Mutation Research, 249 (1991) 201 209
© 1991 Elsevier Science Publishers R.V. 0027-5107/91/$03.50
ADONIS 0027510791001424

MUT 04994

Two benzene metabolites, catechol **and** hydroquinone, produce **a** synergistic induction of micronuclei and toxicity in cultured human lymphocytes

Moire L. Robertson, David **A. Eastniond** * and Martyn T. **Smith**

Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720 (U.S.A.)

(Received 23 **July** 1990)
(Revision received 21 November 1990)
(Accepted 7 January 1991)

Keywords: Benzene; Catechol; Hydroquinone; Phenol; Micronuclei; Synergism

Summary

A mixture of two benzene metabolites, hydroquinone and catechol, produces a striking synergistic genotoxic response in cultured human lymphocytes. This was demonstrated using an anti-kinetochore antibody modification of the micronucleus assay. Treatment with hydroquinone alone or in combination with phenol produced a 3-fold increase in micronucleated cells over background. Treatment with catechol or phenol alone and in combination produced only minor increases in the number of micronucleated cells. In contrast, simultaneous treatment with equimolar (75 μM) concentrations of hydroquinone and catechol resulted in a greater than 16-fold induction of micronucleated cells. Given an additivity model, 20 additional micronucleated cells would be expected (after correcting for background frequencies), yet 140 were observed. Further analysis revealed that over 90% **of** the micronucleated cells stained positively for kinetochores, indicating a high probability that these micronuclei contain entire chromosomes. This synergistic response appears to occur only at equimolar levels of hydroquinone and catechol. These results suggest that these metabolites are acting together to disrupt the mitotic spindle and interfere with chromosome segregation. These data provide further **support** for the hypothesis that multiple nietabolites acting in concert are involved in the benzene-induced genotoxicity and leukemia in humans.

Nonrandom chroninsomal aberrations, principally deletions, translocations and the gain or loss of entire chromosomes, are associated with a variety of human cancers, including leukemia **(Yunis,** 1983; Rowley, 1984). Many of these changes are thought to be primary events critical to the onset of neoplasia (Klein, 1981; Cavanee et al., 1983; Tsutsui et al., 1983). The common industrial solvent, benzene, causes the induction of both structural and numerical chromosome changes (Erdogan and Aksoy, 1973; Ding et al., 1983) in the peripheral blood cells of highly exposed workers as well as myelotoxicity and

* Present address: Environmental **Toxicology Graduate Program**, University **of** California, Riverside, ('A 92521 (U.S.A.).

Correspondence: Dr. **Martyn T. Smith, Department of** Biomedical **and Environmental** Health **Sciences,** School **of** Public Health, University of California, Berkeley, CA **93720** (U.S.A.).

leukemia (IARC, 1988). Benzene, itself, is unlikely to be the actual toxic species, but is converted within the liver to species which exert myelotoxic effects. The primary metabolites are phenol (PH). hydroquinone (HQ), catechol (CAT) and *trans*, trans-muconic acid (Gad-El-Karin1 et al., 1985; Cooper and Snyder, 1988). Both HQ and CAT have been shown to accumulate in rodent bone marrow (Rickert et al., 1979). These phenolic metabolites may then be oxidized to highly toxic quinones by peroxidase enzymes such as myeloperoxidase, present in the bone marrow (Smith et al., 1989). However, the metabolite(s) ultimately responsible for the myelotoxicity and leukemia produced by benzene remains unknown.

In order to study the ability of different benzene metabolites to induce certain types of structural and numerical chromosomal changes, namely chromosome breakage or malsegregation, in human blood cells, we have used a modified niicronucleus assay in isolated peripheral human lymphocytes. This technique employs cytochalasin B to inhibit cytokinesis resulting in the formation of multi-nucleated cells which can easily be scored for the presence or absence of micronuclei (Fenech and Morley, 1985). Chromosome breakage and malsegregatiori events can be differentiated utilizing an anti-kinetochore antibody whereby kinetochore-positive micronuclei have a high probability of containing an entire chromosome. and kinetochore-negative micronuclei contain only chromosomal fragments (Eastmond and Tucker, 1989). Using this technique, we recently demonstrated that several of the phenolic metabolites of benzene and the HQ oxidation product, 1,4-benzoquinone (BQ), induce micronuclei in human lymphocytes (Yager et al., 1990). Based on the slope of the dose–response curves, the relative potencies for micronuclei induction were as follows: BQ > HQ > CAT > PH (Smith et al., 1990). Previous studies in our laboratory have shown that both PH and CAT will stimulate the peroxidase-dependent oxidation of HQ to BQ (Smith et al., 1989) and that the simultaneous administration of PH and HQ produces myelotoxicity similar to that produced by benzene (Eastmond et al., 1987). This work, and that of others (Snyder et al., 1989; Barale et al., 1990), suggests that a combination of metabolites, rather than a single metabolite, is responsible for the myelo- and genotoxic effects of benzene. We have therefore investigated the ability of different combinations of benzene metabolites to induce micronuclei in human lymphocytes. A striking synergistic induction of micronuclei following treatment with HQ and CAT is reported here.

Materials and methods

Cell culture arid treatment conditions. Peripheral human lymphocytes were isolated from the same healthy adult male using Ficoll-Paque (Pharmacia, Piscataway. NJ) density gradients and cultured as described previously (Yager et al, 1990). Briefly, lymphocytes at a density of 5×10^5 cells/ml were cultured for 72 h at 37°C in a 5% CO_2 atmosphere. The total culture volume before addition of the chemicals was 2 nil. Culture medium consisted of RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone, Logan. UT), 2 mM L-glutamine, 100 units/ml penicillin. 100 μg/ml streptomycin (all from Gibco, Grand Island, NY), and 1.5% phytohemagglutinin (PMA) (HA 15, Burroughs-Wellcome, Greenville, NC).

PH (CAS No. 108-95-2), CAT (CAS No. 120-80-9) and HQ (CAS No. 123-31-9), all purchased from Aldrich (Milwaukee, WI), were diluted in phosphate-burrered saline (PBS) (Ca^{2+} and Mg^{2+} free) imtnediately prior to treatment at 24 h in complete medium. Total volumes added did not exceed 90 μl. For experiments with mixtures, the order of addition was as follows: PH, CAT then HQ. Viability was determined at 72 h using the trypan blue dye exclusion technique.

Micronucleus assay. PHA stimulated lymphocytes were cultured for 44 h before the addition of cytochalasin B (Sigma, St. Louis, MO) (3 μg/ml final concentration). Cells were harvested at 72 h onto glass slides using a cytocentrifuge (Shandon, Sewickley, PA). After allowing the slides to air dry, they were fixed in methanol for 15 min. dried and stored desiccated, under a N_2 atmosphere at −20°C until use.

Immunofluorescence. Slides were stained with an anti-kinetochore antibody (Antibodies Itic., Davis, CA) essentially as described (Eastmond

and Tucker, 1989). Briefly, slides were incubated at 37°C in a humidified atmosphere with the anti-kinetochore antibody for 1 h, washed in PBS containing the detergent Tween 20, and incubated for another hour using a fluoresceinated goat anti-human IgG (Antibodies Inc., Davis, CA). After washing, the slides were then stained with 4′,6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO) in an antifade solution (Johnson and Nogueira Araujo, 1981) and coverslipped. Slides were stored refrigerated until use.

Scoring. Randomized and coded slides were scored using a Nikon microscope equipped with epifluorescent illumination using filters for fluorescein (excitation at 470 nm, dichroic at 510 nm, barrier at 520–560 nm) and quinacrine (excitation at 400–440 nm, dichroic at 455 nm, barrier at 470 nm). At least 1000 binucleate lymphocytes (those that have undergone one mitotic division) were scored, 500 from each duplicate culture, for the number of micronuclei. When a micronucleus was located using the quinacrine filter, the presence or absence of a kinetochore spot was noted using the fluorescein filter. Replicative index (R.I.), a measure of cell division kinetics, was calculated by scoring 400 cells per dose (200 per duplicate) (see Table 1 legend).

Statistical analysis. The statistical significance of the results was assessed by analysis of variance (ANOVA), using the Super Anova© program for a MacIntosh.

Results

The induction of micronuclei and micronucleated cells by primary metabolites of benzene alone and in combination is shown in Table 1. PH and CAT alone did not produce significant increases over the PBS control at 1 mM and 75 μM, respectively. While lymphocyte viability was not decreased, CAT treatment lowered the R.I. to 1.4. HQ (75 μM) treatment produced 30 micronuclei and 28 micronucleated cells, a 3-fold increase over background. This treatment also produced slight decreases in both viability and R.I. Simultaneous treatment with PH and CAT did not produce appreciable increases in micronuclei or micronucleated cells over background or their respective controls. However, the addition of PH with HQ produced a borderline significant (described below) increase in the number of micronuclei over that observed from HQ treatment alone. Neither of these mixtures (PH with CAT or PH with HQ) significantly altered the R.I. or viability of the lymphocytes from their respective CAT or HQ controls.

Treatment with equimolar (75 μM) levels of CAT and HQ, however, produced a synergistic increase in both micronuclei and micronucleated

TABLE 1

INDUCTION OF MICRONUCLEI IN CYTOKINESIS-BLOCKED HUMAN LYMPHOCYTES FOLLOWING TREATMENT WITH BENZENE METABOLITES ALONE AND IN COMBINATION

Dose	Number per 1000 BN [ab]		R.I. [c]	Viability
	Micronuclei	Micronucleated cells		
Control (PBS)	10 (2)	9 (1)	2.0	94
Phenol (1 mM)	13 (4)	12 (4)	1.9	96
Catechol (75 μM)	11 (5)	10 (4)	1.4	94
Hydroquinone (75 μM)	30 (4)	28 (4)	1.7	83
Catechol + phenol (75 μM + 1 mM)	13 (4)	13 (4)	1.5	88
Hydroquinone + phenol (75 μM + 1 μM)	35 (2)	28 (4)	1.6	80
Catechol + hydroquinone (75 μM + 75 μM)	200 (3)	149 (20)	1.3	62
Catechol + hydroquinone + phenol (75 μM + 75 μM + 1 mM)	221 (80)	149 (45)	1.3	62

[a] Data represent the mean from duplicate experiments. Numbers in parentheses are the standard deviation.

[b] BN is the number of binucleate cells.

[c] R.I., the replicative index, is defined as: [(1× % mononucleated cells) + (2× % binucleated cells) + (3× % tri and > trinucleated cells)]/100.

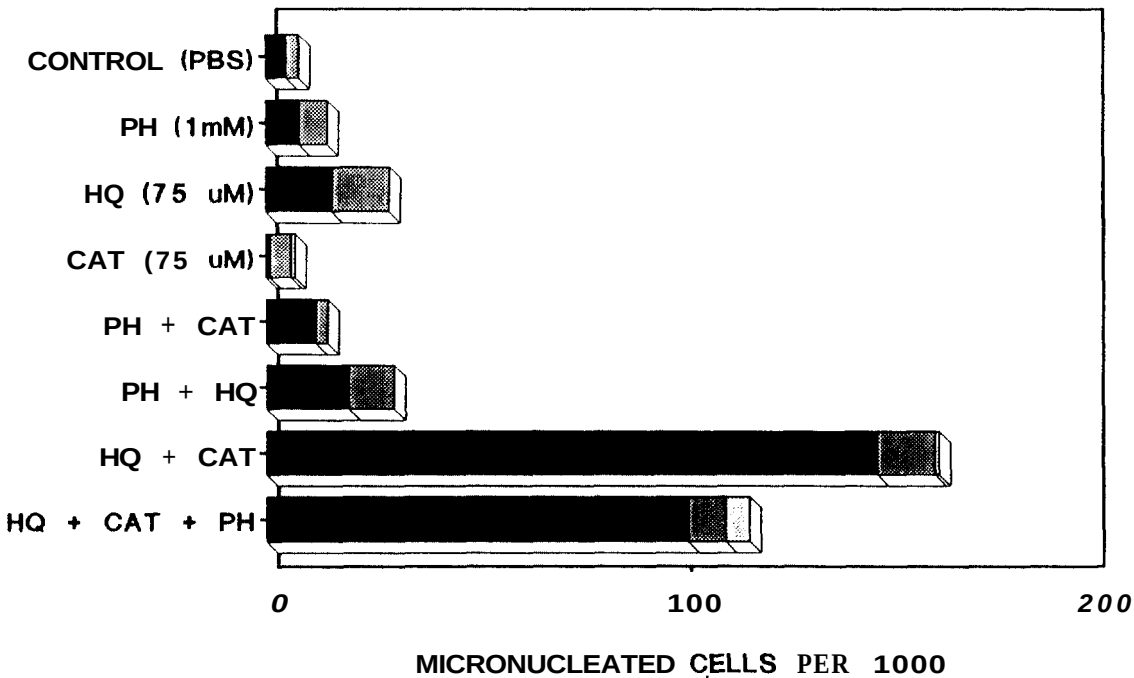

Fig. 1. Induction of kinetochore positive and negative micronucleated cells in cytokinesis blocked human lymphocytes following treatment with benzene metabolites alone and in combination. These data are a subset of the data presented in Table 1. ■ represents the nuinher of micronucleated cells containing at least one kinetochore-positive micronucleus. ▩ represents the nuinher of micronucleated cells containing at least one kinetochore-negative micronucleus. ▨ represents the nuinher of micronuclei unscorable for kinetochores.

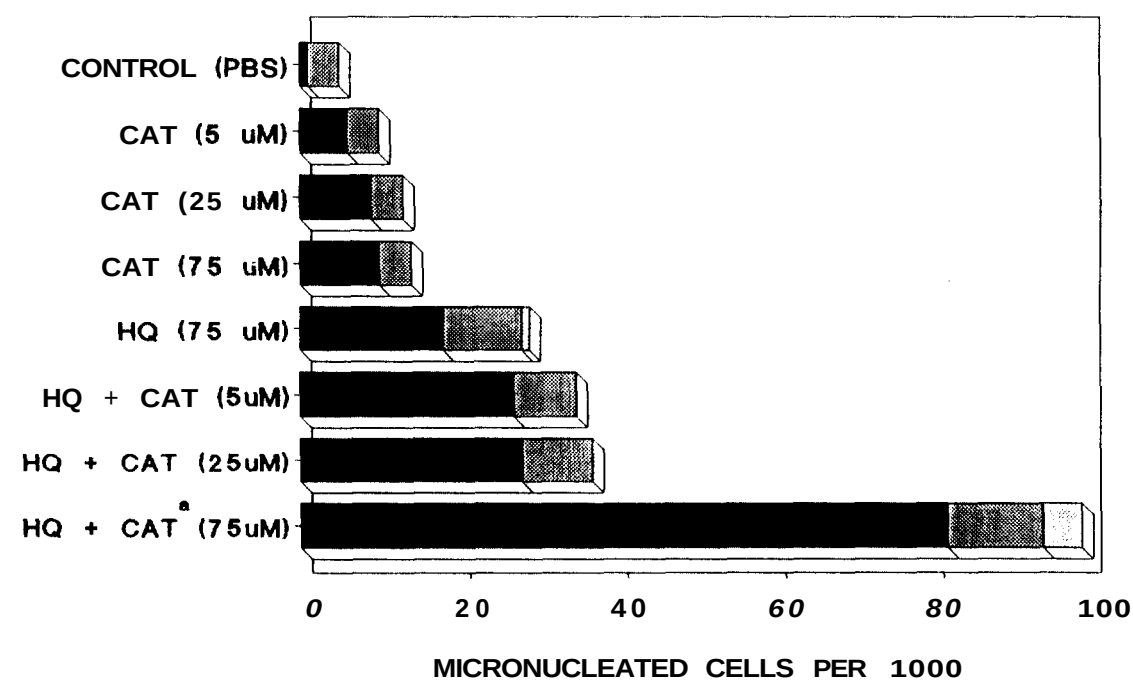

Fig. 2. Induction of kinetochore-positive and -negative micronucleated cells in cytokinesis-blocked human lymphocytes following treatment with 75 μM hydroquinone and varying concentration of catechol. ■ represents the number of micronucleated cells containing at least one kinetochore-positive micronucleus. ▩ represents the number of micronucleated cells containing at least one kinetochore-negative micronucleus. ▨ represents the number of micronuclei unscorable for kinetochores. [a] Only 964 BN cells scored, results normalized to 1000 BN cells.

cells. Simultaneous administration of HQ and CAT produced **149** micronucleated cells per 1000 binucleate cells, a greater than 16-fold increase over background. Given .a model **of** simple additivity, simultaneous exposure to HQ and **CAT** would be expected to produce 20 micronucleated cells above background, yet an additional 140 micronucleated cells were observed over background. This yields a 7-fold increase over that expected given an additivity model. While viability was decreased to 62% with this treatment, a similar decrease was not observed in the R.I. Addition of PH to the mixture of **HQ** and **CAT** produced a small increase of total micronuclei, but did not increase the number of micronucleated cells. The addition of PH to the mixture also did not affect viability or the R.I.

An anti-kinetochore antibody modification of

TABLE 2

DISTRIBUTION OF MICRONUCLEI AND FREQUENCY OF KINETOCHORES CONTAINED WITHIN EACH MICRONUCLEUS [a]

	Nuniber of BN [b] cells with 1 MN [c]	Number of KIN [d] per MN	Number of BN cells with 2 MN	Number of KIN per MN	Number of BN cells with > / = 3 MN	Number of KIN per MN
PBS control	4	0				
	1	3				
CAT (75 μM)	13	0				
	2	1				
	1	2				
HQ (75 μM)	10	0	1	0, 1		
	6	1	1	1, 1		
	2	2				
	1	5				
	2	7				
	2	8				
	1	9				
	1	10				
	2	11				
	1	13				
	1	U [e]				
HQ + CAT	10	0	1	0, 0	1	2, 2, 1
(75 μM + 75 μM)	26	1	1	0, 3	1	U, U, U
	13	2	3	1, 1	1	1, 1, 1, 3
	2	3	3	1, 2	1	0, 1, 2, 2, 3
	1	4	3	1, 3		
	3	5	1	1, 5		
	3	6	1	1, 7		
	2	7	1	1, 9		
	3	8	2	1, 13		
	1	10	1	2, 4		
	2	11	1	2, 5		
	1	14	1	4, 4		
	2	U	1	5, 7		
			1	2, U		
			1	U, U		

[a] Data represent a subset of the data presented in Fig. 2.
[b] BN, binucleate.
[c] MN, micronuclei.
[d] KIN, kinetochores.
[e] U, unscorable for kinetochores.

the micronucleus test (Eastniond and Tucker, 1989) was used to distinguish between micronuclei containing chromosome fragments (kinetochore-negative) and micronuclei containing entire cliromosomes (kinetochore-positive). Fig. 1 shows that for treatment of lymphocytes with the metabolites alone, the proportion of kinetochore-positive vs. -negative did not differ significantly from the control, except for HQ (see below). Treatment of lymphocytes with PH and HQ or PH and CAT also did not alter the proportion of kinetochore-positive and -negative micronucleated cells relative to the controls. Treatment with the equimolar mixture of HQ and CAT, however, produced mostly kinetochore-positive rnicronucleated cells, with > 90% of the total MN containing kinetochores. This treatment has no effect on the number of kinetochore-negative micronucleated cells. The synergistic genotoxic effect of this combination on lymphocytes can he explained almost entirely in terms of an increase in kinetochore-positive micronuclei.

The dose-dependency of micronucleus formation on CAT concentration was investigated by varying the CAT concentrations from 5 to 75 μM while maintaining HQ constant at 75 μM (Fig. 2). The synergistic increase in micronucleated cells was not observed until equimolar concentrations of HQ and CAT were reached. This non-linear effect indicates that a protective mechanism, operational at the lower doses of CAT, may be overwhelmed with equimolar concentrations of CAT and HQ.

Utilizing a subset of the data shown in Fig. 2, the number of kinetochores contained within each micronucleus is shown in Table 2. These data illustrate that the equimolar dose of HQ and CAT result in many cells containing multiple micronuclei and that each micronucleus frequently contains multiple kinetochores.

An analysis of variance was performed after pooling all the data presented in Table I and Figs. 1 and 2. PH or CAT by themselves, or their combinations, have no significant effect on any of the results. HQ alone increases significantly the number of micronucleated cells ($p = 0.022$) and of kinetootiore-negative micronucleated cells ($p < 0.0001$). Although borderline significant ($p = 0.053$), an interaction between PH and HQ is the second most important effect at explaining the number of micronuclei formed. The most important effect is a non-linear interaction between CAT and HQ ($p < 0.0001$). 'This interaction explains most of the results for the number of niicronuclei, micronucleated cells and kinetochore-positive micronucleated cells. It has no effect on the number of kinetochore-negative micronucleated cells.

Discussion

Here we report that simultaneous exposure of human lymphocytes to two benzene metabolites. CAT and HQ, results in a striking synergistic induction of micronuclei in human lymphocytes. Greater than 90% of the micronuclei stained positively for kinetochores, indicating that they arose as a result of chromosome lag events. These data provide considerable support to the growing acceptance that benzene-induced myelotoxicity and genotoxicity is not the effect of a single metabolite acting at a single target site (Tice et al., 1982; Goldstein, 1989; Snyder et al., 1989; Barale et al., 1990), but rather multiple metabolites acting at several target sites, all of which may be critical to the onset of neoplasia. A considerable amount of evidence exists demonstrating that the metabolites of benzene can produce biochemical lesions both individually and in combination. Individually, each of the primary metabolites have been shown to be capable of producing genotoxic activity in vitro (Erexson et al., 1985; Glatt et al., 1989; Yager et al., 1990). Additional evidence supports a role for mixtures of metabolites. Irons and associates (Eastmond et al., 1987) demonstrated that co-administration of PH and HQ resulted in significant reductions of bone marrow cellularity. Snyder et al. (1989) then showed that combinations of PH and HQ or HQ and *trans,trans*-muconaldehyde were synergistic in decreasing iron incorporation into red blood cells. Most recently, Barale et al. (1990) reported that the in vivo coadministration of a mixture of PH and HQ produced a synerpistic induction of niicronucleated erythrocytes in mice. We also observed an interaction between PH and HQ, but of borderline significarice. Additional experiments are

warranted to verify the presence of this interaction in our test system.

These data implicate that PH and HQ acting together in the bone marrow may be responsible for benzene-induced carcinogenicity. However, administration of PH, a metabolic precursor to HQ, fails to reproduce the pancarcinogenicity characteristic of benzene exposure (National Cancer Institute, 1980; Huff et al., 1989). One possible explanation is that administered PH may be metabolized and distributed within the body differently than PH which is generated endogenously from benzene (Subrahmanyam et al., 1990). Another likely explanation for this difference may be that substantially more CAT is formed following benzene administration than following PH administration, as recently demonstrated by Inoue and coworkers using rabbits (Inoue et al., 1989). Since CAT is formed primarily (> 95%) from the oxidation of dihydrodiol precursors following benzene administration (Billings, 1985), and PH is mainly converted to HQ (Sawahata and Neal, 1983), it is likely that more CAT will be formed following benzene than PH administration. Thus, the critical levels of CAT relative to HQ which are observed in the bone marrow of rats exposed to benzene (Rickert et al., 1979), are unlikely to be achieved by the administration of PH alone. Given the marked synergistic genotoxicity of HQ and CAT described here, the numerical chromosome changes observed in the lymphocytes of workers exposed to benzene (Forni et al., 1971; Ding et al., 1983) may therefore be due, at least in part, to the Formation of both HQ and CAT following exposure to benzene.

Notable toxicity (38%), as assessed by trypan blue dye exclusion, was only apparent after treatment with equimolar levels of HQ and CAT. However, this measure of toxicity is likely to underestimate the true overall toxicity since cells dying as a result of treatment at 24 h may be lost to analysis at 72 h. A further indication of toxicity is the marked decrease in replicative index, a measure of cell cycle kinetics. Studies are currently underway in our laboratory focusing on the nature of the interaction between HQ and CAT at doses below 75 μM. These studies will include not only micronucleus formation, but also investigation of the time course of toxicity.

Several mechanisms can be proposed for the observed synergism between HQ and CAT. The most likely explanation is interference with microtubule integrity. Microtubules, composed primarily of tubulin polymers, are essential for the proper segregation of chromosomes into the daughter nuclei during cell division. In vitro treatment with HQ or its oxidation product BQ to isolated tubulin inhibits polymerization by binding at or near the GTP binding site (Irons and Neptun, 1980). This disruption could not be shown with CAT or PH. HQ, BQ and CAT also inhibit lymphocyte mitogenesis and agglutination, processes dependent on microtubule and sulfhydryl integrity (Sherline and Mundy, 1977; Chaplin and Wedner, 1978; Pfeifer and Irons, 1981; Epe et al., 1989). Microtubule disruption can result from chemical blockage of at least 2 of the multiple (4-11) titratable sulfhydryl moieties (Kuriyama and Sakai, 1974; Mellon and Rebhun, 1976; Ikeda and Steiner, 1978). Such sulfhydryl groups provide highly reactive nucleophilic centers for conjugate addition reactions with electrophilic compounds such as HQ or BQ (Pfeifer and Irons, 1983). Thus, these findings suggest that the synergistic induction of kinetochore-positive micronuclei may be occurring from mechanisms whereby one metabolite facilitates the binding of the other to tubulin sulfhydryl moieties. Studies exploring this hypothesis are currently underway in our laboratory. Alternative molecular mechanisms which may be responsible for the observed synergistic effect include the formation of a multi-polar spindle (Sellitto and Kuriyama, 1988), disruption of microtubule-associated proteins and chromosome-to-pole movement (Paschal and Valee, 1987; Koshland et al., 1988) or interference with kinetochore capture of microtubules (Mitchison and Kirschner, 1985). Cleavage of kinetochores, as is seen with caffeine (Brinkley et al., 1988), would result in poleward movement of kinetochores detached from the chromosomes yielding predominately kinetochore-negative micronuclei. Since kinetochores were not observed in the cytoplasm and because the majority of micronuclei observed in this study were kinetochore-positive and contained multiple kinetochores (Table 2), kinetochore cleavage is unlikely to be a plausible mechanism.

Consistent, non-random structural and numeri-

cal chromosomal changes have been observed in various forms of leukemia (Yunis. 1983; Rowley, 1984). The potential role for numerical chromosomal changes in carcinogenesis is becoming niore apparent as the importance of changes in gene dosage and balance, expression of recessive mutations and changes in genetic stability become realized (Oshimura and Barrett, 1986; Epstein, 1988). The role for aneuploidy as a primary event leading *to* carcinogenesis is also being clarified. especially with the recent evidence for the involvement of chromosomal loss in the cascade of events leading to colon carcinogenesis in humans (Vogelstein et al., 1989). The synergistic aneuploidy-inducing effect of HQ and CAT described here may therefore play a key role in the development of benzene-induced leukemia.

Acknowledgements

The authors are indebted to Dr. Janice W. Yager for her expert advice on the micronucleus assay, to Dr. Frederic Bois for the statistical analysis and to Mr. William M. Parasidin for technical assistance. M.L.R. is a trainee of the Health Effects Component of the U.C. Toxic Substances Program. Supportcd by NIH grants 1t2 ES04705 and P30 ES01896.

References

Barale, R., A. Marrazzini, C. Betti, V. Vangelisti, N. Loprieno and I. Barrai (1990) Genotoxicity of two metabolites of benzene: phenol and hydroquinone show strong synergistic effects in vivo, Mutation Res., 244. 15–20.

Billings, R.E. (1985) Mechanisms of catechol formation from aromatic compounds in isolated rat hepatocytes, Drug Metab. Dispos., 13, 287-290.

Brinkley, B.R., Zinkowski, R.P., Mollon, W.L., Davis, F.M., Pisegna. M.A., M. Pershouse and P.N. Rao (1988) Movement and segregation of kinetochores experimentally detached from mammalian chromosomes, Nature (London), 336, 251–254.

Cavanee, W.K.. Dryja, T.P., Phillips, R.A., Benedict, W.F., Gobout, R.. Gallic. R.L., Murphee, A.L.. L.C. Strong and R.L. White (1983) Expression of recessive alleles by chromosomal mechanisms in retinoblastoma, Nature (London), 305. 779–784.

Chaplin. D.D..and H.J. Wedner (1978) Inhibition of lectin-induced lymphocyte activation by diamide and other sulfhydryl reagents, Cell. Immunol.. 36, 303-31I.

Cooper. K.R. and R. Snyder (1988) Benzene metabolism (toxicokinetics and the molecular aspects of benzene toxicity), in: M. Aksoy (Ed.). Benzene Carcinogenicity. CRC Press, Boca Raton, FL, pp. 33–58.

Ding, X., Li, Y., Ding, Y., and H. Yang (1983) Chromosome changes in patients with chronic benzene poisoning, Chin. Med. J.. 96, 681–685.

Eastmond, D.A., Smith, M.T., arid R.D. Irons (1987) An interaction of henzene metabolites reproduces the myelotoxicity produced with benzene exposure. Toxicol. Appl. Pharmacol., 91, X5 95

Eastmond, D.A., and J.D. Tucker (1989) Identification of aneuploidy-inducing agents using cytokinesis-blocked human lymphocytes and an antikinetochore antibody, Environ. Mol. Mutagen.. 13, 34-43.

Epe, B., U.H. Harhig, D. Schiffmann and M. Metzler (1989) Microtubular proteins as cellular targets for carcinogenic estrogens and other carcinogens, in: M.A. Resnick and H.K. Vig (lids.). Mechanisms of Chromosome Distribution and Aneuploidy, Liss, New York, pp. 345–351.

Epstein, C.J. (1988)Mechanisms of the effects of aneuploidy in mammals, Annu. Rev. Genet.. 22, 51–75.

Erdogan, G., and M. Aksoy (1973) Cytogenetic studies in thirteen patients with pancytopenia and leukaemia associated with long-term exposure to benzene, New Istanbul Contrib. Clin. Sci., 10, 230–247.

Erexson, G.L., J.L. Wilmer and A.D. Kligerman (1985)Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites in vitro, Cancer Res.. 45. 2471-2477.

Fenech. M. and A.A. Morley (1985) Measurement of micronuclei in lymphocytes, Mutation Res.. 147. 29-36.

Forni, A., Cappellini, A., Pacifico, E. and E. Vigliani (1971) Chromosome changes and their evolution in subjects with past exposure to benzene, Arch. Environ. Health, 23. 385 391.

Gad-El-Karim, M.M., V.M. Sadagopoda Ramanujam and M.S. Legator (1985) *trans*,*trans*-Muconic acid, an open-chain urinary nietaholite of benzene in mice. Quantification by high pressure liquid chromatography. Xenobiotica, 15, 211 220.

Glatt, H., Padykula, R., Berchtold, G.A., Ludewig, G., Platt, K.L.. Klein. J. and F. Oesch (1989) Multiple activation pathways of henzene leading to products with varying genotoxic characteristics. Environ. Health Perspect., 82, 81-89.

Goldstein, B.D. (1989) Introduction: Occam's razor is dull, Environ. Health Perspect.. 82, 3–6.

Huff. J.E., J.K. Haseman, D.M. DeMarini, S. Eustis, R.R. Maronpot. A.C. Peters. R.L. Pershing. C.C. Chrisp and A.C. Jacobs (1989)Multiple-site carcinogenicity of benzene in Fisher 344 rats and B6C3F1 mice, Environ. Health Perspect.. 82. 125–163.

IARC (1988) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 10, L. Fishbein and I.K. O'Neill (Eds.), Environmental Carcinogens; Methods of Analysis and Exposure Measurement—Benzene and Alkylated Benzenes. Lyon, International Agency for Research on Cancer, pp. 3-18.

Ikeda, Y.. and M. Steiner (1978) 'Sulfhydryls of platelet tubulin: their role in polymerization and colchicine binding, Biochemistry, 17, 3454–3464.

Inoue, O., K. Seiji and M. Ikeda (1989) Pathways for formation of catechol and 1,2,4-benzenetriol in rabbits, Bull Environ. Contam. Toxicol., 43, 220–224.

Irons, R.D.. and D.A. Neptun (1980) Effects of the principal liydroxy-metabolites *of* benzene on *microtubule* polymerization, Arch. Toxicol.. 45, 297–305.

Johnson. G.D., and G.M. de C. Noguiera Araujo (1981) A simple method of reducing the fading of immunofluorescence during microscopy, J. Immunol. Methods, 43, 349–350.

Klein, G. (1981) The role of gene dosage and genetic transpositions in carcinogenesis, Nature (I-andon). 294, 313–316.

Koshland. D.E., T.J. Mitchison and M.W. Kirschner (1988) Polewards chromosome movement driven by microtubule depolymerization in vitro, Nature (London), 331, 499–504.

Kuriyania, R., and **H.** Sakai (1974) Role **of** tubulin –SH groups in polymerization to microtubules, J. Hiocheni., 76, 651–654.

Mellon, M.G., and L.I. Rebhun (1976) Sulfhydryls and the in vitro polymerization of tubulin, J. **Cell Biol.**, 70, 226–238.

Mitchison, T.J., and M.W. Kirschner (1985) Properties of the kinetochore in vitro, II. Microtubule capture and ATP-dependent translocation. J. Cell Biol., 101, 766–777.

National Cancer Institute (1980) Bioassay of phenol for possible carcinogenicity, NTP No. 80–15, **U.S.** Department of Health and Human Services, Technical Report Series, Vol. 203.

Oshimura, M., and J.C. Barrett (1986) Chemically induced aneuploidy in mammalian cella: Mechanisms and biological significance in cancer. Environ. Mutagen., 8, 129–159.

Paschal, B.M., **and** R.B. Vallee (1987) Retrograde transport by the microtubule associated protein MAP1C, Nature (London). 330, 181–183.

Pfeifer, R.W., and R.D. Irons (1981) Inhibition of lectin-stimulated lymphocyte agglutination and mitogenesis by hydroquinone: Reactivity with intracellular sulfhydryl groups, Exptl. Mol. Pathol., 35, 189–198.

Pfeifer. R.W., and R.D. Irons (1983) Alteration of lymphocyte function by quinones through a sulfhydryl-dependent disruption of microtubule assembly, Int. J. Immunopharmacol., 5, 463–470.

Rickert, D.E., Baker, T.S., Bus, J.S., Barrow, C.S. and R.D. Irons (1979) Benzene disposition in the rat after exposure by inhalation, Toxicol. Appl. Pharmacol., 49, 417–423.

Rowley, J.D. (1984) Biological implications **of** consistent chromosome rearrangements in leukemia and lymphoma, Cancer Res., **44,** 3159–3161.

Sawahata, **T.,** and K.A. Neal (1983) Biotransformation **of** phenol to hydroquinone and catechol by rat liver microsomes, Mol. Pharmacol., 23, 453 460.

Sellitto, C., and R. Kuriyama (1988) Distribution **of** pericentriolar material in multipolar spindles induced by colcemid **treatment** in Chinese hamster ovary cells. J. Cell Sci., 89, 57–65.

Sherline. P., and G.R. Mundy (1977) Role of the tubulin-microtubule system in lymphocyte activation. J. Cell. Biol.. 74, 371–376.

Smith, M.T.. Yager, J W., Steinmetz, K.M. and D.A. Eastmond (1989) Peroxidase-dependent metabolism of benzene's phe**nolic** metabolites and its potential role in benzene toxicity and carcinogenicity, Environ. Health Perspect., 82, 23–29.

Smith, M.T., Robertson, M.L., Yager. J.W. arid D.A. Eastmond (1990) Role of metabolism in benzene-induced myelotoxicity and leukemogenesis, in: M.L. Mendelsohn and K.J. Albertini (Eds.), Mutation and the Environment; Part B: Metabolism, Testing Methods, and Chromosomes. Wiley-Liss, New York, pp. 125–136.

Snyder. It.. Dimitriadis, **E.,** Guy, R., Hu, P., Cooper. K., Hauer, H., Witz, G. and B.D. Goldstein (1989) Studies **on** the mechanism of benzene toxicity, Environ. Health Perspect., 82, 31–35.

Subrahnianyam. V.V.. Doane-Setzer, P., Steinmetz, K.L., Ross, **D)** and M.T. Smith (1990) Phenol-induced stimulation of hydroquinone bioactivation in mouse bone marrow in vivo: Possible implications in benzene niyelotoxicity, Toxicology, 62, 107–116.

Tice, R.R., T.F. Vogt and D.L. Costa (1982) Cytogenetic effects **of** inhaled benzene in murine bone marrow, in: R.R. Tice, T.F. Vogt and K.M. Schaich (Eds.), Genotoxic Effects of Airborne Agents, Plenum, New York. pp. 257–275.

Tsutsui, T., Maizumi, H., McLachlan, **J.A.,** and J.C. Barrett (1983) Aneuploidy induction **and** cell transformation by diethylstilbestrol: A possible chromosomal mechanism in carcinogenesis, Cancer Res., 43, 3814–3821.

Vogelstein, **H.,** E.R. Fearon, S.E. Kern, S.R. Hamilton, A.C. Preisinger, Y. Nakamura **and R.** White (1989) Allelotype of colorectal carcinomas, Science, 244, 207–211.

Yager, J.W., Eastmond, D.A., Robertson, M.L., Paradisin, W.M. and M.T. Smith (1990) Characterization of niicronuclei induced in human lymphocytes by benzene metabolites, Cancer Res., 50, 393–399.

Yunis, J.J. (1983) The chromosomal basis of human neoplasia, Science, 221, 227–236.