opsy No. 2 consistently had higher amounts of radioactivity and was the source of more wild-type clones than biopsy No. 1. Although we find that both methods easily distinguish mutant from wild-type cells, they are not refined enough to permit us to interpret the range of values for HGPRT activity.

If random inactivation of loci on the X chromosome in somatic cells of the female has occurred early in development, one would expect to demonstrate two distinct cell phenotypes for X-linked loci in the heterozygote (*5*). Davidson *et al.* (*8*) and DeMars and Nance (*9*) showed two populations of cells in clones from heterozygotes having glucose-6-phosphate dehydrogenases with different electrophoretic mobilities. Danes and Bearn have shown that fibroblasts cloned from females heterozygous for the X-linked recessive form of Hurler's syndrome also fall into two populations, those with increased cellular uronic acid and metachromasia and those with normal uronic acid and tinctorial response (*10*). The HGPRT locus is thus the third for which clones of skin fibroblasts from a female heterozygote show two distinct cell phenotypes.

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Dimethyl Sulfoxide: Interactions with Aromatic Hydrocarbons

Abstract. *Dimethyl sulfoxide (DMSO) enhanced the hypertaurinuria produced by benzene, chlorobenzene, and toluene in rats. Undiluted DMSO was more effective than DMSO diluted with water in potentiating the toxicity of benzene in both rats and mice. Supernatants (9000g) prepared from livers of rats treated with DMSO 24 hours earlier metabolized more benzene than those from control rats.*

The introduction of dimethyl sulfoxide (DMSO) into clinical medicine was accompanied by reports emphasizing its relatively low toxicity and its apparent lack of interaction with a variety of drugs (*1*). Later studies have shown, however, that parenterally administered DMSO may have a greater potential for producing systemic effects than originally suspected (*2*). Of interest is the recent observation that DMSO increased the hepatotoxicity and the lethal effects of carbon tetrachloride (*3*). The studies reported here show that DMSO markedly potentiated the toxicity of several aromatic hydrocarbons and that liver preparations from DMSO-treated rats show an increased capacity to metabolize benzene. These observations suggest that caution is necessary in considering DMSO as simply an inert solvent unlikely to interact with other agents or to produce toxic effects itself.

Although taurine is primarily an intracellular compound, significant quantities are normally found in urine (*4*). Since increased levels of urinary taurine are seen after whole body x-irradiation, toxic doses of colchicine (*5*), or carbon tetrachloride (*6*), hypertaurinuria can be considered an early sign of toxicity. In the experiments reported here taurine excretion was not increased in controls treated with either saline or DMSO alone. After the injection of benzene, chlorobenzene, or toluene without dilution into the peritoneal cavity of rats, urinary taurine excretion increased markedly over the levels seen during the preceding 24-hour control periods (Table 1); however, these doses of the aromatic hydrocarbons produced no deaths. When DMSO, either undiluted or as a 25 percent solution, was injected shortly before the aromatic hydrocarbons, taurine excretion was significantly greater in these rats than in control animals given the aromatic hydrocarbons without DMSO. Furthermore, in rats pretreated with DMSO these doses of aromatic hydrocarbons did produce deaths occasionally, especially when DMSO was given without dilution immediately before the aromatic hydrocarbon.

To study the effect of diluting DMSO on the toxicity of aromatic hydrocarbons, DMSO (5 ml/kg) was given intraperitoneally to rats, either without dilution or as a 25 percent (by volume) solution in water, immediately before benzene (1 ml/kg) was administered by the same route. Mortality was compared with that observed in controls pretreated with saline. Pretreatment with undiluted DMSO produced 100 percent mortality following benzene administration to eight rats, whereas the same dose of DMSO given as a 25 percent solution produced only 11 percent mortality in 18 rats. This dose of benzene (1 ml/kg) did not kill any of 20 rats pretreated with saline, nor does this dose of DMSO (5 ml/kg) given alone produce any deaths.

The observation that closely spaced but separate injections of undiluted DMSO and benzene produced 100 percent mortality led us to explore the toxicity of benzene dissolved in undiluted DMSO. In mice the LD_{50} of benzene administered intraperitoneally with-

Table 1. Enhancement by DMSO of the urinary taurine excretion produced by aromatic hydrocarbons in rats (Wistar strain). Taurine was determined by a method previously described (*14*). Increase in the amount of urinary taurine excreted in the second 24-hour period over the control 24-hour period is expressed as micromoles of taurine excreted per rat, each rat serving as its own control. Taurine excretions in the control 24-hour periods averaged 44.1 micromoles per rat. All injections were given intraperitoneally.

Pretreatment	Treatment	No. of rats	Increase in urinary taurine excreted in 24-hour period after treatment (mean ± S.D.)
Control	Benzene, 0.5 ml/kg	11	37.7 ± 28.3
DMSO	Benzene, 0.5 ml/kg	3	93.7 ± 28.5*
Control	Chlorobenzene, 0.3 ml/kg	3	67.6 ± 4.0
DMSO	Chlorobenzene, 0.3 ml/kg	4	121.9 ± 30.8*
Control	Toluene, 0.5 ml/kg	2	36.9 ± 26.8
DMSO	Toluene, 0.5 ml/kg	2	157.7 ± 9.7*

* Significantly greater than control excretion at 0.03 level of probability.

Table 2. Stimulation of benzene metabolism in 9000*g* supernatant fluid of 33 percent liver homogenates from rats treated with DMSO. Male rats (Wistar strain), weighing about 200 g, received DMSO (5.45 g/kg) as a single oral, intraperitoneal, or subcutaneous dose or were given two percutaneous doses daily for 7 days. With the exception of those animals treated percutaneously the rats were not fed from the time of injection until they were killed. The animals were killed 24 hours after the last dose and ^{14}C-benzene metabolism was studied as previously described (*9*). Differences in phenol and conjugate levels between control and DMSO-treated groups were significant at the 0.05 level of probability or better (except for the intraperitioneal route).

Treatment	No. of rats	Free phenol (mμmole/liver; mean ± S.D.)	Conjugates (mμmole liver; mean ± S.D.)
		Oral dose	
Control	6	983 ± 414	1080 ± 243
DMSO	6	2282 ± 748	2283 ± 751
		Intraperitoneal dose	
Control	6	790 ± 396	965 ± 660
DMSO	6	1703 ± 655	1524 ± 751
		Subcutaneous dose	
Control	12	1046 ± 607	893 ± 614
DMSO	12	2305 ± 1817	1425 ± 1034
		Percutaneous dose	
Control	8	697 ± 367	807 ± 548
DMSO	8	2838 ± 1984	3369 ± 2519

out a vehicle was 0.34 ml/kg (95 percent confidence limits, 0.28 to 0.42 ml/kg), whereas the LD_{50} of benzene dissolved in DMSO was 0.092 ml/kg (95 percent confidence limits, 0.081 to 0.104 ml/kg). By the Litchfield-Wilcoxon method the slope ratio was 1.49 with 95 percent confidence limits of 1.19 to 1.86. This significant departure from parallelism indicates that benzene dissolved in DMSO kills mice by a different mechanism than benzene injected without a vehicle. Although under these conditions no single potency ratio applies over the whole range of responses, a comparison of the LD_{16}, LD_{50}, and LD_{84} values shows that benzene dissolved in DMSO is respectively 2.5, 3.7, and 5.5 times as potent as benzene injected alone.

The toxic effects of benzene poisoning have often been attributed to the formation of free phenol (*7*). Since phenol is known to be the major metabolic product formed from benzene both in vivo (*8*) and in vitro (*9*), we determined whether the 9000*g* supernatants prepared from the livers of rats treated with DMSO 24 hours earlier converted more ^{14}C-benzene to phenol and its metabolites than those prepared from control rats. In the presence of the enzymes and the substrates found in the 9000*g* supernatants of rat liver homogenates approximately half the total phenol formed is in the free form and the remainder is conjugated (*9*). The data in Table 2 show that benzene metabolism in liver supernatant preparations from rats treated with DMSO was about twice that seen in control preparations regardless of whether the DMSO was given orally, intraperitoneally, or subcutaneously. After 7 days of percutaneous application of DMSO, benzene metabolism increased to about four times control levels. Neither 1 day nor 7 days of treatment with DMSO increased the liver weights significantly. The data are expressed as millimicromoles of metabolites per total liver. Increases in the phenol and conjugate fractions were approximately equal and proportional to the overall increase in metabolism in each case. The addition of DMSO in vitro did not stimulate benzene metabolism.

Although we do not know precisely how DMSO potentiated the lethal effects of benzene in these experiments, hepatotoxicity and local damage to the organs in the peritoneal cavity may have contributed to the lethal outcome. Recent evidence indicates that acute administration of either benzene or DMSO can produce morphological changes in the liver. Wirtschafter and Cronyn (*10*) reported that benzene given subcutaneously elevated serum transaminases and produced signs of cytoplasmic degeneration in the liver, while Shilkin *et al.* (*2*) found that undiluted DMSO given intravenously led to severe ultrastructural derangements in hepatocytes within 12 minutes after its administration. In the studies reported here direct access to the liver through intraperitoneal injection seems to be necessary for DMSO to potentiate benzene toxicity, since subcutaneous administration of much higher doses of benzene (10 ml/kg) dissovlved in DMSO (5 ml/kg) were no more toxic than benzene alone, and intravenous administration of benzene (0.300 ml/kg) dissolved in DMSO in a dose that was more than twice the intraperitoneal LD_{99} was found to be completely nonlethal. Undiluted DMSO introduced into the peritoneal cavity probably produced local damage through exothermic reaction of DMSO with water and local toxic actions of DMSO. The local toxicity of DMSO is known to be reduced by diluting it with water prior to administration (*11*). It was noted in the present studies that diluted DMSO (25 percent) did not potentiate benzene toxicity, although it did enhance taurine excretion induced by the aromatic hydrocarbons. Hypertaurinuria seen after the administration of benzene and benzene-DMSO may also have been caused by hepatotoxicity and local damage to abdominal organs.

Another effect of DMSO on the liver was seen in the stimulation of benzene metabolism by liver preparations taken from rats treated with DMSO 24 hours previously. DMSO also stimulated the aromatic hydroxylation of zoxazolamine but not the alicyclic hydroxylation of hexobarbital (*12*). Although phenobarbital increases both aromatic and alicyclic hydroxylation, DMSO seems to be like 3,4-benzpyrene in stimulating only the aromatic variety. Alvares *et al.* (*13*) recently reported that 3,4-benzpyrene induces a cytochrome P-448–containing enzyme different from the cytochrome P-450–containing enzyme induced by phenobarbital. It remains to be demonstrated whether DMSO also stimulates the production of a cytochrome P-448–containing enzyme.

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