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TITLE: ALTERATIONS IN THE METABOLISM OF BENZO[a]PYRENE IN SYRIAN HAMSTER EMBRYO (SHE) CELLS PRETREATED WITH PHENOLIC ANTIOXIDANTS

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ALTERATIONS IN THE METABOLISM OF BENZO[a]PYRENE IN SYRIAN HAMSTER EMBRYO (SHE) CELLS PRETREATED WITH PHENOLIC ANTIOXIDANTS

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INTRODUCTION

Inhibition of chemical- or radiation-induced neoplasia has been observed in animals whose diets were supplemented with antioxidants commonly used as food additives (4,5,28, 29). In a study reported more than a decade ago, inhibition of the carcinogenicity of benzo[a]pyrene (BaP) in the forestomach of mice or of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammory tumors in rats was achieved by the addition of the phenolic antioxidents butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) to the animals' diets (27). The determination of the mechanism(s) by which antioxidants exert their anticarcinogenic effects has been the subject of numerous reports. Various antioxidants have been shown to modulate the metabolism of polycyclic aromatic hydrocarbons (PAH) in such a manner as to drastically reduce the amount of metabolized PAH bound to cellular DNA (1,12, 14,16,23,24). Several hypotheses concerning this effect have ascribed antioxidants as: a) inhibitors of aryl hydrocarbon hydroxylase (AHH) through physical binding to cytochrome P-450 (30,31); b) modulators in the induction and composition of microsomal monooxygenases (12,17,24,26); c) inducers of microsomal epoxide hydratase (3,7,8); and d) inducers of increased conjugation capacity (2,3). Effective reductions in the amounts of reactive, electrophilic metabolites of parent PAH mediated by antioxidants would necessarily affect PAH-DNA binding potential, thus reducing PAH genotoxic potential.

Due to tissue specific differences in the quality and quantity of PAH metabolism and in antioxidant activity, we initiated a study two years ago employing an *in vitro* Syrian hamster embryo (SHE) cell-mediated genotoxicity assay originally described by Huberman and Sachs (15). Our aim was to assess concurrently the influence of the phenolic antioxidant, BHA, on BaP metabolism and genotoxicity *in vitro* (9). In this report we present recent data in which we compare alterations in the metabolism and mutagenicity of

BaP by BHA with two other commonly used antioxidants, BHT and p-methoxyphenol (p-MP).

MATERIALS AND METHODS

Chemicals

BHA, BHT, p-MP and unlabeled BaP were purchased from Aldrich Chemical Co. (Milwaukee, WI). General labeled [^{3}H]-BaP (25 Ci/mmol) was obtained from Amersham (Arlington Heights, IL) and diluted with unlabeled BaP to obtain specific activities of 300-1800 cpm/pmol. All organic solvents were analytical or spectrophotometric grade. Authentic standards of metabolites of BaP were obtained from the NCI Chemical Carcinogen Reference Standard Repository, NCI, NIH (Bethesda, MD). Ultrapure grades of guanidine•HCl and Cs_2SO_4 were from Schwarz/Mann (Orangeburg, NY).

Cells and Culture Conditions

Chinese hamster cells (line CHO-AA8-4) were cultured under conditions described elsewhere (9,25). Cytotoxicity and mutagenicity assays employing CHO as target cells and X-irradiated (4000 r) "feeder" SHE cells have been described in detail elsewhere (9,15,25). Primary SHE cell cultures were prepared by trypsinization of 13-day-old fetuses of randomly bred Syrian hamsters as described elsewhere (9). Near confluent cultures were trypsinized and stored in liquid nitrogen. Second to fourth passages of these cultures were used in experiments designed to measure BaP metabolism, mutagenesis in target CHO cells and BaP-macromolecular binding.

BaP Metabolism

Analysis of extracellular and intracellular metabolites of BaP was performed using X-irradiated SHE cells that were plated at 2 x 10^6 cells per 60-mm dish and incubated at 37° for 24 hours before the addition of antioxidants. After an additional 18 hours of incubation, [^{3}H]-BaP was added to 1 µg/ml and 1 ml aliquots of medium were removed at noted times and immediately extracted twice with 2.5 volumes each of ethyl acetate. Partitioning of [^{3}H]-BaP and [^{3}H]-BaP-metabolites into aqueous (medium) and organic (ethyl acetate) layers was determined by assessing radioactivity of aliquots of each layer in a liquid scintillation spectrophotometer.

ANTIOXIDANT INDUCED ALTERATIONS IN BaP METABOLISM

High pressure liquid chromatography (h.p.l.c.) was performed on vacuum-evaporated aliquots of the ethyl acetate layers. After drying, residues of each sample were solubilized in 100-200 µl of methanol. Samples (20 µl) were injected and eluted with a water:acetonitrile gradient (70:30 to 0:100, v/v) through an Altex reverse phase analytical Ultrasphere-ODS column (4.5 x 150 mm) with 5 µ packing using a Beckman Model 334 h.p.l.c. system as previously described (9). Retention times (R_t) of various BaP metabolites were compared to R_t of authentic BaP standards.

SHE cell-mediated aqueous solubilization of BaP occurs primarily by glucuronide conjugation (10,19,21). Release of these conjugated metabolites was achieved in the following manner. Ethanol precipitates of aqueous extracts were resuspended in 0.1 M phosphate buffer (pH 6.8) in 0.4 ml and incubated at 37° overnight with 1000 Sigma units of E. coli β-glucuronidase type VIII (Sigma Chemical Co., St. Louis MO). Aliquots of these reaction mixes were extracted twice with 2.5 volumes each of ethyl acetate. H.p.l.c. analysis of the organic layers was performed as described above and elsewhere (9).

Analysis of intracellular BaP metabolites and BaP-macromolecular binding was performed using populations of >2 x 10^7 SHE cells. A minimum of ten 60 mm plates with 2 x 10^6 x-irradiated SHE cells per plate was used for each dose of antioxidant tested. After pretreatment with antioxidant and incubation with BaP as described above, cells were harvested by trypsinization and centrifugation. Cytoplasmic extracts and nuclei were prepared on washed cell pellets as described by MacLeod et al. (19). Ethyl acetate soluble BaP metabolites from extracted cytoplasmic fractions were determined by h.p.l.c. as described above.

BaP-Macromolecular Binding

Nuclei preparations were dissolved in 6 M guanidine·HCl, 10 mM EDTA and sonicated. After extraction of the nuclear lysate with ethyl acetate, an aliquot of the aqueous layer was applied to a 2.2 M Cs_2SO_4 solution containing 10 mM EDTA and 9% dimethyl sulfoxide (v/v) and centrifuged according to the procedures established by MacLeod et al. (20). Liquid scintillation spectroscopy of fractionated gradients allowed for the determination of the isopycnic separation of [^{3}H]-BaP-nuclear macromolecules.

RESULTS AND DISCUSSION

Antioxidant-Induced Cytotoxicity in CHO Cells

We previously reported that co-incubation of BHA (to 40 µg/ml) with CHO cells for 48 hours resulted in no significant differences in their colony forming ability compared to control, untreated cells (9). However, BHT and p-MP are toxic to CHO, especially at higher concentrations. For example, incubation of CHO with 20 µg/ml of either BHT or p-MP for 48 hours resulted in 25% or > 80% killing, respectively (data not shown). Phenolic antioxidants have been shown to induce cytotoxicity in other cultured cell systems (18) and recently have been shown to inhibit excision repair synthesis in isolated human peripheral lymphocytes (11). This latter effect, however, was not observed in cultured rodent cells (13).

Inhibition of BaP-Induced Mutagenicity by Antioxidants

Many cultured cell systems, including CHO, lack the capacity to metabolize promutagens/procarcinogens to their active forms. However, target cells, generally chosen for their high plating efficiency, can be co-cultivated with lethally-irradiated "feeder" layers of competent cells (e.g., early passage SHE cells) that provide metabolic capacity. This is the basis of the cell-mediated assay developed previously by Huberman and Sachs (15). Of considerable importance is the recent report by Sebti et al. (22) where it was shown that DNA-BaP adduct formation was both qualitatively and quantitatively similar in target cells compared with the "feeder" cells. This finding adds considerable credibility to the assumption that genotoxicity observed in target cells is a direct consequence of transport of mutagenic metabolites from "feeder" cells to the nuclei of target cells.

In a previous report, we presented data on BHA inhibition of BaP-induced mutagenesis in target CHO cells using a SHE cell-mediated assay (9). In Figure 1 we compare the antimutagenic properties of three antioxidants as a function of their concentration in the culture medium. In control, non-antioxidant-treated cultures ~700 6-thioguanine resistant (6-TG^R) mutants were induced per 10^6 target CHO cells after exposure to BaP at 1 µg/ml for 48 hours. Mutagenicity of BaP can be reduced by 65-70% by prior treatment of the SHE "feeder" cells with 10 µg/ml of p-MP or 20 µg/ml of either BHA or BHT for 18 hours before the addition of BaP.

ANTIOXIDANT INDUCED ALTERATIONS IN BaP METABOLISM

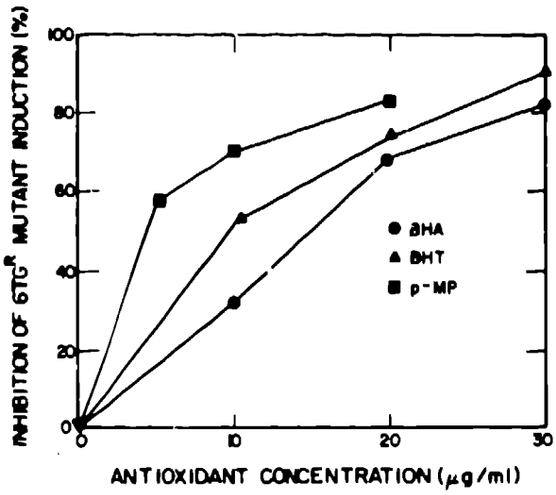


FIGURE 1. Antimutagenic effects of antioxidants in target CHO cells after co-incubation with BaP in the SHE cell-mediated assay. The mutagenicity assay and measurement of 6-TG^R mutants are described elsewhere (9,25).

The extreme cytotoxicity of p-MP at 20 μg/ml necessitated its use at lower concentrations. At the concentrations of antioxidants used, no significant mutagenicity was observed in the target CHO cells in the absence of BaP.

Effects of Antioxidants on BaP Metabolism

As shown above, BHA, BHT and p-MP dramatically suppress the mutagenicity of BaP in target CHO cells in a SHE cell-mediated assay. In addition, we have previously demonstrated that BaP-induced morphological transformation of cultured SHE cells was inhibited ~75% by the addition of 20 μg/ml BHA to the culture medium (9). To determine the biochemical mechanism(s) by which antioxidants protect cells against BaP-induced mutation and transformation *in vitro*, we initiated studies to examine the metabolism of BaP in SHE cells pretreated with p-MP, BHA or BHT.

In Figure 2 we present data concerning the partitioning of [^{3}H]-BaP metabolites into the aqueous phase after aliquots of the extracellular medium were extracted with ethyl acetate. The amount of aqueous-soluble BaP metabolites [presumably glucuronide conjugates of 3-OH- and 9-OH-BaP (10,20)] is reduced in the antioxidant-treated cultures.

ANTIOXIDANT INDUCED ALTERATIONS IN BaP METABOLISM

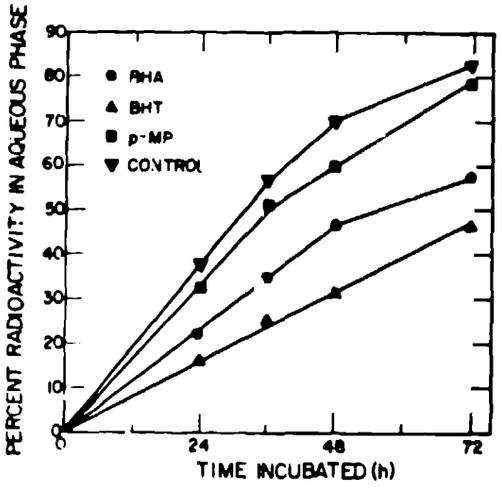
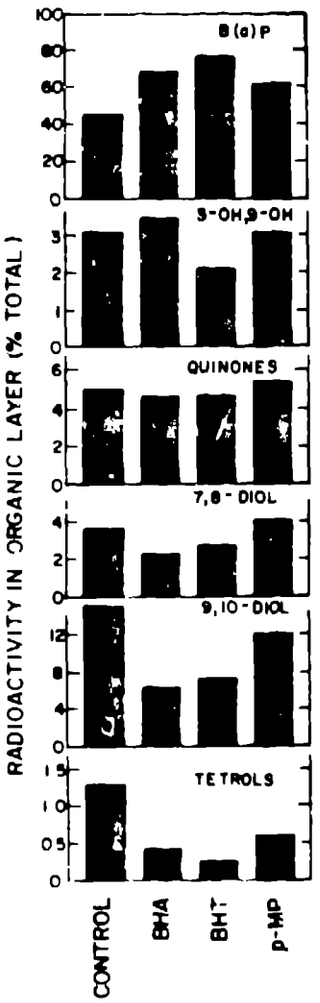


FIGURE 2. Alterations in the metabolism of BaP in SHE cells by various antioxidants. X-irradiated SHE cells were pretreated with antioxidants 18 hours prior to the addition of [^{3}H]-BaP at 1 µg/ml. Aliquots of medium were liquid-liquid extracted at noted times, and the percent of radioactivity partitioning into the aqueous phase was determined. Concentrations of antioxidants used were 10 or 20 µg/ml for p-MP or BHA and BHT, respectively.

This action could be the result of antioxidant-mediated reduction in the overall metabolism of BaP and/or in the conjugation of substrate BaP phenols to aqueous-soluble glucuronides. To distinguish between these possibilities, h.p.l.c. analysis of the ethyl acetate extracts of the extracellular medium was performed on aliquots sampled 36 hours post addition of the BaP. In Figure 3 we show the relative amounts of the various BaP metabolites and parent compound that partitioned into the organic phase as determined by h.p.l.c. The quantity of each metabolite is expressed as a percentage of the total eluting material and was determined from the amount of ^{3}H-cpm at its designated R_t relative to the total ^{3}H-cpm recovered from each chromatograph. The 3-OH- and 9-OH-BaP metabolites are grouped together since their R_t were essentially coincident in our gradient elution conditions. It is apparent from the analysis of this data that overall BaP metabolism mediated by SHE cells is generally reduced by the addition of antioxidants, especially with BHT. In absolute terms, although only 19% of [^{3}H]-BaP exists after 36 hours incubation in the control

ANTIOXIDANT INDUCED ALTERATIONS IN BaP METABOLISM



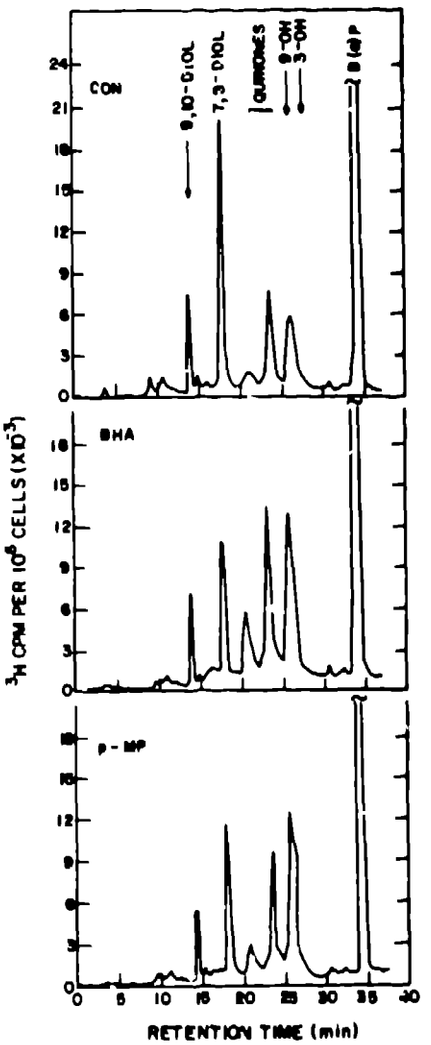


FIGURE 3. (left) Effects of antioxidants on the composition of SHE cell-mediated extracellular, organic-soluble BaP metabolites. Culture conditions for the 36 hour incubation are described in Figure 2 legend and in the Materials and Methods section.

FIGURE 4. (right) H.p.l.c. of intracellular, organic-soluble BaP metabolites in SHE cells pretreated with antioxidants. Culture conditions are the same as described in Figures 2 and 3 legends.

extracellular medium, (i.e., the amount of unmetabolized BaP that partitioned into the organic phase X the amount of organic-soluble BaP and metabolites in the extracellular medium), between 30 and 55% of unmetabolized BaP remains in the extracellular medium containing antioxidants.

Similar h.p.l.c. analysis was performed on the extracellular, aqueous-soluble BaP metabolites after treatment with β-glucuronidase and re-extraction with ethyl acetate. The amount of aqueous-soluble metabolites (primarily 3-OH- and 9-OH-BaP) as glucuronide conjugates was significantly reduced (40-60% of control values) in the extracellular medium for antioxidant-treated SHE cells (data not shown).

Another striking difference in this data is the amount of BaP tetrols (hydrolysis products of unstable but highly reactive BaP-diol-epoxides). In absolute terms, the amounts of extracellular tetrols present are reduced 2-3 fold in antioxidant-treated SHE cells compared to control cultures.

In Figure 4 the results of h.p.l.c. of intracellular, organic-soluble BaP metabolites in SHE cells are shown for control cultures and cultures pretreated with either BHA (20 mg/ml) or p-MP (10 μg/ml). The amount of 7,8-diol-BaP is reduced 30-50% in the antioxidant-treated SHE cultures. However, the amounts of 3-OH- and 9-OH-BaP are greater in the antioxidant-treated cells compared to the control culture.

Our data suggest that in SHE cells antioxidants inhibit the overall metabolism of BaP to its various oxidized moieties including 7,8-diol- and 7,8,9,10-tetrol-BaP [precursor and hydrolysis products, respectively, of the presumptive ultimate carcinogen, 7,8-diol-9,10-epoxide-BaP (6)]. This finding is similar to those reported on antioxidant reduction in BaP metabolism in isolated microsomes from various rodent tissues (17,30,31). A plausible explanation for our results with SHE cells is that the antioxidants interact directly with AHH as suggested by Yang *et al.* (30,31), thus inhibiting AHH metabolic capacity.

As noted above, there are reduced levels of aqueous-soluble BaP metabolites as glucuronide conjugates in the extracellular medium and apparent elevations in the amounts of intracellular BaP monophenols (3-OH- and 9-OH-BaP) in antioxidant-treated SHE cells. If antioxidants induced an increase in the conjugation capacity in SHE cells, as has been observed in isolated rodent hepatic microsomes (2,3), a diminution and not an elevation of these substrates would have been expected.

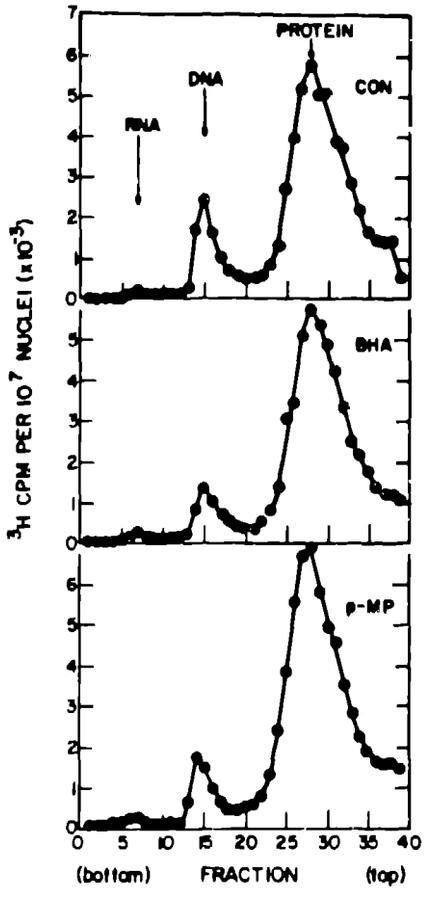


FIGURE 5. Isopycnic sedimentation of [^{3}H]-BaP-labeled macromolecules in Cs_2SO_4. Nuclear macromolecules were isolated from SHE cells treated with antioxidants 18 hours prior to the addition of BaP of 1 μg/ml and further incubation for an additional 36 hours. Sedimentation analysis was performed as described elsewhere (19,20).

Effects of Antioxidants on BaP-Macromolecular Binding

As discussed above, antioxidants reduce SHE cell-mediated metabolism of BaP resulting in lower intra- and extracellular levels of BaP diols and tetrols. Inhibition by antioxidants in the formation of reactive, electrophilic BaP metabolites should result in the reduction of BaP binding to nuclear macromolecules, especially to DNA, the genetic target. In Figure 5, isopycnic sedimentation profiles in Cs_2SO_4 of [^{3}H]-BaP-labeled nuclear macromolecules are

shown. Analysis of nuclear material from SHE cells (+ antioxidants) incubated for 36 hours with BaP at 1 µg/ml was performed according to the procedures of MacLeod et al. (20). Realizing the specific activity of the [^{3}H]-BaP, it is calculated that 4.6, 2.4 and 2.9 pmol BaP are bound to the DNA isolated from 10^7 nuclei of control, BHA-(20 µg/ml) and p-MP-(10 µg/ml) treated cultures, respectively.

A reduction in metabolic capacity by physical binding of antioxidants to cytochrome P-450 (30,31) and/or alterations in the regioselectivity of the microsomal monooxygenase system (26) are plausible explanations for antioxidant-induced reductions in SHE cell-mediated BaP metabolism. A direct consequence of such action is the reduction of BaP binding to nuclear macromolecules that most likely accounts for the suppression of BaP mutagenicity and transformation potential in the SHE cell-mediated assays reported here and elsewhere (9).

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REFERENCES

1. Anderson, M.W., Boroujerdi, M., and Wilson, A.G.E. (1981): Inhibition in vivo of the formation of adducts between metabolites of benzo[a]pyrene and DNA by butylated hydroxyanisole. Cancer Res., 41:4309-4315.

2. Benson, A.M., Batzinger, R.P., Ou, S-Y.L., Bueding, E., Cha, Y-N., and Talalay, P. (1978): Elevation of hepatic glutathione S-transferase activities and protection against mutagenic metabolites of benzo[a]pyrene by dietary antioxidants. Cancer Res., 38:4486-4495.

3. Benson, A.M., Cha, Y-N., Bueding, E., Heine, H.S., and Talalay, P. (1979): Elevation of extrahepatic glutathione S-transferase and epoxide hydratase activities by 2(3)-tert-butyl-4-hydroxyanisole. Cancer Res., 39:2971-2977.

4. Black, H.S. and Chan, J.T. (1975): Suppression of ultraviolet light-induced tumor formation by dietary antioxidants. J. Invest. Dermatol., 65:412-414.

5. Black, H.S., Chan, J.T., and Brown, G.E. (1978): Effects of dietary constituents on ultraviolet mediated carcinogenesis. Cancer Res., 38:1384-1337.

6. Buening, M.K., Wislocki, P.G., Levin, W., Yagi, H., Thakker, D.R., Akagi, H., Koreeda, M., Jerina, D.M., and Conney, A.H. (1978): Tumorigenicity of the optical enantiomers of the diastereomeric benzo[a]pyrene 7,8-diol-9,10-epoxides in newborn mice: exceptional activity of (+)7β, 8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. Proc. Natl. Acad. Sci., USA, 75:5358-5361.

7. Cha, Y-N., Martz, F., and Bueding, E. (1978): Enhancement of liver microsome epoxide hydratase activity in rodents by treatment with 2(3)-tert-butyl-4-hydroxyanisole. Cancer Res., 38:4496-4498.

8. Cha, Y-N., Heine, H.S., and Moldeus, P. (1982): Differential effects of dietary and intraperitoneal administration of antioxidants on the activities of several hepatic enzymes in mice. Drug Metabolism and Disposition, 10:434-435.

9. Chen, D.J., Okinaka, R.T., and Strniste, G.F. (1982): Effects of butylated hydroxyanisole on the metabolism and mutagenic and transformation potentials of benzo-[a]pyrene in cultured mammalian cells. In: Polynuclear Aromatic Hydrocarbons: Formation, Metabolism and Measurement, edited by M. Cooke and A.J. Dennis, pp. 303-317, Battelle Press, Columbus, Ohio.

10. Cohen, G.M., MacLeod, M.C., Moore, C.J., and Selkirk, J.K. (1980): Metabolism and macromolecular biology of carcinogenic and noncarcinogenic metabolites of benzo-[a]pyrene by hamster embryo cells. Cancer Res., 40:207-211.

11. Daugherty, J.P., Davis, S. and Yielding, K.L. (1978): Inhibition by butylated hydroxytoluene of excision repair synthesis and semiconservative DNA synthesis. Biochem. Biophys. Res. Commun., 80:963-969.

12. Dock, L., Cha, Y-N., Jernstrom, B., and Moldeus, P. (1982): Effect of 2(3)-tert-butyl-4-hydroxyanisole on benzo[a]pyrene metabolism and DNA-binding of benzo[a]-pyrene metabolites in isolated mouse hepatocytes. Chem.-Biol. Interactions, 41:25-37.

13. Goodman, J.I., Trosko, J.E., and Yager, J.D., Jr. (1976): Studies on the mechanism of inhibition of 2-acetylaminofluorene toxicity by butylated hydroxytoluene. Chem.-Biol. Interactions, 12:171-182.

14. Harris, C.C., Frank, A.L., van Haaften, C., Kaufman, D.G., Conner, R., Jackson, F., Barrett, L.A., McDowell, E.M., and Trump, B.F. (1976): Binding of [^{3}H]benzo-[a]pyrene to DNA in cultured human bronchus. Cancer Res., 36:1011-1018.

15. Huberman, E. and Sachs, L. (1976): Mutability of different genetic loci in mammalian cells by metabolically activated carcinogenic hydrocarbons. Proc. Natl. Acad. Sci., USA, 73:188-192.

16. Ioannou, Y.M., Wilson, A.G.E., and Anderson, M.W. (1982): Effect of butylated hydroxyanisole on the metabolism of benzo[a]pyrene and the binding of metabolites to DNA, in vitro and in vivo, in the forestomach, lung and liver of mice. Carcinogenesis, 3:739-745.

17. Lam, L.K.T. and Wattenberg, L.W. (1977): Effects of butylated hydroxyanisole on the metabolism of benzo-[a]pyrene by mouse liver microsomes. J. Natl. Cancer Inst., 58:413-417.

18. Leslie, S.W., Gad, S.C., and Acosta,D. (1978): Cytotoxicity of butylated hydroxytoluene and butylated hydroxyanisole in cultured heart cells. Toxicology, 10:281-289.

19. MacLeod, M.C., Cohen, G.M., and Selkirk, J.K. (1979): Metabolism and macromolecular binding of the carcinogen benzo[a]pyrene and its relatively inert isomer benzo[e]-pyrene by hamster embryo cells. Cancer Res., 39:3463-3470.

20. MacLeod, M.C., Mansfield, B.K., Huff, A., and Selkirk, J.K. (1979): Simultaneous preparation of nuclear DNA, RNA, and protein from carcinogen treated-hamster embryo fibroblasts. Anal. Biochem., 97:410-417.

21. Nemoto, N., Hirakawa, T., and Takayama, S. (1979): Metabolism of benzo[a]pyrene in hamster embryo cells. Effects of the concentration of benzo[a]pyrene on its metabolism. Chem.-Biol. Interactions, 25:177-188.

22. Sebti, S.M., Baird, W.M., Knowles, B.B., and Diamond, L. (1982): Benzo[a]pyrene-DNA adduct formation in target cells in a cell-mediated mutation assay. Carcinogenesis, 3:1317-1320.

23. Slaga, T.J. and Bracken, W.M. (1977): The effect of antioxidants on skin tumor initiation and aryl hydrocarbon hydroxylase. Cancer Res., 37:1631-1635.

24. Speier, J.L. and Wattenberg, L.W. (1975): Alterations in microsomal metabolism of benzo[a]pyrene in mice fed butylated hydroxyanisole. J. Natl. Cancer Inst., 55:469-472.

25. Strniste, G.F. and Chen, D.J. (1981): Cytotoxic and mutagenic properties of shale oil byproducts. I. Activation of retort process water with near ultraviolet light. Environ. Mutagenesis, 3:221-231.

26. Sydor, W., Jr., Chow, M.W., Yang, S.K., and Yang, C.S. (1983): Regioselective inhibition of benzo[a]pyrene metabolism by butylated hydroxyanisole. Carcinogenesis, 4:131-136.

27. Wattenberg, L.W. (1972): Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. J. Natl. Cancer Inst., 48:1425-1430.

28. Wattenberg, L.W. (1979): Inhibitors of carcinogenesis. In: Carcinogens: Identification and Mechanisms, edited by A.C. Griffin and C.R. Shaw. pp. 299-316, Raven Press, New York.

29. Wattenberg, L.W., Coccia, J.B., and Lam, L.K.T. (1980): Inhibitory effects of phenolic compounds on benzo[a]-pyrene-induced neoplasia. Cancer Res., 40:2820-2823.

30. Yang, C.S., Strickhart, F.S., and Woo, G.K. (1974): Inhibition of the mono-oxygenase system by butylated hydroxyanisole and butylated hydroxytoluene. Life Sciences, 15:1497-1505.

31. Yang, C.S., Sydor, W., Jr., Martin, M.B., and Lewis, K.F. (1981): Effects of butylated hydroxyanisole on the aryl hydrocarbon hydroxylase of rats and mice. Chem.-Biol. Interactions, 37:337-350.

Key Words (phrases)

Chinese hamster (CHO) cells

Syrian hamster embryo (SHE) cells

BaP metabolism

cytotoxicity, mutagenicity

phenolic antioxidants

benzo[a]pyrene, butylated hydroxyanisole, butylated hydroxytoluene, p-methoxyphenol

β-glucuronidase

DNA, DNA binding

h.p.l.c.

liquid-liquid extraction

isopycnic sedimentation