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Modification of chromium(VI)-induced DNA damage by glutathione and cytochromes P-450 in chicken embryo hepatocytes

(metal carcinogenesis/chromate metabolism/DNA strand breaks/DNA cross-links)

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ABSTRACT **The role of glutathione and cytochrome P-450 in the production of DNA damage by chromium(VI) was examined in chicken embryo hepatocytes by the alkaline elution technique. Cellular levels of glutathione and cytochrome P-450 were altered by treating the hepatocytes with *N*-acetyl-L-cysteine, buthionine sulfoximine, isopentanol, or β-naphthoflavone. A dramatic increase in chromium(VI)-induced DNA strand breaks was observed after increasing glutathione levels in the cells. Chromium(VI)-induced DNA strand breaks were even more numerous when the level of cytochrome P-450 was also increased. Upon depletion of glutathione levels and induction of cytochrome P-450 or cytochrome P-448, little or no DNA strand breaks or DNA interstrand cross-links were observed after chromium(VI) treatment. Chromium(VI)-induced DNA-protein cross-links generally decreased after either increases or decreases in cellular levels of glutathione or cytochrome P-450 or P-448. These results suggest that glutathione enhances chromium(VI)-induced DNA damage through metabolic activation of chromium(VI). The possible production of reactive chromium species upon metabolism by glutathione and cytochrome P-450 or P-448 and their involvement in DNA damage is discussed.**

Chromium(VI) compounds are recognized as carcinogens in humans and animals (1) and as mutagens in bacterial and mammalian cell systems (2). Chromium(VI) causes DNA strand breaks and cross-links *in vivo* and in cultured cells (3–6). It has been proposed that cellular metabolism of chromium(VI) compounds is necessary for DNA damage, since chromium(VI) does not react with isolated DNA under physiological conditions (7). The DNA-damaging ability of other carcinogenic agents has been shown to be modulated by metabolic pathways such as the cytochrome P-450 system, sulfotransferases, and glutathione (GSH) (8). Changes in cellular levels of cytochromes P-450 alter the levels of DNA damage produced by carbon tetrachloride (9), benzo[*a*]pyrene (10), and dimethylnitrosamine (11) in rodents.

In vitro studies have identified several cellular components that are capable of metabolizing chromium(VI) (12–14). Rat liver microsomes contain a NADPH-dependent chromium(VI) reductase activity (15). Induction of cytochrome(s) P-450 by phenobarbital enhances the chromium(VI) reductase activity of liver microsomes; however, induction of cytochrome(s) P-448 by 3-methylcholanthrene has no effect (13). GSH also reduced chromium(VI) *in vitro* under physiological conditions (12, 14). The reaction of GSH with chromium(VI) has been proposed to occur by a two-step mechanism that involves the rapid formation of a chromium(VI) thioester followed by the reduction of the chromium(VI) thioester to chromium(III) (12).

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The present report describes the effect of altering the intracellular concentrations of cytochrome P-450 and P-448 and GSH on chromium(VI)-induced DNA damage in primary cultured chicken embryo hepatocytes. Chromium(VI) treatment is known to produce DNA damage in chicken embryo hepatocytes (5, 6). The hepatocytes are well suited for these experiments because intracellular levels of the different isozymes of cytochrome P-450 can be increased by β-naphthoflavone (16) and isopentanol (17). GSH has been shown to increase in these cells with the induction of cytochrome P-450, but not with induction of cytochrome P-448 (18). Also, GSH can be decreased in chicken embryo hepatocytes by buthionine sulfoximine (18) or increased by *N*-acetyl-L-cysteine (see below). The present report shows that changes in cellular levels of cytochrome P-450 or P-448 and GSH in the hepatocytes resulted in dramatic alterations of the levels and types of chromium(VI)-induced DNA lesions detected by the alkaline elution technique.

MATERIALS AND METHODS

Chemicals. Williams E medium was purchased from Flow Laboratories. Sodium [^{51}Cr]chromate with a specific activity of 0.5 mCi/μg (1 Ci = 37 GBq) was obtained from Amersham. Buthionine sulfoximine was obtained from Chemalog (South Plainfield, NJ). All other chemicals were purchased from Fisher or Sigma.

Preparation and Treatment of Chicken Embryo Hepatocytes. Primary cultures of hepatocytes were prepared from the livers of 16-day embryos and cultured as described previously (5). Approximately 2×10^6 cells were seeded on 3.5-cm polystyrene tissue culture dishes for all experiments, except for the spectral cytochrome P-450 assays, which required approximately 4.4×10^6 cells on 6.0-cm dishes. The medium was replaced with fresh medium 24 hr after the cells had been plated. For all the chromium experiments, 48 hr after the hepatocytes had been plated, the cells were exposed to 5 μM sodium chromate in the medium for 2 hr. Drug pretreatments varied as follows: to increase GSH, hepatocytes were treated for 2 hr with 1 mM *N*-acetyl-L-cysteine and then the *N*-acetyl-L-cysteine was removed and fresh medium was added prior to chromate treatment; to decrease GSH, cells were treated with 0.1 mM buthionine sulfoximine for 20 hr before chromate addition; to increase cytochrome P-448, cells were treated with 15 μM β-naphthoflavone in the presence or absence of 0.1 mM buthionine sulfoximine for 20 hr prior to chromate treatment; to increase cytochrome P-450, cells were treated with 10 mM isopentanol in the presence or absence of 0.1 mM buthionine sulfoximine for 20 hr. After the isopentanol (with or without buthionine sulfoximine) pretreatment, the medium was changed (with or without buthionine sulfoximine) 1 hr prior to chromate treatment, since isopentanol inhibits cytochrome P-450 ac-

Abbreviation: GSH, glutathione.

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tivity and this inhibition is removed by changing the medium (J. Sinclair, personal communication).

Alkaline Elution. After sodium chromate treatment, the hepatocytes were analyzed for DNA damage by using the alkaline elution technique based on the procedure of Kohn *et al.* (19) as described previously (6). The alkaline elution technique measures the size distribution of long single-stranded DNA, assuming that the rate of elution of the DNA from a polyvinylchloride filter depends on the length of the DNA and is altered by filter absorption of proteins linked to the DNA (19). DNA lesions were calculated from the DNA remaining on the filter after 9 hr of elution, by using the equations of Kohn *et al.* (19), and were based on the comparison of DNA elution rates of control and treated cells (with or without x-irradiation).

Assays for Cytochromes P-450. Cytochromes P-450 and P-448 were determined by the CO difference spectral assay (20). Benzphetamine-*N*-demethylase and 7-ethoxyresorufin-*O*-deethylase activities, which are assays of cytochrome P-450 and cytochrome P-448 isozymes, respectively, were measured spectrofluorometrically in cell homogenates as described previously (21).

GSH Assay. GSH was determined spectrofluorometrically according to Hissin and Hilf (22) as described previously (6). Up to 0.1 mM *N*-acetyl-L-cysteine did not interfere with this assay.

Sodium Chromate Uptake. The uptake of chromium(VI) by the hepatocytes was determined by analyzing the concentration of ^{51}Cr remaining in the medium after a 2-hr treatment with 5 μM sodium [^{51}Cr]chromate (1 μCi/μg).

Statistical Analysis. The Student *t* test was used for statistical analysis (23).

RESULTS

Alterations of Cellular GSH and Cytochrome P-450 Levels. The effects of the various drug treatments on GSH and cytochrome P-450 levels and activities in chicken embryo hepatocytes are presented in Table 1. Since the cytochrome P-450 level does not provide information on the specific forms of cytochrome P-450 present, benzphetamine demethylase and ethoxyresorufin deethylase activities, which are specific for phenobarbital- and 3-methylcholanthrene-inducible isozymes, respectively, were determined. None of the drug treatments changed the total amount of protein found in the hepatocytes (0.40 ± 0.02 mg of protein per 10^6 cells). After *N*-acetyl-L-cysteine treatment, GSH was increased 80% while the cytochrome P-450 concentration and benzphetamine demethylase activity were unchanged. The apparent decrease in ethoxyresorufin deethylase activity was probably not significant since the levels in all treatments except for β-naphthoflavone were close to the detection limit (0.5 pmol of resorufin per mg of protein per min). Buthionine sulfoximine treatment caused an 85% decrease in GSH levels with no significant effect on cytochrome P-450 level or activities. Since buthionine sulfoximine did not lower and *N*-acetyl-L-cysteine did not increase benzphetamine demethylase activity, cytochrome P-450 activity did not depend on GSH concentration. Treatment of the hepatocytes with isopentanol resulted in a 3.5-fold increase in cytochrome P-450, associated with a 2-fold increase in benzphetamine demethylase activity, and was accompanied by an 80% increase in GSH. The combined treatment of isopentanol + buthionine sulfoximine increased cytochrome P-450 3.5-fold and benzphetamine demethylase activity 2-fold and decreased GSH 90%. After β-naphthoflavone treatment, cytochrome P-448 was increased 2.5-fold with a 36-fold increase in ethoxyresorufin deethylase activity without a significant increase in benzphetamine demethylase activity ($P > 0.05$) and GSH was decreased by 20% ($P < 0.05$). The combined treatment of β-naphthoflavone + buthionine sulfoximine increased cytochrome P-448 2-fold and ethoxyresorufin activity 40-fold without any significant increase in benzphetamine demethylase activity ($P > 0.05$) and it decreased GSH 85% ($P < 0.001$). GSH concentration did not affect induced cytochrome P-450 activities since addition of buthionine sulfoximine to the inducers isopentanol and β-naphthoflavone did not affect the benzphetamine demethylase or ethoxyresorufin deethylase activity. Addition of 5 μM sodium chromate for 2 hr after treatment of the hepatocytes with the various drugs did not change GSH or cytochrome P-450 levels or activities ($P > 0.05$). After β-naphthoflavone treatment, there was a 20% increase in cytochrome P-448; however, there was no significant change in ethoxyresorufin deethylase activity.

Table 1. Sodium [^{51}Cr]chromate uptake, GSH, and cytochrome P-450 levels and enzymatic activities in chicken embryo hepatocytes after treatment with various drugs with or without an additional 2-hr treatment with 5 μM sodium chromate

Drug treatment	Sodium [^{51}Cr]chromate uptake,*† % ^{51}Cr removed from medium	GSH,*‡ % of control		Cytochrome P-450,*§ pmol/mg protein		Benzphetamine demethylase,¶ nmol HCHO per mg protein per hr		Ethoxyresorufin deethylase,¶ pmol resorufin per mg protein per min	
		Without Cr(VI)	With Cr(VI)	Without Cr(VI)	With Cr(VI)	Without Cr(VI)	With Cr(VI)	Without Cr(VI)	With Cr(VI)
No drug	34 ± 3	100	94 ± 10	37 ± 3	32 ± 7	1.6	1.2	2.0	1.9
N-Acetyl-L-cysteine	34 ± 1	182 ± 19	197 ± 17	39 ± 3	44 ± 2	1.5	1.2	0.5	0.5
Isopentanol	37 ± 1	175 ± 22	193 ± 26	164 ± 20	154 ± 38	4.2	3.8	1.6	—
Isopentanol + buthionine sulfoximine	36 ± 1	8 ± 1	9 ± 2	170 ± 5	134 ± 23	4.0	4.4	0.3	—
Buthionine sulfoximine	31 ± 2	12 ± 3	13 ± 2	29 ± 3	29 ± 3	1.6	1.2	1.4	1.3
β-Naphthoflavone	33 ± 1	83 ± 8	80 ± 3	135 ± 5	159 ± 4‖	1.9	—	72	69
β-Naphthoflavone + buthionine sulfoximine	35 ± 2	18 ± 7	14 ± 2	116 ± 16	103 ± 4	2.3	—	82	53

*Values represent mean ± SEM for $n \geq 4$.
†All values are statistically the same ($P > 0.1$) vs. no drug treatment.
‡GSH level in absence of drug treatment was 17 ± 3 nmol/mg of protein.
§Soret peak for no drug, *N*-acetyl-L-cysteine, buthionine sulfoximine, and isopentanol (with or without buthionine sulfoximine) is 450–452 nm and for β-naphthoflavone (with or without buthionine sulfoximine) it is 449 nm.
¶The error associated with duplicate values was ≤ 20%.
‖$P < 0.05$ vs. no chromium(VI) treatment.

Sodium Chromate-Induced DNA Strand Breaks. Treatment of chicken embryo hepatocytes with 5 μM sodium chromate for 2 hr resulted in 100 rad equivalents (1 rad = 0.01 gray) of DNA strand breaks in the absence of proteinase K digestion (Fig. 1). Upon proteinase K digestion of cell lysates, 140 rad equivalents of protein-associated DNA strand breaks was observed. Pretreatment of the hepatocytes with *N*-acetyl-L-cysteine, increasing GSH 80% with no change in cytochrome P-450, was accompanied by a 50–80% increase in chromium(VI)-induced DNA strand breaks (with or without proteinase K). Isopentanol, which increased GSH 80% and increased cytochrome P-450 3.5-fold, resulted in an approximately 140–170% increase in DNA strand breaks (with or without proteinase K) caused by chromium(VI). After isopentanol pretreatment the level of chromium(VI)-induced strand breaks (with or without proteinase K) was 50–60% greater than after *N*-acetyl-L-cysteine pretreatment ($P < 0.02$). Depletion of GSH with either an increase (isopentanol + buthionine sulfoximine treatment) or no change (buthionine sulfoximine treatment) in cytochrome P-450 decreased chromium(VI)-induced strand breaks approximately 50–75% and completely eliminated protein-associated DNA strand breaks. There was no significant difference between the chromium(VI)-induced DNA strand breaks seen with buthionine sulfoximine or buthionine sulfoximine + isopentanol pretreatments ($P > 0.1$). Pretreatment of hepatocytes with β-naphthoflavone, which caused a 20% decrease in GSH as well as a 2.5-fold increase in cytochrome P-448, resulted in a 50–85% decrease in DNA strand breaks caused by chromium(VI) and eliminated protein-associated DNA strand breaks. Chromium(VI)-induced DNA strand breaks were absent after the combined treatment of β-naphthoflavone + buthionine sulfoximine. This value was significantly less than for buthionine sulfoximine or β-naphthoflavone pretreatment alone ($P < 0.01$).

Sodium Chromate-Induced DNA Cross-Links. Chromium(VI)-induced DNA-protein and DNA interstrand cross-links were affected less by varying cellular levels of cytochrome P-450 and GSH than were DNA strand breaks (Fig. 2). DNA interstrand cross-links were not significantly ($P > 0.05$) affected by changes in either cytochrome P-450 or GSH levels except after the combined pretreatment with β-naphthoflavone + buthionine sulfoximine or isopentanol + buthionine sulfoximine, which resulted in elimination of DNA interstrand cross-links ($P < 0.01$). After isopentanol pretreatment, the level of chromium(VI)-induced DNA interstrand cross-links was greater than after *N*-acetyl-L-cysteine pretreatment ($P < 0.05$). Chromium(VI)-induced total DNA cross-links (mainly DNA-protein cross-links) were reduced approximately 50% ($P < 0.005$) by all the drug treatments except isopentanol alone, with which no change ($P > 0.05$) was observed.

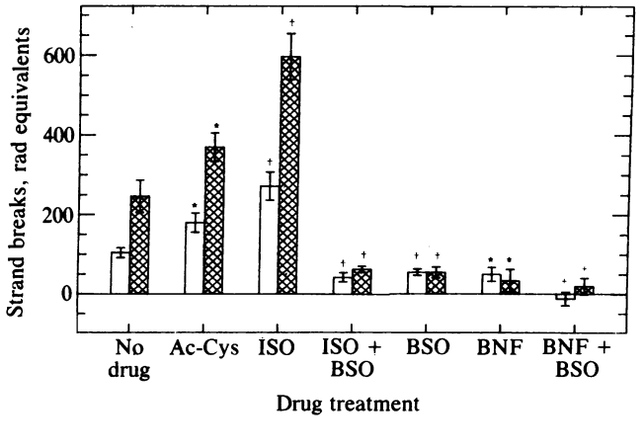

FIG. 1. DNA strand breaks in chicken embryo hepatocytes produced by chromium(VI) after various drug treatments. Cells were pretreated with the indicated drugs and then exposed to 5 μM sodium chromate for 2 hr. Ac-Cys, *N*-acetyl-L-cysteine; ISO, isopentanol; BSO, buthionine sulfoximine; BNF, β-naphthoflavone. DNA strand breaks were measured by the alkaline elution technique, without (open bars) or with (cross-hatched bars) proteinase K digestion. Protein-associated strand breaks are represented by the difference between DNA strand breaks with and without proteinase K digestion of the cell lysates. Calculations of DNA damage caused by chromium(VI) treatment compared cells treated with drugs alone with cells treated with chromium(VI) and drugs. Values represent mean ± SEM for $n \geq 6$.
*$P < 0.01$ vs. no drug treatment.
†$P < 0.005$ vs. no drug treatment.

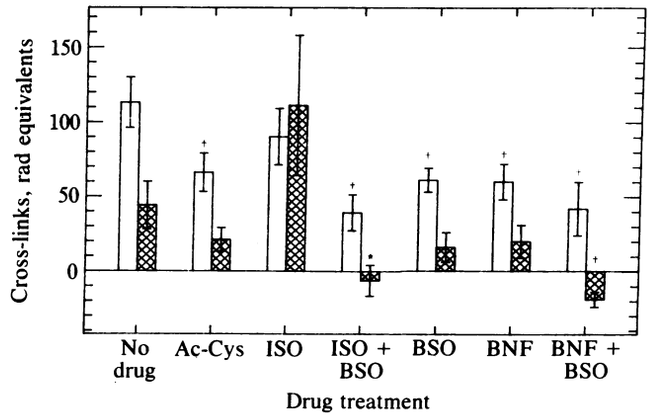

FIG. 2. DNA cross-links in chicken embryo hepatocytes produced by chromium(VI) after various drug treatments. Cells were treated as described in Fig. 1 legend. Open bars, total DNA cross-links (without proteinase K digestion); cross-hatched bars, DNA interstrand cross-links (with proteinase K digestion of the cell lysates). Values represent mean ± SEM for $n \geq 6$.
*$P < 0.01$ vs. no drug treatment.
†$P < 0.005$ vs. no drug treatment.

Effect of Drug Treatments on Chromate Uptake. The uptake of sodium chromate was measured after the various drug treatments to ensure that the changes in chromium(VI)-induced DNA damage were not due to changes in chromium(VI) uptake by the hepatocytes (Table 1). Exposure of hepatocytes to 5 μM sodium chromate for 2 hr resulted in removal of 31–37% of the [^{51}Cr]chromate from the medium under all pretreatment conditions. Thus, pretreatment with the drugs used in these experiments did not alter ($P > 0.1$) the uptake of chromium(VI) by the cells.

Effect of Drug Treatments on DNA. For the effect of the changes of GSH and cytochromes P-450 on chromium(VI)-induced DNA damage to be measured accurately, the drugs used to alter these cell constituents should not damage the DNA. DNA damage resulting from the drug treatments is presented in Table 2. Little or no DNA damage was produced by the various drug treatments. Although buthionine sulfoximine (with or without β-naphthoflavone) pretreatment resulted in a small decrease in the amount of DNA strand breakage when analyzed in the absence of proteinase K, pretreatment with buthionine sulfoximine + isopentanol resulted in a slight increase in DNA strand breaks, and pretreatment with *N*-acetyl-L-cysteine caused no detectable change in DNA strand breaks. However, no DNA strand breaks were observed under any condition upon proteinase K digestion. Thus, there does not appear to be any correlation between GSH levels in the cells and DNA damage. β-Naphthoflavone (with or without buthionine sulfoximine) pretreatment resulted in a small amount of DNA cross-links (mainly DNA-protein cross-links). In calculating the chromium(VI)-induced DNA lesions, drug treatment plus

Table 2. DNA damage in chicken embryo hepatocytes after various drug treatments

Drug treatment	Strand breaks, rad equivalents		Cross-links, rad equivalents	
	Without proteinase K	With proteinase K	Without proteinase K	With proteinase K
N-Acetyl-L-cysteine	14 ± 12	9 ± 10	−6 ± 8	−3 ± 2
Isopentanol	−32 ± 20	7 ± 30	−7 ± 11	−7 ± 35
Isopentanol + buthionine sulfoximine	17 ± 7*	6 ± 9	15 ± 9	6 ± 7
Buthionine sulfoximine	−29 ± 8*	−2 ± 18	−1 ± 10	3 ± 7
β-Naphthoflavone	8 ± 15	−27 ± 47	26 ± 6*	−11 ± 32
β-Naphthoflavone + buthionine sulfoximine	−19 ± 9*	−32 ± 36	19 ± 7*	15 ± 9

Cells were treated and analyzed for DNA damage by using alkaline elution. Calculations of DNA damage compared untreated cells with drug-treated cells. All values represent mean ± SEM for $n \geq 6$.
*$P < 0.05$ vs. zero.

chromium(VI) treatment was always compared to drug treatment alone to eliminate any possible low level of DNA damage resulting from the drug treatments from the data.

DISCUSSION

The level of chromium(VI)-induced DNA strand breaks was dramatically increased in hepatocytes with increased levels of GSH (*N*-acetyl-L-cysteine and isopentanol pretreatments) and dramatically decreased with decreased levels of GSH (buthionine sulfoximine and β-naphthoflavone pretreatments) (Fig. 1). Increased levels of protein-associated DNA strand breaks were observed only with treatments (*N*-acetyl-L-cysteine and isopentanol) that increased GSH levels. Protein-associated strand breaks may be due to chromium(VI)-induced DNA-protein cross-links or to covalent attachment of repair enzymes, topoisomerases, or other nuclear proteins to sites of DNA strand breakage (24). These results suggest that GSH played a key role in the activation of chromium(VI) to species capable of causing DNA strand breaks. Although GSH is usually thought to protect cells from the toxic effects of agents such as acetaminophen (25), benzo[*a*]pyrene (26), and radiation (27), it is also known to activate mutagens such as *N*-hydroxy-3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (28), 1,2-dibromoethane (29, 30), 1,2-dichloroethane (31), *N*-methyl- and *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (29). GSH has been shown to be important for reduction of chromium(VI) in rat liver *in vivo* (14). GSH has been shown to form a chromium(VI) thioester upon reaction with chromium(VI) *in vitro* (12, 32). It is possible that formation of a chromium(VI) thioester in cells could aid in the cellular activation of chromium(VI). The chromium(VI) thioester of GSH has been shown to undergo a redox reaction involving GSH *in vitro*, producing reactive chromium(IV) species (12) that may be capable of damaging DNA (Fig. 3). Even though no change in GSH level was observed after chromium(VI) treatment, the steady-state amount of the chromium(VI) thioester in the cells is expected to be small (12), so the detection of any small change in GSH levels is unlikely. Also, previous studies have shown that chromium(VI) stimulates the synthesis of GSH in chicken embryo hepatocytes (6).

GSH was not the only cellular metabolic system involved in chromium(VI)-induced DNA damage. Induction of cytochromes P-450 and P-448 also altered chromium(VI)-induced DNA damage (Figs. 1 and 2). In the presence of high levels of GSH, isopentanol-induced cytochrome P-450 appeared to act synergistically with GSH and to increase the chromium(VI)-induced DNA strand breaks ($P < 0.01$) and DNA interstrand cross-links ($P < 0.1$). In the presence of GSH, the level of chromium(VI)-induced DNA strand breaks decreased after induction of cytochrome P-448 by β-naphthoflavone. This could be due to the cytochrome P-448 deactivating the chromium(VI) or could be related to the 20% decrease in GSH found upon β-naphthoflavone treatment. Also, β-naphthoflavone could have decreased other cytochrome P-450 isozymes, such as the isozyme found in control cells, which are capable of metabolizing chromium(VI). In chicken embryo liver, β-naphthoflavone treatment decreases the level of the 2-allyl-2-isopropylacetamide-inducible cytochrome P-450 mRNA below the normal level found in untreated controls (34). When cellular GSH was depleted by using buthionine sulfoximine, induction of cytochrome P-450 or P-448 resulted in a dramatic decrease in chromium(IV)-induced DNA damage. Under conditions of high GSH, it is possible that cytochrome P-450 reacts with the chromium(VI) thioester of GSH and forms species such as chromium(IV) that can damage DNA (Fig. 3). Chromium(V) species have been detected *in vitro* as intermediates in the microsomal reduction of chromium(VI) (33) and in the reaction of chromium(VI) with stoichiometric amounts of GSH (32). Therefore, under conditions of low GSH and high cytochrome P-450 or P-448, chromium(VI) may be metabolized to chromium(V) species, which are rapidly inactivated within the cell (Fig. 3). It is also possible that these species react with DNA to form adducts such as intrastrand cross-links or monoadducts that are not detectable by the alkaline elution technique.

The effect of GSH and cytochrome P-450 or P-448 on chromium(VI)-induced DNA interstrand cross-links was more difficult to detect than the effect on DNA strand breaks, since the level of DNA interstrand cross-links was ½ to ⅓ of the level of DNA strand breaks. However, high levels of cytochrome P-450 or P-448 combined with low GSH resulted in a detectable decrease in DNA interstrand cross-links. A high level of cytochrome P-450 combined with high GSH resulted in a detectable increase in DNA interstrand cross-links compared with that seen under high GSH conditions.

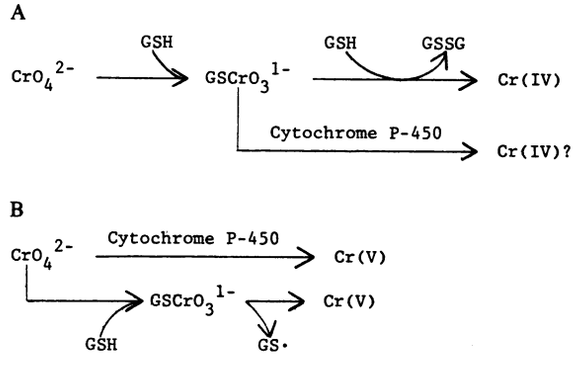

FIG. 3. Possible metabolic pathways of chromium(VI) in cells. (*A*) Under conditions with high GSH levels. (*B*) Under conditions with low GSH levels. GSSG, glutathione disulfide; GS·, glutathione radical. See ref. 12 for the GSSG pathway in *A* and refs. 33 and 32, respectively, for the cytochrome P-450 and GSH pathways in *B*.

These results were similar to those observed with chromium(VI)-induced DNA strand breaks.

Total DNA cross-links (mainly DNA-protein cross-links) produced by chromium(VI) were not significantly changed by the isopentanol pretreatment (high cytochrome P-450 with high GSH). Both an increase and a decrease in GSH without any change in cytochrome P-450 or P-448 resulted in a 40% decrease in chromium(VI)-induced DNA-protein cross-links. It appeared that DNA-protein cross-links may be formed by a different mechanism than DNA strand breaks or DNA interstrand cross-links. Chromium(V) intermediates or chromium(III) complexes formed as the final products of metabolism may be responsible for the formation of DNA-protein cross-links. It is possible that other cellular components such as GSH transferases may be involved in producing some chromium(VI)-induced DNA lesions. Along with GSH, GSH transferases have been shown to be important for the formation of DNA adducts with 1,2-dibromoethane (30) and *N*-hydroxy-3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (28). GSH transferases are altered in rats by 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treatment (10). Another complication with the chromium(VI)-induced DNA-protein cross-links was that the level of cross-links might have been rising at the time examined. Previous work with chicken embryo hepatocytes has shown that chromium(VI)-induced DNA-protein cross-links reached a maximal level 3 hr after removal of chromium(VI) following a 2-hr treatment with 5 μM chromium(VI) (6). In contrast, DNA interstrand cross-links and strand breaks are maximal immediately after the 2-hr chromium(VI) treatment (6).

In conclusion, certain forms of cytochrome P-450 in the presence of GSH appeared to activate chromium(VI) to species capable of producing DNA strand breaks and DNA interstrand cross-links. However, upon depletion of GSH and induction of cytochrome P-450 or P-448 little or no chromium(VI)-induced DNA strand breaks or interstrand cross-links were observed. These studies indicate that GSH might play a key role in chromium(VI) metabolism and chromium(VI)-induced DNA damage, possibly through the formation of a chromium(VI) thioester. Chromium(VI)-induced DNA-protein cross-links were decreased upon induction or depletion of GSH and were unchanged by cytochrome P-450 in the presence of GSH, suggesting that these lesions may be formed by a different mechanism than DNA strand breaks and interstrand cross-links.

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