

# Studies on the Mechanism of Benzene Toxicity

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Using the  $^{59}\text{Fe}$  uptake method of Lee et al. it was shown that erythropoiesis in female mice was inhibited following IP administration of benzene, hydroquinone, *p*-benzoquinone, and muconaldehyde. Toluene protected against the effects of benzene. Coadministration of phenol plus either hydroquinone or catechol resulted in greatly increased toxicity. The combination of metabolites most effective in reducing iron uptake was hydroquinone plus muconaldehyde. We have also shown that treating animals with benzene leads to the formation of adducts of bone marrow DNA as measured by the  $^{32}\text{P}$ -postlabeling technique.

## Introduction

Previous studies from this laboratory have used the incorporation of radioactive iron into red cell hemoglobin in male mice as a measure of benzene toxicity (1-3). Since benzene inhibited neither hemoglobin synthesis nor the maturation of reticulocytes to erythrocytes, the extent of  $^{59}\text{Fe}$  uptake was used as a measure of proliferation of bone marrow erythroid cells. Using this technique, we demonstrated that the ability of benzene to retard bone marrow function could be manifested as a decrease in  $^{59}\text{Fe}$  uptake.

Recent studies by Eastmond et al. (4) showed that treating animals with combinations of phenol and hydroquinone, which are two metabolites of benzene, produced decreases in bone marrow cellularity similar to those produced by benzene. We decided to investigate the effects of benzene metabolites, both singly and in combination, on  $^{59}\text{Fe}$  uptake in mice, to determine whether similar effects were observed using this indication of marrow function and to extend the range of compounds studied. Thus, we also studied effects of toluene, catechol, muconaldehyde, and *p*-benzoquinone.

## Methods

$^{59}\text{Fe}$  uptake was measured using a modification of the method of Lee et al. (1,2). Female Swiss albino mice (25-30 g) were placed on a treatment schedule that in-

involved three treatments with prospective bone marrow depressants on Monday at 5 P.M. and Tuesday at 9 A.M. and 5 P.M. The mice were given 1  $\mu\text{C}$  of  $^{59}\text{Fe}$  ( $^{59}\text{FeCl}_3$ , Amersham) IV at 9 A.M. on Thursday, and the blood was sampled for counting at 9 A.M. on Friday. Calculations were based on the assumption that the blood volume equals 6% of the body weight. DNA adducts were detected using the  $^{32}\text{P}$ -postlabeling technique of Randerath and co-workers (5-7) and the HPLC technique of Haseltine (8) for the separation of the adducts.

## Results and Discussion

Transit time between the progenitor cells (pronormoblast, normoblast, reticulocyte and erythrocyte) is 24 hr for each stage in the mouse. We had previously reported that the pronormoblast was the most susceptible form to benzene, and the normoblast was also susceptible, but under these experimental conditions no effects were observed on the reticulocyte or the erythropoietin responsive stem cells (1-3). By focusing treatment around a point 48 hr prior to administering the iron, we could concentrate on the effects of the benzene metabolites on the pronormoblast.

In these studies, unlike those of Lee et al. (1-3), female mice were used and all treatments were given IP, several controls were required. Benzene was given IP 24 hr prior to iron and no effect was observed on  $^{59}\text{Fe}$  uptake, confirming that benzene inhibited neither hemoglobin synthesis nor the maturation of the reticulocytes (data not shown). However, Figure 1 shows that when given 48 hr prior to measuring  $^{59}\text{Fe}$  uptake, benzene produced a dose-dependent decrease in erythroid cells. As shown previously in male mice (3), toluene, which is a competi-

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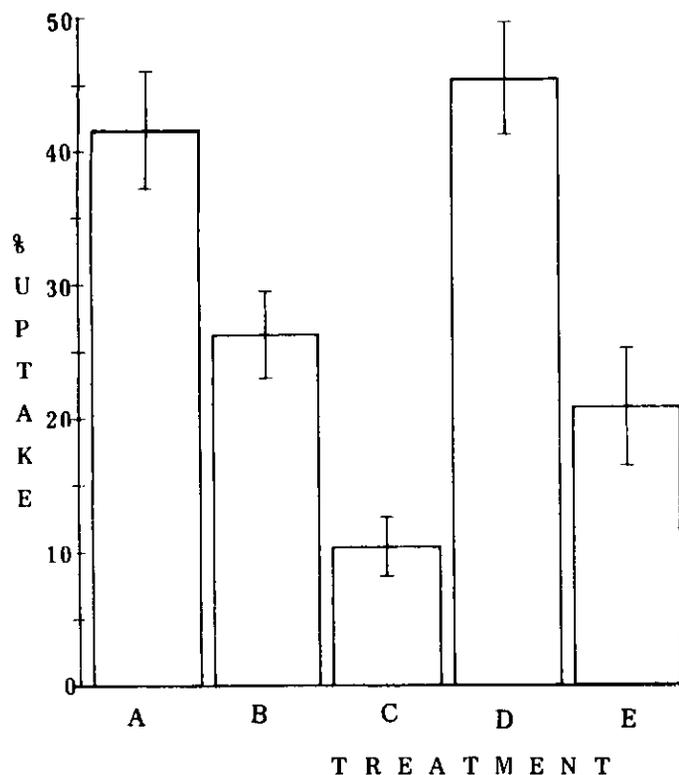


FIGURE 1. Effect of benzene on  $^{59}\text{Fe}$  uptake into erythrocyte hemoglobin in female mice and its alteration by toluene. The ordinate represents the percent of the administered dose of  $^{59}\text{Fe}$  found in circulating red cells 24 hr after IV administration. Column A,  $^{59}\text{Fe}$  uptake in control animals given corn oil; columns B and C, effects of benzene given at 150 and 300 mg/kg, toluene coadministered with 150 mg/kg, respectively, column D, effect of 300 mg/kg toluene coadministered with 150 mg/kg of benzene, and column E, effect of 600 mg/kg toluene coadministered with 300 mg/kg benzene.

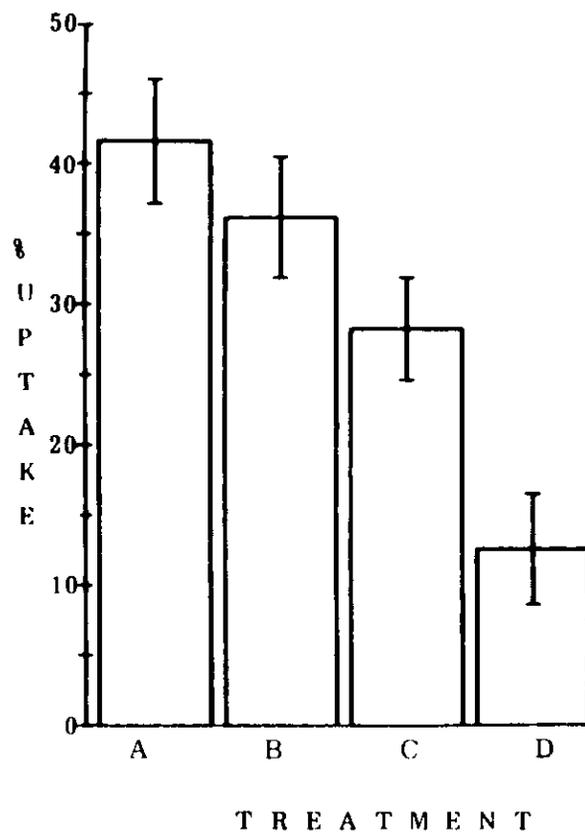


FIGURE 2. Effects of hydroquinone on  $^{59}\text{Fe}$  uptake into erythrocyte hemoglobin in female mice. Column A, animals given normal saline; columns B, C, and D, animals given hydroquinone at 50, 75, and 100 mg/kg IP, respectively.

tive inhibitor of benzene metabolism, protected against benzene toxicity. Phenol given alone had little or no effect on  $^{59}\text{Fe}$  uptake.

Figure 2 shows that hydroquinone also inhibited  $^{59}\text{Fe}$  uptake. Figures 3A and 3B show that phenol potentiates the reduction in  $^{59}\text{Fe}$  uptake produced by hydroquinone. Catechol, given alone, had little or no effect on  $^{59}\text{Fe}$  uptake. Figure 4 shows that when given with phenol,  $^{59}\text{Fe}$  uptake was decreased. The former confirms the results of Eastmond et al. (4), and the latter suggests that this is a general phenomenon regarding the effect of phenol on dihydroxybenzenes.

It is suggested that this effect results from the free radical oxidation of phenol to phenoxy radical, which in turn oxidizes hydroquinone in two steps to form *p*-benzoquinone, which may be closer to the ultimate toxic agent than hydroquinone. Figure 5 shows the effect of *p*-benzoquinone on  $^{59}\text{Fe}$  uptake. The effect is achieved at doses in the range of 1 to 5% of the doses of hydroquinone. It is important to note that toluene was not effective in protecting against hydroquinone or *p*-benzoquinone, suggesting that cytochrome P-450 was not involved in these steps.

Muconaldehyde, a ring-opened product of microsomal

benzene metabolism, was also effective in reducing  $^{59}\text{Fe}$  uptake in the low milligram dose range (Fig. 6). The most striking observation, however, was that when given in combination with hydroquinone, at doses that did not reduce  $^{59}\text{Fe}$  uptake when given alone, i.e., 1 mg/kg of muconaldehyde and 50 mg/kg of hydroquinone, a dramatic decrease in  $^{59}\text{Fe}$  uptake was observed, and complete cessation was observed at 2 mg/kg of muconaldehyde and 50 mg/kg hydroquinone (Fig. 7).

These observations suggest that exceedingly low doses of benzoquinone and muconaldehyde are effective in reducing bone marrow function. They also indicate that it is likely that benzene toxicity is the result of an interactive effect of benzene metabolites. Their effects may occur at a variety of targets in the cell.

To determine the effect of benzene on DNA as a target, the  $^{32}\text{P}$ -postlabeling method (8) was applied to bone marrow DNA isolated from benzene-treated rats (1 mL/kg). The separation of the postlabeled nucleotides is shown in Figure 8. The single peak following the deoxyadenosine monophosphate standard is more hydrophobic than any of the normal nucleotides, hence, the longer retention time. This suggests that it is larger and bulkier, with perhaps additional rings.

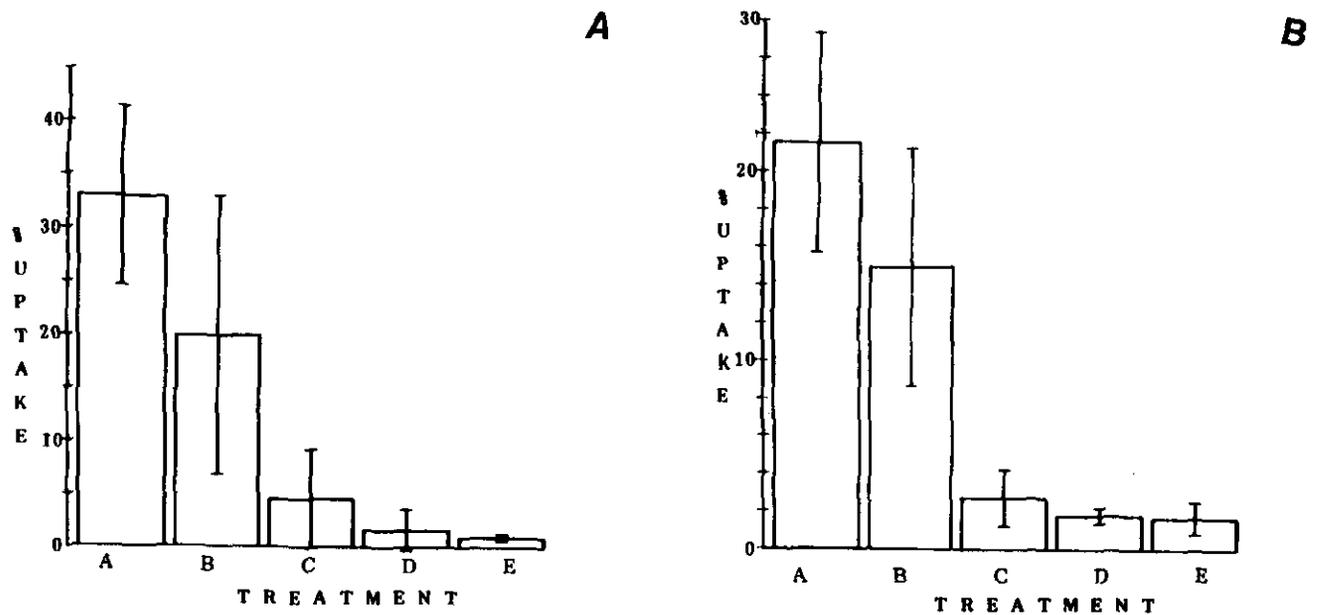


FIGURE 3. Interactive effect of hydroquinone and phenol on  $^{59}\text{Fe}$  uptake into erythrocyte hemoglobin in female mice in which (A) the dose of hydroquinone was varied and the dose of phenol held constant, (B) the dose of phenol was varied and the dose of hydroquinone was held constant. Column A, the saline controls; columns B, C, D, and E, results from animals given (A) hydroquinone or (B) phenol at 25, 50, 75, and 100 mg/kg, respectively, plus in each case 50 mg/kg of (A) phenol or (B) hydroquinone, IP.

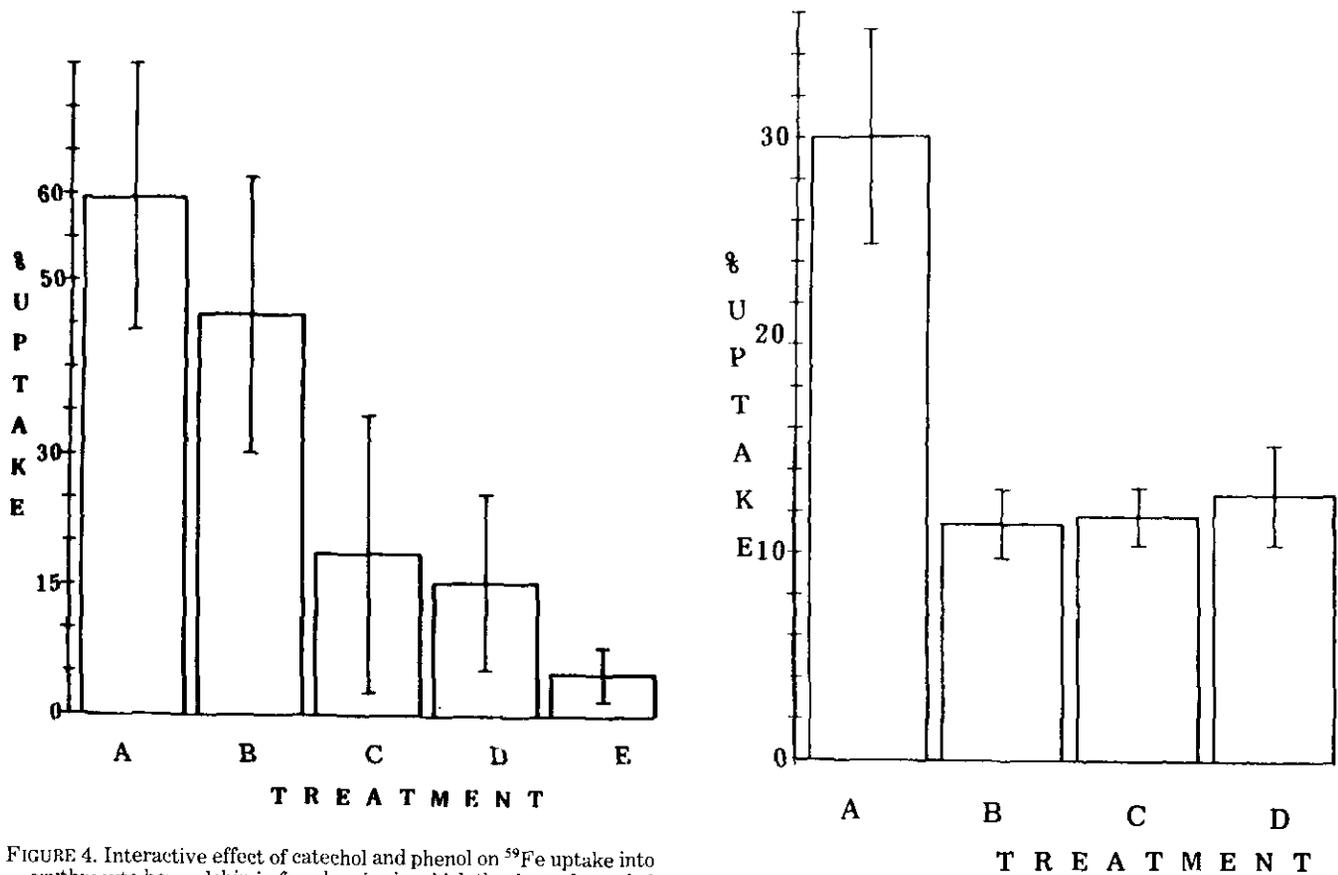


FIGURE 4. Interactive effect of catechol and phenol on  $^{59}\text{Fe}$  uptake into erythrocyte hemoglobin in female mice in which the dose of catechol was varied and the dose of phenol was held constant. Column A, saline controls; columns B, C, D, E, animals given catechol at 25, 50, 75, and 100 mg/kg, respectively, plus in each case 50 mg/kg of phenol, IP.

FIGURE 5. Effect of *p*-benzoquinone on  $^{59}\text{Fe}$  uptake into erythrocyte hemoglobin in female mice. Column A, animals given normal saline; columns B, C, D, effects of *p*-benzoquinone given at doses of 1, 2, and 4 mg/kg, IP, respectively.

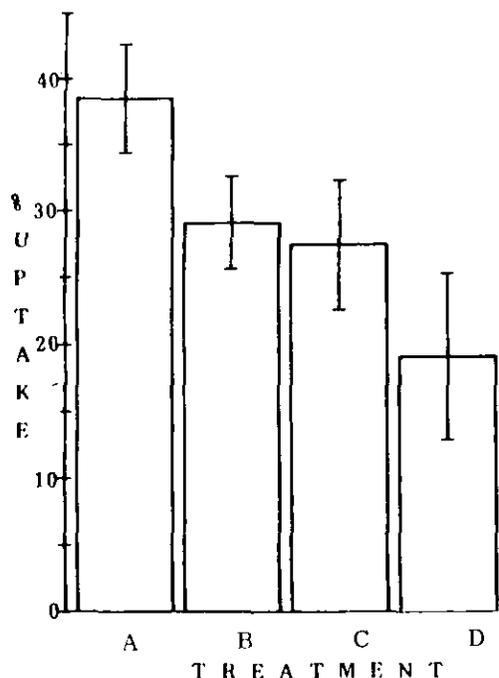


FIGURE 6. Effect of muconaldehyde on  $^{59}\text{Fe}$  uptake into erythrocyte hemoglobin in female mice. Column A, animals given normal saline; columns B, C, D, effects of muconaldehyde given at doses of 1, 2, and 4 mg/kg, IP, respectively.

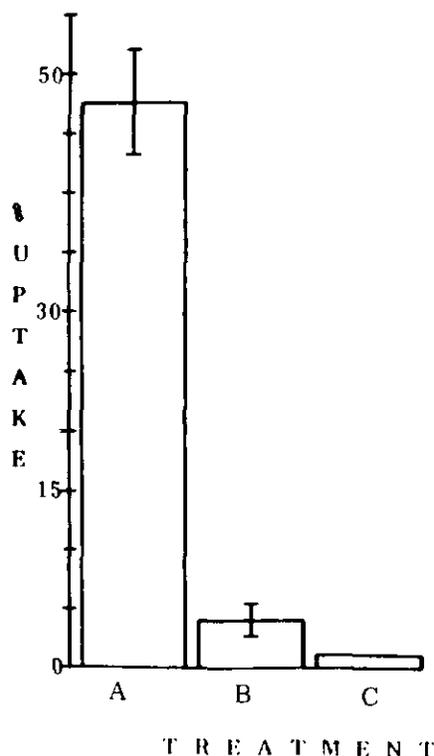


FIGURE 7. Interactive effect of muconaldehyde and hydroquinone on  $^{59}\text{Fe}$  uptake into erythrocyte hemoglobin in female mice. Column A, saline controls; column B, animals given muconaldehyde at 1 mg/kg, and hydroquinone at 50 mg/kg; column C, animals given muconaldehyde at 2 mg/kg and hydroquinone at 50 mg/kg, IP.

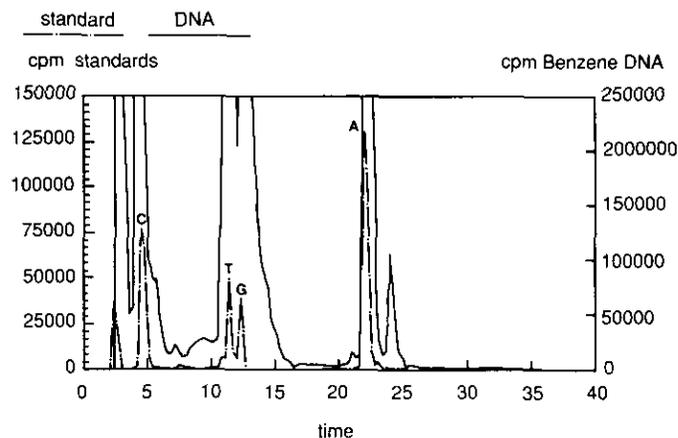


FIGURE 8. HPLC of bone marrow DNA from rats treated with benzene, 1 mL/kg, once daily, for 4 days. The DNA was hydrolyzed and  $^{32}\text{P}$ -postlabeled prior to chromatography. A, AMP, C, CMP, G, GMP, T, TMP.

The indication that *p*-benzoquinone is a more potent suppressor of  $^{59}\text{Fe}$  uptake is consistent with the fact that *p*-benzoquinone can exist as an electrophilic resonance form, which could directly react with the critical biomolecules such as DNA, RNA, and enzymic protein. The peak in evidence in Figure 11 indicates that covalent binding to DNA occurs *in vivo*. The indication that other targets are involved is the suggestion by Irons and Neptun (9,10) that tubulin may be a significant target for benzene metabolites as well as the report by Schwartz et al. (11) pinpointing DNA polymerase as a site at which hydroquinone may react. Furthermore, Rushmore et al. (12) showed that DNA was an important target for benzene metabolites in mitochondria *in vitro*. The combined impact of benzene metabolites on these key cellular sites may be the ultimate cause of failure of bone marrow cells to proliferate under the influence of benzene.

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