JOURNAL OF BACTERIOLOGY, Apr. 1996, p. 2131–2135 Vol. 178, No. 7
0021-9193/96/$04.00+0

Bacterial Glutathione: a Sacrificial Defense against Chlorine Compounds†

JASON A. CHESNEY,[1] JOHN W. EATON,[2] AND JOHN R. MAHONEY, JR.[2,3]*

Picower Institute, Manhassett, New York 11030,[1] and Division of Experimental Pathology, Department of Pathology and Laboratory Medicine,[2] and Department of Anesthesiology,[3] Albany Medical College, Albany, New York 12208

Received 21 September 1995/Accepted 16 January 1996

Aerobic organisms possess a number of often overlapping and well-characterized defenses against common oxidants such as superoxide and hydrogen peroxide. However, much less is known of mechanisms of defense against halogens such as chlorine compounds. Although chlorine-based oxidants may oxidize a number of cellular components, sulfhydryl groups are particularly reactive. We have, therefore, assessed the importance of intracellular glutathione in protection of *Escherichia coli* cells against hydrogen peroxide, hypochlorous acid, and chloramines. Employing a glutathione-deficient *E. coli* strain (JTG10) and an otherwise isogenic glutathione-sufficient *E. coli* strain (AB1157), we find that glutathione-deficient organisms are approximately twice as sensitive to killing by both hydrogen peroxide and chlorine compounds. However, the mode of protection by glutathione in these two cases appears to differ: exogenous glutathione added to glutathione-deficient *E. coli* in amounts equal to those which would be present in a similar suspension of the wild-type bacteria fully restored resistance of glutathione-deficient bacteria to chlorine-based oxidants but did not change resistance to hydrogen peroxide. Furthermore, in protection against chlorine compounds, oxidized glutathione is almost as effective as reduced glutathione, implying that the tripeptide and/or oxidized thiol undergo further reactions with chlorine compounds. Indeed, in vitro, 1 mol of reduced glutathione will react with ~3.5 to 4.0 mol of hypochlorous acid. We conclude that glutathione defends *E. coli* cells against attack by chlorine compounds and hydrogen peroxide but, in the case of the halogen compounds, does so nonenzymatically and sacrificially.

Aerobic organisms have numerous and often redundant defenses against oxidants. The natural abundance of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) makes superoxide dismutase, catalase, and peroxidases preeminently important oxidant defenses. These enzymatic systems, along with antioxidants such as tocopherols, retinoids, and ascorbate, provide effective protection against reasonable challenges with activated oxygen species. There are, however, other oxidants against which no obvious defenses are known. Important among these are the chlorine compounds hypochlorous acid (HOCl), monochloramine (NH_2Cl), and taurine monochloramine, which are generated by mammalian neutrophils (10, 20, 27). Hypochlorous acid, in particular, is a strong electrophile and will oxidize a large number of biological molecules, especially those containing thiols, thioethers, and conjugated double bonds (5, 25).

Therefore, it is no surprise that both mammalian neutrophils and water treatment facilities use chlorine and its reactive derivatives as effective bactericides (22). In tests on dilute bacterial suspensions (free of other organics), HOCl has a 50% lethal dose (LD_{50}) of 0.2 to 3.0 μM against most bacteria tested, and there is evidence that chlorine generated by the mammalian neutrophil myeloperoxidase (MPO)-H_2O_2-chloride system is equally effective (24). Interestingly, no significant bacterial resistance to chlorine compounds (with the possible exception of *Cryptosporidium parvum* [13]) has been found, despite decades of water treatment with powerful chlorine-based oxidants. Previous studies have identified several potential bacterial targets of chlorine oxidants such as the electron transport chain (2), iron sulfur centers (15), the membrane-associated origin of DNA replication (16), adenine nucleotides (1–3), important metabolic enzymes such as succinate oxidase (17), and unsaturated fatty acids within membranes (26).

Knowing of the very facile reactions between hypochlorous acid and sulfur compounds (18, 24), we hypothesized that glutathione (GSH), the predominant intracellular thiol compound in *Escherichia coli* and many other bacteria (8), might represent an important defense against attack by hypohalous acids such as hypochlorous acid. We have tested this idea, employing an otherwise isogenic pair of *E. coli* strains, one of which lacks GSH (16). The results affirm the importance of GSH as a defense against chlorine compounds (and, expectably, H_2O_2). Interestingly, exogenous GSH—both reduced and oxidized—appears as effective as intracellular GSH in protection of bacteria against chlorine compounds (but not H_2O_2). These results suggest that GSH is an important but sacrificial defense against chlorine compounds but that the protective reactions do not require the collaboration of intracellular enzymes.

MATERIALS AND METHODS

Preparation of solutions. All solutions were prepared in phosphate-buffered saline (PBS) (1.06 mM KH_2PO_4, 0.6 mM Na_2HPO_4, 154 mM NaCl [pH 7.2]). Stock solutions of hypochlorous acid (HOCl) were prepared immediately before use by dilution of reagent sodium hypochlorite (stock concentration, ~680 mM) in PBS. Stock solutions of hydrogen peroxide (H_2O_2) (~8.8 M) were also diluted in PBS. For preparation of chloramine compounds, 9 parts of 11.1 mM taurine or 11.1 mM ammonium chloride (also in PBS) were admixed with 1 part of 100 mM sodium hypochlorite. Solutions of 6 mM ascorbic acid and 1 mM GSH were freshly prepared in deionized water. Concentrations of stock solutions of HOCl, NH_2Cl, and taurine monochloramine were determined spectrophotometrically by using the known molar absorptivities of 350 M^{-1} cm^{-1} at 292 nm (14, 20), 424 M^{-1} cm^{-1} at 242 nm (27), and 398 M^{-1} cm^{-1} at 250 nm (23), respectively. In addition, concentrations of these chlorine compounds were independently determined by coupled oxidation of 5-thio-2-nitrobenzoic acid to 5,5′-dithiobis-(2-

* Corresponding author. Mailing address: Division of Experimental Pathology, A-81, Department of Pathology and Laboratory Medicine, Albany Medical College, 47 New Scotland Ave., Albany, NY 12208. Phone: (518) 262-6268. Fax: (518) 262-5927. Electronic mail address: MahoneyAMC@aol.com.

† Dedicated to Eugene Ogan on the occasion of his retirement.

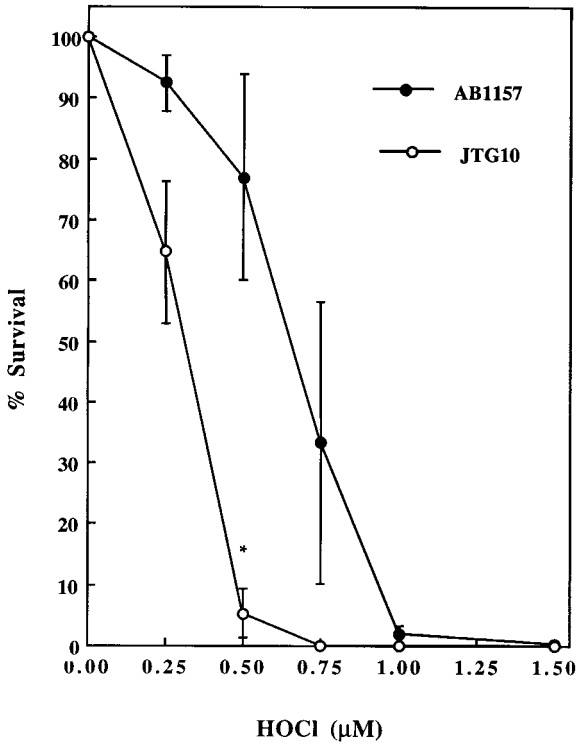

FIG. 1. HOCl toxicity to dilute suspensions of GSH-replete (●) and -deficient (○) *E. coli* cells resuspended to a concentration of 10^6 cells per ml. At time zero, HOCl was added to give the final concentration as indicated. Survival of bacteria after 20 min was assessed by removing aliquots and duplicate pour plating. CFU were enumerated after 36 h of growth at 37°C. $n = 3$; *, $P < 0.01$.

nitrobenzoic acid) (DTNB) or ascorbic acid to dehydroascorbic acid as previously described (4, 7).

Bacterial stains and growth conditions. *E. coli* AB1157 and the GSH-deficient strain JTG10 (derived in Bruce Demple's laboratory, Harvard University, Boston, Mass) were provided by James Fuchs, University of Minnesota, St. Paul. JTG10 and AB1157 are isogenic strains, except that JTG10, as a result of transposon insertion in the *gshA* gene, is completely lacking in GSH (11). Both strains were grown to stationary phase for 18 h at 37°C in brain heart infusion broth (BHI) (Gibco Laboratories, Madison, Wis.). Before performance of the killing assays or analyses of GSH concentrations, the bacteria were washed twice, and resuspended, in cold PBS (4°C). We experimentally verified that these particular *E. coli* strains did not show diminished CFU after exposure to this temperature. Bacterial concentrations were estimated by measuring the A_{600} of bacterial suspensions.

Incubation of *E. coli* cells with oxidants. For killing assays on dilute *E. coli* suspensions, 100 μl was added to 1,930 μl of PBS (25°C), for a final concentration of 10^6 bacteria per ml. After removing 50-μl aliquots for bacterial enumeration (see below), 20 μl of either H_2O_2, HOCl, monochloramine, or taurine monochloramine was added. Further incubation was carried out at 25°C for 20 min or 4 h as indicated. For killing assays on more concentrated suspensions, 100 μl of appropriately diluted *E. coli* suspensions was added to 900 μl of PBS (25°C), for a final concentration of 10^{10} bacteria per ml. After removing 50-μl aliquots for time zero control samples, 10 μl of HOCl solution was added, and the incubation was carried out at 25°C for 20 min.

Assay of bacterial viability. After exposure of bacteria to oxidants, aliquots of the suspensions were appropriately diluted in PBS. A 50-μl aliquot of each sample was placed into a petri dish, and molten Luria-Bertani agar (Gibco Ltd., Paisley, Scotland) was poured over it. The petri dishes were rotated (70 rpm) as the agar solidified. Bacterial viability was measured as the number of colonies (Artek automatic plate counter) appearing after incubation at 37°C for 36 h.

Determination of GSH concentration. Bacterial GSH concentration was determined colorimetrically on the basis of the absorbance of the reaction product of GSH and DTNB (7). Although this technique is unable to differentiate between GSH and other low-molecular-weight thiol-containing compounds, GSH constitutes the overwhelming fraction of acid-soluble thiols in *E. coli*. Bacterial suspensions (1 ml) at a concentration of 5×10^{10} were exposed to various concentrations of HOCl for 15 min. Precipitation of bacteria was accomplished by the addition of 4 ml of 0.08 N H_2SO_4 with vigorous vortexing and incubation for 10 min at 25°C. An aliquot of 0.3 M tungstate (0.5 ml) was added, and the solution was vortexed and left at 25°C for 10 min. Following centrifugation (1,000 × relative centrifugal force for 10 min), the concentration of GSH was determined by measuring the reduction of DTNB (a molar absorptivity coefficient of $13{,}600 \times 10^5$ M^{-1} cm^{-1} at 412 nm was used).

MPO-mediated killing of AB1157 and JTG10 cells. Partially purified human MPO was generously provided by Beulah Gray, University of Minnesota, Minneapolis. MPO (final activity of 7 mU/ml, determined by guiaicol reduction) was added to suspensions of 10^6 *E. coli* cells per ml in PBS. Hydrogen peroxide (0 to 3.0 μM) was added, and bacterial survival was estimated following 30 min of incubation at 25°C.

Statistics. Differences between the two bacterial strains were analyzed by Student's *t* test. A *P* value of <0.05 was used as the minimum for statistical significance.

RESULTS

We reaffirmed that strain JTG10 lacks detectable GSH, whereas AB1157 has roughly normal levels of GSH for *E. coli* cells (132 ± 15 nmol/10^{10} organisms or ~8 μmol/g of bacterial protein) (8). In support of the idea that cellular GSH may protect against killing by chlorine compounds, dilute suspensions of GSH-deficient *E. coli* are much more susceptible to killing by HOCl than are the GSH-replete organisms (Fig. 1). The LD_{50} for the GSH-deficient *E. coli* JTG10 (~0.31 μM HOCl) is more than two times lower than the LD_{50} for the wild type (~0.66 μM HOCl). Because these two strains are otherwise isogenic, the variation is almost certainly due to different GSH statuses. In fact, generation of HOCl via the MPO-H_2O_2-chloride system also revealed even greater differential susceptibility between AB1157 and JTG10 (Fig. 2). These results further demonstrate that enzymatically generated HOCl is nearly as effective at killing *E. coli* cells as reagent HOCl (assuming stoichiometric conversion of H_2O_2 by MPO).

Bacterial GSH content also powerfully affects susceptibility to killing by monochloramine (NH_2Cl; LD_{50}s are ~2.1 and ~4.9 μM for GSH-deficient and wild-type bacteria, respec-

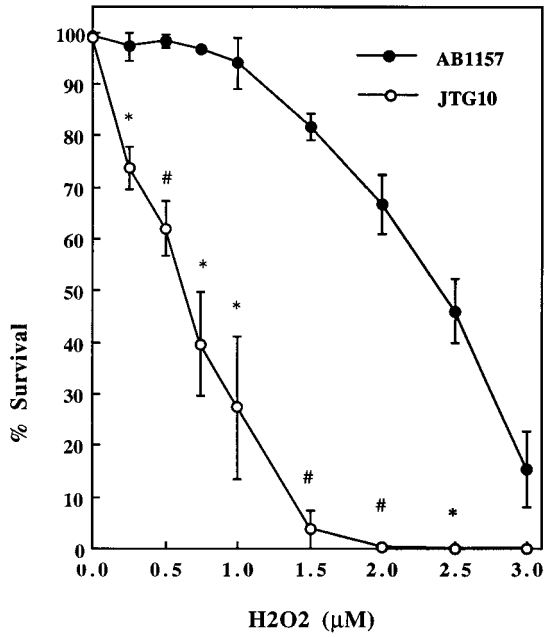

FIG. 2. MPO-H_2O_2-chloride-dependent killing of AB1157 and JTG10 cells. Both strains of bacteria (10^6 cells per ml) were incubated with 7 mU of MPO per ml with increasing concentrations of hydrogen peroxide in Hanks' buffer at pH 7.2 for 20 min. Following the incubation, enumeration of the surviving bacteria was performed by pour plating in nutrient agar. The number of colonies was determined after 36 h of incubation at 37°C. $n = 3$; *, $P < 0.05$; $P < 0.01$.

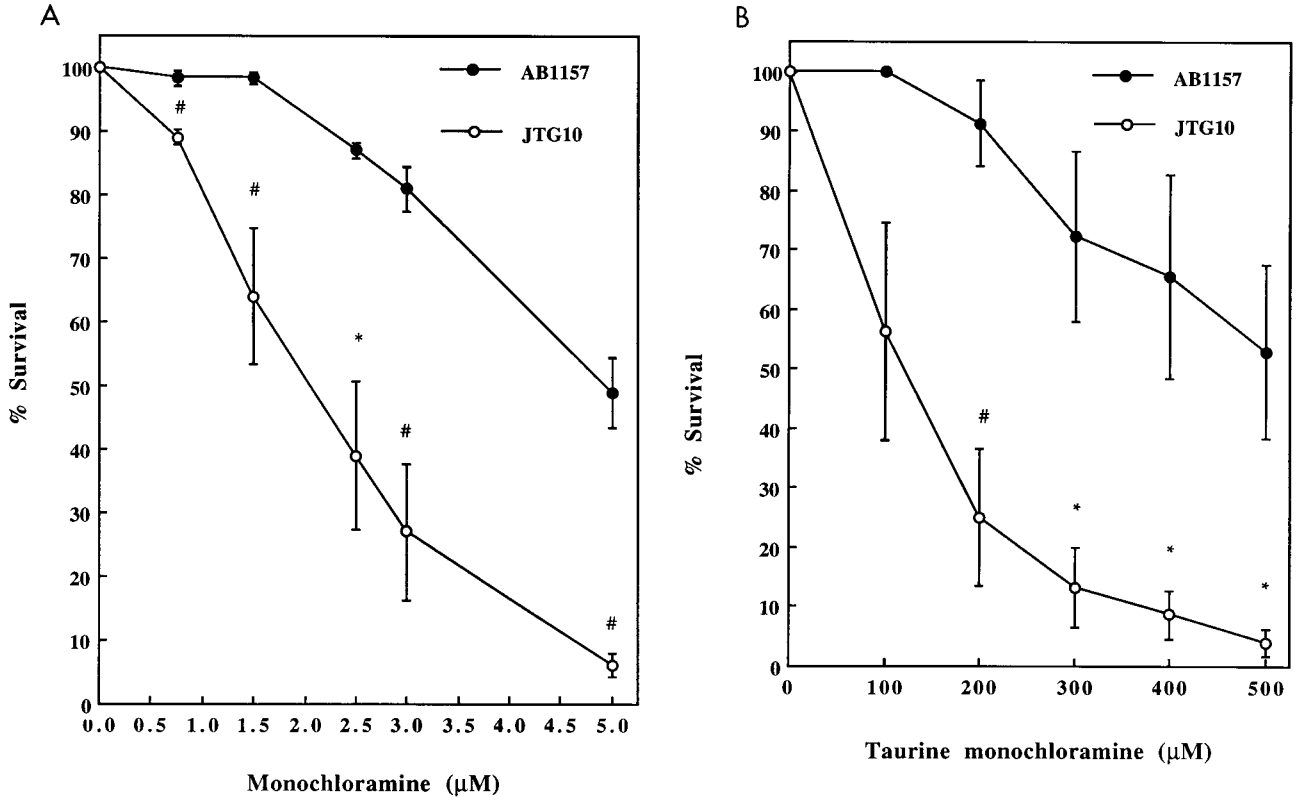

FIG. 3. (A) Monochloramine killing of GSH-replete (●) and -deficient (○) *E. coli* cells. Cells were grown overnight in BHI, washed twice in Hanks' buffer, and resuspended to a concentration of 10^6 cells per ml. At time zero, monochloramine was added to give the final concentration as indicated. Survival of bacteria after a 20-min exposure was assessed by removing an aliquot and pour plating. CFU were enumerated after 36 h of growth at 37°C. $n = 3$; *, $P < 0.05$; #, $P < 0.01$. (B) Taurine chloramine killing of GSH-replete (●) and -deficient (○) *E. coli* cells. Cells were grown overnight in BHI, washed twice in Hanks' buffer, and resuspended to a concentration of 10^6 cells per ml. At time zero, taurine monochloramine was added to give the final concentration as indicated. Survival of bacteria after a 4-h exposure was assessed by removing an aliquot and pour plating. CFU were enumerated after 36 h of growth at 37°C. $n = 3$; *, $P < 0.05$; #, $P < 0.01$.

tively [Fig. 3A]) and taurine monochloramine (LD_{50}s are ~120 and ~500 μM for GSH-deficient and wild-type bacteria, respectively [Fig. 3B]). As might be expected, GSH-deficient *E. coli* cells are also more readily killed by H_2O_2. As shown in Fig. 4, there is a roughly twofold difference in the LD_{50} of H_2O_2 for wild-type organisms compared with that for GSH-deficient organisms. Interestingly, and as previously reported (11), there is no difference in susceptibility to H_2O_2 in wild-type and GSH-deficient bacteria when killing is assessed in organisms in log-phase growth (data not shown). It should be noted that GSH levels are significantly higher in stationary-phase cultures of *E. coli* cells (8).

The relative resistance of GSH-replete *E. coli* cells to killing by chlorine compounds might be due to the -SH group of bacterial GSH acting as a sink for reactive chlorine. If so, a more or less stoichiometric HOCl-mediated loss of bacterial GSH might be expected. However, in intact wild-type *E. coli* cells, GSH is oxidized in a ratio of 1 GSH to 4 HOCl, suggesting that HOCl reacts with other targets within the bacteria or that bacterial GSH is oxidized to a higher sulfur oxidation state than the disulfide (22). Nonetheless, intracellular GSH still provides *E. coli* cells with substantial protection against HOCl challenge. Even when 70% of the GSH in concentrated (10^{10} cells per ml) wild-type *E. coli* cells has been oxidized (by 34 nmol of HOCl), ~95% of the organisms in the incubation still survive (Fig. 5). Additional experiments indicate that the intracellular GSH is acting to directly (i.e., nonenzymatically) detoxify chlorine compounds. A concentration of exogenous GSH similar to that found in the wild-type *E. coli* cells (132 nmol of GSH per 10^{10} organisms) was added to a suspension of GSH-deficient *E. coli* cells. This exogenous GSH is as protective of the GSH-deficient *E. coli* cells as the intracellular GSH of the wild-type organisms. Because external GSH is not able to penetrate the outer membrane of *E. coli* cells (10a), it is unlikely that the increased tolerance of the GSH-deficient organisms to HOCl upon the addition of exogenous GSH is explained by intracellular GSH-dependent reactions. Indeed, added GSH fails to correct the enhanced sensitivity of these GSH-deficient *E. coli* cells to H_2O_2 (i.e., the LD_{50} remains at ~4). This implies that, in defense against H_2O_2, GSH is part of an enzymatically driven redox system [hydroperoxidase-GSH reductase-NAD(P)H]), whereas such cycling of GSH is evidently not involved in protection against chlorine compounds.

DISCUSSION

Since most bacteria do not possess GSH, other thiol-containing compounds such as thioredoxin, glutaredoxin, and cysteine must be able to substitute for a variety of reactions requiring sulfhydryls. Indeed, there are no major apparent differences in the GSH-deficient bacteria with respect to growth and replication in nutrient medium (data not shown). One recognized physiologic difference in the GSH-deficient *E. coli* cells is an altered glutathione-gated potassium efflux (9). It should also be emphasized that the exact mechanisms by which

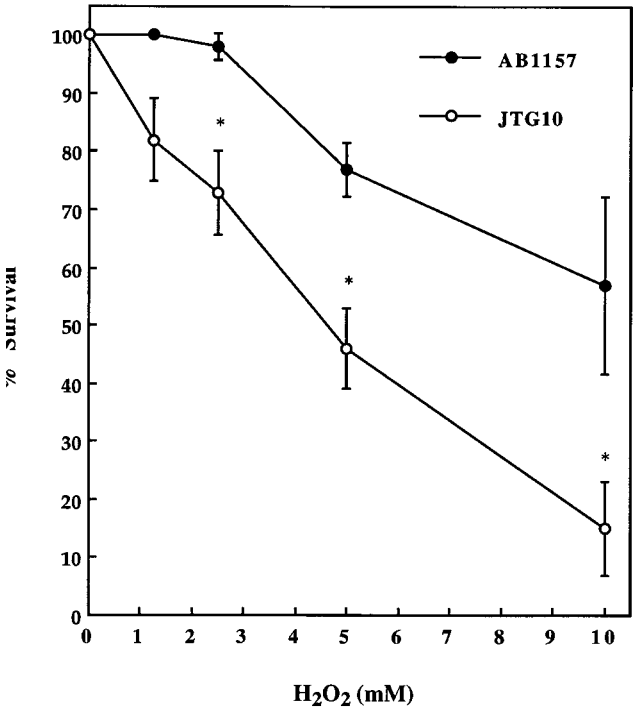

FIG. 4. Hydrogen peroxide killing of GSH-replete (●) and -deficient (○) *E. coli* cells. Cells were grown overnight in BHI, washed twice in Hanks' buffer, and resuspended to a concentration of 10^6 cells per ml. At time zero, H_2O_2 was added to give the final concentration as indicated. Survival of bacteria after a 20-min exposure was assessed by removing an aliquot and pour plating. CFU were enumerated after 36 h of growth at 37°C. $n = 3$; *, $P < 0.05$.

GSH provides protection against oxidative stress are as yet incompletely understood.

Because aerobic microorganisms are subject to relentless oxidant stress, a number of enzymatic defenses against oxidation have evolved. The majority of the earlier work on cellular oxidant defenses has focused on systems which might protect against partially reduced forms of oxygen such as O_2^- and H_2O_2. Little is known of mechanisms whereby cells and unicellular organisms might prevent damage from halogens such as chlorine compounds. In an effort to clarify this issue, we have compared the sensitivities of two otherwise isogenic *E. coli* strains to reagent HOCl and chloramines.

Overall, our results indicate that intracellular GSH provides quantitatively important protection of *E. coli* cells against chlorine compounds. Indeed, earlier investigations of the etiology of hemolytic disease in patients hemodialyzed against chlorinated tap water revealed that HOCl exposure of erythrocytes did cause oxidation of intracellular GSH (6). However, the extent to which the GSH might be protecting the target cells was not investigated. As can be seen by comparing Fig. 1 and 4, HOCl is much more toxic (500 to 1,000 times) to *E. coli* cells than is H_2O_2. Nonetheless, in the case of both HOCl and H_2O_2, the GSH-deficient *E. coli* cells are roughly twice as sensitive to oxidant killing.

It is thought that a significant fraction of the HOCl produced by the MPO system of activated neutrophils may react with endogenous amines (e.g., ammonia, taurine, and other amino acids) to yield derivatives containing nitrogen-chlorine bonds (12, 19). In contrast to an earlier study (21), we find that the bactericidal potency of NH_2Cl is slightly less, rather than more, when compared with that of HOCl. As might be expected, GSH-replete *E. coli* cells also were significantly more resistant to killing when exposed to NH_2Cl (Fig. 3A). The reaction between taurine and HOCl forms taurine monochloramine, a higher-molecular-weight, polar molecule which does not readily penetrate the cell membrane. Since, at pH 7.2, taurine monochloramine slowly dissociates to reactive HOCl, intracellular GSH should still be vulnerable to oxidation. As expected, the GSH-deficient bacteria are also more susceptible to taurine chloramine toxicity (approximately fourfold difference in LD_{50}; Fig. 3). It should be noted that the concentrations of taurine monochloramine required to effect bacterial killing are far greater than those required for HOCl.

The difference in viability between the strains after taurine monochloramine exposure is substantially larger than that seen with the other reagent oxidants (Fig. 1, 2, 3A, and 4). Because bacterial killing by taurine monochloramine is markedly slower (4-h compared with 20-min incubation), GSH reductase may have the necessary time to catalyze the reduction of oxidized GSH by NADPH as GSH is being oxidized. Interestingly, almost all bacterial GSH can be oxidized by HOCl without affecting bacterial viability, suggesting that HOCl may be preferentially oxidizing the GSH. If so, GSH may be acting as a trap for oxidants in order to protect more vital cellular constituents. Furthermore, GSH would appear to be a particularly efficient trap, in that the observed stoichiometry between HOCl added and bacterial GSH oxidized is ~4:1, suggesting that the GSH sulfur is being oxidized to a higher oxidation state than the disulfide.

In conclusion, GSH appears to play a significant role in the resistance of *E. coli* cells to chlorine oxidants. The protection afforded by GSH is due to a rapid reaction with HOCl oxidants

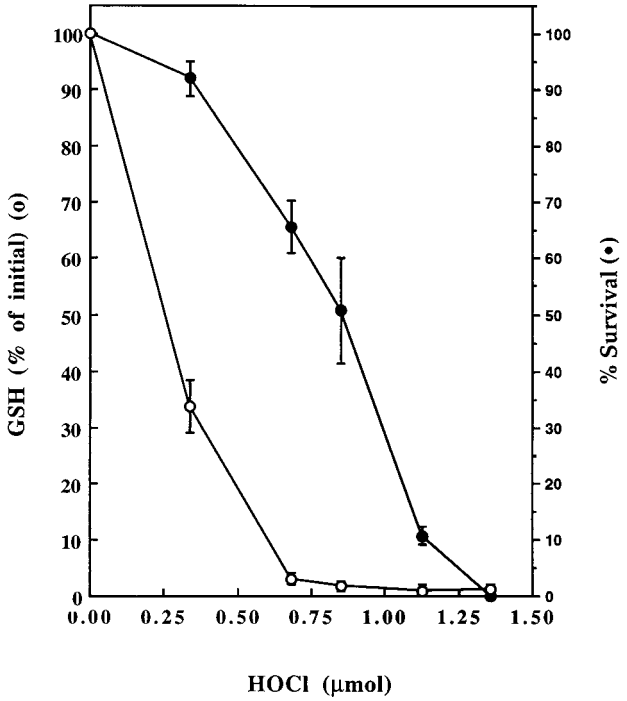

FIG. 5. Relationship between the extent of GSH oxidation and survival of HOCl-stressed wild-type *E. coli* cells. Cells were grown overnight in BHI, washed twice in PBS, and resuspended to a concentration of 10^{10} cells per ml. At time zero, HOCl was added to each incubation to give the final concentration as indicated. After 20 min, a portion of each incubation was immediately extracted with sulfuric acid (○) for GSH determination, and a separate portion was pour plated (●) ($n = 3$).

and, in the case of less-reactive chlorine compounds, may also involve protection via intracellular recycling of oxidized GSH to GSH.

This work was supported by a grant from the National Institutes of Health, AI-25625 (J.W.E.), and a grant-in-aid from the American Heart Association, 91-1317 (J.R.M.).

REFERENCES

1. **Albrich, J. M., J. H. Gilbaugh, K. B. Callahan, and J. K. Hurst.** 1986. Effects of putative neutrophil-generated toxin, hypochlorous acid, on membrane permeability and transport systems of *Escherichia coli*. J. Clin. Invest. **78:** 177–184.
2. **Albrich, J. M., C. A. McCarthy, and J. K. Hurst.** 1981. Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leukocyte myeloperoxidase. Proc. Natl. Acad. Sci. USA **78:**210–214.
3. **Bernofsky, C.** 1991. Nucleotide chloramines and neutrophil-mediated cytotoxicity. FASEB J. **5:**295–300.
4. **Chesney, J. A., J. R. Mahoney, Jr., and J. W. Eaton.** 1991. A spectrophotometric assay for chlorine-containing compounds. Anal. Biochem. **196:**262–266.
5. **Downs, A. J., and C. J. Adams.** 1973. Comprehensive inorganic chemistry. Pergamon, Oxford.
6. **Eaton, J. W., C. F. Kolpin, H. S. Swofford, C.-M. Kjellstrand, and H. S. Jacob.** 1973. Chlorinated urban water: a cause of dialysis-induced hemolytic anemia. Science **181:**463–464.
7. **Ellman, G. L.** 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. **82:** 70–77.
8. **Fahey, R. C., W. C. Brown, W. B. Adams, and M. B. Worsham.** 1978. Occurrence of glutathione in bacteria. J. Bacteriol. **133:**1126–1129.
9. **Ferguson, G. P., A. W. Munro, R. M. Douglas, D. McLaggan, and I. R. Booth.** 1993. Activation of potassium channels during metabolite detoxification in *Escherichia coli*. Mol. Microbiol. **9:**1297–1303.
10. **Foote, C. S., T. E. Goyne, and R. I. Lehrer.** 1983. Assessment of chlorination by human neutrophils. Nature (London) **301:**715–716.

10a. **Fuchs, J.** Personal communication.

11. **Greenberg, J. T., and B. Demple.** 1986. Glutathione in *Escherichia coli* is dispensable for resistance to H_2O_2 and gamma radiation. J. Bacteriol. **168:** 1026–1029.
12. **Grisham, M. B., M. M. Jefferson, D. F. Melton, and E. L. Thomas.** 1984. Chlorination of endogenous amines by isolated neutrophils. Ammonia-dependent bactericidal, cytotoxic and cytolytic activities of chloramines. J. Biol. Chem. **259:**10404–10413.
13. **Korich, D. G., J. R. Mead, M. S. Madore, N. A. Sinclair, and C. R. Sterling.** 1990. Effects of ozone, chlorine dioxide, chlorine and monochloramine on *Cryptosporidium parvum* oocyst viability. Appl. Environ. Microbiol. **56:**1423–1428.
14. **Morris, J. S.** 1966. The acid ionisation constant of HOCl from 5 to 35°C. J. Phys. Chem. **70:**3798–3805.
15. **Rosen, H., and S. J. Klebanoff.** 1982. Oxidation of *Escherichia coli* iron centers by the myeloperoxidase-mediated microbicidal system. J. Biol. Chem. **257:**13731–13735.
16. **Rosen, H., J. Orman, R. M. Rakita, B. R. Michel, and D. R. VanDevanter.** 1990. Loss of DNA-membrane interactions and cessation of DNA synthesis in myeloperoxidase-treated *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**10048–10052.
17. **Rosen, H., R. M. Rakita, A. M. Waltersdorph, and S. J. Klebanoff.** 1987. Myeloperoxidase-mediated damage to the succinate oxidase system of *Escherichia coli*. Evidence for selective inactivation of the dehydrogenase component. J. Biol. Chem. **262:**15004–15010.
18. **Ross, D.** 1988. Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity and glutathione-dependent protection. Pharmacol. Ther. **37:**231–249.
19. **Test, S. T., M. B. Lampert, P. J. Ossanna, and S. J. Weiss.** 1984. Generation of nitrogen-chlorine oxidants by human phagocytes. J. Clin. Invest. **74:**1341–1349.
20. **Test, S. T., and S. J. Weiss.** 1986. The generation and utilization of chlorinated oxidants by human neutrophils. Free Radical Biol. Med. **2:**91–116.
21. **Thomas, E. L.** 1979. Myeloperoxidase, hydrogen peroxide, chloride antimicrobial system: nitrogen-chlorine derivatives of bacterial components in bactericidal action against *Escherichia coli*. Infect. Immun. **23:**522–531.
22. **Thomas, E. L.** 1979. Myeloperoxidase-hydrogen peroxide-chloride antimicrobial system: effect of exogenous amines on antibacterial action against *Escherichia coli*. Infect. Immun. **25:**110–116.
23. **Weiss, S. J., R. Klein, A. Slivka, and M. Wei.** 1982. Chlorination of taurine by human neutrophils. Evidence for hypochlorous acid generation. J. Clin. Invest. **70:**598–607.
24. **Winterbourn, C. C.** 1985. Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of the oxidant to hypochlorite. Biochim. Biophys. Acta **840:**204–210.
25. **Winterbourn, C. C.** 1990. Neutrophil oxidants: productions and reactions. S. Karger, Basel.
26. **Winterbourn, C. C., J. J. M. van der Berg, E. Roitman, and F. A. Kuypers.** 1992. Chlorohydrin formation from unsaturated fatty acids reacted with hypochlorous acid. Arch. Biochem. Biophys. **296:**547–555.
27. **Zgliczynski, J. M., T. Stelmaszynska, J. Domanski, and W. Ostroski.** 1971. Chloramines as intermediates of oxidation reaction of amino acids by myeloperoxidase. Biochim. Biophys. Acta **235:**419–424.