Hydroquinone and Catechol Reduce the Frequency of Progenitor B Lymphocytes in Mouse Spleen and Bone Marrow

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Abstract: Hydroquinone and catechol are two metabolites of benzene that are potential inducers of hematotoxicity. We investigated the in uiuo toxicity of these metabolites toward the deuelopment of polyclonal. plaque-forming cells (PC-PFC) from progenitor B lymphocytes. Dextran sulfate (DxS), lipopolysaccharide (LPS), or the two mitogens combined (DxS + LPS) were used to induce proliferation and maturation of these progenitors to PC-PFC. Groups of 4 C57BL/6 mice were exposed to 2 daily doses. either intravenously or intraperitoneally. of hydroquinone (100 mg/kg) or catechol (75mg/kg) for 3 consecutiue days. Spleen and marrow cells were harvested for culture 1 day later. The results demonstrated that both metabolites were cytotoxic to spleen cells. Hydroquinone (100 mg/kg) also reduced marrow cellularity, whereas catechol (75mg/kg) did not significantly affect marrow cellularity. Each compound reduced the frequency of PC-PFC developed from the spleens and marrows of treated mice, but only catechol selectively inhibited the maturation of LPS-activated marrow progenitors into end-stage PC-PFC. These experiments demonstrate the immunotoxic potential of hydroquinone and catechol in uiuo through the reduction of progenitor B lymphocytes and suggest that inhibition of precursor cell maturation may play a significant role in the hematotoxicity observed after chronic exposure to benzene.

Key Words: Hydroquinone: Catechol: Progenitor cells: B lymphocytes: Proliferation; Maturation; Spleen: Bone marrow

INTRODUCTION

Exposure to benzene, a compound widely used as a solvent and as an intermediate in the production of a variety of chemicals. can result in multiple clinical disorders in man and animals that are related to the general hematotoxicity of benzene. These disorders include leukopenia, lymphocytopenia. erythrocyte macrocytosis. reduced serum immunoglobulin. acute myelogenous leukemia. lymphoma. and aplastic anemia (Tabershaw and Cooper, 1974 Cohen et al., 1978). The development of the latter disorder, aplastic anemia. is of primary toxicological concern in light of the preferential distribution of benzene (Schrenk et al., 1941) and some benzene metabolites to the bone marrow (Rusch et al., 1977; Greenlee et al., 1981). Benzene

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itself is not considered the ultimate toxicant but is transformed in vivo into metabolites that react with cellular macromolecules and thereby interfere with normal cellular functions (Snyder and Kocsis. 1975). The most likely cellular functions affected are the normal proliferation and maturation of bone marrow precursor cells (Lee et al.. 1974 Snyder et al., 1977; Uyeki et al., 1977).

Among the many metabolites of benzene. hydroquinone and catechol have received increased scrutiny due to a number of observations: First. hydroquinone and catechol localize and covalently bind in rat bone marrow and lymphoid tissue (Greenlee et al.. 1981); second. decreases in hydroquinine concentrations in vivo, due to increased hepatic metabolism of benzene to less toxic conjugates, produces a concomitant reduction in leukopenia (Greenlee et al., 1980); and third. both of these metabolites are known to inhibit rat lymphocyte function (Irons et al.. 1981a.b) and induce sister chromatid exchanges in human lymphocytes (Morimoto and Wolff. 1980) in vitro.

As mentioned previously. catechol and hydroquinone may damage various hemopoietic progenitor cells. Only limited evidence. however. links hydroquinone and catechol directly to in vivo progenitor cell toxicity. Bolcsak and Nerland (1980) demonstrated suppression of erythropoiesis in mice given injections of phenol. catechol. or hydroquinone. which corroborates the study by Lee et al. (1974). showing suppressed erythropoiesis after acute benzene exposure. In contrast. Mitchell (1971) observed no hematotoxicity in rats given 0.25–0.75 mg/kg subcutaneously of either catechol, phenol. or benzoquinone daily. for 1 week. In a similar experiment. Nomiyama (1965) observed significant leukopenia only with catechol (30 mg/kg) and no toxicity with hydroquinone (50 mg/kg) administered subcutaneously. Recently. Chavin et al. (1979) observed that subcutaneous injections of hydroquinone could suppress the development of experimentally induced melanoma in mice. Together these studies suggest that hydroquinone or catechol potentially interfere with precursor cell function in vivo.

In the present study. we investigated the in vivo toxicity of hydroquinone and catechol toward mouse progenitor B lymphocytes. Progenitor cells from both the spleen and bone marrow (femur) compartments were assessed for their ability to develop into mature. polyclonal plaque-forming cells (PC-PFC). The bone marrow was of interest because it is the target organ in benzene toxicity and also is the site of rapid generation of small, progenitor B lymphocytes (Osmond and Nossal. 1974). The spleen is an important peripheral lymphoid organ that serves as a reservoir for migrating progenitor B lymphocytes from the bone marrow and also provides an environment for further differentiation of these cells into end-stage B lymphocytes (Brahim and Osmond. 1970; Melchers et al.. 1975). The rapid generation and maturation of progenitor B cells (Osmond. 1979) renders them highly susceptible to toxic agents that affect dividing cells and these characteristics provide a model for examining in vivo drug effects on cell function (Pazdernik and Corbett. 1979; Wierda and Pazdernik. 1979a.b). Previous studies (Gronowicz and Coutinho. 1974; Melchers and Andersson. 1974; Andersson et al.. 1979 Phillips and Melcher. 1979) have shown that progenitor B lymphocytes can be stimulated to proliferate and develop in vitro into PC-PFC after exposure to mitogenic concentrations of lipopolysaccharide (LPS). Other investigators have discovered that another mitogen. dextran sulfate (DxS) can selectively activate progenitor B lymphocytes that are ontogenetically more immature than LPS-responsive B cells (Gronowicz and Coutinho. 1974 Gronowicz et al.. 1976 Ulla et al.. 1977; Pazdernik and Nishimura, 1978). Both of these mitogens have been utilized in this study to examine the in vivo effects of hydroquinone and catechol on proliferation and maturation of

Abbreviations. Con A: **concanavalin A**; DxS: **dextran sulfate**; ^{3}H-TdR: **tritiated thymidine**; LPS: **lipopolysaccharide**; **PC-PFC: polyclonal plaque-forming cell**; **PFC: plaque-forming cell**; **TNP-SRBC: trinitrophenylated sheep red blood cells**: **iv: intravenously**; **ip: intraperitoneally**; CNS: **central nervous system**; Ig: **immunoglobulin**; ANOVA: **analysis of variance**; **dpm: disintegrations per minute**

progenitor lymphocytes from mouse bone marrow and spleen. These studies revealed that both hydroquinone and catechol were cytotoxic toward progenitor B lymphocytes and that at least one benzene metabolite. catechol. could selectively inhibit the maturation of bone marrow progenitor B lymphocytes into PC-PFC.

METHODS

Animals

Male C57BL/6 CRlBR mice were obtained from the Charles River Breeding Laboratories, Hinston. NY. All animals were 6 weeks old on arrival. The mice were housed four per cage with hardwood bedding and had free access *to* food (Wayne Certified Lab Blox. Allied Mills. Inc.. Chicago. IL) and water A 12 hr light cycle was maintained.

Drug Treatment

Hydroquinone was obtained from Aldrich Chemical Co. (Milwaukee. WI) and catechol was purchased from Sigma (St. Louis. MO). Groups of 4 mice each were injected with the appropriate metabolite either intravenously (iv) or intraperitoneally (ip). twice daily at 7 hr intervals (at 0900 hr and 1600 hr) for 3 consecutive days. This regime was modeled after the study by Hodgson et al. (1975) which showed that a two dose schedule of hydroxyurea injected into mice markedly reduced proliferating cells in bone marrow. Dosing was limited to 3 days since benzene. the parent compound of hydroquinone and catechol. induced significant myelotoxicity in mice when administered in single. daily doses over a 3 day period (Wierda et al., 1980). Doses of hydroquinone (100 mg/kg) or catechol (75 mg/kg) were the maximally tolerated concentrations when given slowly iv; both compounds were central nervous system (CNS) stimulants. Both routes of administration were employed to rule out pharmacokinetic differences. since the lack of toxicity observed in previous studies of these metabolites (Nomiyama. 1965; Mitchell. 1971) may have been in part due to the subcutaneous route of injection.

Organ Cellularity

Mice were killed by cervical dislocation 1 day after the last injection of metabolite and the spleen and left femur from each mouse were excised. Single cell suspensions were teased from spleens in RPMI 1640 medium (Gibco). Marrow cells were obtained by flushing the femurs with 4 ml of medium. The cell suspensions were triturated through Pasteur pipets. placed in sterile test tubes. and debris allowed to sediment by gravity. Nucleated cell counts for individual organs from each mouse were determined by counting the cell suspension (lysed with Zap-oglobin. Coulter. Hialeah, FL) with a Coulter counter. Model ZF,.

Polyclonal Proliferation **Assays**

Spleen cells from 4 individual mice in each group were pooled and cultured for 3 days in flat-bottomed microtiter trays (Linbro) in 0.2 ml of medium (1×10^5 cells/well). The medium for all tissue culture assays in this study consisted of RPMI 1640 medium supplemented with 10% fetal calf serum (Microbiological Associates, Walkersville, MD). 2 mM glutamine. 5×10^{-5} M 2-mercaptoethanol (Sigma). 100 IU/ml penicillin, and 100 μg/ml streptomycin (Gibco). Polyclonal activators. concanavalin A (Con A. 2.5 μg/ml). or *E. coli* lipopolysaccharide 055:B5 (LPS; 10 μg/ml). purchased from Sigma. were added *to* quadruplicate cultures *to* stimulate T and B lymphocytes. respectively

Bone marrow cells were also pooled and cultured for 4 days in 0.2 ml of medium at 4×10^5 cells/well. Dextran sulfate (20 μg/ml; mol wt = 500.000). LPS (10 μg/ml). or a combination of the *two* (DxS + LPS) were used as polyclonal activators of progenitor B lymphocytes. DxS and LPS were purchased from Sigma (St. Louis. MO).

Cultures were pulsed 6 hr prior to harvest with 0.2 μCi ^{3}H-thymidine (^{3}H-TdR, specific activity = **6.7** Ci/mmol; New England Nuclear) to assess the degree of polyclonal proliferation. Nonadherent cells were subsequently harvested with an automated harvestor (Microbiological Associates) onto glass fiber filters. The filters containing radioactive material were counted in ACS scintillation fluid (Amersham. Arlington Heights. IL) using a Packard Tricarb. Model 460 CD. liquid scintillation spectrometer. The culture responses for each group were converted into the response per organ by multiplication of the average culture response by the number of cells per individual organ.

Polyclonal Plaque-forming Cell Assay

Polyclonal antibody secreting cells (PFC), generated from progenitor B cells after stimulation by LPS or LPS + DxS combined. were assayed using a modification of the original method described by Pazdernik and Nishimura (1978). Spleen cells were removed from control and drug-treated mice and cultured, in triplicate, in 1 ml of medium with mitogenic concentrations of LPS (10 μg/ml) for 4 days in 24 well (Costar) tissue culture plates (2×10^5 cells/well). Bone marrow cells (2×10^5 cells/well) were also cultured in 24 well plates with LPS; in addition. DxS (20 μg/ml) was added to separate triplicate cultures that also contained LPS (DxS + LPS). Addition of DxS + LPS to bone marrow cultures has been shown to stimulate small. noncycling progenitor lymphocytes that are neogenically less mature than those cells stimulated by LPS alone (Pazdernik and Nishimura. 1978). Preliminary experiments demonstrated that DxS alone induced **no** PFC formation in either bone marrow or spleen cultures.

Heavily labeled. trinitrophenyl-coupled sheep red blood cells (TNP-SRBC) were prepared according to the procedure of Rittenberg and Pratt (1969). Use of SRBC that are heavily labeled with hapten in a hemolytic plaque assay will detect B cells that are secreting polyclonal immunoglobulin M (IgM) antibodies (Coutinho. 1976; Coutinho et al.. 1977) and some cells secreting IgG antibodies (Pasanen and Makela. 1969). SRBC alone. i.e.. without TNP-hapten labeling. gave a range of background PFC between 10–180 PFC per culture. The total number of PFC per femur or spleen was calculated by multiplying the mean frequency of PFC in culture by the number of cells per individual organ. Statistical significance for all experimental assays were determined at the 5% level of significance using a one-way analysis of variance (ANOVA) and Dunnetts *t* test.

RESULTS

Kinetics of B-Cell Proliferation and Maturation after Mitogen Stimulation

To examine the effects of catechol and hydroquinone on the proliferation and maturation of progenitor B cells. it was first necessary to characterize the responses of bone marrow and spleen progenitor B lymphocytes from normal mice to stimulation by DxS. LPS. and a combination of the two (DxS + LPS). As depicted in Table 1. the rates of proliferation in response to LPS or DxS + LPS were qreater in spleen cell cultures than in marrow cell cultures. with the amount of ^{3}H-TdR accumulated peaking on day 3 in spleen cultures compared with day 4 or 5 for bone marrow cultures. Peak ^{3}H-TdR accumulation in spleen cultures was 4.5 and 2.3 times greater in LPS and DxS + LPS cultures, respectively. than in the comparable bone marrow cell cultures. Addition of DxS alone to cultures of either spleen cells or marrow cells caused no significant increase in ^{3}H-TdR accumulation above background (not shown). When

Table 1 *Kinetics of lymphocyte proliferation in vitro after LPS* or *DxS + LPS activation*

	Mean *dpm/2 x 10^5 cells*[a]		
Organ	*Day*[b]	*LPS (10 μg/ml)*	*LPS + DxS (20 μg/ml)*
Marrow	3	8.190 ± 375	54.885 ± 1.998
Marrow	4	14.074 ± 356	66.765 ± 6.468
Marrow	5	19.257 ± 1.867	46.652 ± 598
Marrow	6	8.751 ± 962	8.761 ± 2.124
Spleen	2	32.780 ± 2.549	70.634 ± 12.001
Spleen	3	92.812 ± 3.752	156.611 ± 998
Spleen	4	39.843 ± 2.820	53.711 ± 12.051

[a] Average amount of ^{3}H-TdR accumulated ± SD during a 6 hr pulse in triplicate cultures.
[b] Days in culture.

the induction of spleen and marrow cell maturation of PC-PFC by mitogens was assessed. we observed that DxS potentiated the number of LPS-induced PC-PFC formed in bone marrow cultures but not in spleen cultures (Table 2). The lack of LPS-induced PC-PFC potentiation by DxS in spleen cultures occurred despite a concomitant increase in ^{3}H-TdR uptake (Table 1). Another observation noted was that peak PC-PFC responses in spleen or marrow cultures (Table 2) always occurred 1 day after peak proliferation (Table 1).

Effect of Hydroquinone and Catechol Administration on Cellularity

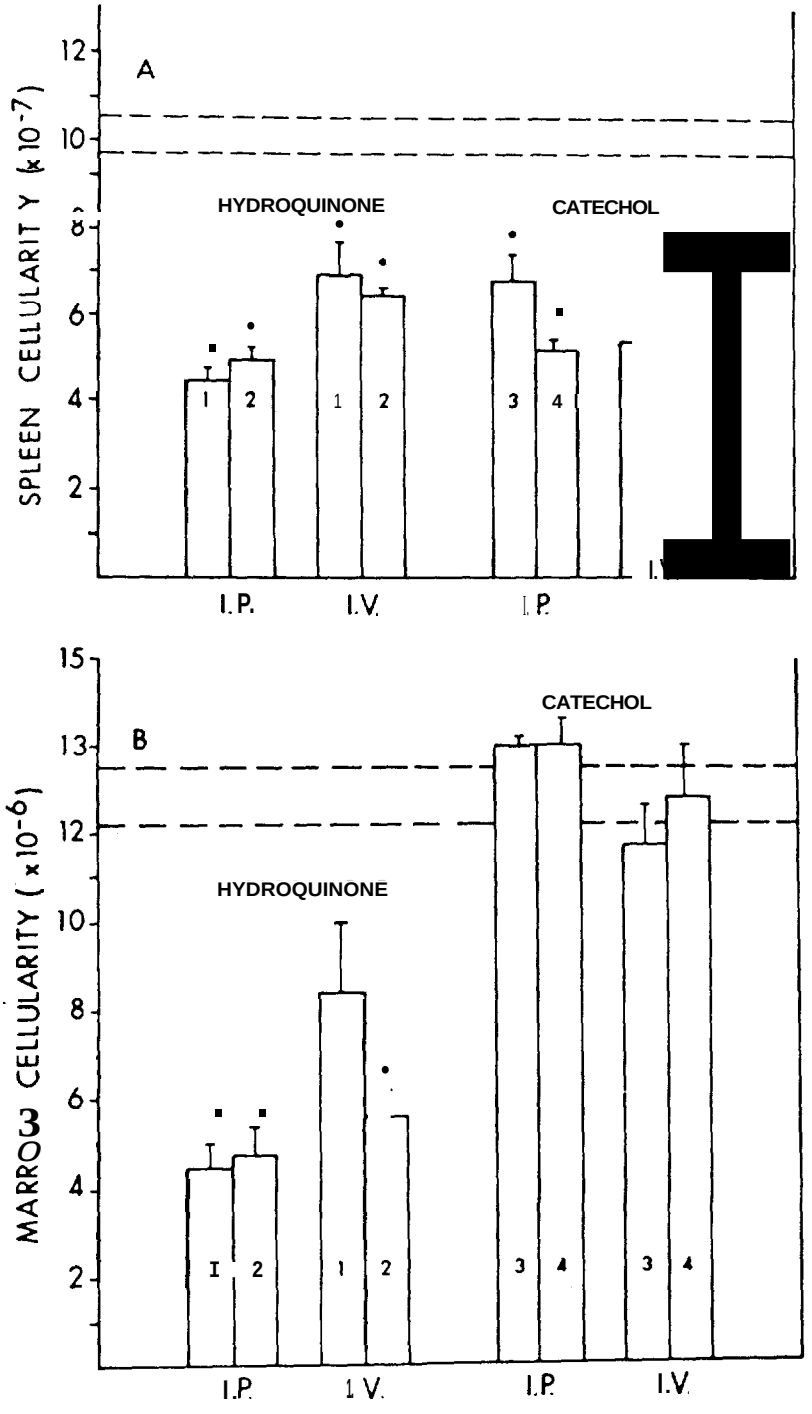
Spleen and femur nucleated cell counts were determined after iv or ip administration of either hydroquinone (100mg/kg) or catechol (75mg/kg) to C57BL/6 mice (Fig. 1). Spleen cellularity was significantly reduced in each experiment by both hydroquinone and catechol. This reduction occurred approximately 1 day after a 3 day. 6 dose treatment regime. Bone marrow cellularity (Fig. 1B) was also significantly reduced by hydroquinone: in contrast. catechol had no affecton bone marrow cellularity. This difference in bone marrow cytotoxicity may be attributed to the greater dose of hydroquinone (100 mg/kg) given relative to catechol (75mg/kg). These doses were the highest iv concentrations of these compounds that the mice could tolerate. as revealed by preliminary experiments.

Table 2 *Kinetics of PFC generation in spleen and marrow cultures after LPS or DxS + LPS activation*

	Average *PFC/2 x 10^5 cells*[a]		
Organ	*Day*[b]	*LPS (10 μg/ml)*	*LPS + DxS (20μg/ml)*
Marrow	3	48 ± 21	230 ± 15
Marrow	4	349 ± 91	1.253 ± 132
Marrow	5	648 ± 52	1.510 ± 190
Marrow	6	1.130 ± 326	810 ± 30
Spleen	2	94 ± 20	133 ± 21
Spleen	3	942 ± 121	909 ± 71
Spleen	4	1.713 ± 272	1.573 ± 320

[a] Mean frequency of anti-trinitrophenylated sheep red blood cells ± SD in triplicate culture.
[b] Days in culture.

Figure 1 Effect of hydroquinone and catechol on spleen and femur cellularity. Organ cellularities were determined 1 day after a 3 day, 6 dose treatment regime with the indicated metabolites. Hydroquinone (100mg/kg) or catechol (75 mg/kg) was given io or ip to groups of 4 mice. Each experiment was repeated twice. Numerals enclosed within the bars indicate experiment number. The results are depicted as mean cellularity ±SD. The dotted lines enclose the range of organ cellularities for the control group, Asterisks denote statistical significance at $p < 0.05$. *(A) Spleen cellularity; (B) femur cellularity.*



Effect of Hydroquinone and Catechol on Spleen Lymphocytes

Hydroquinone and catechol administration reduced the frequency of immature B cells in the spleen that develop into PC-PFC after LPS stimulation (Fig. 2). The reduction was independent of the route of administration and directly paralleled the loss of spleen cellularity (Figure 1A). Mitogen activation of these spleen cells for 72 hr with the T- and B-cell mitogens. Con A and LPS. was also assessed in vitro (data not shown). The number of splenic lymphocytes responding to either mitogen was significantly reduced (range 25–75% of control) by both metabolites; the decreased responses again paralleled the reduction in spleen cellularity. No evidence for preferential suppression between T- and B-cell responses was observed. In comparison to the reduced LPS mitogen responses for each group. the PC-PFC responses (Figure 2) were inhibited. on a percent basis. to a greater degree than the proliferative response. as assessed by ^{3}H-TdR. No preferential suppression (or enhancement) of the proliferative responses to either T- or B-cell mitogens was observed.

Alteration of Bone Marrow Cell Proliferation

One of the consequences of adding DxS, LPS. or DxS + LPS. to a sufficient cell concentration of marrow lymphocytes is that immature B cells proliferation is stimulated. Figure 3 illustrates the proliferation observed when femur cells from hydroquinone- or catechol-treated mice were cultured 4 days with the various B-cell mitogens. Hydroquinone significantly reduced the frequency of the LPS-induced PC-PFC response and the potentiation of LPS-induced PC-PFC by DxS in mice given the metabolite either ip or iv. In contrast. catechol caused a significant reduction of these same responses only when injected intravenously. DxS stimulates a very weak proliferative response (equal to or 2 times background) in cell cultures containing 2×10^{5} cells/ml or less. Figure 3 illustrates that following pretreatment with hydroquinone or

Figure 2 *Effect of* hydroquinone and catechol on spleen *PC-PFC* response. Spleen cells *from* hydroquinone- *or* catechol-treated *(iv* or *ip)* mice were cultured in triplicate with *LPS* and assayed for *PC-PFC* formation. The results are expressed as percent of control and represent the mean *PC-PFC* frequency per organ. Asterisks denote statistical significance at $p < 0.05$. Each experiment was repeated twice.

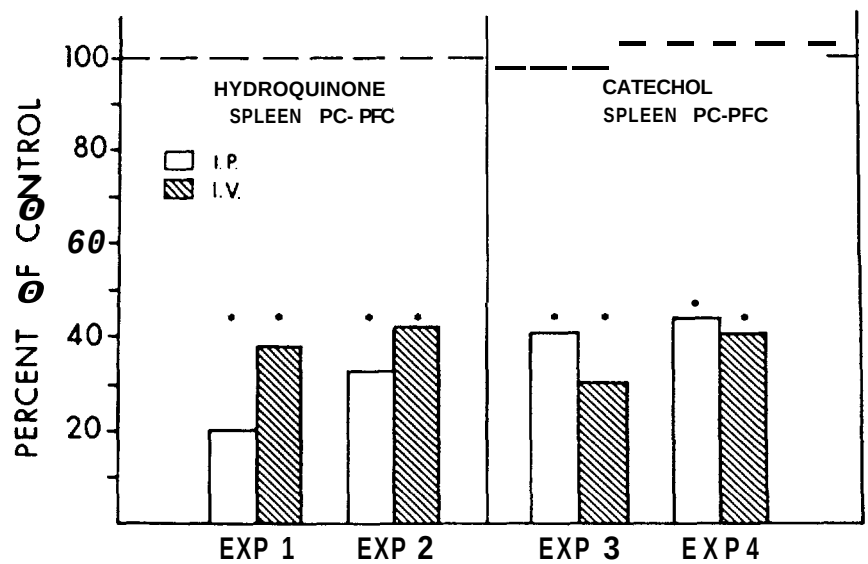
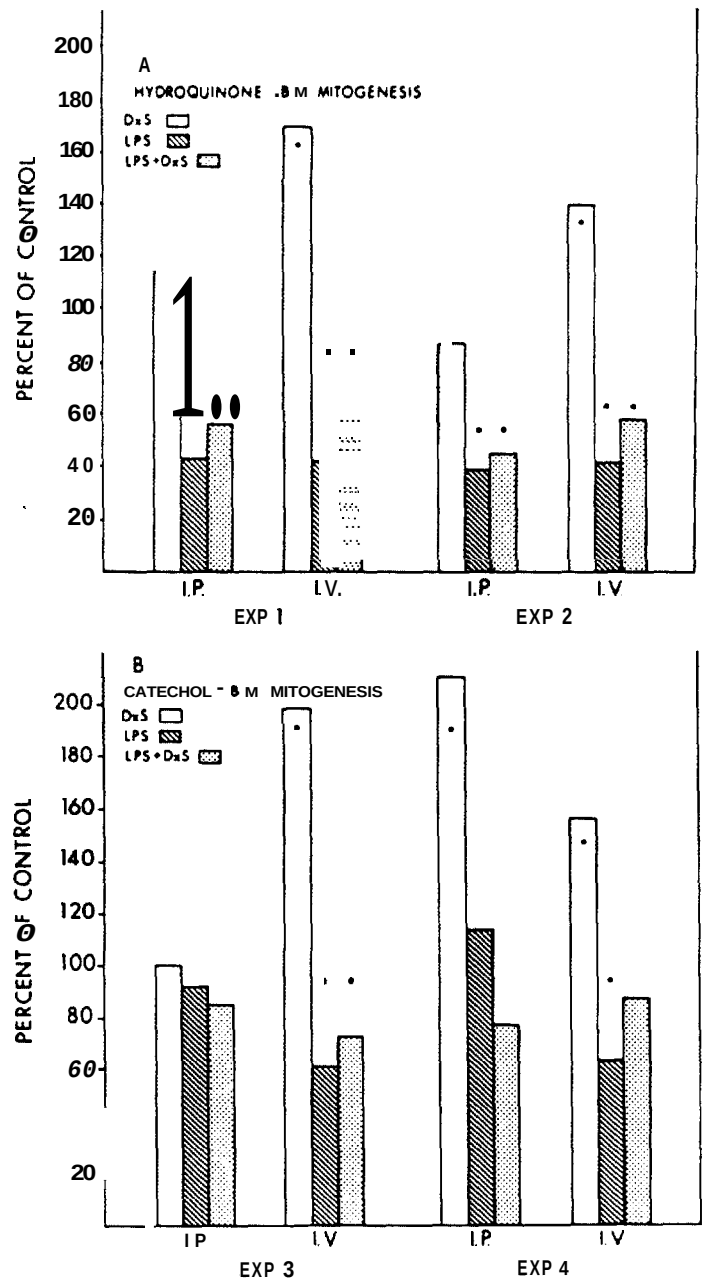


Figure 3 Effect of hydroquinone and catechol on marrow cell proliferation to mitogen stimulation. Marrow cells from metabolite-treated mice were cultured in quadruplicate for 3 days with DxS, LPS, or DxS + LPS and assayed for ^{3}H-TdR accumulation. The results are expressed as percent of control and represent the mean ^{3}H-TdR accumulated (dpm) per organ. Each experiment was repeated twice. Asterisks denote statistical significance at $p < 0.05$. (A) Hydroquinone; (B) catechol.



catechol. the low-level DXS response was in most cases unaffected or enhanced above the DXS response of control cultures. With catechol. alterations in mitogen-induced proliferation occurred despite any significant effect on marrow cellularity.

Reduction of Bone Marrow PC-PFC

Since catechol and hydroquinone decreased the frequency of LPS- and (DxS+LPS)-proliferating cells obtained from bone marrow (Fig. 3). it follows that the number of PC-PFC that arise from these cells would also be reduced. As shown in Figure 4A. iv or ip administration of hydroquinone decreased both PC-PFC responses (induced by LPS alone or with DxS+LPS) in a pattern that again paralleled the decrease in bone marrow cellularity (Fig. 1B). Catechol, on the other hand. induced a different pattern of toxicity toward bone marrow progenitor cells (Fig. 4B). Catechol significantly reduced the frequency of PC-PFC (DxS+LPS) in only one experiment when given iv. In contrast. catechol treatment by either route reduced the number of bone marrow PC-PFC (LPS) in all 4 experiments without producing any reduction in bone marrow cellularity. PC-PFC (LPS) responses were inhibited to a much greater degree in all cases than the proliferative responses to LPS (Fig. 3). Normally. in the generation of PC-PFC (LPS) from bone marrow cells. there is characteristically a paucity of proliferation (Tables 1 and 2). An apparent resistance of PC-PFC formation in (DxS+LPS) cultures to hydroquinone or catechol was also observed (Fig. 4). Similarly. cultures containing DxS alone did not show any reduction in proliferation after catechol and in 3 of the 4 treatment groups the amount of ^{3}H-TdR taken up was actually enhanced (Fig. 3).

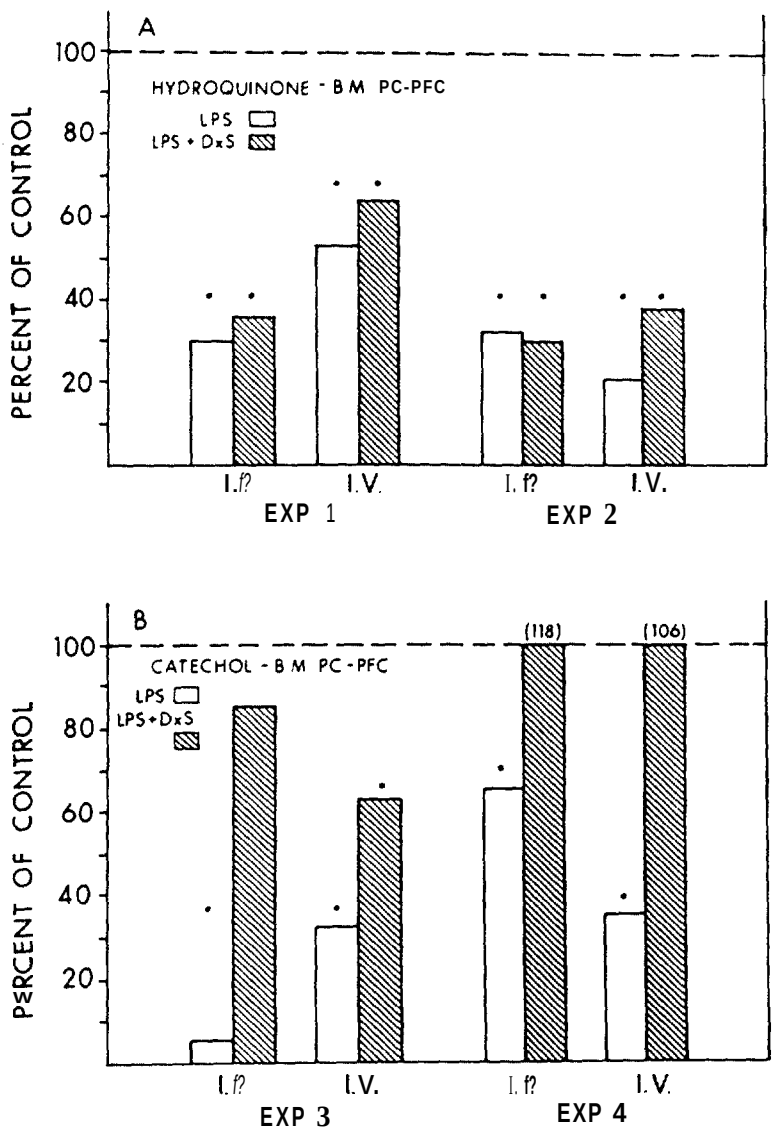
DISCUSSION

Catechol and hydroquinone are metabolites usually found in the urine of humans and animals exposed to benzene (Snyder and Kocsis. 1975; Rusch et al.. 1977) and have recently been shown to localize in the bone marrow and other lymphoid organs of the rat (Greenlee et al., 1981; Irons et al.. 1981a) after benzene exposure. Direct evidence for the toxicity of catechol and hydroquinone to lymphocytes in vivo has been relatively limited (Nomiyama. 1965; Mitchell. 1971; Bolcsak and Nerland. 1980). The present results demonstrate the in vivo toxicity of catechol and hydroquinone, administered to mice either iv or ip. toward immature lymphocytes in the bone marrow and spleen. Thus these compounds are not only myelotoxic, but irnmunotoxic as well, due to the reduction of progenitor B lymphocytes. which are precursors of mature. antibody-producing cells.

In the present studies, LPS and DxS were employed as T-cell independent polyclonal activators to assess the functional capabilities of B cells separately from the cytotoxic effect of hydroquinone and catechol on T helper cells. We cannot rule out the possibility, however, that some of the effects observed in this study resulted from drug action on various suppressor cells, macrophages, or even the hematopoietic microenvironment. Thus any discussion of metabolite toxicity to certain cell populations must be done with the implicit reservation of metabolite effects in vivo on various accessory cells. In the present study we examined the ability of progenitor B lymphocytes from hydroquinone- and catechol-treated mice to proliferate and mature into PC-PFC. The term "progenitor" is used in the context described by Phillips and Gearhard (1979) to represent a nondividing, small. immature lymphocyte at various stages of differentiation. based on such markers as cytoplasmic or surface IgM, Ia antigens, or Fc receptors.

Our results further substantiate the observation that DxS potentiates proliferation and maturation of bone marrow PC-PFC in cultures containing LPS (Gronowicz and Coutinho, 1974; Bona et al.. 1978: Pazdernik and Nishimura. 1978). Alone, DS induced no significant proliferation as assessed by ^{3}H-TdR and no PC-PFC were formed when 2×10^{5} cells/ml were plated. The latter observation is supported by Melchers (1977). who demonstrated that the

Figure 4 *Effect of hydroquinone and catechol on marrow PC-PFC responses. Marrow cells from metabolite-treated mice were cultured in triplicate with LPS or DxS + LPS and assayed for PC-PFC formation. The results are expressed as percent of control and represent the mean PFC frequency per organ. Each experiment was repeated twice. Asterisks denote statistical significance at* $p < 0.05$. *(A) Hydroquinone: (B) catechol.*



number of DxS-induced PFC in cultures of fetal liver cells. another source of progenitor B cells. was only 10% of the response obtained with LPS and often was the same as the number obtained without any mitogen. Ulla et al. (1977) also demonstrated that no splenic B-cell proliferation to DxS occurred at cell concentrations below 2×10^5 cells. ml.

In contrast to bone marrow cells. spleen cells showed no enhancement in PC-PFC formation if DxS was also added to cultures in combination with LPS (Table 2). This lack of potentiation can be ascribed to the presence of more mature. LPS-responsive cells in the spleen that are refractory to DxS stimulation. while. in companson. the bone marrow has more immature DxS-responsive progenitor B cells. Support for this conclusion comes from studies demonstrating that newly formed bone marrow lymphocytes, which are originally unresponsive to LPS. undergo spontaneous maturation in the absence of mitogens and subsequently become LPS-reactive (Burrows et al.. 1978: Phillips and Melchers. 1979). Spleen cells apparently do not undergo this transformation in culture as these immature B-cells are already LPS-responsive (Burrows et al.. 1978). Alternatively. because DxS did potentiate LPS-induced ^{3}H-TdR uptake in spleen cell cultures. the results may indicate that DxS (in the presence of LPS) activates a population of splenic B cells which secrete antibodies other than IgM. These antibodies would not be detected by the TNP-SRBC assay for PC-PFC used in the present study. This hypothesis remains to be tested.

The primary toxic effect of hydroquinone observed in these studies was the reduction of nucleated cells in the spleen and marrow. Reductions in functional progenitor cells were evident in all compartments examined and the reduction paralleled decreases in organ cellularity With lower doses of hydroquinone it may be possible to duplicate the catechol-induced effects discussed below. Indeed. we have subsequently found that 75 mg/kg of hydroquinone. administered twice a day for 3 consecutive days. did not affect bone marrow cellularity. although spleen cellularity was significantly reduced (data not shown): a response identical to the one observed with 75 mg/kg of catechol (Fig. 1). These results also demonstrated that the observed toxicities of catechol and hydroquinone were. for the most part. independent of the route of administration (i.e.. iv or ip).

Catechol was unique in that it did not reduce bone marrow cellularity but definitely inhibited the LPS-induced PC-PFC response. To interpret this response a number of factors must be considered: First. the generation of bone marrow PC-PFC (LPS) is primarily a maturational event. particularly in light of little cellular proliferation in these cultures (Table 1). Quintans and Lefkovits (1974) have shown that final effector-cell maturation (PFC) can take place in the absence of proliferation. The results are also in total agreement with the study of Rusthoven and Phillips (1980). showing that marrow progenitor B cells respond by maturation to PFC after LPS stimulation. with the maximum response occurring on days 5–6. Second. the reduction in bone marrow PC-PFC (LPS) occurred without a concomitant reduction in femur cellularity (Fig. 2). Taken together, these results indicate that either a selective decrease in LPS-responsive. progenitor B cells occurred or the ability of these cells to mature into end-stage PFC had been compromised. We favor the latter hypothesis because the generation of PC-PFC (LPS) is primarily a maturational event and because the mitogen-induced proliferative responses (Fig. 3B) were not as severely affected as the PC-PFC (LPS) response (Fig. 4B). DxS alone did not induce PFC formation and induced only minimal proliferation; however. bone marrow cultures from catechol-treated mice showed an increase in basal DxS-induced proliferation above control cultures (Fig. 3B). This property was shared with bone marrow cells from mice given hydroquinone (Fig. 4A). This increase in ^{3}H-TdR accumulation probably reflects an enrichment of DxS-activated progenitor B cells from the marrows of metabolite-treated mice. However. Ulla et al. (1977) have reported that DxS activates splenic macrophages in vitro to secrete factors that induce B cells to proliferate. This effect has not been reported to occur in bone marrow cells. but it cannot be ruled out that the increased ^{3}H-TdR accumulation in DxS cultures may also reflect an increase in marrow macrophages.

In contrast to the toxicity of catechol toward LPS-induced PC-PFC. the potentiation of LPS-induced PC-PFC by DxS was relatively unaffected (Fig. 4B). The cause for this dichotomy remains unknown: however. one explanation may be that the progenitor B-cell population activated by DxS is selectively resistant to the dose of catechol given in vivo. Alternatively, because these earlier progenitors occur in greater frequency in the marrow than LPS-induced progenitors (Burrowset al.. 1978; Osmond. 1979). a slight reduction in frequency of these cells would not yield a significant reduction in the frequency of PC-PFC in DxS + LPS cultures. Another explanation is that the presence of DxS in culture actually reverses catechol-induced maturational arrest. The mechanism for the postulated reversal is unknown; however, DxS can affect such growth processes as the synthesis and distribution of cellular, sulfated glycosaminoglycans that may play a role in cellular growth control (Ehrlich and Murray, 1979).

A possible mechanism for the toxicity of hydroquinone and catechol has been provided by studies demonstrating that both metabolites can interfere with microtubule assembly and lectin-induced blastogenesis in vitro (Irons and Neptun. 1980: Pfeifer and Irons. 1981; Irons et al.. in press). Metabolite interaction with the cytoskeleton can result either in a concentration-dependent cytotoxicity or an inhibition of lymphocyte function in the absence of cell death.

In summary. the results showed that at least one metabolite of benzene (catechol) has the potential to inhibit progenitor B-cell maturation in mouse bone marrow. Both hydroquinone and catechol were cytotoxic to the spleen. whereas hydroquinone was cytotoxic to bone marrow at high doses. and neither agent reduced marrow cellularity at lower doses. Both compounds compromised immune function by reducing progenitor B lymphocytes in the spleen and bone marrow. Thus the immunotoxic potential of hydroquinone and catechol in vivo suggests that interference with precursor cell maturation may play a significant role in the hematotoxicity observed after chronic exposure to benzene.

REFERENCES

Andersson J. Coutinho A. Melchers F (1979) Mitogen-activated B-cell blasts reactive to more than one mitogen. J Exp Med 149:553.

Bolcsak LE. Nerland DE (1980) Inhibition of erythropoiesis by benzene and benzene metabolites. *Pharmacology* 22:173A.

Bona C. Yano A. Dimitria A. Miller RG (1978) Mitogenic analysis of murine B-cell heterogeneity. J *Exp* Med 148:136.

Brahim F. Osmond DG (1970) Migration of bone marrow lymphocytes demonstrated by selective bone marrow labeling with thymidine-H^3. *Anat Rec* 168:139.

Burrows PD. Keamey JF. Lawton AR. Cooper MD (1978) Pre-B cells. Bone marrow persistance in anti-μ-suppressed mice. conversion to B lymphocytes. and recovery after destruction by cyclophosphamide. J *Immunol* 120:1526.

Chavin W. Jelonek EJ. Reed AH, Binder LR (1980) Survival of mice receiving melanoma transplants is promoted by hydroquinone. Science 208:408.

Cohen HS. Freedman ML. Goldstein BO (1978). The problem of benzene in our environment: clinical and molecular considerations. *Am* J Med *Sci* 275:124.

Coutinho A (1976) Genetic control of B-cell responses. 11. Identification of the spleen B-cell defect in C3H/HeJ mice. *Scand* J Immunol 5:129.

Coutinho A. Forni L. Melchers. F. Watanabe T (1977) Genetic defect in responsiveness to the B-cell mitogen lipopolysaccharide. *Eur* J *Immunol* 7:325.

Ehrlich K. Murray M (1979) Effect of DEAE-dextran and dextran sulfate on sulfate incorporation into glycosaminoglycans of normal and SV40 virus-transformed 3T3 cells. *Life Sci* 25:1557.

Greenlee WF. Rickert DE. Chism JP. Irons RD (1980) Effect on the toxicity and metabolism of benzene. *Toxicol Appl Pharmacol* 53:134A.

Greenlee WF. Gross EA. Irons RD (1981) A study of the disposition of ^{14}C-labelled phenol. catechol and hydroquinone in the rat using whole body autoradiography. Chem *Biol* Interaction 33:285.

'ronowicz E. Biberfeld P. Wahren B. Coutinho A (1976) Characterization of dextran-sulfate sensitive cells. Scan *J Immunol* 5:573.

Gronowicz E. Coutinho A (1974) Selective triggering of B cell subpopulations by mitogens. *Eur J Immunol* 4:771

Hodgson GS. Bradley TR. Martin RF. Summer M. F y P (1975) Recovery of proliferating haemopoietic progenitor cells after killing by hydroxyurea. Cell Tissue Kinet 8:51.

Irons RD. Neptun DA (1980) Effects of the principal hydroxymetabolites of benzene on microtubule polymerization. Arch Toxicol 45:297.

Irons RD. Greenlee WF. Wiarda D. Bus JS (1981a) Relationship between benzene metabolism and toxicity: a proposed mechanism for the formation of reactive intermediates from polyphenol metabolites. In Biological Reactive fntermediates *2*. A Second fnternational Symposium. (Eds.. R Snyder. DV Parke. JJ Kocsis. DJ Jollow. New York: Plenum Press. in press.

Irons RD. Neptun DA. Pfeifer RW (1981b) Sulfhydryl-dependent inhibition of lymphocyte growth and microtubule assembly by quinone metabolites of benzene. *J*. Reticuloendo. *Soc*. 30:359.

Lee EW. Kocsis JJ. Snyder R (1974) Benzene: acute effect on ^{59}Fe incorporation into circulating erthrocytes. Toxicol Appl Pharmacol 27:431.

Melchers F (1977) B lymphocyte development in fetal liver. l. Development of reactivities to B cell mitogens in vivo and in vitro. Eur J frnrnunol 7:476.

Melchers F. Andersson J (1974) The kinetics of proliferation and maturation of mitogen-activated bone marrow-derived lymphocytes. Eur. J. frnrnunol. 4:687.

Melchers F. von Boehmer H. Phillips RA (1975). B-lymphocyte subpopulations in the mouse. Organ distribution and ontogeny of immunoglobin-synthesizing and of mitogen-sensitive cells. Transplant Rev 25:26.

Mitchell JR (1971) Mechanism of benzene-induced aplastic anemia. Fed Am *Soc* Exp Biol 30:561.

Morimoto K. Wolff S (1980) Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res 40:1189.

Nomiyama K (1965) Studies on poisoning by benzene and its homologues. Toxicity of benzene metabolites to hemopoiesis. *Ind* Health 3:53.

Osmond DG (1979) Generation of B lymphocytes in the bone marrow. In Lymphocytes in **the** Immune Response. (Eds., MD Cooper. DE Mosier. I Scher. ES Vitetta.) Amsterdam: Elsevier-North Holland. p. 63.

Osmond DG. Nossal GJV (1974) Differentiation of lymphocytes in mouse bone marrow. II. Kinetics of maturation and renewal of antiglobulin-binding cells studied by double labeling. Cell frnrnunol 13:132.

Pasanen VJ. Makela O (1969) Effect of the number of haptens coupled to each erythrocyte on haemolytic plaque formation. Immunology 16:399.

Pazdernik TL. Uyeki EM (1974) Carrier- and hapten-antibody producing cells in vitro. II. Effects of purine antimetabolites. Agents Actions 4:15.

Pazdernik TL. Nishimura T (1978) A new technique for detecting the immediate precursor of the antigen reactive cell in mouse bone marrow J frnrnunol Meth 23:363.

Pazdernik TL. Corbett JD (1979) Effects of chloramphenicol products on hemopoietic precursor cells in vitro. Pharmacology 19:191.

Pfeifer RW. Irons RD (1981) Inhibition of lectin-stimulated lymphocytes agglutination and mitogenesis by hydroquinone: reactivity with intracellular sulfhydryl groups. *Exp Mol Pathol* 35:189.

Phillips RA. Gearhart PJ (1979) Biased Summary: Ontogeny and differentiation of B cells. In Lymphocytes in *the* fmmune Response. Eds. MD Cooper. DE Mosier. I Scher. ES Vitetta. Amsterdam: Elsevier North-Holland. p. 107.

Phillips RA. Melchers F (1979) Reduced responsiveness of immature B cells to the B cell mitogen, lipoprotein. J Irnmunol 122:1473.

Quintans J. Lefkovitz I (1974) Clones of antibody-forming cells in pokeweed mitogen-stimulated microcultures. I. Requirement for the induction of the antibody response of nude spleen cells. *Eur* J Irnmunol 4:613.

Rittenberg MB. Pratt KL (1969) Antitrinitrophenyl (TNP) plaque assay. Primary response of Balb/c mice to soluble and particulate immunogen. Proc *Soc Exp Biol Med* 132:575.

Rusch GM. Leong BKJ. Laskin S (1977) Benzene metabolism. J *Toxicol Environ Health* (Suppl(2):23.

Rusthoven JJ, Phillips RA (1980) Hydroxyurea **kills** B cell precursors and markedly reduces functional B cell activity in mouse bone marrow. J Irnmunol 124:781.

Schrenk HH. **Yant** WP. Pearce SJ et al (1941) Absorption, distribution and elimination of benzene by body tissues and fluids of dogs exposed to benzene vapor. *J In Hyg Toxicol* 23:20.

Snyder R. Lee EW. Kocsis JJ. Witmer CM (1977) Bone marrow depressant and leukemogenic actions of benzene. Life *Sci* 21:1709.

Snyder R. Kocsis JJ (1975) Current concepts of chronic benzene toxicity. *CRC Crit* Rev *Toxicol* 3:265.

Tabershaw IR. Cooper WC (1974) A mortality study of petroleum refinery workers. Am Petroleum Inst Med Res Report #EA7402. September 15.

Ulla C. Persson I. Lenmart L. Hammarstram G. Smith CIE (1977) Macrophages are required for the dextran-sulfate induced activation of B lymphocytes. J *Immunol* 119:1138.

Uyeki EM. El-Ashkar A. Shoeman DW. Bisel TU (1977) Acute toxicity of benzene inhalation to hemopoietic precursor cells. Toxicol **Appl** Pharmacol 4049.

Wierda D. Pazdernik TL (1979a) Effects of cyclophosphamide **on** hemic precursor cells in mouse bone marrow and spleen. *J Immunopharmacol* 1:357.

Wierda D. Pazdemik TL (1979b) Toxicity of platinum complexes on hemopoietic precursor cells. *J Phamacol Exp* Ther 211:531.

Wierda D. Greenlee WF. Irons RD (1980) Immunotoxicity of benzene metabolites in C57BL/6 mice. Pharmacology 22:207A.