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# Superoxide Dismutase in Anaerobic Bacteria of Clinical Significance

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Twenty-two anaerobic bacteria isolated from infected sites and normal fecal flora were assayed for superoxide dismutase (SOD). The organisms were also classified according to their oxygen tolerance into aerotolerant, intermediate, and extremely oxygen-sensitive groups. There was a correlation between the enzyme level and the oxygen tolerance, in that the aerotolerant and intermediate organisms had SOD, whereas the extremely oxygen-sensitive isolates had low or undetectable enzyme. Among the oxygen-tolerant organisms, gram-negative bacteria had higher levels of SOD than gram-positive organisms. Oxygen was shown to induce SOD production in a strain of *Bacteriodes fragilis* grown in minimal medium under continuous-culture conditions. Enzyme levels in this isolate grown under static conditions were lower in minimal medium than in complex medium, indicating that other components in the complex medium were stimulating the production of SOD. Our data suggest that the variation in oxygen tolerance of anaerobes is usually related to their level of SOD. It is postulated that SOD may be a virulence factor that allows pathogenic anaerobes to survive in oxygenated tissues until the proper reduced conditions are established for their growth.

The pathogenic potential of anaerobic bacteria was initially recognized by Veillon and Zuber in 1898 (23). Over the ensuing 70 years, oxygen-sensitive forms have received a modicum of attention, but their part in disease processes has clearly been overshadowed by the emphasis on facultative microorganisms. We are currently witnessing a renaissance of interest in the role of anaerobic bacteria in suppurative infections (6). Yet, despite the recent recognition of anaerobes in serious infections, there is a dearth of information on their pathogenic mechanisms.

Evidence from our laboratory has suggested that a prerequisite to virulence in an anaerobe is its capacity to tolerate small concentrations of oxygen (4, 21). This supposition, admittedly a paradox at first glance, is based on the observation that anaerobic bacteria isolated from infected sites are almost invariably oxygen tolerant.

Further insight into the question of oxygen tolerance may be gained from studies of the enzyme superoxide dismutase (SOD), which has been proposed as the basis of anaerobiosis by McCord et al. (15). SOD protects cells from the toxic effects of superoxide radicals generated from molecular oxygen. According to this theory, clinical anaerobic isolates previously demonstrated to be oxygen tolerant should possess the enzyme. The purposes of our study were to survey various species of anaerobic bacteria recovered from both clinical infections and the normal flora for the presence of SOD and to correlate these results with their tolerance to oxygen.

## MATERIALS AND METHODS

**Isolation of anaerobes from clinical specimens.** Clinical specimens (such as pus and infected tissues) were transported expeditiously to the laboratory in oxygen-free transport tubes (2) and immediately processed in an anaerobic chamber with an atmosphere of 80% $N_2$, 10% $H_2$, and 10% $CO_2$ (1). Portions of each specimen were cultured on brain heart infusion (BHI) plates supplemented with 5% sheep erythrocytes, 0.1% hemin, and 10 $\mu$g of vitamin $K_1$ per ml (BHIS) and on additional selective plates containing BHIS with 75 $\mu$g of kanamycin per ml. Individual colonies were streaked for purity and tested for anaerobiosis, after which they were identified by criteria outlined in the Virginia Polytechnic Institute (VPI) *Anaerobic Laboratory Manual* (10).

**Isolation of anaerobes from normal flora.** Freshly passed stool specimens were transferred immediately into the anaerobic chamber, and all subsequent manipulations were done in this anaerobic environment. A 0.1-g sample obtained from a deep part of the stool was diluted 1:100 in VPI salts solution (10). Four serial 100-fold dilutions were made, and 0.1-ml portions were pipetted onto the

surfaces of agar plates to yield final dilutions of $10^{-3}$, $10^{-5}$, $10^{-7}$, $10^{-9}$, and $10^{-11}$. The following media, freshly prepared and prereduced in the chamber, were used to plate each dilution: (i) BHI agar supplemented with 5% laked sheep blood, 0.5% yeast extract, 10 $\mu$g of vitamin $K_1$ per ml, and 0.05% cysteine (BHISL); (ii) BHIS with 75 $\mu$g of kanamycin and 7.5 $\mu$g of vancomycin per ml; and (iii) BHIS with 50 $\mu$g of rifampin per ml.

**Oxygen tolerance studies.** Oxygen tolerance of anaerobic organisms was determined by a modification of a previously described method (21). The organisms were grown for 18 h in prereduced BHI broth with 5% peptic digest of sheep blood, 0.5% yeast extract, 0.5% cysteine, and 0.1 $\mu$g of vitamin $K_1$ per ml (BHISB). The inoculum was adjusted to approximately $10^6$ to $10^7$ colony-forming units/ml. Twelve prereduced BHISL plates were inoculated with approximately $10^5$ colony-forming units by a Steers replicator (20). The anaerobic control plate was kept in the chamber. Twelve plates were removed from the chamber; one was used for an aerobic control and another for a microaerophilic control. Ten plates were exposed to room air in unsealed, vented anaerobic jars to determine oxygen tolerance. The exposure periods were 10, 30, and 45 min and 1, 2, 4, 5, 24, 48, and 72 h. At each time interval, the plates were returned to the chamber and incubated for 48 h at 35°C. The end points were plates with no growth or less than five colonies. The definition of oxygen tolerance is the period of time the isolates can survive exposure to room air.

**Batch growth for enzyme assay.** Organisms to be assayed for levels of SOD were inoculated into 100 ml of BHISB. The culture was incubated at 35°C in the anaerobic chamber and grown to heavy turbidity of approximately $5 \times 10^9$ cells. Growth was stopped by cooling to 4°C; then, the bacteria were centrifuged at 10,000 rpm for 15 min. The cells were washed twice in 10 ml of 0.1 M potassium phosphate buffer, pH 7.8, with $10^{-4}$ M ethylenediaminetetraacetate. Cells were resuspended in 10 ml of the potassium phosphate buffer in an ice bath and sonically treated with three 45-s bursts on a Biosonic III sonic oscillator (Bronwill Scientific Inc., Rochester, N. Y.) with the medium-sized probe. The sonic extract was centrifuged at 4°C at 15,000 rpm for 15 min, and the supernatant was separated from the pellet. The supernatant was used for the enzyme and protein assays.

**SOD assay.** SOD was measured in the supernatant of disrupted cells by a modification of the method of McCord and Fridovich (14). The assay was performed in 3 ml of 0.1 M potassium phosphate buffer, pH 7.8, containing $10^{-4}$ M ethylenediaminetetraacetate in a 1.0-cm cuvette at 20°C. The reaction mixture contained $10^{-5}$ M ferricytochrome *c*, $5 \times 10^{-5}$ M xanthine, and sufficient xanthine oxidase to produce a rate of reduction of ferricytochrome *c* at 420 nm of 0.067 absorbance unit/min. Under these defined conditions, the amount of SOD required to inhibit the rate of reduction of ferricytochrome *c* by 50% was defined as 1 U of activity. If there was less than a 50% inhibition in the rate of reduction of cytochrome *c*, the amount of enzyme could not be quantitated accurately because of the non-linearity of the enzyme reaction; such values were recorded as "low level." If no reduction of cytochrome *c* was noted, the organisms were said to have "no detectable enzyme." The specific activity for the organism was defined as units per milligram of protein in the bacterial extract. The protein concentration was measured according to the method of Lowry et al. (13). The consistency of the enzyme assay was tested each day by determining the activity of a fixed amount of purified SOD (Truett Labs, Dallas, Tex.).

**Continuous culture for enzyme assay.** Chemostat cultures of *Bacteriodes fragilis* strain 339 were grown in minimal medium, using a model C30 Bioflow fermentor (New Brunswick Scientific Co., New Brunswick, N. J.) modified to maintain an anaerobic environment, as previously described (16). This culture was allowed to grow for 24 h at 37°C with agitation. A flow of sterile medium was then started to give a dilution rate (Dc) of 0.16/h. The dilution rate was calculated from the formula: Dc = flow rate (milliliters per hour)/fermentor volume (milliliters). Experimental studies were initiated after determining that a stable population of viable cells was established. After the organism had been in continuous culture for 24 h, it was exposed to oxygen by bubbling the gas into the culture vessel, with adjustment of the concentration via a dissolved-oxygen electrode. Samples were obtained for SOD assay at various time intervals by collecting the effluent from the fermentation.

The *B. fragilis* minimal medium was prepared as described by Varel and Bryant (22), modified by deleting cysteine and reducing the medium in the anaerobic chamber. The medium was freshly prepared for each study.

**Effect of medium on SOD levels.** *B. fragilis* strain 8 was cultured in BHIS broth in the anaerobic chamber under static conditions to determine the effect of optimum anaerobiosis on the concentration of SOD. The organism was subcultured every 2 to 3 days over a period of 60 days. At weekly intervals, a portion was subcultured into 100 ml of prereduced medium for SOD assay.

*B. fragilis* 339 was grown under static conditions in the chamber to determine the levels of enzyme in minimal medium versus prereduced enriched medium. The culture was transferred daily in the test medium, and at various intervals it was subcultured into 100 ml of the test medium for SOD assay.

## RESULTS

**Survey of anaerobes for SOD.** Twenty-two anaerobic bacteria were surveyed for SOD, including 14 clinical isolates, 6 bacteria from normal fecal flora, and 2 reference strains (Table 1). Three aerobic organisms were included as controls. Two of the normal flora isolates were obtained from Sydney M. Finegold, Wadsworth Anaerobic Laboratory (WAL), Los Angeles, Calif.; and the two clostridia reference strains were obtained from V. R. Dowell, Center for Disease Control, Atlanta, Ga.

TABLE 1. *Number and source of the anaerobic bacteria assayed for SOD and oxygen tolerance*

| Organism | Strain | Source[a] | SOD (U/mg of protein) | Oxygen tolerance |
|---|---|---|---|---|
| Aerotolerant | | | | |
| *Bacteroides fragilis* | 8 | C | 2.98 | >72 h |
| *B. fragilis* | 339 | C | 2.7 | >72 h |
| *B. fragilis* | 364 | C | 3.3 | >72 h |
| *B. fragilis* | 366 | C | 4.6 | >72 h |
| *B. vulgatus* | 10 | NF | 7.0 | >72 h |
| *B. ovatus* | WAL[b] | NF | 5.7 | >72 h |
| *Bacteroides* sp. | 365 | C | 13.5 | >72 h |
| *Eubacterium limosum* | 388 | C | 1.9 | >72 h |
| *E. rectale* | 386 | C | 4.0 | >72 h |
| *Bifidobacterium* sp. | WAL[b] | NF | ND[c] | >72 h |
| *Propionibacterium acnes* | 540 | C | 19.6 | >72 h |
| *Clostridium perfringens* | 19 | C | 0.44 | >72 h |
| *C. perfringens* | 322 | C | Low[d] | >72 h |
| *C. ramosum* | BSI | NF | 2.4 | >72 h |
| Intermediate | | | | |
| *Fusobacterium varium* | 486 | C | 2.2 | 30 h |
| *F. nucleatum* | 506 | C | 1.5 | 2 h |
| *Clostridium clostridiiformis* | 538 | C | Low | 24 h |
| *C. clostridiiformis* | 539 | C | Low | 24 h |
| EOS | | | | |
| *Clostridium novyi* type B | 6388 | Ref | Low | <10 min |
| *C. haemolyticum* | 14344 | Ref | Low | <10 min |
| *Bifidobacterium adolescentis* var. A | BS2 | NF | ND | <10 min |
| *Butyrivibrio fibrisolvens* | BS4 | NF | ND | <10 min |
| Aerobic controls | | | | |
| *Escherichia coli* | 474B5 | Ref | 17.9 | |
| *Klebsiella* sp. | 188 | Ref | 12.9 | |
| *Bacillus globigii* | | Ref | 13.7 | |

[a] C, Clinical isolate; NF, normal flora isolate; Ref, reference strain.
[b] Originally isolated as EOS from fecal specimen.
[c] ND, None detectable.
[d] Less than 50% inhibition of cytochrome *c* reduction.

The isolates were classified according to their oxygen tolerance: aerotolerant, survival for 72 h or more; intermediate, survival for periods of 10 min to 48 h; and extremely oxygen sensitive (EOS), inability to survive a 10-min exposure. There was a reasonable correlation between the enzyme levels and the oxygen tolerance of the organisms (Fig. 1). In general, the organisms surviving longer than 72 h had higher levels of SOD than the isolates with intermediate tolerance. The four EOS isolates had very low or undetectable levels of SOD activity. They included two clostridia reference strains that had been subcultured frequently and two fresh isolates from normal feces (a *Bifidobacterium adolescentis* and a *Butyrivibrio fibrisolvens*). The direct relation of SOD to oxygen tolerance was not universal, in that a strain of *Clostridium perfringens* and a *Bifidobacterium* sp. survived longer than 72 h while possessing low or undetectable levels of SOD (Table 1). Gram-negative anaerobic organisms seem to have increased levels of enzyme as compared to gram-positive bacteria in the tolerant groups. The enzyme levels of the three facultative organisms used as controls were two to five times higher than those of most of the anaerobic isolates.

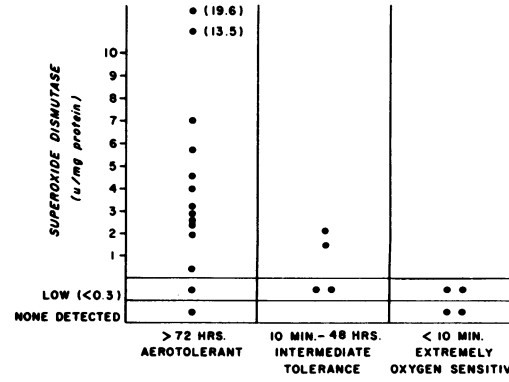


FIG. 1. *SOD levels and oxygen tolerances of 22 anaerobic bacteria.*

The two WAL isolates, *B. ovatus* and *Bifidobacterium* sp., were originally isolated as EOS organisms, but on subsequent testing they survived 72 h of oxygen exposure. This observation, plus the knowledge that anaerobic organisms may increase their oxygen tolerance on subculture in the laboratory, prompted the following experiments on induction of SOD.

**Effects of culture conditions on the induction of SOD.** *B. fragilis* strain 339 was grown anaerobically in minimal medium under continuous culture conditions and subsequently exposed to 10 and 25% atmospheric oxygen. Previous experiments by Onderdonk et al. had demonstrated that oxygen had a bacteriostatic effect on *B. fragilis* under these conditions (16). There was no detectable SOD when the organism was in steady-state growth under strict anaerobiosis (Table 2). Three hours after exposure to 10% oxygen, a low level of enzyme was detected; at 6 h, the level of enzyme was 4.0 U/mg of protein. An increase in the level of SOD was also noted with exposure to 25% oxygen.

Using another strain of *B. fragilis* (no. 8), SOD levels remained relatively constant when cultured under static conditions for 60 days in BHIS broth in an anaerobic chamber. The enzyme concentration in this isolate ranged from 2.4 to 5.8 U/mg of protein. The divergent results with these two strains, one in minimal medium under continuous culture conditions and the other in complex medium under static conditions, suggested that the medium may affect the level of enzyme. Therefore, *B. fragilis* 339, used in the continuous-culture experiments, was grown under static conditions in an anaerobic chamber. There was no detectable enzyme in minimal medium, whereas in the complex medium (BHIS) this organism produced 2.7 U/mg of protein. These results indicate that the production of SOD can be induced by components in a complex medium as well as by the addition of oxygen. An alternative explanation is that there was more oxygen trapped in the complex medium than in the minimal medium.

TABLE 2. *Effect of oxygen exposure on SOD levels in B. fragilis grown in continuous culture*

| Oxygen concn (%) | Time (h) | SOD (U/mg of protein) |
|---|---|---|
| 10 | 0 | ND[a] |
| | 1 | ND |
| | 3 | Low |
| | 6 | 4 |
| 25 | 0 | Low |
| | 4 | 1.23 |

[a] ND, None detectable.

## DISCUSSION

Variations in oxygen tolerance of anaerobes is not only of academic interest, but also may be important in the pathogenicity of certain anaerobic bacteria that are members of our microflora. Evidence to support the concept that oxygen tolerance is a virulence factor is derived from microbiological and clinical observations. Loesche, using stock cultures, assigned anaerobic bacteria to three categories by their ability to survive specific periods of oxygen exposure: strict, moderate, and microaerotolerant (12). All organisms in the strict group were components of the normal flora, whereas the other two groups included isolates from infected sites as well as the microflora. In studying the oxygen tolerance of 57 fresh anaerobic clinical isolates, Tally et al. found that all organisms survived for at least 8 h, and 44 of the 57 tolerated 48 to 72 h of oxygen exposure; furthermore, no EOS organisms were encountered in these clinical specimens, despite the use of anaerobic chamber techniques (21). Previous reports using optimal anaerobic techniques and carefully collected specimens, which avoided contamination by normal flora, also failed to recover EOS organisms from infected sites (18, 19). These findings are provocative, because in the course of studying anaerobic infections, it becomes obvious that the infecting organisms originate in the normal flora of the host, an ecological setting in which EOS organisms are known to reside as well (3).

The conundrum is best illustrated in intra-abdominal sepsis that results from spillage of colonic contents. *B. fragilis*, several clostridial species, and anaerobic cocci were the most common isolates from infected sites (7). Certain components of the normal flora were conspicuously absent, including bifidobacteria, eubacteria, ruminococcus, veillonella, lactobacilli, and butyrivibrio (3). Similar observations have been made in an animal model of intra-abdominal sepsis (17). The inoculum of cecal contents was known to contain EOS organisms, but none were recovered from the subsequent abscesses.

Oxygen tolerance appears to be responsible for this selection process. This characteristic would allow the pathogens to survive in oxygenated tissues until the proper milieu, a low oxidation-reduction potential, is established. Our data indicate that the enzyme SOD, present in all anaerobes of clinical significance included in this survey, may be the biochemical basis of this tolerance.

SOD, an enzyme first described by McCord and Fridovich in 1969, catalyzes the conversion of toxic superoxide radicals to $H_2O_2$ (14). Super-

oxide radicals are generated in the reduction of molecular oxygen, during which four electrons are accepted either univalently or in pairs. The stepwise reduction results in the production of superoxide ($O_2^-$) radicals which, although having short half-lives, are extremely reactive and may cause cell damage (5). The toxicity of superoxide radicals has been postulated to be important in the deleterious effect of oxygen on anaerobes. SOD is an enzyme that would protect cells by the conversion of $O_2^-$ to $H_2O_2$ and $O_2$.

In a limited survey of eucaryotic and procaryotic cells, McCord et al. were unable to detect SOD in anaerobic bacteria, and they proposed that SOD was the enzymatic basis of anaerobiosis (15). Subsequently, two laboratories have reported the presence of the enzyme in anaerobic microorganisms. Lindmark and Muller studied two anaerobic flagellates that were known to tolerate oxygen exposure, *Tritrichomonas foetus* and *Monocercomonas* sp., and detected SOD at levels of 25 and 10% of those obtained from *Escherichia coli* (11). Hewitt and Morris surveyed various species of clostridia and found a distribution of SOD that seemed to correlate with the oxygen tolerance of the organisms (9). These investigators commented that the level of SOD may have an influence on whether oxygen exposure has a bactericidal or bacteriostatic effect.

At face value, these reports tend to refute the original theory of McCord et al.; the results of our study, however, reconcile the divergent results. Anaerobic organisms, as discussed above, vary in their oxygen susceptibility from aerotolerant to extremely sensitive. When examining the distribution of SOD in our isolates of anaerobic bacteria, it appears that aerotolerant and moderate organisms possess SOD, whereas strict anaerobes have very low or undetectable enzyme. The organisms tested in the two cited studies (9, 11) were relatively aerotolerant and were found to possess SOD. Those examined by McCord et al. (15) were strict anaerobes, and thus lacked SOD.

In the course of converting $O_2^-$ to $H_2O_2$ by SOD, peroxide may be important in auto-assassination of those organisms lacking catalase. The recent discovery of catalase in *B. fragilis* suggests the mechanism by which this pathogen protects itself against the toxic products of oxygen reduction (C. Flen and J. Rosenblatt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C16, p. 28).

It is unlikely that SOD is the sole basis of an organism's ability to withstand oxygen exposure, since a strain of bifidobacteria that lacked detectable enzyme was able to survive for 72 h in room air. Although this strain had been isolated originally as an EOS organism from a fecal specimen, the subculture we tested was aerotolerant. There are two possible explanations for this phenomenon. First, the organisms may not have the capability to utilize oxygen and produce superoxide radicals. Gregory and Fridovich have found that such a situation exists with *Lactobacillus plantarum*, an organism closely related to bifidobacteria (8). Their studies showed that *L. plantarum* did not consume oxygen when tested with a Clark electrode, whereas the *E. coli* control did. The lack of oxygen consumption by this bifidobacterium would explain its oxygen tolerance, but the reason for its oxygen susceptibility when initially isolated from feces would remain unexplained. The second possible explanation is that the organism possesses low levels of SOD in the matrix that were not detected in our assay. Two distinct enzymes have been shown to exist in *E. coli*: a mangano enzyme in the cell matrix and an iron enzyme, which is in the periplasmic space as determined by its release by osmotic shock (5). In our studies, sonic disruption should have released the enzyme in the matrix if it were present, but we did not test the cellular debris for SOD activity; therefore, this possibility still exists.

The results of our survey revealed two further observations that deserve comment. The two reference strains of clostridia were considered EOS organisms, although low levels of SOD were detectable. This may reflect the vagaries in classifying bacteria as EOS. We used the criteria of Attebery et al., taking 10 min of exposure as the reference point (3). However, some anaerobists use a stricter definition of EOS, i.e., an organism that dies after exposure to oxygen for 30 s or less. The two isolates with low levels may not fulfill the stricter criterion.

The second observation is that a strain of *B. fragilis* originally isolated as an EOS organism had become oxygen tolerant when tested in our laboratory and was found to possess significant levels of SOD. The development of oxygen tolerance in anaerobic bacteria is a phenomenon long recognized by microbiologists. If SOD is the basis of defense against the toxic products of oxygen, then its inducibility would be a plausible explanation for acquiring such tolerance. This enzyme has been demonstrated to be inducible in *E. coli* and *Streptococcus faecalis* on exposure to hyperbaric oxygen (5). In our studies with *B. fragilis* in continuous culture, we found an increase in the amount of SOD after exposure to oxygen, demonstrating that the organism has the ability to regulate the concentration of SOD in response to a proper stimulus.

Under static conditions in an anaerobic chamber, *B. fragilis* continued to synthesize SOD at a fairly constant rate for 60 days. Further investigations in complex and minimal media indicated that oxygen is not the only stimulus to SOD synthesis. It is likely that other substances present may function as inducers. The complex medium contained many compounds that could produce superoxide ions in the presence of oxygen, e.g., flavins, ferredoxin, and hemoproteins (5). Microorganisms that recognize the lethal potential of these compounds are forced to maintain adequate levels of SOD. Since these compounds are not present in minimal medium (22), which is also devoid of oxygen, there would be no justification for production of the enzyme. However, once a stimulus such as oxygen is present, organisms possessing the genetic capability rapidly synthesize SOD. An alternative hypothesis for our observations is that enzyme production is suppressed by the lack of nutrients in minimal medium, so that the organism synthesizes only essential macromolecules. These and other questions hopefully will be resolved in future experiments.

## LITERATURE CITED


1. **Aranki, A., S. A. Syed, E. B. Kenney, and R. Freter.** 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. 17:568–576.
2. **Attebery, H. R., and S. M. Finegold.** 1969. Combined screw-cap and rubber-stopper closure for Hungate tubes (pre-reduced anaerobically sterilized roll tubes and liquid media). Appl. Microbiol. 18:558–561.
3. **Attebery, H. R., V. L. Sutter, and S. M. Finegold.** 1972. Effect of a partially chemically defined diet on normal human fecal flora. Am. J. Clin. Nutr. 25:1391–1398.
4. **Bartlett, J. G., N. Sullivan-Sigler, T. J. Louie, and S. L. Gorbach.** 1976. Anaerobes survive in clinical specimens despite delayed processing. J. Clin. Microbiol. 3:133–136.
5. **Fridovich, I.** 1974. Superoxide dismutases, p. 35–97. *In* A. Meister (ed.), Advances in enzymology. John Wiley & Sons, Inc., New York.
6. **Gorbach, S. L., and J. G. Bartlett.** 1974. Anaerobic infections. N. Engl. J. Med. 290:1177–1184, 1237–1245, 1289–1294.
7. **Gorbach, S. L., H. Thadepalli, and J. Norsen.** 1974. Anaerobic microorganisms in intraabdominal infections, p. 399–407. *In* A. Balows, R. M. DeHaan, V. R. Dowell, Jr., and L. G. Guze (ed.), Anaerobic bacteria: role in disease. Charles C Thomas, Publisher, Springfield, Ill.
8. **Gregory, E. M., and I. Fridovich.** 1974. Oxygen metabolism in *Lactobacillus plantarum*. J. Bacteriol. 117:166–169.
9. **Hewitt, J., and J. G. Morris.** 1975. Superoxide dismutase in some obligately anaerobic bacteria. FEBS Lett. 50:315–318.
10. **Holdeman, L. V., and W. E. C. Moore.** 1972. Anaerobic laboratory manual. Virginia Polytechnic Institute and State University, Blacksburg, Va.
11. **Lindmark, D. G., and M. Muller.** 1974. Superoxide dismutase in the anaerobic flagellates, *Tritrichomonas foetus* and *Monocercomonas sp.* J. Biol. Chem. 249:4634–4637.
12. **Loesche, W. J.** 1969. Oxygen sensitivity of various anaerobic bacteria. Appl. Microbiol. 18:723–727.
13. **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.
14. **McCord, J. M., and I. Fridovich.** 1969. Superoxide dismutase. An enzyme function for erythrocuprein (hemocuprein). J. Biol. Chem. 244:6049–6055.
15. **McCord, J. M., B. B. Keele, and I. Fridovich.** 1971. An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc. Natl. Acad. Sci. U.S.A. 68:1024–1027.
16. **Onderdonk, A. B., J. Johnston, J. W. Mayhew, and S. L. Gorbach.** 1976. Effect of dissolved oxygen and Eh on *Bacteroides fragilis* during continuous culture. Appl. Environ. Microbiol. 31:168–172.
17. **Onderdonk, A. B., W. M. Weinstein, N. M. Sullivan, J. G. Bartlett, and S. L. Gorbach.** 1974. Experimental intra-abdominal abscesses in rats: quantitative bacteriology of infected animals. Infect. Immun. 10:1256–1259.
18. **Rosenblatt, J. E., A. Fallon, and S. M. Finegold.** 1973. Comparison of methods for isolation of anaerobic bacteria from clinical specimens. Appl. Microbiol. 25:77–85.
19. **Spaulding, E. H., V. Vargo, T. C. Michaelson, and R. M. Swenson.** 1972. A comparison of two procedures for isolating anaerobic bacteria from clinical specimens, p. 37–46. *In* A. Balows, R. M. DeHaan, V. R. Dowell, Jr., and L. G. Guze (ed.), Anaerobic bacteria: role in disease. Charles C Thomas, Publisher, Springfield, Ill.
20. **Steers, E., E. L. Foltz, and B. S. Graves.** 1959. An inocula-replicating apparatus for routine testing of bacterial susceptibility of antibiotics. Antibiot. Chemother. (Washington, D. C.) 9:307–311.
21. **Tally, F. P., P. R. Stewart, V. L. Sutter, and J. E. Rosenblatt.** 1975. Oxygen tolerance of clinical anaerobic bacteria. J. Clin. Microbiol. 1:161–164.
22. **Varel, V. H., and M. P. Bryant.** 1974. Nutritional features of *Bacteroides fragilis* subsp. *fragilis*. Appl. Microbiol. 28:251–257.
23. **Veillon, M. M., and Z. Zuber.** 1898. Recherches sur quelques microbes strictement anaerobies et leur role en pathologie. Arch. Med. Exp. Anat. Pathol. 10:517–545.