Selective trapping by glutathione conjugation of the benzo(a)pyrene-7,8-diol-9,10-epoxide moeties which bind to DNA

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ABSTRACT

The role of glutathione (GSH) conjugation in protecting nuclear DNA from the electrophilic attack of reactive benzo[a]pyrene (BaP) metabolites was examined in a variety of mammalian cell lines which differ in their sensitivity to the toxic effects of BaP. Reactive BaP metabolites were generated intracellularly or extracellularly using rat liver microsomes as activation system. The results suggest that 3 major types of cell line can be distinguished according to the capacity for GSH conjugation and DNA binding of extracellularly generated BaP metabolites. The first cell type is represented by HepG2 cells which conjugated BaP metabolites only very poorly with GSH. The number of DNA adducts in these cells reached about 10 pmol/mg DNA, which was taken to be the maximum DNA binding in 'unprotected' cells. The second type of cell line, e.g. V79 and NCI-H322, was also 'unprotected' as indicated by maximum DNA binding. However, this cell type was capable of conjugating BaP metabolites with GSH. The third type of cell, e.g. 2sFou and H4IIEC3/G$^-$, showed only very little DNA binding and was positive for GSH conjugation. When GSH conjugation in these apparently 'protected' cells was suppressed by depletion of GSH, the level of DNA binding increased to that found in 'unprotected' lines. GSH depletion did not substantially affect DNA binding in the latter.

As demonstrated in 2sFou cells, GSH conjugation also 'protected' against the DNA binding of reactive metabolites generated intracellularly from BaP or trans-7,8-dihydroxy-7,8-dihydro-BaP (BaP-7,8-diol). Thus GSH depletion increased DNA binding of BaP and BaP-7,8-diol metabolites more than 10fold in these cells. In contrast to the marked GSH-dependent difference in DNA binding, GSH conjugation trapped at most 1/3 of the BaPDE generated from BaP-7,8-diol.

Taken together, the results suggest that BaPDE is contained in more than one intracellular pool. BaPDE species which bind to DNA appear to be contained in a relatively small pool where they are accessible to GSH conjugation in 'protected' cells only and not in 'unprotected' cells.

INTRODUCTION

Benzo(a)pyrene (BaP) has been studied extensively for its metabolism and biological effects using a large variety of experimental systems (1,2). However, the distinct tissue specificity noted for this carcinogen is not well understood. It is not known, for example, why rat liver is resistant to the toxic effects of BaP whereas extrahepatic tissues of rats such as skin, forestomach or mammary gland are susceptible.

BaP is activated to cytotoxic and genotoxic products by cytochrome P-450-dependent monooxygenases (3). The ultimate carcinogenic form of BaP is thought to be BaP-7,8-diol-9,10-epoxide (BaPDE), specifically the (+)enantiomer of r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene (anti-BaPDE) (4-6). The diolepoxide binds covalently to DNA, primarily to the N2-position of guanine (7,8). Anti-BaPDE, once formed, may be enzymatically conjugated with glutathione (GSH) (9), spontaneously hydrolyzed to tetraols or reduced to triols (10,11).

As both BaP sensitive and insensitive tissues are capable of activating the chemical (2), their different susceptibilities are probably attributable to different defense mechanisms. A number of observations indicate that GSH conjugation counteracts the formation of anti-BaPDE-DNA adducts in preparations of rat hepatocytes (12-14). These

findings support the hypothesis that the trapping of BaPDE by GSH conjugation protects the liver of adult rats against the carcinogenic effect of BaP (13,15). However, there is reason to doubt that it is merely the overall capacity for GSH conjugation which determines the susceptibility of cells to DNA binding of BaPDE. For example, we have observed that mouse fibroblasts C3H/10T1/2 are not protected against the formation of BaPDE-DNA adducts (16), although they are able to form substantial amounts of BaPDE-GSH conjugates (16,17). Moreover, protected cells, such as hepatocytes, conjugate only part of the BaPDE formed from the proximate carcinogen (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene (BaP-7,8-diol) (18).

In view of these discrepancies we have re-examined the role of GSH conjugation in the cell-specific defense against the binding of BaPDE to DNA. To this end, we suppressed GSH conjugation by depleting intracellular GSH and determined whether and to what extent this increases DNA binding of reactive metabolites formed from BaP or BaP-7,8-diol. As a model for 'protected' cells we used the well-differentiated variant line of rat hepatoma H4IIEC3 cells, 2sFou (19). These cells were chosen because they are, like hepatocytes, capable of metabolizing BaP (20), yet are highly resistant to the cytotoxic and genotoxic effects of the polycyclic hydrocarbon (21-23).

'Protected' cells such as 2sFou and 'unprotected' cells such as C3H/10T1/2 (16) might differ qualitatively and

quantitatively in pathways of BaP metabolism other than that of GSH conjugation, potentially obscuring the true role of GSH conjugation in the protection against DNA binding. For this reason we tested whether 2sFou cells are also protected against reactive BaP metabolites generated extracellularly, employing rat liver microsomes as activation system. Using this system, we monitored the effect of GSH depletion in several cell lines which were selected on the basis of their known sensitivity to the toxic effects of BaP. For example, among the cells chosen, V79, NCI-H322 and HepG2 have been shown to be susceptible to BaP toxicity (24-26), whereas H4IIEC3/G$^-$ and H5 appear to be largely protected against the polycyclic hydrocarbon (22,21). Another criterion for the selection was the capacity for GSH conjugation. HepG2 cells were of particular interest in this respect, since they have unusually low GSH transferase activities (Krupski-Brennstuhl, PhD thesis, München 1989) and may thus serve as a genuine example of cells not protected by GSH conjugation.

MATERIALS AND METHODS

Chemicals and enzymes were obtained from the following sources: [G-^{3}H]BaP (specific activity 15-40 Ci/mmol) and [7,10-^{14}C]BaP (specific activity 30-50 mCi/mmol) from Amersham Buchler GmbH, Braunschweig; [^{3}H]BaP-7,8-diol (specific activity 416 mCi/mmol); [^{3}H](±)anti-BaPDE (specific activity 479 mCi/mmol) from the NCI Chemical Carcinogen Reference Standard Repository, a function of the Division of Cancer Etiology, NCI, NIH, Bethesda, Maryland, 20892, USA; BaP from Carl Roth, Karlsruhe; 1-fluoro-2,4-dinitrobenzene from Serva, Heidelberg; 1-chloro-2,4-dinitrobenzene (CDNB) from Sigma Chemie, Deisenhofen; NADPH and all enzymes from Boehringer, Mannheim; Ultrasphere ODS and Spherisorb-NH_2 columns (21 cm x 4 mm) from Beckman Instruments, Munich, and Besta, Heidelberg, respectively, all F.R.G.; tissue culture supplies from Biochrom KG, Berlin, F.R.G.; buthionine sulfoximine (BSO) from Chemical Dynamics Cooperation, New Jersey, USA.

Cell cultures: 2sFou, H4IIEC3/G^{-} (are referred to as 'H4IIE' in the following), and H5 cells are derived from the Reuber hepatoma (27). They were kindly provided by Dr. M.C. Weiss (Institut Pasteur, Paris, France). The cells have been well characterized with regard to their expression of liver specific functions (28-30) including the expression of

cytochromes P-450 (31). WRC cells were obtained from American Type Tissue Culture Collection (Biochrom), 208F cells from Dr. Schmidt, Department of Molecular Cell Pathology of the GSF, V79 cells from Flow Laboratories, Meckenheim, F.R.G., NCI-H322 cells from Dr. Schuller, Dept. Pathobiol., Coll. Vet. Med. Knoxville, Tenn, USA and HepG2 cells, from Dr. B. Knowles, Wistar-Institute, Philadelphia, USA. Cultures were grown in Hams F12 : NCTC 135 (1:1) media containing 5% fetal calf serum (rat hepatoma cells), Dulbecco's Modified Eagle's medium (WRC, 208F, V79), Eagle's Minimal Essential Medium (HepG2), or RPMI 1640 (NCI-H322), all supplemented with 10% fetal calf serum. Penicillin (100 units/ml) and streptomycin (100 µg/ml) were added to all media. Cells were grown at 37°C in 7% CO_2 :93% air. They were passaged every 3-8 days by replating 1-2 x 10^5 cells/100 mm plate.

Depletion of GSH: Cells were treated with BSO (0.2 mM) and CDNB (2 µM), as previously described (16). The treatment with BSO (0.2 mM) was continued during the 2 h and 8 h incubations with radiolabelled substrates when [^{3}H]BaP or [^{3}H]BaP-7,8-diol were activated intracellularly. In the following, cells treated with BSO and CDNB are referred to as 'GSH-depleted' cells.

Incubation of cells with radiolabelled substrates: In order to activate [^{3}H]BaP and [^{3}H]BaP-7,8-diol in 2sFou cells,

monooxygenase activity was induced by pretreating the cells with benz(a)anthracene (20 µM) for 18 h (31), and cells were then incubated with [^{3}H]BaP (4 µM) (sp. act. 4.5 Ci/mmol) or [^{3}H]BaP-7,8-diol (4 µM) (sp. act. 0.37 Ci/mmol) in DMSO for 2 or 8 h as described earlier (16).

Activation of [^{3}H]BaP by microsomes: For microsome-mediated activation of [^{3}H]BaP the cells (4-8 x 10^6 cells/plate) were preincubated with 7,8-benzoflavone (5 µM) for 60 min and then co-incubated with microsomes from 3-methylcholanthrene pre-treated rats (1.6 mg protein/plate) as described previously (16). Incubations with [^{3}H]BaP (10 µM) (sp. act. 4.5 Ci/mmol) and NADPH (1 mM) lasted for 1 h. The capacity of the microsomal preparations for activating [^{3}H]BaP to DNA binding species was monitored by incubating microsomes with calf thymus DNA (500 µg/4 ml).

Metabolism of [^{3}H]BaP and [^{3}H]BaP-7,8-diol: Metabolites of [^{3}H]BaP and [^{3}H]BaP-7,8-diol were extracted from the growth media and separated by HPLC as described previously (16). [^{3}H]BaP-7,8-diol and its organic-extractable metabolites were analyzed by reversed phase HPLC on an Ultrasphere column. GSH conjugates were determined in the aqueous phase by ion exchange HPLC on a 5µ Spherisorb-NH_2 column. Total metabolism of [^{3}H]BaP was determined according to the method of van Cantfort (32).

Analysis of DNA binding: The procedure for the isolation of the DNA by phenol extraction, purification using hydroxyapatite, separation of the modified nucleosides by reversed phase HPLC and characterization of the adducts, has been described previously (16).

Determination of GSH and protein: Reduced GSH was assayed in protein-free acid extracts of cells following the fluorimetric method of Brehe and Burch (33). The amount of cellular or microsomal protein was quantified using the procedure of Lowry et al. (34).

RESULTS

Depletion of glutathione

Intracellular GSH levels of the cell lines tested ranged from 25 to 75 nmol/mg protein (Table I). Treatment with BSO and CNDB decreased the GSH content in most of the cell lines below 1% of the controls (Table I). Residual amounts of GSH were as low as 0.01-0.2 nmol/10^6 cells (Table I). GSH-depleted cells did not show any sign of cytotoxicity during the 24 h observation period as judged by light microscopy. In human lung tumour cells NCI-H322 and hepatoma HepG2 treatment with BSO and CDNB lowered the GSH levels to only 30% and 8% of that in controls, respectively, (Table I). However, when the concentration of BSO was increased 50-fold and that of CDNB 10-fold, the GSH levels in NCI-H322 cells were suppressed to 7% of that in controls (Table I). No attempts were made to further lower the GSH levels in HepG2 cells, which possess very low GSH transferase activities (cf. below and Table IV).

Metabolism of [^{3}H]BaP and [^{3}H]BaP-7,8-diol in 2sFou cells.

2sFou cells metabolized 80% of 4 µM [^{3}H]BaP within the first 2 hours of incubation. The [^{3}H]BaP metabolism was almost complete after 8 h (Figure 1A). The amount of GSH conjugates increased continuously during the 8 h observation period (Figure 1B) amounting to 56 and 132 pmol/10^6 cells

after 2 and 8 h, respectively (Table II). This corresponded to 4% of the total metabolites. When the water soluble [^{3}H]BaP metabolites were separated by HPLC, 65% of total BaP-GSH conjugates co-eluted with an anti-BaPDE-GSH standard (16) (data not shown). GSH depletion completely suppressed the formation of GSH conjugates (Figure 1B) but did not alter the total metabolism of BaP (Figure 1A).

The metabolism of [^{3}H]BaP-7,8-diol was considerably faster than that of [^{3}H]BaP (Figure 2A). The pattern of ethyl acetate extractable metabolites formed during 30 min of incubation is shown in Figure 3. The largest peak co-eluted with r-7,c-10,t-8,t-9-tetrahydroxy-7,8,9,10-tetrahydro-BaP (tetraol I) (16). At least three more smaller peaks were discernible, of which the one following tetraol I coeluted with r-7,t-8,t-9,t-10-tetrahydroxy-7,8,9,10-tetrahydro-BaP (tetraol II) (Figure 3).

After completion of BaP-7,8-diol metabolism at 2 h, tetraol I comprised 10-20% of total metabolites (Figure 2B). GSH depletion did not significantly alter the rate of total BaP-7,8-diol metabolism nor the pattern of metabolites. If any change occured, there was a minor decrease in the formation of tetraol I.

GSH conjugates formed from BaP-7,8-diol metabolites reached a maximum value of 240 pmol/10^6 cells after 1 h. They decreased slowly during the following 6 hours of incubation (Figure 2B). The GSH conjugates eluted as one single peak together with an anti-BaPDE-GSH standard (16), when analysed

by HPLC (data not shown). No GSH conjugates of BaP-7,8-diol metabolites were detectable in GSH-depleted cells (Figure 2B).

Formation of DNA adducts following intracellular activation of [^{3}H]BaP and [^{3}H]BaP-7,8-diol in 2sFou cells: Dependence on GSH depletion.

2sFou cells were incubated with 4 µM [^{3}H]BaP and the binding of [^{3}H]-labelled material to DNA determined after 2 and 8 h of exposure. After 2 h the radioactivity covalently bound to DNA was below the limits of detection (Table III). After 8 h of exposure 0.7 pmol BaP-molecules/mg DNA were bound (Table III). Following DNA digestion, however, all the radioactivity remained in the aqueous phase, which contains only unmodified nucleosides . In GSH-depleted cells substantial amounts of radioactive material were found to be associated with DNA, corresponding to 5.7 and 6.8 pmol/mg DNA after 2 and 8 h of exposure to [^{3}H]BaP (Table III). In this case 70 to 80% of the radioactivity eluted with the fraction of modified nucleosides (data not shown).

Analysis of the modified nucleosides by HPLC yielded two major peaks (Figure 4A). The first peak 'a', which co-eluted with (+)-anti-BaPDE-deoxyguanosine (16) comprised about 30% of the total radioactivity applied to the column. Peak 'b', presumably a syn-BaPDE-DNA adduct (35), was not further characterized. The ratio of radioactivity in peaks 'a':'b' was 1.0:0.7.

When 2sFou cells were exposed to [^{3}H]BaP-7,8-diol for 8 h, radioactive material, corresponding to 7.4 pmol/mg DNA, was covalently bound to DNA (Table III). Upon GSH depletion, DNA binding increased 10-fold and 80% of the total radioactivity was located in peak 'a' and 'b', at a ratio of 1.0:0.7 (Figure 4B). In GSH-containing cells the ratio was 1.0:0.3.

Metabolism and binding of [^{3}H]BaP to DNA of 2sFou cells following extracellular activation.

Rat liver microsomes added to 2sFou cells as an extracellular source of reactive BaP species, metabolised 40 nmoles [^{3}H]BaP/plate within 60 min. Only a small fraction, i.e. 105 pmol/10^6 cells corresponding to about 1% of the total metabolites, consisted of GSH conjugates (Table IV). About half of these GSH conjugates co-eluted with anti-BaPDE-GSH (data not shown). In GSH-depleted cells, GSH conjugation was suppressed to 4% of the controls, i.e. 4 pmol/10^6 cells (Table IV).

DNA binding of extracellularly generated [^{3}H]BaP metabolites showed a similar dependence on GSH levels as did that of intracellularly generated metabolites. Less than 2 pmol radioactive products/mg DNA were found in GSH-containing cells and about 10 pmol/mg DNA in GSH-depleted cells (Table V). The HPLC profiles of the modified nucleosides isolated from control and GSH-depleted cells were qualitatively similar to those observed after intracellular

activation of BaP or BaP-7,8-diol (Figure 4C). However, after extracellular activation the great majority of the radioactivity was found in peak 'a'.

DNA binding and formation of GSH conjugates in various cell lines following extracellular activation of [^{3}H]BaP.

Table V shows the binding of extracellularly formed BaP metabolites in several cell lines (cf. Table I). The hepatoma cells H4IIE and H5 formed low amounts of DNA adducts, similar to the amount in 2sFou cells. The two non-hepatic rat cell lines WRC and 208F formed 2-3 times more adducts than 2sFou cells. DNA binding in Chinese hamster V79, and in human NCI-H322 and HepG2 cells was considerably higher, corresponding to 10-14 pmol/mg DNA. DNA adducts formed by V79, NCI-H322 and HepG2 cells were analyzed by HPLC. The patterns of adduct peaks were similar to those previously found with C3H/10T1/2 cells (16) and to those found in GSH-depleted 2sFou cells (cf. Figure 4C). About 40% of the total DNA adducts eluted with anti-BaPDE-deoxyguanosine (data not shown).

GSH depletion caused a 6-7 fold increase in DNA binding in H4IIE and H5 cells (Table V) and a twofold increase in WRC. GSH depletion did not - or only slightly - increase DNA binding in V79, NCI-H322, 208F or HepG2 cells, which in the presence of GSH, already contained relatively high levels of adducts.

GSH conjugates (Table IV) ranged from 34 pmol/10^6 cells in V79 to 132 pmol/10^6 cells in H4IIE cells. Depletion of GSH

to less than 1% of that in controls suppressed GSH conjugates to various degrees. Whereas GSH conjugates were no longer detectable in H4IIE, WRC and H5 cells, V79 cells still formed 20% of the GSH conjugates in controls. In NCI-H322 cells, the amount of GSH conjugates formed was reduced by only 40% when the GSH levels were suppressed to 7% of that in controls (cf. Table I).

DISCUSSION

The present results strongly suggest that 2sFou hepatoma cells are protected against DNA binding of the ultimate carcinogen (+)-anti-BaPDE primarily by GSH conjugation. Thus the number of DNA adducts with BaP metabolites was very low in 2sFou cells containing 'normal' GSH levels, but increased drastically when GSH was depleted and GSH conjugation suppressed. From previous observations made by others and us (13,15,36) we conclude that GSH functions as a co-factor of GSH transferases, and not per se in conjugating electrophilic BaP metabolites. The GSH-dependent protection of 2sFou cells against DNA binding appeared not to be tied to cell-specific pathways or subcellular sites of BaP activation, since the phenomenon was also observed when the reactive BaP species were generated by an extracellular activation system.

The findings made with 2sFou cells are in general agreement with those using isolated rat liver nuclei (13) or freshly isolated hepatocytes (14). However, they bring out an important new aspect. Previous observations (14) suggested that liver cells are protected against DNA binding of BaP metabolites because they are able to quantitatively remove reactive BaP species by GSH conjugation. The present results indicate that other, more specific factors might be essential for protecting hepatic cells against the formation of BaP-DNA adducts.

The first argument for this idea stems from the observation that protection against DNA binding did not correlate with the degree of GSH conjugation in the various cell lines tested. Among these lines HepG2 were unique in that they virtually lacked the ability for conjugating BaPDE to GSH. By definition they are 'unprotected' and may be taken as a reference for 'maximum' DNA binding of reactive BaP metabolites. The fact that DNA binding in BaP-sensitive lines such as V79, NCI-H322 or C3H/10T1/2 (16), which are proficient in GSH conjugation, was about as high as in HepG2 cells, suggests that BaPDE species binding to DNA escape detection by GSH transferases in these lines. It is fitting that suppression of GSH conjugation failed to substantially increase the pre-existant high levels of binding. In the light of these observations it is striking that the hepatoma lines 2sFou or H4IIE are protected to such a high degree, even though their capacity for conjugating BaP metabolites to GSH was similar to that of 'unprotected' cells such as C3H/10T1/2 (16). In both 2sFou and C3H/10T1/2 cells (16), about 50% of the total conjugates were tentatively identified as BaPDE-GSH-conjugates (Cumpelik and Hesse, unpublished observations). The results may be interpreted as demonstrating that certain cells, notably hepatic cells, are able to specifically trap those BaPDE moieties which bind to DNA ($BaPDE_{DNA}$). Other cell lines, e.g. rat mammary gland cells WRC, are able to at least partially prevent DNA binding by GSH conjugation.

A second argument for the existence of specific mechanism(s) of protection other than total inactivation of reactive BaPDE derives from quantitative aspects of GSH conjugation with BaPDE. These become most apparent when anti-BaPDE is generated by the metabolism of its precursor BaP-7,8-diol in 2sFou cells. Estimating the total pool of anti-BaPDE from its two major primary products, tetraol I and GSH conjugates, it can be seen that BaP-tetraols comprise about 2/3 and BaPDE-GSH-conjugates 1/3 of this pool (Figure 3). If $BaPDE_{DNA}$ species were part of the total pool of BaPDE, suppression of GSH conjugation would increase DNA binding at most by 1/3. In fact, DNA binding increased by a factor of 10.

The results are best explained by the hypothesis that $BaPDE_{DNA}$ species constitute a small pool separate from the majority of intracellular BaPDE. At present the nature of this pool, i.e. its function and subcellular location, can only be a matter of speculation. We think it most likely that the pool relates to the transfer of BaPDE from its site of formation, endoplasmatic reticulum or extraneous 'microsomes', to its site of binding, nuclear DNA.

In principle, highly lipophilic electrophiles such as BaPDE may move within the cell either by lateral diffusion through the phospholipid bilayers of intracellular membranes or by transport through the cytosol attached to proteins containing hydrophobic binding sites (38-41). We assume that the critical step in $BaPDE_{DNA}$ transfer takes place in the

cytosol and not - as thought previously (16) - in the membrane network. The assumption is primarily based on the observation that the pool of $BaPDE_{DNA}$ is relatively small, whereas the membrane associated BaPDE pool is bound to be large. The majority of BaPDE has been shown to partition into the lipid phase of cellular membranes (40,42,43). Also, there are no indications that BaPDE contained in hepatic membranes is subject to GSH conjugation and could be the direct source of the observed BaPDE-GSH conjugates. On the one hand, non-polar electrophiles, once they are taken up into membranes, are in general inaccessible to soluble GSH transferases (44). On the other hand, substantial activities of membrane-bound GSH transferase, specifically directed against BaP products, have not been found in either adult rat hepatocytes (45) or differentiated hepatoma cells (unpublished observation).

In contrast to the conditions in the lipophilic environment of membranes, BaPDE species which are transported through the aqueous phase of the cytosol to nuclear DNA may react with nucleophiles or be intercepted by GSH transferases if they are not shielded by their transport proteins. A number of proteins in the cytosol and serum have been described which would qualify as 'transporters' of $BaPDE_{DNA}$ (39,46-50). These proteins are capable of stabilizing electrophiles (49,50) and facilitate their transport within the cells (47,51).

Since maximum (GSH-independent) binding of BaPDE was similar in 'protected' and 'unprotected' cells, the capacity

for transfer of the diolepoxide to DNA appears to be of the same order of magnitude in the two cell types. It remains open to question why in some cells such as 2sFou or H4IIE, but not in others, the presumed $BaPDE_{DNA}$ carrier complex is accessible to GSH conjugation. In view of the fact that mammalian tissues contain different forms of GSH transferases (41), the specificity of the response might reside in these enzymes. However, the phenomenon could also be due to the existence of cell specific transport proteins.

To summarize, the results suggest that BaPDE species which bind to DNA are contained in a specific cellular compartment. This compartment probably consists of cytosolic proteins which mediate the transport of the diol epoxides to their targets. The cell-specific and - presumably - tissue-specific response to BaP might be attributable to a different accessibility of carrier-bound BaPDE to GSH transferases.

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ABBREVIATIONS

BaP	benzo(a)pyrene
BaPDE	BaP-7,8-diol-9,10-oxide
anti-BaPDE	r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetra-hydrobenzo(a)pyrene
GSH	glutathione
BaP-7,8-diol	(±)-trans-7,8-dihydroxy-7,8-dihydro-benzo(a)pyrene
CDNB	1-chloro-2,4-dinitrobenzene
BSO	buthionine sulfoximine
tetraol I	r-7,c-10,t-8,t-9-tetrahydroxy-7,8,9,10-tetra-hydrobenzo(a)pyrene
tetraol II	r-7,t-8,t-9,t-10-tetrahydroxy-7,8,9,10-tetra-hydrobenzo(a)pyrene

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Table I: Depletion of intracellular GSH by BSO and CDNB in various cell lines

Cell line	Tissue/Species	Treatment[a] BSO (mM)	Treatment[a] CDNB (µM)	GSH content (nmol/mg protein)[b] Control cells	GSH content (nmol/mg protein)[b] GSH-depleted cells	% of Controls
2sFou	hepatoma, rat	0.2	2	54.4 ± 4.2	0.03 ± 0.01	<1
H4IIE	hepatoma, rat	0.2	2	24.7 ± 2.9	0.2 ± 0.05	<1
H5	hepatoma, rat	0.2	20	73.3 ± 0.9	<0.01	<1
WRC	mammary gland, rat	0.2	2	29.2 ± 10.0	<0.01	<1
208F	fibroblasts, rat	0.2	2	49.3 ± 4.5	<0.01	<1
V79	lung, Chinese hamster	0.2	2	29.5 ± 2.8	0.05 ± 0.05	<1
NCI-H322	lung carcinoma, man	0.2	2	69.5 ± 3.3	18.9 ± 0.7	27.0
		5.0	20	63.3 ± 2.7	4.4 ± 0.5	7.0
HepG2	hepatoma, man	0.2	2	45.8 ± 1.8	3.5 ± 0.3	7.6

[a] Cells were exposed to BSO for 18 hour. CDNB was added during the last h of BSO exposure.

[b] Reduced GSH was assayed in protein-free acid extracts of cells as described in MATERIALS AND METHODS.

Table II: Total metabolism and GSH conjugation of [^{3}H]BaP and [^{3}H]BaP-7,8-diol in 2sFou cells[a]

		Metabolites (pmol/10^6 cells)				
			GSH conjugates			
Substrate	Incubation time (h)	Total	Control cells		GSH-depleted cells	
BaP	2	1600	56 ± 1.5[b]	(4)[c]	<2	(<1)[c]
BaP	8	3300	132 ± 13.3	(4)	<2	(<1)
BaP-7,8-diol	2	3200	241 ± 19.5	(8)	<2	(<1)

[a] Incubation conditions and determination of metabolites as described in MATERIAL AND METHODS.

[b] Mean and range of duplicate determinations.

[c] Percent of total metabolites.

Table III: Effect of GSH-depletion on DNA-binding of metabolites formed from [^{3}H]BaP or [^{3}H]BaP-7.8-diol in 2sFou cells[a]

Substrate	Incubation time (h)	DNA binding (pmol/mg DNA)[b] Control cells	(n)	GSH-depleted cells	(n)
BaP	2	< 0.1	(3)	5.7 ± 1.0[c]	(3)
BaP	8	0.7 ± 0.6	(3)	6.8 ± 1.4	(3)
BaP-7,8-diol	8	7.4 ± 1.5	(2)	76.9 ± 10.2	(2)

[a] Cells were incubated with 4 µM [^{3}H]BaP or [^{3}H]BaP-7,8-diol for the times indicated. DNA was isolated and purified using hydroxyapatite as described previously (19).

[b] Total radioactivity bound to DNA.

[c] Mean and standard deviation for triplicate determinations and range for duplicate determinations. The number of determinations is given in parentheses.

Table IV: Effects of GSH-depletion on the formation of GSH conjugates during extracellular activation of [^{3}H]BaP in hepatoma line 2sFou and other cell lines[a]

	GSH conjugates (pmol/10^6 cells)		
Cell line	Control cells	GSH-depleted cells	(percent of controls)
2sFou	105 ± 33[b]	4 ± 4	(4)
H4IIE	132 ± 26	<2	(<2)
H5	61 ± 17	<2	(<2)
WRC	55 ± 6	<2	(<4)
208F	128 ± 29	53 ± 9	(41)
V79	34 ± 3[c]	6 ± 3[c]	(18)
NCI H322	64 ± 9	37 ± 12[c d]	(58)
Hep G2	4 ± 0.1	<2	-

[a] Cells (4-8 x 10^6 per plate) were co-incubated with rat liver microsomes in the presence of [^{3}H]BaP (10 µM) and NADPH (1 mM) for 1 h. GSH conjugates were determined as decribed in MATERIALS AND METHODS.

[b] Mean and range of two independent experiments.

[c] Mean of duplicate determinations of a single experiment.

[d] GSH levels were decreased to 7% of controls (see Table 1).

Table V: Formation of DNA adducts in 2sFou and other cell lines after extracellular activation of [^{3}H]BaP: dependence on GSH-depletion[a]

Cell line	DNA binding (pmol/mg DNA)[b]		GSH-depleted/ Controls
	Controls	GSH-depleted	
2sFou	1.8 ± 0.15[c]	9.8 ± 2.4	5.4
H4IIE	2.3 ± 0.7	15.4 ± 1.2	6.7
H5	1.1 ± 0.4	9.3 ± 1.3	7.1
WRC	4.1 ± 0.6	9.8 ± 3.1	2.5
208F	5.7 ± 1.0	7.9 ± 1.7	1.3
V79	13.0 ± 1.5	18.2 ± 6.2	1.4
NCI-H322,	10.4 ± 0.8	12.7[d] ± 1.2	1.3
Hep G2	10.2 ± 1.1	10.2 ± 0.8	1.0

[a] Conditions for incubations and isolation of DNA as described in MATERIALS AND METHODS.

[b] Total radioactivity bound to DNA.

[c] Mean and standard deviation of triplicate determinations.

[d] GSH levels were decreased to 7% of controls (cf. Table 1).

LEGENDS TO FIGURES

Fig. 1 Metabolism of [^{3}H]BaP in 2sFou cells.

GSH depletion, incubation with 4 µM [^{3}H]BaP, and determination of unreacted substrate and GSH conjugates in the medium by solvent extraction and HPLC were performed as described in MATERIALS AND METHODS.
Disappearance of substrate (A) and formation of GSH-conjugates (B); control cells (open symbols) and GSH-depleted cells (closed symbols).

Fig. 2 Metabolism of [^{3}H]BaP-7,8-diol in 2sFou cells.

GSH depletion, incubation with 4 µM [^{3}H]BaP-7,8-diol, and determination of unreacted substrate and metabolites in the medium by solvent extraction and HPLC were performed as described in MATERIALS AND METHODS.
Disappearance of substrate (A) and formation of metabolites (B); control cells (open symbols) and GSH-depleted cells (closed symbols).
(,) GSH conjugates, (,) tetraols I.

Fig. 3 HPLC profile of organic-extractable metabolites of [^{3}H]BaP-7,8-diol formed by 2sFou cells.

Cells were exposed to 4 µM [^{3}H]BaP-7,8-diol for 30 min. The organic extract was analyzed by HPLC as described in MATERIALS AND METHODS. Arrows give the position of tetraol I, tetraol II and BaP-7,8-diol fluorescence standards.

Fig. 4 Formation of DNA adducts in 2sFou cells during intra- or extracellular activation of [^{3}H]BaP or [^{3}H]BaP-7,8-diol: dependence on GSH depletion.

HPLC profiles of modified nucleosides after intracellular activation of [^{3}H]BaP (A), intracellular activation of [^{3}H]BaP-7,8-diol (B), or extracellular activation of [^{3}H]BaP by rat liver microsomes (C). Procedures for incubations and separation of modified nucleosides are given in MATERIALS AND METHODS. The arrows show the elution position of a (+)-anti-BaPDE-deoxyguanosine standard (19).
White areas under the lines represent modified nucleosides from GHS-depleted cells, the black areas those from control cells.

FIGURE 1

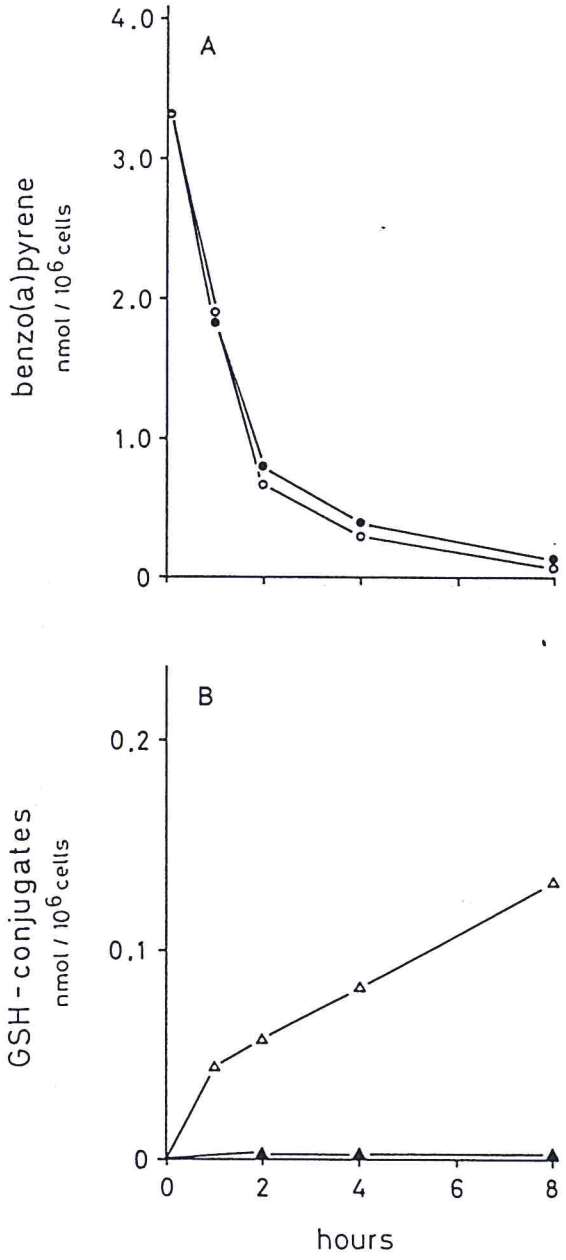

A
B
benzo(a)pyrene
nmol / 10^6 cells
GSH - conjugates
nmol / 10^6 cells
4.0
3.0
2.0
1.0
0
0.2
0.1
0
0
2
4
6
8
hours

FIGURE 2

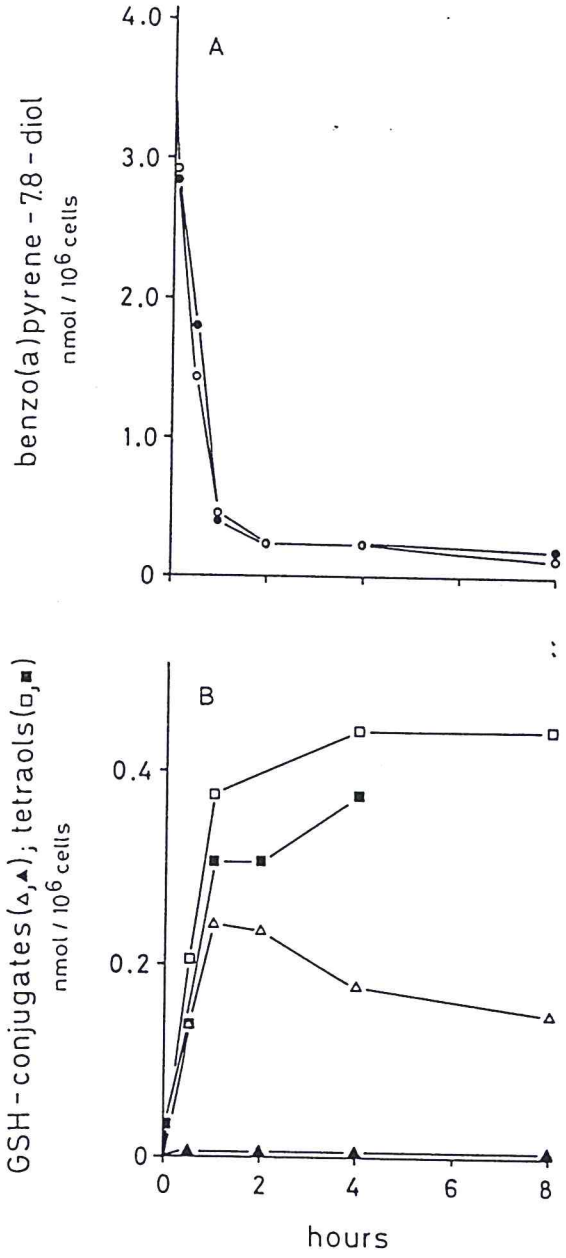

4.0
3.0
2.0
1.0
0
A
benzo(a)pyrene - 7.8 - diol
nmol / 10^6 cells
B
0.4
0.2
0
0
2
4
6
8
hours
GSH - conjugates (Δ,▲); tetraols (□,■)
nmol / 10^6 cells

FIGURE 3

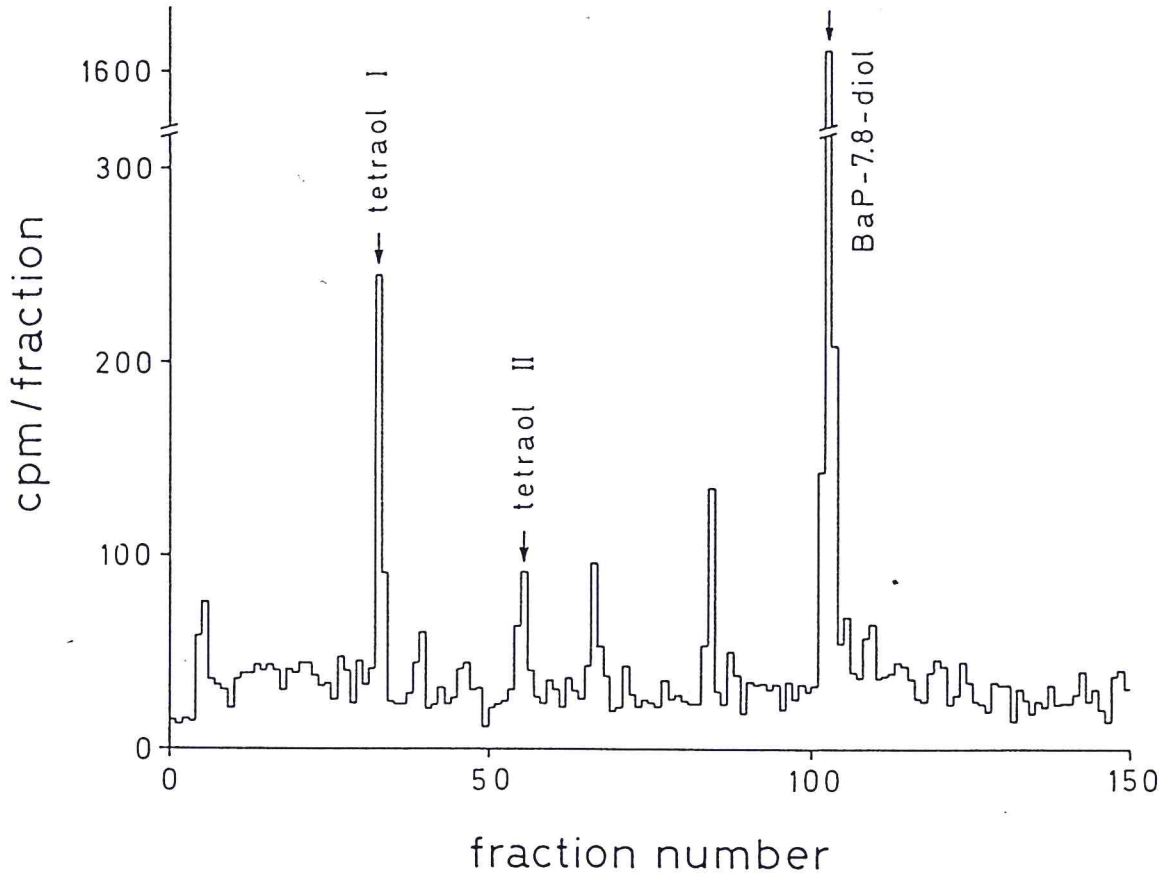
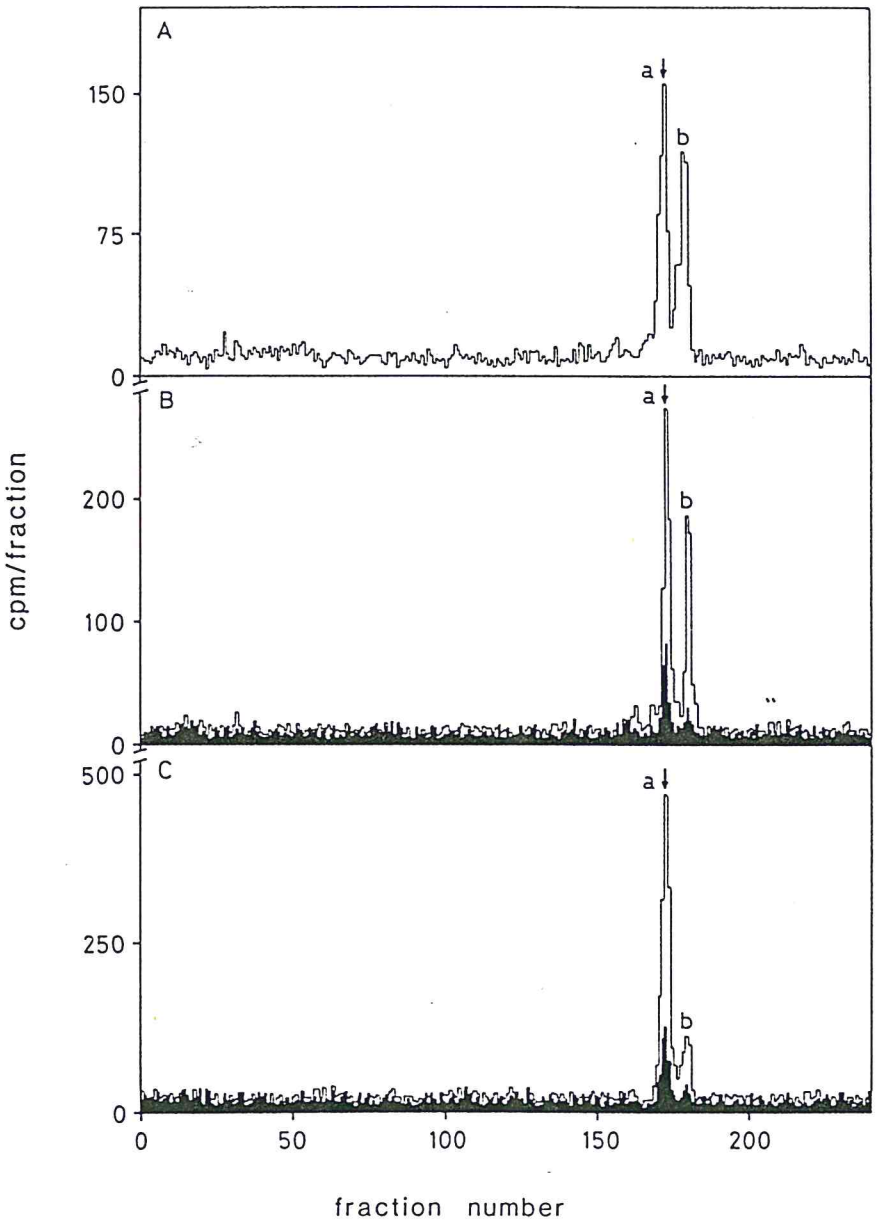

1600
300
200
100
0
cpm/fraction
tetraol I
tetraol II
BaP-7.8-diol
0
50
100
150
fraction number

FIGURE 4


A
a
b
150
75
0
B
a
b
200
100
0
C
a
b
500
250
0
0
50
100
150
200
cpm/fraction
fraction number