INFECTION AND IMMUNITY, July 1989, p. 2173–2178
0019-9567/89/072173-06$02.00/0

Vol. 57, No. 7

Oxidant Stress in *Neisseria gonorrhoeae*: Adaptation and Effects on L-(+)-Lactate Dehydrogenase Activity

KAI-SONG FU,[1] DANIEL J. HASSETT,[1,2] AND MYRON S. COHEN[1,3]*

Departments of Microbiology and Immunology[1] and Medicine,[3] University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, and Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710[2]

Received 19 December 1988/Accepted 2 April 1989

***Neisseria gonorrhoeae*, an obligate human pathogen, is subjected to oxidant stress when attacked by O_2 reduction products formed by neutrophils. In this study, exposure of gonococci to sublethal concentrations of superoxide and hydrogen peroxide (and related O-centered radicals) resulted in phenotypic resistance to oxidant stress. Adaptation required new protein formation but was not related to increases in superoxide dismutase or catalase. We have previously demonstrated that gonococci use phagocyte-derived L-(+)-lactate. Oxidant stress of greater magnitude than that required for adaptation led to a generalized increase in bacterial metabolism, particularly in L-(+)- and D-(−)-lactate utilization and lactate dehydrogenase activity. Increased lactate utilization required new protein synthesis. These results suggest the possibility that lactate metabolism is of importance to *N. gonorrhoeae* subjected to oxidant stress. Use of *lct* mutant organisms unable to use L-(+)-lactate should allow examination of this hypothesis.**

Oxidant stress represents a significant threat to both procaryotic and eucaryotic organisms (15). Most bacteria produce antioxidant enzymes of documented importance for the endogenous stress associated with aerobic metabolism and exogenous stress resulting from exposure to extracellular oxidants (19, 38), such as that which occurs during the attack of phagocytic cells (5). *Escherichia coli* (10, 13, 18–21), *Salmonella typhimurium* (11, 40), and *Bacillus subtilis* (35) exposed to sublethal concentrations of hydrogen peroxide (H_2O_2) adapt so as to survive subsequent exposure to considerably higher concentrations of this oxidant. Adaptation has been linked to induction of antioxidant enzymes (18), competent DNA repair (13), and formation of a variety of new proteins whose functions are now being explored (32). New proteins resulting from oxidant stress may overlap with those produced by heat shock (11, 32) and other forms of stress.

Neisseria gonorrhoeae is an unusual bacterial pathogen. It is strictly limited to human hosts (8). Although oxygen can be used by gonococci as a terminal electron acceptor (34), the organism forms little or no superoxide dismutase (SOD) (36) and this enzyme cannot be induced by redox stress (2, 12, 13). *N. gonorrhoeae* is, therefore, an attractive and appropriate model for the study of oxidant stress.

Most bacteria possess both cytoplasmic NAD-dependent lactate dehydrogenases (LDHs), which convert pyruvate to lactate, and NAD-independent membrane-bound enzymes, which prefer lactate as a substrate (16). Since humans form only L-(+)-lactate, we have focused on L-(+)-NAD-independent LDH activity. Gonococci bound to human neutrophils use neutrophil-derived L-(+)-lactate, leading to a remarkable increase in oxygen consumption (6, 9) and the creation of an anaerobic environment in vitro (7). Gonococcal L-(+)-LDH is believed to be relatively resistant to environmental stress (3) and could be important to the survival of bacteria exposed to phagocytes. This study was undertaken to examine the ability of gonococci to adapt to oxidant stress and to determine the effects of such stress on L-(+)-LDH activity.

MATERIALS AND METHODS

Reagents. Xanthine, D-(−)-lactate, L-(+)-lactate, cytochrome *c*, phenazine methosulfate, chloramphenicol, buttermilk xanthine oxidase, bovine erythrocyte SOD, and bovine liver catalase were purchased from Sigma Chemical Co., St. Louis, Mo. Concentrations of glucose, pyruvate, and lactate in solution were determined by standard kits obtained from Sigma. All other reagents were of the highest grade available.

Bacteria. *N. gonorrhoeae* FA1090, a clinical isolate provided by P. Frederick Sparling (University of North Carolina at Chapel Hill), was subcultured daily on gonococcal culture broth (GCB) agar (GC medium base; Difco Laboratories, Detroit, Mich.) containing 1 and 0.5% (vol/vol) Kellogg supplements I and II (27), respectively. Broth cultures (GCB containing 2% supplement I and 5 mM sodium bicarbonate) were inoculated from overnight cultures and grown to log phase on a platform shaker at 135 cycles/min. Cell density was monitored with a Klett-Summerson colorimeter (Klett Manufacturing Co., New York, N.Y.) equipped with a 540-nm-pore-size filter. A reading of 35 Klett units indicated the presence of approximately 3×10^8 CFU/ml. All cultures were incubated at 37°C in an atmosphere of 5% CO_2. In some experiments, bacteria were suspended in a defined medium supplemented with hypoxanthine and uracil (30, 33).

Gonococcal oxygen consumption. Oxygen consumption was measured with a Clark polarographic oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio) at 37°C with a 1.0-ml volume containing approximately 10^8 organisms per ml as previously described (9). Gonococci were grown to log phase, exposed to xanthine oxidase-xanthine (a superoxide-generating system described below), centrifuged at $500 \times g$ for 10 min, and suspended in Hanks balanced salt solution (without glucose). The effects of oxidative stress (described below) and the utilization of L-(+)-lactate, glucose, or pyruvate were examined. Organisms killed with a higher concentration of enzymatically

* Corresponding author.

generated free radicals or UV light were unable to utilize carbon (unpublished data); accordingly, results were expressed as nanomoles of O_2 consumed per minute and normalized to 10^6 viable organisms (see below).

Exposure of gonococci to stress. Gonococci were grown to log phase as described above and exposed to 0.45 mM xanthine and various concentrations of xanthine oxidase (0.005 to 0.2 U/ml) at 37°C with shaking (135 cycles/min) for 1 h. Within 1 h, 0.005 and 0.2 U of xanthine oxidase generated 0.16 and 2.18 mM H_2O_2, respectively. The pH of the reaction mixture was the same as that of the mixture without the enzyme. In some experiments, chloramphenicol (100 μg/ml) was included in the reaction mixture. This concentration was sublethal but inhibited incorporation of mixed amino acids (data not shown; 22). Heat shock was achieved by incubating gonococci at 41°C for 2 min. Bacterial viability was determined by diluting organisms 100-fold in GCB, plating them onto GCB agar plates, and counting colonies after incubation at 37°C for 48 h with 5% CO_2.

Other assays. LDH was measured at 25°C as previously described (25), by using intact gonococci or membrane fractions from cells which had been disrupted with sonication. Protein was measured by the method of Lowry et al. (29) with bovine serum albumin as the standard. SOD and catalase were measured as previously described (2, 14). The concentration of H_2O_2 formed by xanthine oxidase-xanthine was measured as described by Homan-Muller et al. (23). The inhibitory effect of chloramphenicol on gonococcal protein synthesis was determined by the uptake of ^{14}C-labeled mixed amino acids. The assay was performed as previously described (6).

Statistics. Data were compared by the Student *t* test. Differences at $P < 0.05$ were considered significant.

RESULTS

The effect of enzymatically generated O-centered radicals and H_2O_2 on the viability of *N. gonorrhoeae* was evaluated by using various concentrations of xanthine oxidase acting aerobically on a saturated solution of xanthine. A linear relationship between the $\log_{10}$ xanthine oxidase concentration and the survival of *N. gonorrhoeae* was demonstrated (Fig. 1). The 50% lethal dose of xanthine oxidase was 0.065 U/ml. A xanthine oxidase concentration of 0.005 U/ml was sublethal.

Bacteria were exposed to 0.005 U of xanthine oxidase per ml, washed, and then exposed to 0.2 U of this enzyme. Sublethal pretreatment resulted in a significant increase in bacterial survival, suggesting adaptation (Fig. 2). To determine whether new protein synthesis was required for adaptation, bacteria were incubated in the presence of a concentration of chloramphenicol (100 μg/ml) that had a minimal effect on survival but was adequate to inhibit 50% of new protein synthesis (unpublished data; 22). Chloramphenicol inhibited the expected adaptation (Fig. 2). After treatment with 0.005 U of xanthine oxidase per ml, SOD remained undetectable and the catalase concentration decreased relative to that of untreated control cells from 656 to 479 U/mg. Since heat shock and oxidant stress in *S. typhimurium* resulted in production of similar new proteins (32), we tested the effect of oxidant adaptation on gonococcal survival to heat shock. Incubation of gonococci at 41°C for 2 min following exposure to 0.005 U of xanthine oxidase per ml resulted in increased survival compared with that of organisms not exposed to xanthine oxidase (Fig. 2).

Our previous studies (7, 9) have suggested that L-(+)-lactate metabolism by gonococci plays an important role in their interaction with neutrophils. Gonococci were exposed to the xanthine oxidase superoxide-generating system, and their oxygen consumption in response to a variety of carbon sources and their LDH activity were examined. Exposure of gonococci to 0.01 U of xanthine oxidase or less per ml did not result in increased oxygen consumption (Fig. 3). After exposure to higher concentrations of xanthine oxidase, a general increase in metabolic activity was observed (Fig. 3). When L-(+)-lactate was used as a substrate, gonococci exposed to xanthine oxidase demonstrated a sixfold increase in O_2 consumption. Increases of smaller magnitude were noted when glucose and pyruvate were used as substrates (Fig. 3). Maximal effects were noted when 0.1 U of xanthine oxidase per ml was used for 60 min (data not shown).

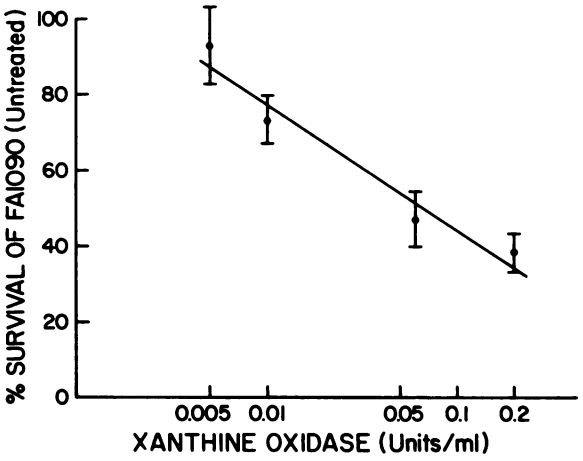

FIG. 1. Survival of *N. gonorrhoeae* FA1090 after exposure to a superoxide-generating system. Gonococci were grown as described in Materials and Methods and exposed to xanthine-xanthine oxidase-generated oxygen reduction products for 1 h, after which the suspensions were serially diluted and bacterial viability was assessed. The results are means, and the brackets represent standard errors; 5 to 16 separate experiments were performed, each in duplicate.

As expected, exposure of gonococci to xanthine oxidase led to increased LDH activity (Fig. 4). We noted a significant increase ($P < 0.05$) in L-(+)-LDH activity up to a concentration of 0.2 U of xanthine oxidase per ml. This effect was not specific, since D-(−)-LDH activity was also increased (data not shown). Experiments were conducted to determine the mechanism(s) by which stress increased LDH activity. Chloramphenicol (100 μg/ml) inhibited the LDH activity induced by redox stress (Fig. 5). To determine whether increased LDH activity was a stable hereditary trait, L-(+)-lactate usage was examined in organisms harvested after stress and grown to stationary phase (Fig. 4); no increase in LDH activity was noted in this population.

Experiments were conducted to examine L-(+)-lactate utilization by stressed bacteria in a milieu in which other physiologic substrates were available. In these experiments, medium carbon utilization by log-phase organisms over 10 min was measured. Gonococci offered glucose, lactate, and pyruvate at physiologic concentrations (33, 34) preferentially used L-(+)-lactate (Fig. 6). Similar results were obtained when the carbon sources were used at equimolar concentrations or the experiments were conducted in defined medium (30, 33) rather than buffer (data not shown). The pyruvate concentration in the medium increased, as expected on the basis of lactate conversion to pyruvate (16). A small increase

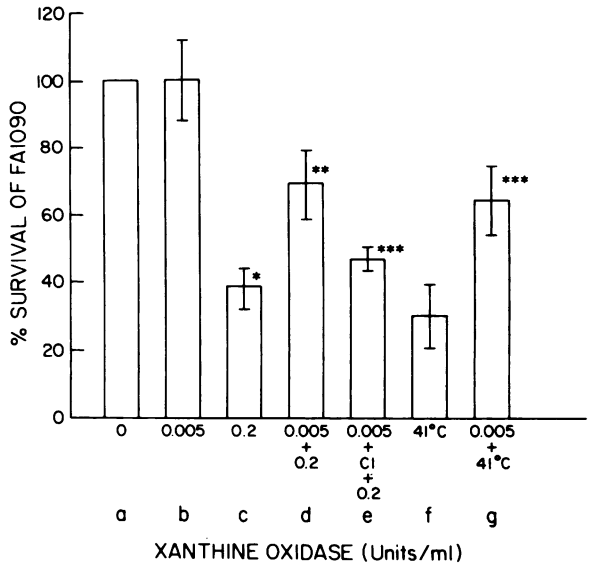

FIG. 2. Adaptation of *N. gonorrhoeae* FA1090 to oxidant stress. Gonococci were prepared as described in the legend to Fig. 1 and treated as follows: a, no treatment; b, treatment with 0.005 U of xanthine oxidase; c, treatment with 0.2 U of xanthine oxidase; d, as in b plus 0.2 U of xanthine oxidase; e, as in d plus 100 μg of chloramphenicol (Cl) per ml; f, treatment at 41°C for 2 min; g, pretreatment with 0.005 U of xanthine oxidase and then incubation at 41°C. The results represent the means and standard errors of three to six separate experiments, each performed in duplicate. For statistical comparisons, condition a was compared with b (no significant difference), a was compared with c ($P < 0.01$ designated *), c was compared with d ($P < 0.01$ designated **), d was compared with e ($P < 0.05$ designated ***), and f was compared with g ($P < 0.05$ designated ***). Chloramphenicol treatment of bacteria allowed a 50% reduction in amino acid incorporation relative to that of the control, with no significant decrease in bacterial viability (data not shown).

in L-(+)-lactate utilization was seen in organisms exposed to xanthine oxidase at a concentration (0.1 U/ml) which reduced their viability (Fig. 6), consistent with the increased metabolism of surviving organisms (Fig. 3). Gonococci grew

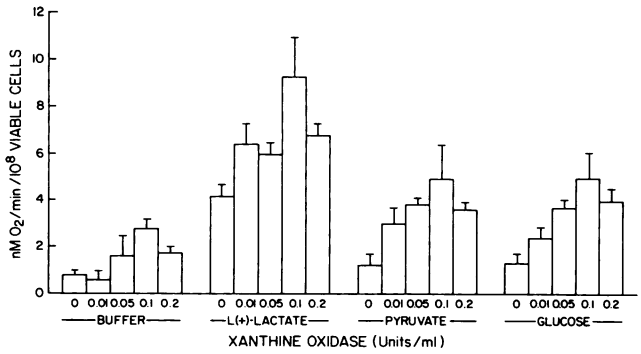

FIG. 3. Oxygen consumption by *N. gonorrhoeae* provided different carbon sources. Gonococci were pretreated with various concentrations of xanthine oxidase, after which the cells were washed with Hanks balanced salt solution, pH 7.4 (minus glucose), and suspended in Hanks balanced salt solution (minus glucose). L-(+)-Lactate, pyruvate, or glucose was added to cell suspensions at final concentrations of 20 μg/ml, 20 μg/ml, and 1 mg/ml, respectively. The bars represent the means and the brackets represent the standard errors of three separate experiments.

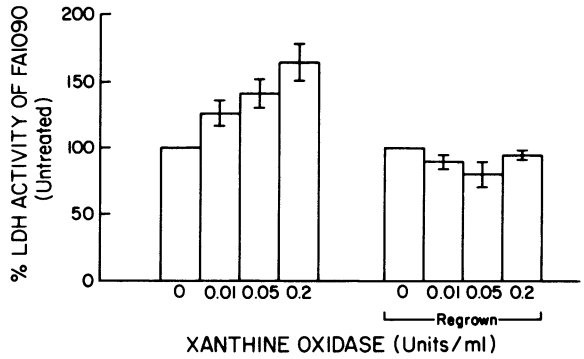

FIG. 4. L-(+)-LDH activity in viable gonococci exposed to oxidant stress. Gonococci were treated with the xanthine oxidase-xanthine superoxide-generating system as in the experiment whose results are shown in Fig. 1. L-(+)-LDH activity was examined at 1 h after exposure. Regrown gonococci represent organisms treated with xanthine oxidase-xanthine, diluted in GCB (plus supplements), and grown to log phase for reexamination of LDH activity. The bars represent the means and the brackets represent the standard errors of four separate experiments, each performed in duplicate.

somewhat faster with L-(+)-lactate (physiologic concentration) in a defined medium than when provided pyruvate or glucose (Fig. 7).

DISCUSSION

The ability of pathogenic organisms such as gonococci to evade host defenses has been intensively studied (8). In general, constitutive structural proteins have received the greatest attention (8). However, rapid alterations at the phenotypic level can lead to changes allowing survival of adapted populations of cells. For example, heat shock proteins can be viewed as such a response, and the regulation and some effects of these proteins have been characterized (24, 32). More recently, the possibility that metabolic alterations directly favor survival has been suggested (7, 9).

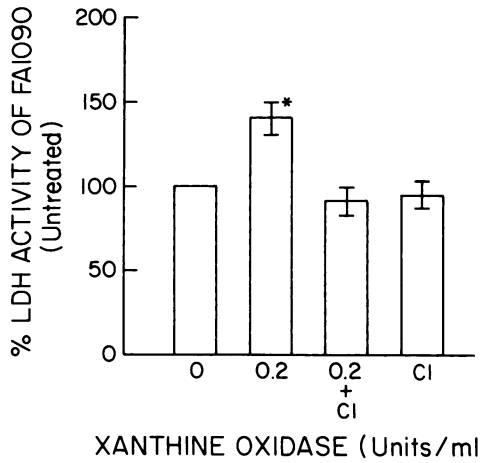

FIG. 5. L-(+)-LDH activity after oxidant stress in viable organisms treated with chloramphenicol (Cl; 100 μg/ml). The bars represent the means and the brackets represent the standard errors of five separate experiments, each performed in duplicate. *, $P < 0.05$ as determined with the Student t test. Chloramphenicol-treated organisms demonstrated a 50% reduction in amino acid incorporation (data not shown).

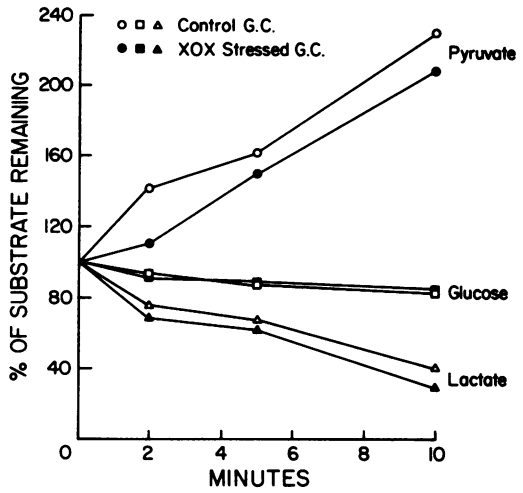

FIG. 6. Consumption of various carbon sources by *N. gonorrhoeae* in 0.02 M phosphate buffer, pH 7.4. The initial concentrations of carbon sources were as follows: L-(+)-lactate, 11.5 mg/dl; glucose, 100 mg/dl; and pyruvate, 1.1 mg/dl. Gonococci (G.C.) were treated (closed symbols) with 0.1 U of xanthine oxidase in the presence of xanthine (XOX) for 1 h at 37°C. The results are typical of three separate experiments, each performed in duplicate.

In this study, we found that gonococci exposed to sublethal concentrations of superoxide, H_2O_2, and/or HO· (generated by the action of xanthine oxidase on xanthine) exhibited increased survival when confronted with lethal concentrations of oxidants or heat shock. Survival depended on new protein synthesis, since chloramphenicol-treated bacteria could not adapt properly. These results are similar to those reported by Christman et al. (11), who worked with *S. typhimurium* exposed to H_2O_2. We used the xanthine oxidase-xanthine model because it allowed gonococci to be exposed to gradually increasing concentrations of O_2^- and H_2O_2, more closely paralleling the attack by phagocytes to which these bacteria are subjected (reviewed in reference 8).

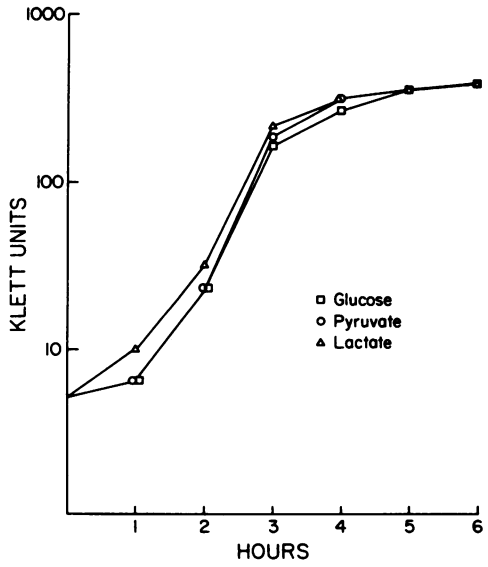

FIG. 7. Growth of *N. gonorrhoeae* in a defined medium with different carbon sources. The results are typical of three separate experiments.

The concentration of H_2O_2 formed by 0.005 U of xanthine oxidase per ml and 0.45 mM xanthine is consistent with the pretreatment concentrations used in earlier stress studies (11, 24, 35, 40). Furthermore, standard buffers and media may provide Fe^{3+} in concentrations adequate to catalyze the formation of HO· in the presence of O_2^- and H_2O_2, and we are currently exploring this hypothesis.

The mechanism for gonococcal adaptation to oxidant stress is unknown. Neither catalase nor SOD increased in response to xanthine oxidase, as would be predicted on the basis of earlier work (2, 12, 36). Indeed, we noted a decrease in catalase activity, perhaps related to the injurious effects of oxidants on catalase previously reported (12, 28). We have not studied the concentration of other antioxidant proteins (e.g., peroxidases) which might play a role in protection of *N. gonorrhoeae* (2). Christman and co-workers described nine new proteins in *S. typhimurium* after oxidant stress under control of an *oxyR1* gene, and *oxyR1* mutants were not able to adapt to oxidant stress (11). We have examined new protein formation on one-dimensional polyacrylamide gels after oxidant stress (unpublished data), but the results have not been conclusive.

A variety of experiments were undertaken to determine whether the metabolic responses of gonococci—in particular carbohydrate utilization—could be linked to adaptation. Organisms exposed to sublethal concentrations of xanthine oxidase demonstrated no alteration in metabolic activity. However, bacteria treated with a higher concentration of this enzyme showed a significant increase in L-(+)-LDH activity, and stressed organisms used L-(+)-lactate more readily than other carbon sources provided. These results are consistent with the detailed studies of Barron and Hastings, who demonstrated the resistance of gonococcal LDH to a variety of adverse environmental conditions (3). Using the guinea pig chamber model, Goldner and coworkers (17) noted the utilization of lactate by gonococci concomitant with an influx of neutrophilic phagocytes. Lysko and Morse (30) reported that lactate utilization by gonococci was not susceptible to the inhibitory effects of gonadal steroids present in vaginal secretions, suggesting another situation in which lactate utilization might be adaptive. Most recently, Anderson and Farkas (1) reported that mammalian cells subjected to heat shock or anoxia form a new isoenzyme of LDH important in adaptation to stress.

Although the function of NAD-independent L-(+)-LDH in gonococci is unknown, this activity has been linked to proton motive force and substrate transport in other bacterial species (26). The increased O_2 utilization observed with lactate as a substrate could, in part, represent more efficient use of glucose than lactate. However, lactate utilization also allowed faster growth than did other substrates. In *E. coli*, growth on lactate increases catalase formation (18), and we have made a similar observation with gonococci (unpublished data). An increase in catalase in bacteria exposed to phagocytes (which generate both lactate and H_2O_2) might be particularly advantageous.

Experiments with chloramphenicol suggested that new protein synthesis was required for increased lactate utilization. However, we were unable to document increased LDH activity in membranes harvested from gonococci exposed to a concentration of xanthine oxidase which stimulated increased lactate utilization in viable organisms (unpublished data). Such negative results are difficult to interpret, because LDH activity could have been artifactually lost in a variety of ways. It is also possible that cytoplasmic (presumably NAD-dependent) LDH plays a role in the increases ob-

served. Beyond the formation of more LDH, the metabolic responses observed could reflect greater access to the substrate, altered enzyme kinetics, or induction of a cytochrome complex with enhanced affinity for oxygen (31). The increased metabolism of glucose and pyruvate observed in stressed organisms is consistent with the latter hypotheses. Of further interest, growth of *E. coli* in lactate increases formation of the cytochrome *d* complex (31), which may be particularly resistant to oxidant damage because of a lack of iron-sulfur centers (31, 39).

The results of this study demonstrate the similarity between gonococci and other bacteria in their responses to oxidant stress. Given the unique and limited milieu of gonococci, it is possible to argue that adaptation is necessary for their sustained viability under oxidant stress. The increase in metabolic activity we observed seems to represent a phenotypic rather than a genotypic event. A relationship between growth conditions and resistance to oxidant stress has recently been demonstrated in *E. coli* in which glucose starvation increases resistance to H_2O_2 (24). Our studies, however, do not relate lactate metabolism to adaptation or survival. Indeed, the magnitude of stress (e.g., xanthine oxidase concentration) required for metabolic stimulation was considerably higher than that which allowed adaptation, although this difference could relate to the sensitivities of the assays used. Available data suggest that L-(+)-LDH formation is under the regulation of the *lct* gene (37). Viable *E. coli* (37) and *B. subtilis* (4) *lct* mutants unable to use L-(+)-lactate have been generated. By using a direct selection technique involving gonococcal isolates inhibited by the metabolism of phenyllactate, mutants have been isolated whose outer membranes cannot utilize L-(+)- or D-(−)-lactate (R. J. Bhatnager, A. T. Hendry, K. T. Shanmugam, and R. A. Jensen, Mol. Microbiol., in press). We have found that these mutant organisms consume O_2 at a rate considerably slower than that of the wild type with L-(+)-lactate as the carbon source (M. S. Cohen, K. Bean, R. K. Bhatnagar, A. T. Hendry, and R. A. Jensen, Clin. Res. **37**:562A, 1989). These *lct* mutants should allow us to test the relative importance of L-(+)-lactate metabolism in adaptation to oxidant stress and in the pathobiology of *N. gonorrhoeae*.

ACKNOWLEDGMENTS

We thank Bradley Britigan for comments on this work and P. Frederick Sparling and Janne Cannon for critical reading of the manuscript.

This work was supported by Public Health Service grants AI-5036-11, 2T32AI07001-09, and AI07151-06 from the National Institutes of Health and by the Program in the Chemistry of Life Sciences of the National Science Foundation (DCB-8616115).

LITERATURE CITED

1. **Anderson, G. R. J., and B. K. Farkas.** 1986. The major anoxic stress response protein p34 is a distinct lactate dehydrogenase. Biochemistry **27**:2187–2193.
2. **Archibald, F. S., and M.-N. Duong.** 1986. Superoxide dismutase and oxygen toxicity defenses in the genus *Neisseria*. Infect. Immun. **51**:631–641.
3. **Barron, E. S. G., and A. B. Hastings.** 1933. Studies on biological oxidations. II. The oxidation of lactic acid by alpha-hydroxy oxidase and its mechanism. J. Biol. Chem. **100**:155–182.
4. **Barstow, D. A., A. R. Clark, W. N. Chia, D. Wigley, A. F. Sharman, J. J. Holbrook, T. Atkinson, and N. P. Minton.** 1986. Cloning, expression and complete nucleotide sequence of the *Bacillus stearothermophilus* L-lactate dehydrogenase gene. Gene **46**:47–55.
5. **Beaman, B. L., C. M. Black, F. Doughty, and L. Beaman.** 1985. Role of superoxide dismutase and catalase as determinants of pathogenicity of *Nocardia asteroides*: importance in resistance to microbicidal activities of human polymorphonuclear neutrophils. Infect. Immun. **47**:135–141.
6. **Britigan, B. E., Y. Chai, and M. S. Cohen.** 1985. Effects of human serum on the growth and metabolism of *Neisseria gonorrhoeae*: an alternative view of serum. Infect. Immun. **50**:738–744.
7. **Britigan, B. E., and M. S. Cohen.** 1986. Effects of human serum on bacterial competition with neutrophils for molecular oxygen. Infect. Immun. **52**:657–663.
8. **Britigan, B. E., M. S. Cohen, and P. F. Sparling.** 1985. Gonococcal infection: a model of molecular pathogenesis. N. Engl. J. Med. **312**:1683–1694.
9. **Britigan, B. E., D. Klapper, T. Svendsen, and M. S. Cohen.** 1988. Phagocyte derived lactate stimulates oxygen consumption by *Neisseria gonorrhoeae*. J. Clin. Invest. **81**:318–324.
10. **Carloiz, A., and D. Touati.** 1986. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? EMBO J. **5**:623–630.
11. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell **41**:753–762.
12. **Cohen, M. S., Y. Chai, B. E. Britigan, W. McKenna, J. Adams, T. Svendsen, K. Bean, D. J. Hassett, and P. F. Sparling.** 1987. Role of extracellular iron in the action of the quinone antibiotic streptonigrin: mechanisms of killing and resistance of *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **31**:1507–1513.
13. **Demple, B., and J. Halbrook.** 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. Nature (London) **304**:466–468.
14. **Elstner, E. F., and A. Heupel.** 1976. Inhibition of nitrite formation from hydroxyammonium-chloride: a simple assay for superoxide dimutase. Anal. Biochem. **70**:616–620.
15. **Fridovich, I.** 1978. The biology of oxygen radicals. Science **201**:875–880.
16. **Garvie, E. I.** 1980. Bacterial lactate dehydrogenase. Microbiol. Rev. **44**:106–139.
17. **Goldner, M., C. W. Penn, S. C. Sanyal, D. R. Veale, and H. Smith.** 1979. Phenotypically determined resistance of *Neisseria gonorrhoeae* to normal human serum: environmental factors in subcutaneous chambers in guinea pigs. J. Gen. Microbiol. **114**:169–177.
18. **Hassan, H., and I. Fridovich.** 1977. Enzymatic defenses against the toxicity of oxygen and of streptonigrin in *Escherichia coli*. J. Bacteriol. **129**:1574–1583.
19. **Hassan, H., and I. Fridovich.** 1977. Physiological function of superoxide dismutase in glucose-limited chemostat cultures of *Escherichia coli*. J. Bacteriol. **130**:805–811.
20. **Hassan, H., and I. Fridovich.** 1978. Regulation of the synthesis of catalase and peroxidase in *Escherichia coli*. J. Biol. Chem. **253**:6445–6450.
21. **Hassan, H., and I. Fridovich.** 1979. Superoxide, hydrogen peroxide, and oxygen tolerance of oxygen-sensitive mutants of *Escherichia coli*. Rev. Infect. Dis. **1**:357–369.
22. **Hebeler, B. H., and S. A. Morse.** 1976. Physiology and metabolism of pathogenic *Neisseria*: tricarboxylic acid cycle activity in *Neisseria gonorrhoeae*. J. Bacteriol. **128**:192–201.
23. **Homan-Muller, J. W. T., R. S. Weening, and D. J. Roos.** 1975. Production of hydrogen peroxide by phagocytizing human granulocytes. J. Lab. Clin. Med. **85**:198–201.
24. **Jenkins, D. E., J. E. Schultz, and A. Martin.** 1988. Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli*. J. Bacteriol. **170**:3910–3914.
25. **Johnston, K. H., and E. C. Gotschlich.** 1974. Isolation and characterization of the outer membrane of *Neisseria gonorrhoeae*. J. Bacteriol. **119**:250–257.
26. **Kaback, H. R.** 1974. Transport studies in bacterial membrane vesicles. Science **186**:882–892.
27. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Perkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274–1279.

28. **Kono, Y., and I. Fridovich.** 1982. Superoxide radical inhibits catalase. J. Biol. Chem. **257**:5751–5754.
29. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
30. **Lysko, P. G., and S. A. Morse.** 1980. Effects of steroid hormones on *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **18**:281–288.
31. **Miller, M., and R. B. Gennis.** 1987. The purification and characterization of the cytochrome *d* terminal oxidase complex of the *Escherichia coli* aerobic respiratory chain. J. Biol. Chem. **258**:9159–9165.
32. **Morgan, R. W., M. F. Christman, G. Jacobson, G. Storz, and B. N. Ames.** 1986. Hydrogen peroxide inducible proteins in *Salmonella typhimurium* overlap with heat shock and stress proteins. Proc. Natl. Acad. Sci. USA **83**:8059–8063.
33. **Morse, S. A., and T. J. Fitzgerald.** 1974. Effect of progesterone on *Neisseria gonorrhoeae*. Infect. Immun. **10**:1370–1377.
34. **Morse, S. A., S. Stein, and J. Hines.** 1974. Glucose metabolism in *Neisseria gonorrhoeae*. J. Bacteriol. **120**:702–714.
35. **Murphy, P., B. C. A. Dowds, D. J. McConnell, and K. M. Devine.** 1987. Oxidative stress and growth temperature in *Bacillus subtilis*. J. Bacteriol. **169**:5766–5770.
36. **Norrod, P., and S. A. Morse.** 1979. Absence of superoxide dismutase in some strains of *Neisseria gonorrhoeae*. Biochem. Biophys. Res. Commun. **90**:1287–1294.
37. **Pascal, M. C., M. J. Puig, and M. Lepelletier.** 1969. Étude génétique d'une mutation affectant l'activité L-lactate déhydrogenase chez *Escherichia coli* 12. C.R. Acad. Sci. **268**:737–739.
38. **Root, R. K., and M. S. Cohen.** 1981. Microbicidal mechanisms of human neutrophils and eosinophils. Rev. Infect. Dis. **3**: 565–598.
39. **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. **242**:15004–15010.
40. **Winquist, L., U. Rannug, A. Rannug, and C. Ramel.** 1984. Protection from toxic and mutagenic effects of hydrogen peroxide by catalase induction in *Salmonella typhimurium*. Mutat. Res. **141**:145–147.