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Lethal and Mutagenic Actions of *N*-Methyl-*N'*-Nitro-*N*-Nitrosoguanidine Potentiated by Oxidized Glutathione, a Seemingly Harmless Substance in the Cellular Environment

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Both the lethal and the mutagenic actions of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) on cells of *Streptococcus pneumoniae* were greatly potentiated by a component of yeast extract added to the cellular environment. This component was found to be an oxidation product of glutathione, glutathione disulfide (GSSG). At low concentrations in the medium, both GSSG and glutathione potentiated MNNG action, but at high concentrations, glutathione (and other sulfhydryl compounds) abolished the effect. Point mutations in a cellular gene conferred resistance to the potentiating effect, and they blocked uptake of either GSSG or glutathione into the cells as well. This gene apparently encodes a component of the system for glutathione transport in *S. pneumoniae*. The mechanism by which GSSG, an apparently innocuous substance in the environment, renders low levels of MNNG genotoxic and cytotoxic thus depends on its transport into the cell, where it is reduced by glutathione reductase and then activates intracellular MNNG. Also, it was observed that mutants of *S. pneumoniae* defective in DNA mismatch repair are more resistant to MNNG than are wild-type cells by a factor of 2.5.

The Hex DNA mismatch repair system of *Streptococcus pneumoniae* acts on heteroduplex DNA formed during genetic transformation and on incorrectly synthesized DNA made during chromosomal replication (for a review, see reference 6). The latter activity prevents mutations. Defects in the *hex* genes block such repair. The sequences of the *hexA* gene (29) and of the related *mutS* gene of *Salmonella typhimurium* (10) were recently used to detect a homologous human mismatch repair gene, defects of which were shown to cause human colon cancer (8, 22). Some time ago, it was observed that mutants with defective *hex* genes accumulated in cultures of *S. pneumoniae* mutagenized by repeated cycles of treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (15). Whereas mutations in other genes of *S. pneumoniae* were found with a frequency of ~0.2%, the *hex* mutant frequency was as high as 50%. This suggested that the *hex* mutants might be relatively resistant to MNNG and led to the studies reported here.

The carcinogen MNNG is known to exert its mutagenic and lethal effects by methylation of DNA. Although its most prominent products are 7-methylguanine and 3-methyladenine (21), the formation of O_6-methylguanine appears to be responsible for its mutagenic action (7, 24). The Mut mismatch repair system of *Escherichia coli*, which is related to the Hex system (6, 10, 29), recognizes mismatches of O_6-methylguanine with T or C (7, 12). To exert its action, MNNG must be converted to an active intermediate, methyldiazohydroxide; this occurs slowly in aqueous solution, but the rate of activation can be increased 100-fold by addition of sulfhydryl compounds (21).

While investigating the susceptibility of cells of *S. pneumoniae* to MNNG, we observed a powerful enhancement of mutagenic and lethal action by addition of yeast extract (YE) to the medium. A seemingly innocuous substance was found to be responsible. The potential hazard of such interactions between chemical substances placed into our environment should not be overlooked.

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MATERIALS AND METHODS

Chemicals. MNNG, glutathione (GSH), glutathione disulfide (GSSG), thioglycolic acid, and NADPH were obtained from Sigma Chemical Co. Solutions of MNNG and GSH were prepared immediately before use at 10 mg/ml in dimethyl sulfoxide and water, respectively. Glycine-2-[^{3}H]GSH, 1.0 Ci/mmol, in 10 mM dithiothreitol, was obtained from Dupont. To prepare [^{3}H]GSSG from this material, dithiothreitol was extracted according to the method of Butler et al. (3), and the remaining [^{3}H]GSH was oxidized in air according to the method of Heijn et al. (11). Attempts to convert small amounts of [^{3}H]GSH to [^{3}H]GSSG with hydrogen peroxide by the method of Akerboom et al. (1) apparently produced a further oxidation product, which was eluted between GSSG and GSH in the fractionation procedure described below.

YE was prepared by crumbling 1 lb (ca. 0.5 kg) of fresh baker's yeast into 1 liter of water and heating the suspension to the boiling point. After cooling and removal of the sediment by centrifugation, the supernatant fluid was sterilized by filtration.

Fractionation of GSH, GSSG, and YE. Various mixtures of labeled and unlabeled GSH and GSSG with or without YE were applied to a column (1.5 by 45 cm) of polyacrylamide beads (BioGel-P2 [Bio-Rad]) and eluted with 50 mM NaCl–10 mM sodium phosphate (pH 5.8). Fractions of 2 ml were collected. Labeled compounds were detected by scintillation counting, bulk GSH or GSSG was detected by A_{202}, and MNNG potentiating activity in YE was detected by bioassay.

Bacterial strains. Strains of *S. pneumoniae* are derivatives of R6 and include 216 (*malM597*), 706 (*malM597 hexA4 nov*), and 533 (*sul str bry nov ery*). Strains 1277 to 1282 are spontaneous mutants of 216 or 706 isolated as colonies resistant to MNNG together with GSSG, GSH, or YE as follows: from strain 216, 1277 (GSSG), 1278 (GSH), 1281 (YE), and 1282 (YE); from strain 706, 1279 (GSSG) and 1280 (GSH). Strains 1283 to 1288 are *str* transformants with DNA from strain 533 of strains 1277 to 1282, respectively. Unless otherwise specified, cells of strain 216 were used in the experiments.

Growth and transformation of bacteria. Cultures of *S. pneumoniae* were grown in a semisynthetic medium and transformed as described previously (14). To select transformants resistant to MNNG and GSSG, cells were plated in a bottom agar layer containing MNNG at 30 μg/ml and GSSG at 10 μg/ml. They were covered with a top agar layer containing one-third of the volume and triple the concentrations of MNNG and GSSG to allow gradual diffusion of additional amounts of the active agents into the bottom layer. To select for resistance to

MNNG and GSH or YE, GSH at 10 μg/ml or 3% (vol/vol) YE replaced the GSSG in the bottom layer and triple these concentrations replaced GSSG in the top layer. Spontaneous mutants were obtained from platings of cells not treated with DNA.

Assays for survival and mutagenesis. Portions of cultures grown to an optical density at 650 nm of 0.2 (approximately 10^8 CFU/ml) were placed in tubes containing various amounts of MNNG and potentiating agents, and 0.1-ml samples were taken after various periods of incubation at 37°C and diluted into 0.9 ml of medium at 0°C. Survival was determined by plating appropriate dilutions in agar (14). For routine assays, samples were taken only at 0 and 60 min. Potentiating activity in fractions was determined in the presence of MNNG at 15 μg/ml, a concentration that gives no killing by itself. Lethal units in the tested sample were calculated as $-\ln(S/S_0)$, where S/S_0 is the surviving fraction at 60 min.

For mutagenesis experiments, 1-ml portions of culture were treated as described above and diluted into 1 ml of chilled medium. A sample was taken for measurement of survival, and 0.8 ml was diluted into 7.2 ml of medium containing 0.2% sucrose and thymidine at 50 μg/ml. After incubation for 3 h at 37°C for expression of the mutations, the cells were centrifuged, washed with 2 ml, and suspended in 0.9 ml of medium without sucrose. Samples were plated in medium with 0.2% maltose to detect Mal^+ mutants (14); in medium with 0.2% sucrose, thymidine at 50 μg/ml, and trimethoprim at 20 μg/ml to detect Tmp^r mutants (presumably defective in the gene for thymidine synthetase [16, 27]); and in medium with 0.2% sucrose for the total count of CFU.

Measurement of GSH and GSSG uptake. Cultures were grown to an optical density at 650 nm of 0.2. After addition of [^{3}H]GSH or [^{3}H]GSSG at 50 nCi and 10 μg/ml, 1-ml portions were incubated at 37°C for various periods and filtered on Millipore disks. The cells were washed three times with 5 ml of chilled medium, the disks were dried and placed in scintillation fluid, and the radioactivity was counted.

GSH reductase assay. Cell extracts were prepared as described previously (19), except that no dithiothreitol was added. Enzyme activity was determined spectrophotometrically according to the method of Carlberg and Mannervik (4).

RESULTS

Resistance of *hex* mutants to MNNG. To test the relative resistance to MNNG conferred by a *hex* mutation, we compared an isogenic pair of strains, 216 and 706. Strain 216 contains *mal597* and is wild type for *hex*. Strain 706 contains *hexA4* in addition to *mal597*. (It also contains *nov*, which was used to introduce the *hex* mutation into strain 216 [18].) Survival of these two strains at two different MNNG concentrations is shown in Fig. 1. The logarithmic killing rate in the Hex^- strain was approximately 40% of that in the Hex^+ strain at both concentrations. The *hexA4* mutant tested presumably carries a missense mutation at a single site in the gene, because in multiple dosage it shows a partial Hex^+ phenotype (2). Similar enhancement of survival was obtained (data not shown) for a lengthy insertion mutation, *hexA*::*cat-9* (29), and for the *hexB3* mutation, which occurred in another *hex* gene identified in *S. pneumoniae* (2, 6). Several other isogenic pairs of Hex^+ and Hex^- strains also showed the same differential killing (data not shown).

In examining the above survival data, we were puzzled by the apparent resistance of the wild-type strains to MNNG, because in the original experiments of mutagenesis with MNNG (15), we found that survival was reduced to 0.1% at 60 min even with an MNNG concentration of only 20 μg/ml. Those experiments were different, however, in that YE had been added to the medium. That the addition of YE remarkably affects the survival rate is shown in Fig. 2. When MNNG was added at 15 μg/ml to the medium in the absence of YE, no killing was observed in this time period (data not shown). In the presence of YE, survival at 60 min was reduced to 0.1% with this concentration of MNNG.

Identification of the factor in YE potentiating MNNG. Column fractionation of the potentiating activity in YE by gel filtration and anion-exchange chromatography showed it to behave predominantly as a single substance (data not shown). From the sizing column, it appeared to have a molecular weight between 500 and 2,000. Because sulfhydryl compounds

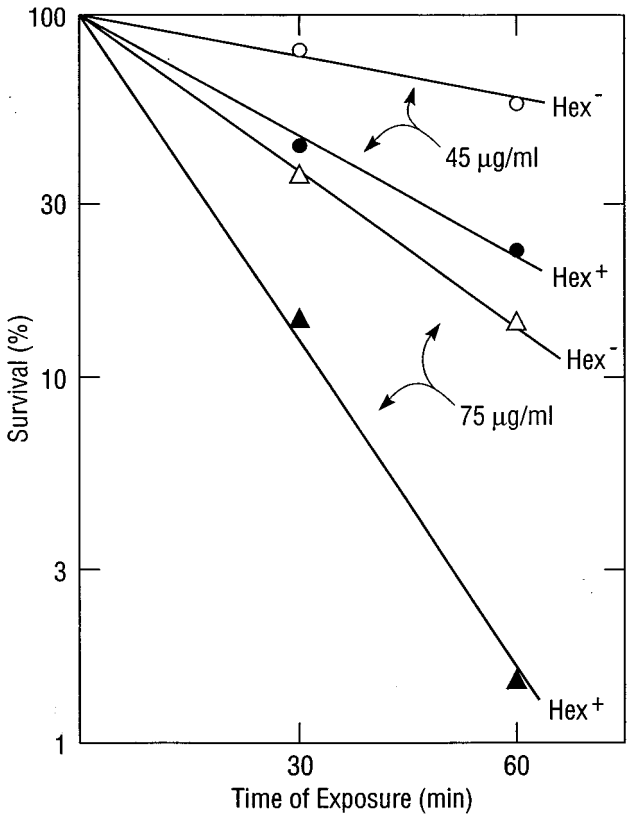

FIG. 1. Sensitivity of Hex^+ and Hex^- cells to MNNG. Closed symbols, Hex^+ strain 216; open symbols, Hex^- strain 706. Circles, MNNG at 45 μg/ml; triangles, MNNG at 75 μg/ml.

can activate MNNG (21, 25) and the oxidized form (GSSG) of GSH has a molecular weight (612) in this range, we tested the effect of these compounds on killing by MNNG. Concentrations of either GSH or GSSG as low as 1 μg/ml greatly reduced survival in the presence of MNNG (see Fig. 4, below).

To see whether the YE factor corresponded to GSSG, we compared its behavior with GSSG on a gel filtration column (BioGel-P2 [Bio-Rad]). We prepared labeled [^{3}H]GSSG by air oxidation of [^{3}H]GSH and saw that it eluted at the position of authentic GSSG (Fig. 3a); GSH was retained significantly longer by the column. Fractionation of YE together with tracer amounts of [^{3}H]GSSG showed that most of the potentiating activity eluted as a single peak at the position of GSSG, although some activity eluted later, as a shoulder of the peak (Fig. 3b). This behavior was reproducible. The major active component of YE appears to be GSSG; the minor component may be a further oxidation product of GSH (see Materials and Methods).

Potentiation of both lethal and mutagenic effects of MNNG. YE and GSSG both enhance the mutagenic effect of MNNG as well as its lethal effect, as demonstrated in Table 1. Two types of mutations were examined. The mutations to Mal^+ represent back-mutations of *malM597* from G:C to A:T at position 2572 in the amylomaltase gene of *S. pneumoniae* (5). The mutations to Tmp^r, which confer trimethoprim resistance in the presence of thymidine, correspond for the most part to forward mutations in the gene for thymidylate synthetase (16, 27). They relieve the biosynthetic demand for folate compounds, the production of which is reduced by inhibitors of dihydrofolate reductase, such as aminopterin or trimethoprim. The frequen-

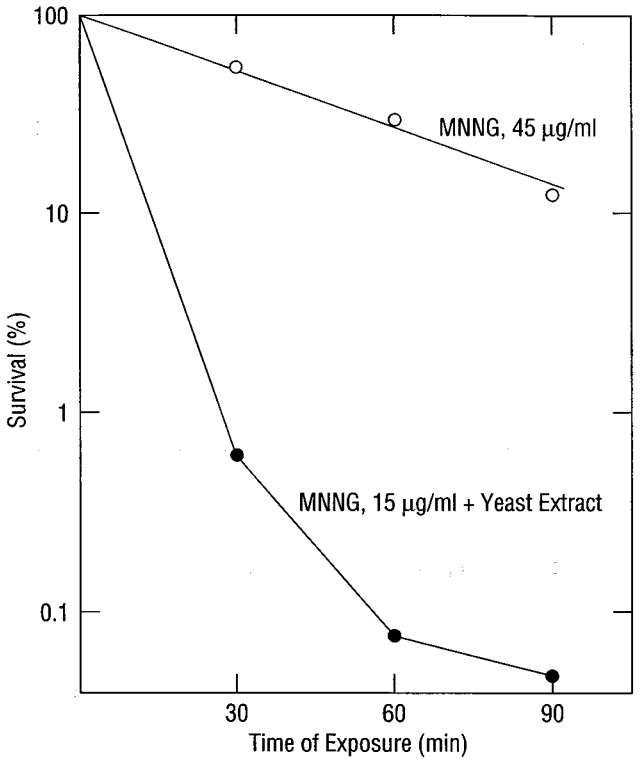

FIG. 2. Potentiation of MNNG action by YE. Open circles, MNNG at 45 μg/ml; closed circles, MNNG at 15 μg/ml with YE at 3% (vol/vol). In the absence of YE, MNNG at 15 μg/ml gave no detectable killing.

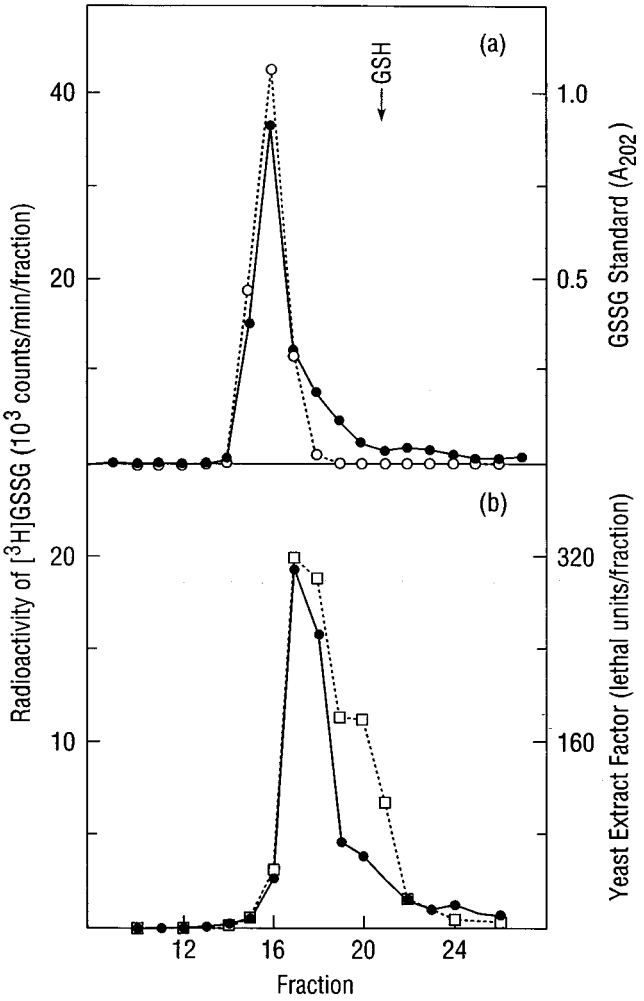

FIG. 3. Gel filtration of GSSG and MNNG-potentiating activity in YE. (a) Correspondence of [^{3}H]GSSG preparation with authentic GSSG. Arrow indicates position of elution of GSH. (b) Elution of MNNG-potentiating activity of YE. Closed circles, radioactivity; open circles, A_{202}; squares, lethal units of potentiating activity.

cies of both types of mutants, which were barely affected by MNNG alone at 15 μg/ml, were considerably increased by addition of either YE or GSSG. The frequencies observed were comparable to those obtained with MNNG alone at 60 μg/ml; that is, at a given level of survival similar frequencies were found. This is evident from the similarity in mutant frequencies per lethal hit (Table 1) and is consistent with a common activated form of MNNG in the presence and absence of potentiation.

A comparison of the effects of various concentrations of sulfhydryl compounds and GSSG on potentiation of the lethal effect of MNNG gave insight into the mechanism of potentiation. Although the lethal effect remained strong as the concentration of GSSG increased 300-fold, high concentrations of GSH reversed the effect of low concentrations and eliminated the lethal effect (Fig. 4). This is interpreted to indicate that, at low concentrations, GSH is taken up and concentrated by the cells, where it activates internalized MNNG, but at high concentrations outside the cells, GSH can precociously activate external MNNG, which decays without access to its DNA target. GSSG, which does not directly interact with MNNG, does not show this effect. Another sulfhydryl compound, thioglycolic acid, cannot potentiate the action of MNNG, as shown in Fig. 4. However, when MNNG is potentiated by addition of YE, the further addition of higher concentrations of thioglycolic acid reduces and eliminates the potentiation. These results can be explained by the inability of the cells to take up thioglycolate (in contrast to GSH) and by its action similar to that of GSH outside the cells.

Mutants resistant to potentiation of MNNG. Mutants resistant to the potentiating effects of YE, GSSG, and GSH were readily obtained by plating cells of *S. pneumoniae* in the presence of any of these agents and MNNG. In all cases tested, the mutants were simultaneously resistant to the potentiating effect of all three agents, but the mutants retained the same sensitivity to MNNG alone at concentrations of >30 μg/ml (data not shown). In the Hex$^+$ strain 216, the mutant frequency was 10^{-6}. In the Hex$^-$ strain 706, it was 4×10^{-5}; the higher value presumably results from the absence of mismatch repair. Both of these background mutation frequencies are higher than the usual spontaneous mutation frequencies in *S. pneumoniae* because the selective agent itself is a mutagen.

Transformation of *S. pneumoniae* cells by the marker conferring resistance could be observed and its frequency could be measured by the same selection procedure used to obtain the mutants. Analysis of the transformation frequencies in Hex$^+$ and Hex$^-$ recipients demonstrated that point mutations in a single gene could give rise to the resistant phenotype. It is known that the Hex mismatch repair system recognizes only base mismatches or very small deletions or insertions (6, 23). Therefore, if the Hex system affects the frequency of transfer of a marker, the marker must be a point mutation (or at most a deletion or insertion of 5 nucleotides). Table 2 lists the

TABLE 1. Potentiation of MNNG mutagenesis by YE and GSSG

Addition(s) (per ml)	Time (min)	Survival (S/S_0)	No. of mutants/ml[a]		Mal^+ mutant frequency	
			Tmp^r	Mal^+	Per survivor	Per lethal hit[b]
None	0	1.00	10	10	3.8×10^{-8}	
15 μg of MNNG	5	0.97	20	16	6.2×10^{-8}	ND[c]
15 μg of MNNG	10	1.02	70	20	7.4×10^{-8}	ND
15 μg of MNNG + 30 μl of YE	5	0.64	695	813	7.5×10^{-6}	1.7×10^{-5}
15 μg of MNNG + 30 μl of YE	10	0.11	1,150	688	4.8×10^{-5}	2.2×10^{-5}
15 μg of MNNG + 15 μg of GSSG	5	0.63	3,550	1,282	7.7×10^{-6}	1.6×10^{-5}
15 μg of MNNG + 15 μg of GSSG	10	0.05	1,350	426	3.0×10^{-5}	1.0×10^{-5}
60 μg of MNNG	5	1.09	760	564	2.0×10^{-6}	ND
60 μg of MNNG	10	0.67	2,380	1,874	1.1×10^{-5}	2.7×10^{-5}

[a] Tmp^r, resistant to trimethoprim in the presence of thymidine; Mal^+, able to use maltose.
[b] Lethal hits were calculated as $-\ln(S/S_0)$.
[c] ND, not determined because of insignificant measurement of killing at low doses.

frequencies of transformation obtained with DNA from mutants selected for resistance to potentiation of MNNG killing by YE, GSSG, or GSH. A streptomycin resistance mutation was introduced into the DNA donors to serve as a reference marker, since individual cultures can vary in competence for transformation (17). With the Hex^- recipient, the Ntg^r/Str^r ratios were all approximately equal to 1.0, which indicates that these mutations to resistance did not correspond to large deletions (14) and, therefore, that each one occurred in a single gene. It is possible that the mutations all affected the same gene. With the Hex^+ recipient, the ratios were much lower, 0.05 to 0.17, which indicates that the heteroduplex mismatches were recognized by the Hex system, and so the loss of potentiation resulted from point mutations. Transformants were tested and found to have the same properties as the original mutants with regard to resistance to MNNG and, as described below, transport of GSH.

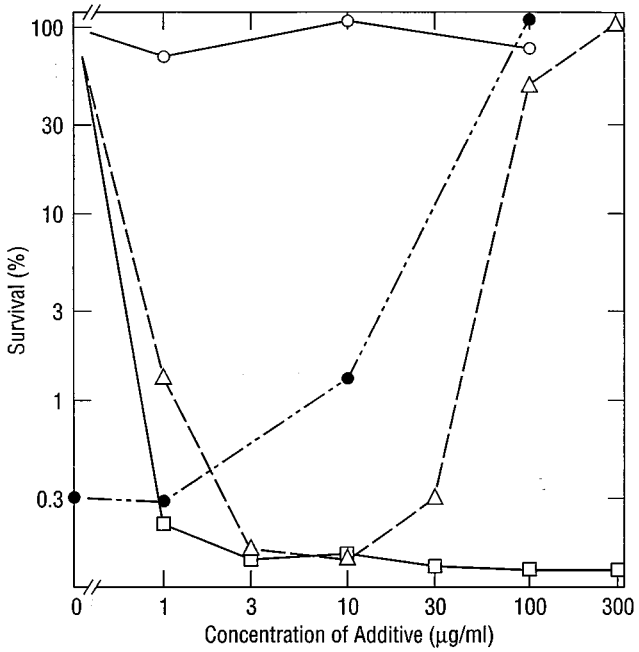

FIG. 4. Potentiation of MNNG action by GSH and GSSG. Survival is shown after 60 min with MNNG at 15 μg/ml and additives as follows: triangles, GSH; squares, GSSG; open circles, thioglycolic acid; closed circles, 3% (vol/vol) YE and thioglycolic acid as indicated.

Mutants defective in GSH transport. Wild-type cells of *S. pneumoniae* can readily take up and accumulate both GSH and GSSG (Fig. 5). Uptake abilities for both of these compounds are almost completely lost in the mutants resistant to potentiation of MNNG (Fig. 5). This was true whether the mutant was originally selected for resistance to potentiation by GSSG, GSH, or YE. The mutants shown in Fig. 5 contain *str*, but this marker had no effect on the uptake phenotype (data not shown). We conclude that a common gene product is required for transport of GSH and GSSG and that the mutants are defective in a gene required for making this product. We call this gene *glt*, for glutathione transport. Most likely, the product is a protein that acts as a permease for GSH and its derivatives.

Extracts of wild-type and mutant cells were assayed for glutathione reductase, the enzyme that converts GSSG to GSH by reduction with NADPH as the electron donor. This enzyme activity was present in both wild-type strains and in all the MNNG-resistant mutants and transformants tested. The observed activities ranged between 10 and 20 nmol/min/mg of protein.

Mechanism of potentiation of MNNG by GSSG. The proposed mechanism of potentiation by GSSG of MNNG killing and mutagenesis is summarized here. At low concentrations of MNNG in the medium, the amounts taken into the cell are too slowly activated to show much of an effect. However, when GSSG is added to the medium even at low concentrations, it is taken up and concentrated by the cell and reduced to GSH by the intracellular glutathione reductase. The sulfhydryl com-

TABLE 2. Frequencies of resistant colonies in transformation of Hex^+ and Hex^- strains by DNA from mutants resistant to potentiation of MNNG by GSSG, GSH, and YE[a]

DNA donor strain[b]	Recipient strain resistance frequency					
	216 (Hex^+)			706 (Hex^-)		
	Ntg^r	Str^r	Ratio[c]	Ntg^r	Str^r	Ratio[c]
None	1.1×10^2			4.6×10^3		
1285 (GSSG)	1.2×10^4	2.4×10^5	0.05	2.8×10^5	3.2×10^5	0.87
1286 (GSH)	1.0×10^4	2.0×10^5	0.05	2.8×10^5	3.3×10^5	0.84
1288 (YE)	3.4×10^4	2.0×10^5	0.17	3.7×10^5	3.4×10^5	1.08

[a] Frequencies are numbers of colonies per milliliter of transformed culture. Ntg^r, resistant to MNNG plus GSSG.
[b] Potentiating agent used in selection of resistant mutation is shown in parentheses.
[c] Ratio of Ntg^r to Str^r transformants for each combination of donor and recipient.

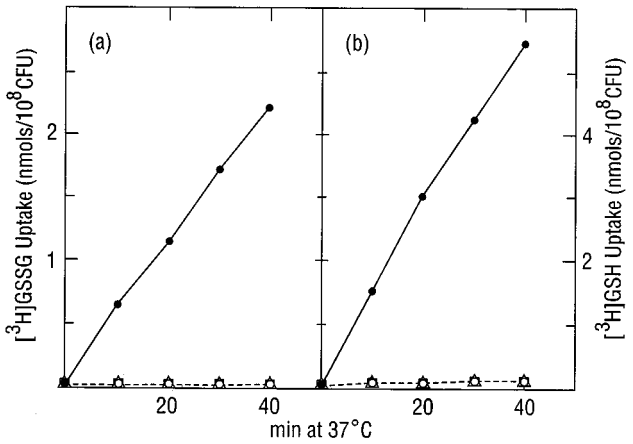

FIG. 5. Uptake of GSSG and GSH by wild-type and MNNG potentiation-resistant mutants of *S. pneumoniae*. (a) Uptake of [^{3}H]GSSG. (b) Uptake of [^{3}H]GSH. Strains are as follows: closed circles, 216 (wild type); open circles, 1287; triangles, 1284; squares, 1283. The three mutant strains were selected in the presence of YE, GSH, and GSSG, respectively.

pound will activate intracellular MNNG so that it methylates DNA, thereby exerting its lethal and mutagenic effects. Low concentrations of GSH in the medium will be accumulated by the same transport system and activate MNNG directly in the cell; at high concentrations, however, GSH activates and destroys MNNG outside the cell. Sulfhydryl compounds that are not taken up, such as thioglycolate, are not effective. The potentiation is specific for MNNG; another DNA methylating agent, methyl methane sulfonate, was not affected by the addition of either GSSG or GSH (data not shown). Mutants that lack the GSH transport system do not show the potentiation. Two other bacterial species tested, *Bacillus subtilis* and *E. coli*, showed neither potentiation of MNNG action nor uptake of GSH (data not shown).

DISCUSSION

A significant, albeit limited (2.5-fold) resistance of mismatch repair system mutants to MNNG was found in *S. pneumoniae*. A possible mechanism for the relative sensitivity of Hex$^+$ cells is the formation of irreparable double-strand breaks in DNA by attempts to repair DNA simultaneously on opposite strands. In this case, recognition by the Hex system of O_6-methylguanine mismatches with thymine in replicating DNA could remove a segment of the nascent strand. Removal of a *3*-methyladenine lesion in the opposite strand could give rise to a strand break by successive action of a *3*-methyladenine DNA glycosylase similar to the enzyme reported in *E. coli* (33) and an AP-endonuclease, such as ExoA in *S. pneumoniae* (30). This general type of mechanism was proposed for the sensitivity of *dam* mutants of *E. coli* to MNNG (12), but in this case, the difference between MutS$^+$ and MutS$^-$ was far greater and presumably resulted from simultaneous removal of length segments from opposite strands triggered by action of MutH in nicking (20) both nascent and parental unmethylated DNA strands. In the case of human cells, however, a great difference in sensitivity to MNNG was reported for the MT1 mutant (9), which has been shown to be defective in mismatch repair (13). It is not obvious why the human system differs so much from *S. pneumoniae*, inasmuch as no role for DNA methylation or a MutH-like protein has been found in either of these two systems, and strand targeting is presumably due only to breaks in the nascent DNA strands.

Overshadowing the effect of *hex* mutations on MNNG sensitivity was the dramatic effect of GSH. When added to the culture medium in either oxidized or reduced form, GSH increased killing and mutagenesis of the bacteria by MNNG at least 100-fold (Fig. 4 and Table 1). The mechanism of this potentiation is apparently intracellular activation of MNNG by GSH that is either taken up directly by the cell or produced by glutathione reductase from GSSG taken up by the cell. *S. pneumoniae* appears to have a common transport mechanism for GSH and GSSG, which can be rendered defective by a single point mutation. Although it has been reported before that intracellular levels of GSH affect sensitivity to MNNG (21, 31, 32), this is the first instance shown for an indirect effect of GSSG and for implication of GSH and GSSG transport in the activation process. In oxidative environments, which most commonly occur, GSH would be present in the form of GSSG. This was true for the case of the YE added to the bacterial medium, in which we first observed the potentiating effect.

This study points up the danger of an apparently innocuous substance in the environment, GSSG, serving as a strong activating agent for a potential mutagen or carcinogen that may be added to or already in the environment. It shows that, as well as considering the directly harmful action of a substance added to the environment, it is advisable to consider also the possible interaction with other substances present. The interaction may be quite subtle, and apparent only after uptake of the substances by living cells, as in the present case.

Not all cells can transport GSH. We observed that *B. subtilis* and *E. coli* lack this ability. Thus, it is not surprising that addition of GSH to the medium protects *E. coli* from mutagenic action rather than potentiating it (28). In general, GSH protects mammalian cells, by acting as an antioxidant (26). Some mammalian cells can take up GSH (31), and mammalian membrane vesicles have been shown to transport GSSG (1, 11), although the latter transport would be out of the cell. The fact that *glt* mutations in the GSH uptake system are readily selected in *S. pneumoniae* by their conferring of resistance to the combination of MNNG and GSSG should facilitate analysis of the genetics and molecular biology of GSH transport.

Cells of *S. pneumoniae* may be unusual among bacteria in their ability to accumulate GSH. It might be possible to use this property of the *S. pneumoniae* bacterium to treat cases of pneumonia, particularly when caused by antibiotic-resistant strains. Although the obvious treatment with a combination of GSSG and MNNG may be too harsh to body tissues and dangerously mutagenic, it is conceivable that the GSH transport system might be harnessed to introduce a lethal agent specific for bacteria.

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REFERENCES

1. **Akerboom, T. P. M., V. Narayanaswami, M. Kunst, and H. Sies.** 1991. ATP-dependent *S*-(2,4-dinitrophenyl)GSH transport in canalicular plasma membrane vesicles from rat liver. J. Biol. Chem. **266:**13147–13152.
2. **Balganesh, T. S., and S. A. Lacks.** 1985. Heteroduplex DNA mismatch repair system of *Streptococcus pneumoniae*: cloning and expression of the *hexA* gene. J. Bacteriol. **162:**979–984.
3. **Butler, J., S. P. Spielberg, and J. D. Schulman.** 1976. Reduction of disulfide-containing amines, amino acids, and small peptides. Anal. Biochem. **75:**674–675.
4. **Carlberg, I., and B. Mannervik.** 1985. Glutathione reductase. Methods En-

zymol. **113:**484–490.
5. **Chen, J.-D., and S. A. Lacks.** 1991. Role of uracil-DNA glycosylase in mutation avoidance by *Streptococcus pneumoniae*. J. Bacteriol. **173:**283–290.
6. **Claverys, J. P., and S. A. Lacks.** 1986. Heteroduplex deoxyribonucleic acid base mismatch repair in bacteria. Microbiol. Rev. **50:**133–165.
7. **Eadie, J. S., M. Conrad, D. Toorchen, and M. D. Topal.** 1984. Mechanism of mutagenesis by O^6-methylguanine. Nature (London) **308:**201–203.
8. **Fishel, R., M. K. Lescoe, M. R. S. Rao, N. G. Copeland, N. A. Jenkins, J. Garber, M. Kane, and R. Kolodner.** 1993. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell **75:**1027–1038.
9. **Goldmacher, V. S., R. A. Cuzick, and W. G. Thilly.** 1986. Isolation and partial characterization of human cell mutants differing in sensitivity to killing and mutation by methylnitrosourea and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. J. Biol. Chem. **261:**12462–12471.
10. **Haber, L. T., P. P. Pang, D. I. Sobell, J. A. Mankovich, and G. C. Walker.** 1988. Nucleotide sequence of the *Salmonella typhimurium mutS* gene required for mismatch repair: homology of MutS and HexA of *Streptococcus pneumoniae*. J. Bacteriol. **170:**197–202.
11. **Heijn, M., R. P. J. Oude Elferink, and P. L. M. Jansen.** 1992. ATP-dependent multispecific organic anion transport system in rat erythrocyte membrane vesicles. Am. J. Physiol. **262**(Cell Physiol. **31**)**:**C104–C110.
12. **Karran, P., and M. G. Marinus.** 1982. Mismatch correction at O^6-methylguanine residues in *E. coli* DNA. Nature (London) **296:**868–869.
13. **Kat, A., W. G. Thilly, W.-H. Fang, M. J. Longley, G.-M. Li, and P. Modrich.** 1993. An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. Proc. Natl. Acad. Sci. USA **90:**6424–6428.
14. **Lacks, S.** 1966. Integration efficiency and genetic recombination in pneumococcal transformation. Genetics **53:**207–235.
15. **Lacks, S.** 1970. Mutants of *Diplococcus pneumoniae* that lack deoxyribonucleases and other activities possibly pertinent to genetic transformation. J. Bacteriol. **101:**373–383.
16. **Lacks, S., and B. Greenberg.** 1973. Competence for deoxyribonucleic acid uptake and deoxyribonuclease action external to cells in the genetic transformation of *Diplococcus pneumoniae*. J. Bacteriol. **114:**152–163.
17. **Lacks, S., and R. D. Hotchkiss.** 1960. A study of the genetic material determining an enzyme activity in pneumococcus. Biochim. Biophys. Acta **39:**508–517.
18. **Lacks, S. A., J. J. Dunn, and B. Greenberg.** 1982. Identification of base mismatches recognized by the heteroduplex-DNA-repair system of *Streptococcus pneumoniae*. Cell **31:**327–336.
19. **Lacks, S. A., and S. S. Springhorn.** 1984. Cloning in *Streptococcus pneumoniae* of the gene for *Dpn*II DNA methylase. J. Bacteriol. **157:**934–936.
20. **Lahue, R. S., K. G. Au, and P. Modrich.** 1989. DNA mismatch correction in a defined system. Science **245:**160–164.
21. **Lawley, P. D., and C. J. Thatcher.** 1970. Methylation of deoxyribonucleic acid in cultured mammalian cells by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Biochem. J. **116:**693–707.
22. **Leach, F. S., et al.** 1993. Mutations of a *mutS* homolog in hereditary non-polyposis colorectal cancer. Cell **75:**1215–1225.
23. **Lopez, P., M. Espinosa, B. Greenberg, and S. A. Lacks.** 1987. Sulfonamide resistance in *Streptococcus pneumoniae*: DNA sequence of the gene encoding dihydropteroate synthase and characterization of the enzyme. J. Bacteriol. **169:**4320–4326.
24. **Loveless, A.** 1969. Possible relevance of *O*-6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. Nature (London) **223:**206–208.
25. **McCalla, D. R.** 1968. Reaction of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide with DNA in vitro. Biochim. Biophys. Acta **155:**114–120.
26. **Meister, A.** 1994. Glutathione-ascorbic acid antioxidant system in animals. J. Biol. Chem. **269:**9397–9400.
27. **Okada, T., K. Yanagisawa, and F. J. Ryan.** 1961. A method for securing thymineless mutants from strains of *Escherichia coli*. Z. Vererbungsl. **92:**403–412.
28. **Owens, R. A., and P. E. Hartman.** 1986. Glutathione: a protective agent in *Salmonella typhimurium* and *Escherichia coli* as measured by mutagenicity and by growth delay assays. Environ. Mutagen. **8:**659–673.
29. **Priebe, S. D., S. M. Hadi, B. Greenberg, and S. A. Lacks.** 1988. Nucleotide sequence of the *hexA* gene for DNA mismatch repair in *Streptococcus pneumoniae* and homology of *hexA* to *mutS* of *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **170:**190–196.
30. **Puyet, A., B. Greenberg, and S. A. Lacks.** 1989. The *exoA* gene of *Streptococcus pneumoniae* and its product, a DNA exonuclease with apurinic endonuclease activity. J. Bacteriol. **171:**2278–2286.
31. **Romert, L., and D. Jenssen.** 1987. Mechanism of *N*-acetylcysteine (NAC) and other thiols as both positive and negative modifiers of MNNG-induced mutagenicity in V79 Chinese hamster cells. Carcinogenesis **8:**1531–1535.
32. **Romert, L., S. Swedmark, and D. Jenssen.** 1991. Thiol-enhanced decomposition of MNNG, ENNG and nitrosocimetidine: relationship to mutagenicity in V79 Chinese hamster cells. Carcinogenesis **12:**847–853.
33. **Thomas, L., C.-H. Yang, and D. A. Goldthwait.** 1982. Two DNA glycosylases in *Escherichia coli* which release primarily 3-methyladenine. Biochemistry **21:**1162–1169.