include *trans-trans* muconic acid (336) and 1-phenylmercapturic acid (319, 374).

The first major series of studies on the metabolic fate of benzene in animals was initiated in 1949 by Williams and co-workers. They found that, following oral administration of benzene to rabbits, 21% of the dose was excreted as phenols. This consisted of a mixture of phenol, catechol, quinol, and hydroxyquinol. A small amount of a nonaromatic material, *trans-trans*-muconic acid, was also found (350). Virtually all of the excreted phenols were conjugated either as glucuronides or as ethereal sulfates (354). Over 95% of the phenol was eliminated during the first 2 days. During this same period only 60% of the dihydroxyphenols, catechol, and quinol were eliminated. The hydroxyquinol elimination did not reach a maximum until the third day. It was therefore suggested that the dihydroxyphenols were subsequent oxidation products of phenol and that the trihydroxyphenol was an even later oxidation product (355).

In a series of experiments, phenol (325), catechol (901), resorcinol (902), and quinol (902) were administered to rabbits via a stomach tube.

The urine was then examined for these compounds and for possible metabolites. In all cases, the major metabolites were conjugated monoglucuronides. Ethereal sulfates were also formed. Only the urine of rabbits treated with catechol contained detectable amounts of hydroxyquinol, even though it could have been formed from further oxidation of all three dihydroxyphenols. Based on this evidence, the authors proposed the metabolic scheme shown in Fig. **1.**

Using radioactive labeling, Parke and Williams **(351)** studied the tissue distribution of benzene in the rabbit. **A** total of **16%** of the initial dose was recovered from the tissues 1 day after administration. The majority of the radioactivity was detected in the voluntary muscle (57%), the involuntary muscle (9%), and the blood *(5%).* No radioactivity was detected in the bone marrow, spleen, or brain. In contrast to this study, results using rats in our laboratory show considerable concentration of benzene in bone marrow and spleen immediately following inhalation.

Parke and Williams **(532)** also administered [$^{14}$C] benzene **(0.34–0.50** g/kg) orally to rabbits. They recovered **84–89%** of the original dose as radioactivity in the expired air, urine, feces, and body tissue. In the expired air, **43%** was recovered as unchanged benzene and **1.5%** as $^{14}CO_2$. The elimination of the $^{14}CO_2$ began **12–18** hours after administration of the benzene, and continued for several days. The urine contained **34.5%** of the original radioactivity, phenol accounting for **23.5%,** quinol for 4.8%, catechol for **2.2%,** hydroxyquinol for **0.3%,** *trans-trans*-muconic acid for **1.3%,** and phenylmercapturic acid for 0.5%. These determinations were based on samples collected over a **3** day period. After **3** days 5–10% of the original dose was still in the animal distributed throughout the tissues. In a subsequent study, Williams and co-workers found that 0.8% of the benzene was excreted in the bile **(302).**

The distribution of metabolic products resulting from administration of benzene to the rat has not been studied in as much detail as that in rabbits. Cornish and Ryan **(312)** observed that administration of benzene

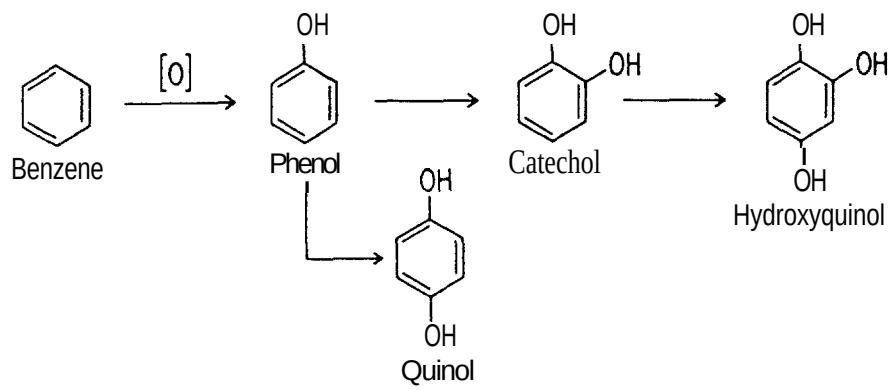


**FIGURE 1. Proposed *in vivo* benzene metabolic scheme.**

to rats (88 mg/kg, ip) produced a 23% yield of phenolic metabolites in the urine. Of these, 70% were conjugated as sulfates, 17% were conjugated as glucuronides, and the balance were free phenols. Gerarde and Ahlstrom (326) also observed a high level of organic sulfates in the urine of benzene-treated rats. Van Rhees (370) determined that on ip administration of 2–4 mg of benzene to rats metabolism was complete within 8 hours. Bakke and Scheline (7) reported finding 3–4% of an oral dose of benzene (100 and 1,000 mg/kg) in the urine as conjugated phenol. Traces of catechol and quinol were also detected. The low yield of urinary phenols was attributed to the strain of rat or to the method of administration of the benzene. It was noted that the rats developed diarrhea from the treatment.

It has been reported (888) that when mice were treated with benzene (880 mg/kg, sc in oil) over 70% of the benzene was recovered within 8 hours in the expired air. The major metabolite found in the urine was phenol. Traces of catechol were also present. These phenols were predominantly excreted as glucuronides (50–65%) with ethereal sulfates accounting for 26–38%. About 5% was found to be unconjugated phenol.

Few investigations of benzene toxicity have involved species other than rabbits, rats, and mice. Horses given benzene were said to eliminate potassium phenylsulfate in the urine (308). Callow and Helle (310) reported that dogs receiving 1.56 g benzene had a higher output of organic sulfur in the urine. In a subsequent study (362), dogs were given inhalation exposures to benzene (500–1,300 ppm) during a period of 8–22 days. At the conclusion of the exposure, the animals were sacrificed and the distribution of benzene in the various tissues was determined. Over 83% of the recovered benzene was found distributed between the fat, bone marrow, and urine. Of the remaining amount, about 1% was found in the blood. These results, along with those previously noted, emphasize the marked difference in distribution depending on different routes of administration.

More recent work by Oehme (912) involved several species of animals. He reported that on treatment of dogs with benzene (10–100 mg/kg, iv) 55–80% of the dose was recovered from the urine as a mixture of phenylglucuronides and phenylsulfates. In a similar experiment using cats, 80% was excreted as phenylsulfate and the balance was divided between free phenol and the glucuronide. In the pig, the major conjugate obtained was the glucuronide *(60%)* along with 30% free phenol and only traces of phenylsulfate. In contrast, the urine of goats exposed to benzene contained predominantly phenylsulfate (75%), most of the remainder being the glucuronide (25%) with only about 1% free phenol.

The metabolism in humans is essentially the same as that in animals. Teisinger et al. (390) exposed human subjects to approximately 100 ppm (340 mg/liter) benzene for 5 hours daily. It was observed that the average retention of inhaled vapor was 46.3%. Of the retained benzene, approxi-

mately 12% was subsequently expired unchanged and approximately 0.1–0.2% was excreted in urine. The remainder was metabolized to various phenolic compounds previously described for animals.

Treatment of animals with benzene metabolites has also been used in investigation of the metabolic pathways. Work by Williams has shown that the major metabolic fate of phenol given to several species was the formation and elimination of conjugates. Aii species studied except the cat and pig produced large amounts of both glucuronides and ethereal sulfates. The pig excreted the total dose as phenylglucuronide and the cat as phenylsulfate (87%) and quinol sulfate (13%) (311).

It would appear that, regardless of the route of administration, benzene is eliminated both in the expired air and in the urine. In the expired air, one finds predominantly unchanged benzene with some carbon dioxide. In the urine, one finds the conjugated metabolic oxidation products of benzene, typically large quantities of phenol accompanied by smaller amounts of catechol, hydroquinol, and hydroxyhydroquinol. The liver appears to be the major site of both oxidation and conjugation.

## *IN VITRO* BENZENE METABOLISM

More recent work has suggested that catechol is not derived from phenol, but may result from dehydrogenation of 1,2-dihydro-1,2-dihydroxybenzene (DDB). When synthetic DDB was given to rabbits, both phenol and catechol were isolated in the urine (907). Also, when extracts of rabbit liver powder were treated with *trans*-DDB in the presence of triphosphopyridine nucleotide (TPN), catechol was obtained. Phenol was not found in the reaction mixture (306). When the cis isomer was administered, large amounts of phenol as well as catechol were obtained (367). In a study reported by Sato et al. (360), the urine of rabbits treated with benzene (0.50 ml in 0.50 ml olive oil, intraperitoneally) was examined specifically for cis- and *trans*-DDB. The *trans*-DDB and a glucuronide that yielded trans-DDB on treatment with 1-glucuronidase were found by paper chromatography and electrophoresis. Cis-DDB was not detected.

In separate studies by Nomiyama, 7-week-old Donryu rats were given subcutaneous injections of benzene or one of its metabolites. The compounds tested included benzene (1.0 g/kg), phenol (0.20 g/kg), catechol (0.03 g/kg), quinol (0.05 g/kg), hydroxyquinol (0.005 g/kg), *trans*, trans-muconic acid (0.013 g/kg), and phenylmercapturic acid (0.01 g/kg). The doses that were administered approximated the metabolic yield expected from 1.0 g/kg of benzene. The animals receiving the phenol developed leukocytosis. Animals receiving catechol developed a significant leukopenia. Since benzene induces leukopenia, it would appear that the metabolism of benzene to catechol proceeds directly, probably through benzene glycol. Otherwise, phenol would have induced a similar leukopenic response (346).

It has been suggested that benzene metabolism is initiated by a free radical attack of H-O$^{\bullet}$ or H-O-O$^{\bullet}$ on the ring, in the same manner as that observed when benzene is treated with hydrogen peroxide and ferrous ion (393). A second, and possibly related, theory postulates the formation of benzene epoxide as one of the initial steps in benzene metabolism. Enzymatically, benzene oxide may be hydrated to a dihydrodiol by the action of epoxide hydrase; subsequent breakdown of this material could lead to the products isolated from the metabolism of benzene (907, 913, 991). Jerina and co-workers (908) showed that incubation of mammalian liver homogenates with benzene epoxide led to the spontaneous generation of phenol. The microsomal fraction of rabbit liver was shown to contain an epoxide hydrase that was capable of converting arene epoxides to *trans*-dihydrodiols. The soluble fraction, from the liver homogenate, contained a glutathione-5-epoxide transferase that was capable of catalyzing the addition of glutathione to the ring. The premercapturic acid, S-(1,2,-dihydro-2-hydroxyphenyl)glutathione, was also isolated on treatment of the soluble fraction *of* rat liver homogenate with benzene epoxide and glutathione. Thus, benzene epoxide could be a transient intermediate, which could isomerize directly to yield phenol, undergo enzymatic addition of water to form a dihydrodihydroxybenzene, or by enzymatic addition of glutathione yield a premercapturic acid. Attempts to trap [$^{14}$C] benzene epoxide formed from [$^{14}$C] benzene incubation with rabbit liver microsomes were unsuccessful. The failure to isolate this intermediate could be attributed to its half-life of only *2* minutes in aqueous systems. However, the oxide of naphthalene, a homolog of benzene, was recovered when naphthalene was incubated with liver microsomes (369). Also, Hamilton has proposed that, on theoretical grounds, it would be expected that mixed function oxidation reactions proceed through arene oxide intermediates (330).

It has been shown that microsomes isolated from rabbit or dog liver are capable of converting benzene to phenol (917). **Also,** both liver homogenates and microsomal preparations obtained from rabbits, rats, and mice were active (365). It was found that the microsomal fractions were capable of both hydroxylation of benzene *to* phenol and subsequent conjugation to give either phenylsulfate or phenylglucuronide. Conjugation was dependent on available ATP, as was the rate of benzene metabolism. In a subsequent study, however, it was demonstrated that the rate of conjugation does not affect the rate of metabolism. Therefore, the increased rate of benzene metabolism in the presence of added ATP is a function *of* the effect of the ATP on benzene hydroxylase activity **(418).**

During incubation of benzene with rat liver slices approximately two-thirds of the phenol formed was conjugated as the sulfate with the remainder metabolized *as* the glucuronide (315). Thus, in the rat, sulfonation is the major pathway for conjugation of phenol. Ikeda (333) measured the levels of the sulfonating enzymes in rats during growth and

compared them with the susceptibility of the animals to benzene poisoning. He observed that the enzyme activity paralleled growth of the animal. Also, the levels were higher in male rats than in female rats. He found that the higher the level of the sulfonating enzyme activity, the greater resistance the rat had toward benzene toxicity. In similar experiments, he did not observe a parallel correlation between aryl 4-hydroxylase activity or UDPglucuronyltransferase activity and benzene toxicity. However, both enzyme levels were shown to increase during moderate benzene intoxication.

Based on the available experimental findings, the metabolism and elimination of benzene would appear to be as follows. Approximately 40% is eliminated unchanged in the expired air. The remainder is oxidized to benzene oxide in the liver by the aryl hydrocarbon hydroxylase enzyme system. The benzene oxide then breaks down via one of three pathways. First, it can spontaneously rearrange to yield phenol. Second, it can be acted on by epoxide hydrase, giving benzene glycol. The benzene glycol then yields predominantly catechol via enzymatic dehydrogenation, and some trans, trans-muconic acid from subsequent oxidation. Third, the benzene oxide can react with glutathione in the presence of epoxytransferase to produce phenylmercapturic acid. The majority of the phenol formed undergoes subsequent conjugation to yield phenylsulfates and phenylglucuronides. However, some of the phenol undergoes subsequent oxidation to produce hydroquinol, which is then conjugated and eliminated. Finally, the majority of the catechol is conjugated and eliminated, with a small amount being oxidized to hydroxyhydroquinol. These pathways are shown in Fig. 2.

## INDUCTION AND INHIBITION OF BENZENE METABOLISM IN ANIMALS

Fasting can reduce the ability of the liver to hydroxylate a variety of substances. Cornish and Ryan **(312)** investigated the effect of fasting on the ability of male Sprague-Dawley rats to metabolize benzene. Fasted and nonfasted rats were injected with benzene (88 mg, ip). The fasted animals exhibited an increase in glucuronide excretion that was approximately three times the increase obtained with nonfasted animals. The fasted control animals showed a slight reduction in glucuronide excretion. The benzene-treated groups, both fasted and nonfasted, showed large increases in free phenol excretion. Additional groups of nonfasted animals were pretreated with SKF **525-A** (0-diethylaminoethyl diphenylpropylacetate hydrochloride, a well-known inhibitor of microsomal enzymes). This resulted in a marked depression of both free phenol and glucuronide excretion in rats receiving benzene. It also extended the clearance time of the phenols from 24 hours to several days. Thus, while SKF **525-A** inhibits glucuronide formation, fasting appears to induce it in this system.

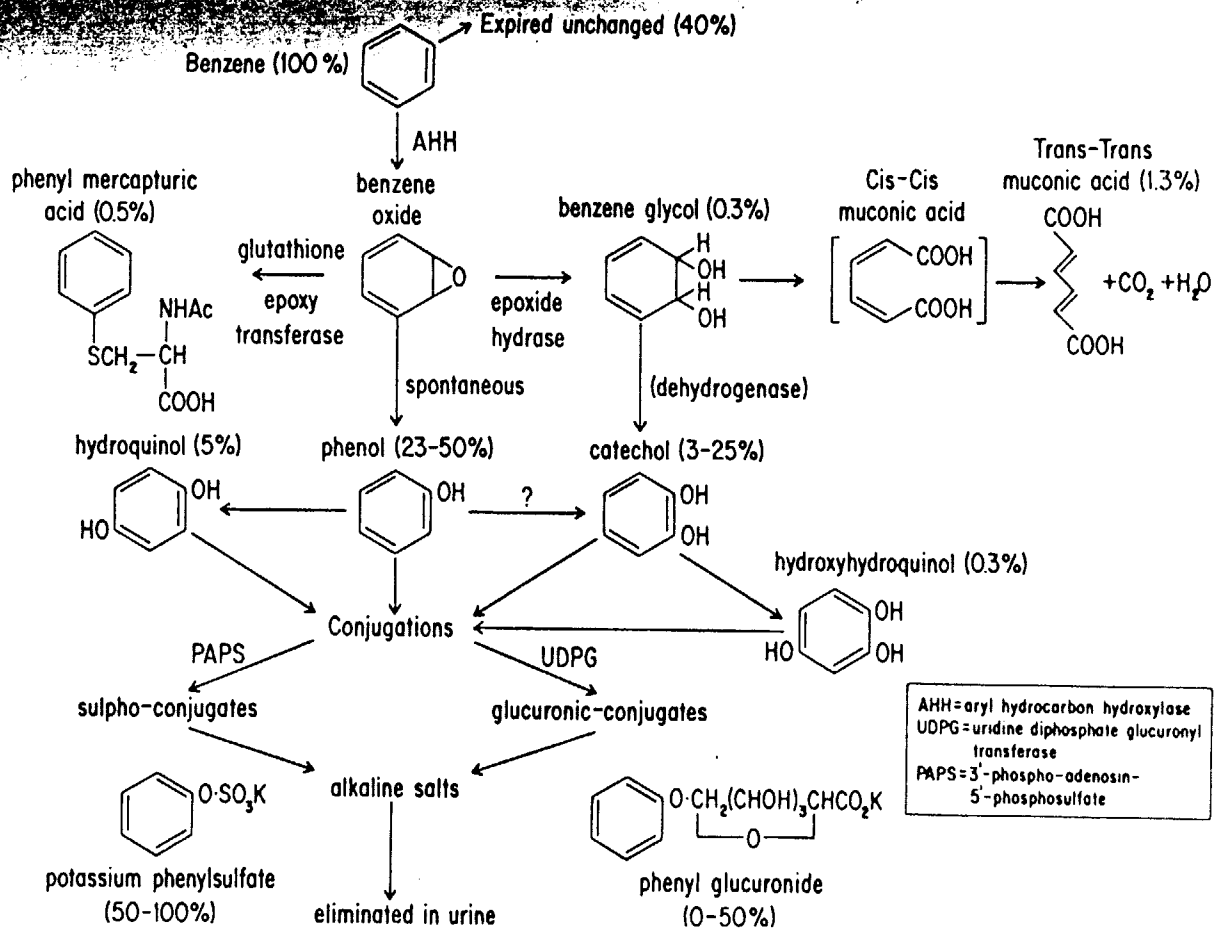


FIGURE 2. Pathways of benzene metabolism and elimination.

Preexposure of rabbits and rats to a single dose of benzene (1.1 g/kg) was shown to stimulate subsequent benzene metabolism (365). However, when repeated injections of *50* mg/kg were given 5 days per week for 1 month, no stimulation was observed (371). A subsequent study, was performed to investigate the possibility that the stimulation observed with the single benzene pretreatment was caused by the phenolic metabolites (356). Groups of male rats were pretreated with phenol, catechol, resorcinol, quinol, or hydroxyquinol and then given a single subcutaneous injection of benzene. No effect, was observed in the phenol and catechol groups. In the resorcinol, quinol, and hydroxyquinol groups, a slight decrease in metabolic rate was observed. The level of cytochrome P-450 in the liver was also measured, as it is a mixed function oxidase system and could be involved in the metabolism of benzene. No elevation in the level *of* cytochrome P-450 was observed, even in rats receiving up to 14 daily injections of benzene (1.1 g/kg, sc). In contrast to these results, Norpoth and co-workers (347) found that inhalation exposure of male Wistar rats to a concentration of 450 ppm of benzene, 5 hours per day for 10 or 28 days, resulted in a 75% increase in the cytochrome P-450 content in the liver and a significant increase in the level of aminopyrine demethylase. The livers in the animals receiving ten exposures were also enlarged. Thus, it would appear that a large dose of benzene is necessary to stimulate subsequent benzene metabolism.

Treatment of animals with phenobarbital (PB) enhances some of the oxidative enzymes in the liver. Liver microsomes obtained from rats and rabbits pretreated with either benzene or PB had an increased rate of metabolism for benzene when compared with microsomes obtained from untreated animals (365). The changes were most marked in the benzene-treated animals. Liver microsomes obtained from these animals were capable of metabolizing benzene three times as fast as similarly prepared control microsomes. Increases were observed for production of both conjugated and unconjugated phenol. The stimulation in the PB-treated groups was not as great as in the benzene-treated group, and two doses of animal pretreatment were required to elicit an effect. Again, the production of both conjugated and unconjugated phenol was increased, but in this case the effect was most pronounced in the production of conjugated phenol. When rats were given benzene and concurrent doses of radioactive L-leucine, these exposed animals exhibited a greater uptake of radioactivity in the liver than did the controls. This implies that metabolic stimulation was derived from synthesis of additional enzymes, not from activation of those already present.

Ikeda and Ohtsuji (335) observed stimulation of aryl hydroxylase activity in Wistar rats and guinea pigs receiving PB treatment and subsequent exposure to benzene. Urinary phenol levels were measured at *2* hour intervals postexposure. During the first 2 hour period, the rats in the PB-treated group excreted more than double the phenol obtained from the

control group. By 10 hours postexposure, the urinary phenol levels for both groups were normal. The activities of the two conjugating enzyme systems, phenolic sulfonation and glucuronidation, were not appreciably stimulated. The PB-treated rats exhibited a greater resistance to the leukopenic action of benzene.

In subsequent work, Mikulski (340) observed only a slight increase in total phenol excretion by PB-pretreated Wistar rats compared to rats receiving benzene alone. The comparison was based on the total phenol output during a 48 hour postexposure period. These results do not conflict with those of Ikeda and Ohtsuji, since they measured the rate of excretion and used shorter time spans: *2,* 4, and 6 hours. Mikulski observed a threefold increase in the total excretion of glucuronates, implying a stimulation of that enzyme system.

Recently, Gut (329) reported that PB pretreatment of male Wistar rats increased the rate of metabolism of benzene sixfold in hepatic microsomal preparations obtained from these animals, compared with similar preparations obtained from control animals, However, PB did not influence benzene blood levels following oral, intraperitoneal, or subcutaneous benzene administration. Phenol levels in blood and urine following oral and intraperitoneal administration of benzene to pretreated animals were elevated during the first 3 hours postexposure compared to those in control animals receiving benzene without pretreatment. These levels rapidly returned to the levels observed in the control animals and were not nearly as high as would have been predicted based on the *in vivo* experiment. Furthermore, neither blood nor urine phenol levels were elevated in the pretreated animals receiving subcutaneous administration of benzene. This observation was attributed to a slower rate of benzene absorption following subcutaneous administration, when compared to oral and intraperitoneal administration. The author attributes the low expression of microsomal induction observed in the *in vivo* studies to inhibition of the enzymes by phenol and an insufficient biological energy supply. These results illustrate the caution that must be exercised when comparing *In vivo* studies to *in **vitro*** studies.

Gill and co-workers (327) found that PB pretreatment increased the rate of free phenol excretion in male Sprague-Dawley rats following subcutaneous benzene administration. The fraction of phenol eliminated in conjugated form, however, was lower in the PB group. They also observed that the PB pretreatment apparently protected the animals from the leukopenic action of benzene observed in the control group. Additional groups of animals received 3-methylcholanthrene (MCH) or SKF **525-A** followed by injection of benzene. The animals in the MCH group also exhibited an elevated level of phenol excretion; however, no protection from leukopenia was observed in this group. In the SKF 525-A group, phenol excretion was lowered and the benzene-induced leukopenia was similar to that observed in the control group.

Drew et al. (316, 317, 318) found that PB pretreatment did not alter the acute toxicity of benzene. They exposed PB-pretreated and untreated female CD rats to benzene atmospheres in exposure chambers and measured the $LC_{50}$ for each group. The PB treatment did not alter the observed $LC_{50}$. In addition, they measured metabolic rate (of benzene) and aryl hydroxylase activity in rat liver and lung preparations obtained from animals that had received PB, chlorpromazine (CP), and MCH. In the liver preparations, the PB elicited a tenfold increase and the MCH yielded a threefold increase, while no effect was observed in the CP group. In the lung preparations, the only enhancement of aryl hydroxylase activity observed was in the group that had received CP. This would suggest that different hydroxylase systems are involved in the liver and lung.

Gonasun and co-workers (328) found that microsomes from Swiss mice metabolized benzene more rapidly than did similar microsomal preparations from rats and rabbits. Treatment of mice with benzene increased benzene metabolism *in vitro* without increasing cytochrome P-450 levels. Conversely, treatment of the mice with PB increased cytochrome P-450 levels but did not affect the rate of *in vitro* benzene metabolism. Aniline, metyrapone, aminopyrine, and SKF 525-A, all of which inhibit the metabolism of other compounds by cytochrome P-450 or interact with cytochrome P-450, inhibited benzene metabolism. Also, cytochrome c, which inhibits mixed function oxidase reactions (apparently by diverting electrons from cytochrome P-450) (973), also inhibits benzene metabolism. Benzene metabolism was also inhibited by potassium cyanide, which inhibits cytochrome oxidase but not cytochrome P-450. Finally, benzene metabolism was inhibited by carbon monoxide, which normally inhibits cytochrome P-450 mediated reactions. This inhibition was reversible by light at a wavelength of 450 nm. From these data it was concluded that benzene hydroxylase is a form of mixed function oxidase.

It has been suggested by Gigon et al. (973) that the rate-limiting step in drug metabolism is the rate at which the cytochrome P-450 substrate complex can be reduced. Thus the actual rate-limiting factor may not be the level of cytochrome P-450, but that of a second, as yet uncharacterized, substance.

Mitchell (341) compared the toxic effect of benzene with the toxic effects of its major metabolites. Groups of adult male rats were injected with benzene (0.88 g/kg, sc), phenol (0.25–0.75 g/kg, *sc),* catechol (0.25–0.75 g/kg, sc), or quinol (0.25–0.75 g/kg, sc) daily for 1 week. The dose level of the phenols was high enough to kill 50% of the animals, yet no hematopoietic toxicity was observed. The animals receiving benzene developed aplastic anemia. In other studies, piperonyl butoxide (which blocks benzene metabolism), PB, and 75% hepatectomy all protected against benzene-induced aplastic anemia. Thus it would appear that some form of benzene metabolism by hepatic microsomal enzymes is a requisite for benzene-induced hematopoietic toxicity. While the identification of the

active metabolite has not been accomplished, the results of this experiment would tend to imply that an intermediate metabolite is the toxic agent. However, the findings do differ somewhat from the results of Nomiyama cited above (346).

Various sulfur-containing compounds are known to inhibit biological oxidation. The effects of a series of these compounds—cystine, cystamine and methionine, and the synthetic antioxidant propyl gallate—on benzene metabolism was investigated using rat liver homogenates and rabbits (303). In all cases, the rat liver showed an inhibition of benzene metabolism. This was also true for rabbits receiving pretreatment with methionine or simultaneous administration of benzene with any of the four antioxidants. It was further observed that the myelotoxic action of the benzene was reduced by administration of the above compounds.

Dimethyl sulfoxide (DMSO) pretreatment enhances benzene metabolism and toxicity (337). Wistar male rats were pretreated with DMSO (5 ml/kg) and then with benzene (1 ml/kg) or with benzene alone. The DMSO rats experienced higher levels of urinary phenols, and the mortality in that group was 100%. No mortality was observed in the control animals receiving the same dose of benzene, but without DMSO. Similar results were observed in mice. These results were obtained when the DMSO was administered intraperitoneally, and are attributed to local organ damage and hepatotoxicity of the DMSO. When the DMSO was administered subcutaneously, these effects were not observed.

Administration of 3-amino-1,2,4-triazole to male and female Donryu rats inhibited their ability to metabolize benzene (1.0 g/kg, ip) (344, 529). These observations were true when measurements were made on the *in vivo* production of phenol or on the ability of liver homogenates from animals pretreated with triazole to metabolize benzene. This inhibition of benzene metabolism was accompanied by a lowering of chronic benzene toxicity.

In summary, chronic benzene toxicity appears to be related to the levels and amounts of benzene metabolites present in the animal. Treatments that increase the rate of benzene metabolism, such as phenobarbital pretreatment, also increase the rate of elimination of the metabolites, decreasing the total exposure and the resulting toxicity. Also, treatments that decrease the rate of metabolism, such as partial hepatectomy or pretreatment with cystine, cystamine, methionine, propyl gallate, SKF 525-A, or 3-amino-1,2,4-triazole, decrease the amount or level of the metabolites and, in some cases, the chronic toxicity. This could result from increased elimination of unchanged benzene in the expired air or production of a lower level of the metabolites for a longer period of time. The observation that pretreatment does not alter the acute toxicity of benzene is not surprising. As an acute toxin, benzene is a central nervous system depressant, and the levels necessary to elicit this effect are many times higher than the levels used to study chronic toxicity.

## MECHANISM OF BENZENE TOXICITY

With some understanding of the metabolic products of benzene, one may be able to examine the possible mechanism of benzene toxicity on the hemopoietic tissues. One proposed mechanism (855) was direct action of the phenolic metabolites on the nucleus and chromosomes. This could result in the arrest of maturation of bone marrow cells or the inhibition of cell division in the erythrocytic system or a combination of both processes. Interference in the maturation of erythrocytes was suggested by the dose-dependent decrease in $^{59}$Fe incorporation into the circulating blood cells in mice given a single subcutaneous injection of benzene. A dose of 88 mg/kg of benzene did not affect $^{59}$Fe uptake, while a dose of 440 mg/kg caused a 27% decrease (515). Subsequent studies found that benzene selectively damaged pronormoblasts and normoblasts without affecting stem cells or reticulocytes (514). Evidence of cell division inhibition by benzene was found in experiments showing mitotic rate reduction (520), production of chromosomal aberrations (537) and inhibition of **DNA** and **RNA** synthesis in hemopoietic cells of rats (506) and rabbits (503, 504). Following the identification of the various metabolites of benzene, it was suggested that the phenolic metabolites might be responsible for benzene toxicity. The rationale was that young rats, which metabolize benzene at a higher rate than old rats, are more susceptible to the effect of benzene. On the other hand, inhibition of benzene metabolism by 3-amino-1,2,4-triazole (344, *529)* or competitive inhibition by toluene (888) reduced benzene toxicity.

Hydroxylated metabolites of benzene were more toxic than benzene, while the sulfate conjugates were less toxic (345). Benzene had little effect on bone marrow cultures of rats, guinea pigs, or rabbits, while the phenols were highly cytotoxic (408). Phenolic intermediates, thus, are suggested as the primary agents causing blood dyscrasias.

Further *in vitro* studies showed that phenol, catechol, and hydroquinol were indeed mitotic toxins (915). In the studies of Nomiyama (346) discussed previously, where benzene metabolites were administered to rats at dose levels similar to those observed in metabolic studies, it was suggested that the formation of catechol via benzene oxide and benzene glycol was the probable cause of hemopoietic disturbance in chronic benzene poisoning.

Another postulated mechanism for the inhibition of cell maturation and division involves the detoxication of benzene via sulfoconjugation. This may lead to depletion of glutathione in bone marrow and subsequently interfere with redox reactions leading to bone marrow depression (855). Following exposure to benzene vapors, a rapid and marked decrease in the percentage of inorganic sulfates (with a corresponding increase in ethereal sulfate) was observed in dogs (373). This suggested that the inorganic sulfates, which are usually derived from sulfur amino acids, were utilized in the conjugation of the phenolic benzene metabolites.

Ethereal sulfate and glucuronide levels in livers of benzene-treated rats and rabbits were determined (333). It was observed that there was a definite correlation between benzene toxicity and the rate of ethereal sulfate formation, but not the rate of glucuronide formation.

So far none of the aforementioned experimental results provide unequivocal support for either mechanism, direct action by phenolic metabolites on chromosomes or an indirect effect due to depletion of sulfate.

## BENZENE METABOLISM IN **HUMANS**

Metabolic studies in humans have dealt almost exclusively with measurements of the rate of elimination of unchanged benzene in expired air (292, 383, 384, 387, 388, 801) and the rate of phenol elimination in the urine (288, 292, 309, 383, 388, 392). The level of benzene in the expired air rapidly drops off, although traces can be detected as much as 24 hours after the exposure (382). Phenol elimination in the urine reaches a maximum approximately 2 hours after the exposure (382). It is found conjugated as phenylsulfate almost exclusively until the concentration of phenol in the urine reaches 400 mg/liter, at which point phenylglucuronide is also observed (291). This demonstrates that sulfonation is the primary route of conjugation and that the glucuronide is formed only when this system becomes saturated.

Teisinger et al. reported finding small amounts of catechol and hydroquinone in the urine of subjects exposed for 5 hours to 100 ppm of benzene (390). In this study, an average of 46% of the benzene was retained. Of the retained benzene, 26% was subsequently eliminated unchanged in the expired air, 61% was eliminated as phenol, 6.3% as catechol, and 2.4% as hydroquinone in the urine. The balance could easily have been eliminated in the feces or as carbon dioxide, as observed in animal studies.

From these observations, it appears that benzene metabolism in humans follows pathways similar to those observed in animals.

## BENZENE METABOLISM IN BACTERIAL SYSTEMS

Additional substantiation for the diol intermediate in benzene metabolism has been shown through studies of bacterial systems. Bacteria in culture can oxidize benzene and catechol at the same rate, but consume phenol at a much lower rate (401, 403). Furthermore, *Pseudomonus aeruginasa* produces catechol when grown on benzene, but cannot produce catechol from phenol (402). Also, when a *Pseudomonus putidu* strain was grown on toluene, it could rapidly oxidize benzene and ethylbenzene. Washed cell suspensions of these bacteria oxidized benzene, catechol, and *cis*-DDB at equal rates. However, phenol and *trans*-DDB were oxidized at much lower rates. Finally, when a variant strain of *P. putida* was grown on

glucose and benzene, cis-DDB was isolated (397). These results, while not necessarily identical to the observations made for animals and animal cell culture systems, strongly support arguments for the dihydroxydiol intermediate in the metabolism of benzene to various phenols.

## SUMMARY

The metabolism and elimination of benzene in humans and all animal species studied appears to follow very similar pathways. It must be noted, however, that the distribution of benzene metabolites in humans has not been thoroughly investigated.

The major differences appear to arise not in the metabolism, but in the conjugation of the final metabolites. Some species, such as the pig, show a preference for glucuronide formation, and others, such as humans and dogs, show a preference for sulfate formation. With humans and dogs there is evidence that sulfonation is the predominant form of conjugation when exposure levels are low, and glucuronide formation is observed only when the sulfonation route is heavily utilized. Evidence that this may be related to chronic toxicity can be inferred from studies with rats. As the rat matures, the levels of sulfonating enzymes in the liver increase. This is accompanied by an increased resistance to benzene poisoning. It should be mentioned, however, that this area has received little attention and subsequent definitive studies are needed.

It has been observed that tissue distribution patterns may vary depending on the route of administration. This has also been noted with many other materials, and is associated with the circulatory sequence from the point of administration to the metabolic target organ, which in the case of benzene is the liver. This must be considered when one attempts to compare data from different experiments or to extrapolate from animal studies to humans.

Finally, chronic benzene toxicity appears to be related to the levels and amounts of benzene metabolites present in the animal. Treatments that increase the rate of benzene metabolism, such as phenobarbital pretreatment, also increase the rate of elimination of the metabolites, decreasing the total exposure and the resulting toxicity. Also, treatments that decrease the rate of metabolism, such as partial hepatectomy or pretreatment with cystine, cystamine, methionine, propyl gallate, SKF 525-A, or 3-amino-1,2,4-triazole, decrease the amount or level of the metabolites and, in some cases, the chronic toxicity. This could result from increased elimination of unchanged benzene in the expired air or production of a lower level of the metabolites for a longer period of time. The observation that pretreatment does not alter the acute toxicity of benzene is not surprising. As an acute toxin, benzene is a central nervous system depressant, and the levels necessary to elicit this effect are many times higher than the levels used to study chronic toxicity.