BACTERIOSTATIC AND BACTERICIDAL EFFECTS OF ANTIBIOTICS ON *BACTEROIDES*

by

Benjamin James Veltri

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APPROVED:

T. D. Wilkins, Chairman

J. L. Johnson

E. M. Gregory

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INTRODUCTION AND LITERATURE REVIEW

There has been a surge of interest in the field of anaerobic microbiology in recent years. Although the existence of anaerobes has been known for over a century their involvement in human disease, with the exception of certain clostridial infections, was largely overlooked. The reason for this increased interest in anaerobes is largely the result of technical advances in the collection and cultivation of these bacteria. As a result, classification schemes have been developed for anaerobic bacteria and their important role in human disease has been realized.

Clinical Aspects

There is now no doubt that certain anaerobes are capable of causing disease in humans. Moore et al. (52) examined 300 specimens from infections of the lung, urogenital tract, brain, blood, and other sites. Specimens were collected anaerobically and cultured in prereduced anaerobically sterilized (PRAS) media. Eighty-five percent of the specimens contained obligately anaerobic bacteria. The majority of specimens contained several anaerobes. Using anaerobic jars instead of PRAS media Martin (48) found that 49% of 27,588 specimens contained anaerobes only. Holland et al. (33) in a similar study reported that anaerobic bacteria were in 48.8% of 826 specimens.

Anaerobic bacteria are capable of causing infection in almost any area of the body. Moore et al. (52) and Gorbach (27) reported anaerobes in 90 to 93% of a diverse group of intra-abdominal septic

processes. In a study involving 12 cases of liver abscesses, Altemeier (1) reported that anaerobes were present in every case. Anaerobes have also been shown to play major roles in adnexal infections, biliary tract infections, pelvic abscesses, cerebral abscesses, Bartholin-Gland abscesses, post-partum infection, post-abortion sepsis, and chest infections (27). These bacteria have been found to be frequent pathogens in upper respiratory tract infections, skin and soft tissue infections, and infrequently in urinary tract infections, bone and joint infections, and endocarditis (27).

Bacteroides fragilis

It is now generally agreed that the single most common anaerobic organism isolated from clinical specimens is Bacteroides fragilis, a gram-negative, non-spore forming rod. The name B. fragilis, at one time represented a group of bacteria containing five subspecies as well as a group called "other" (32). These were differentiated according to sugar fermentation patterns and indole production. The named subspecies included ss. fragilis, ss. thetaiotaomicron, ss. distasonis, ss. vulgatus, and ss. ovatus. These subspecies have recently been elevated to species rank based on DNA homology studies by Cato and Johnson (10). When referred to collectively in this thesis I have used the designation "B. fragilis group."

The B. fragilis group is found in the environment as part of the normal flora of the large intestine. In a study of the fecal flora of 20 Japanese-Hawaiians, Moore and Holdeman (53) found that members

of this group of organisms constituted the greatest percentage (20%) of the cultivable flora. Table 1 lists the percentage and rank of isolation of the individual species of B. fragilis.

The incidence of these bacteria in clinical infections is the reverse of that seen in the normal flora. Holland et al. (33) isolated 408 anaerobes from 153 clinical specimens over a nine year period. These isolates were identified according to current criteria. Bacteroides was the most frequently isolated genus, accounting for 42% of the anaerobes isolated. The B. fragilis group was isolated most frequently, accounting for 24.3% (60% of Bacteroides isolates) of the anaerobes isolated. B. fragilis represented 11% of the isolates, B. thetaiotaomicron, 7%; B. distasonis, 2%; B. vulgatus, 1%; and B. ovatus, 0.002%. Results similar to this have been reported by several other workers (7, 37, 63).

Bactericidal versus Bacteriostatic

Various reports have appeared in the literature citing the need for antimicrobial agents which are bactericidal to B. fragilis. Such drugs are of use in certain clinical situations to be mentioned later. The terms bactericidal and bacteriostatic are frequently used, but subject to various interpretation. Lewis (46) defined bactericidal as the ability to kill bacteria. Bacteriostatic was defined as the ability to prevent bacteria from multiplying. These definitions may seem straightforward but even cell death is subject to various interpretations. Some investigators define a cell as dead

Table 1. Incidence of the B. fragilis group in the normal flora of the large intestine

Organism	Rank of Isolation	Percentage of Flora
B. vulgatus	1	12.10
B. thetaiotaomicron	6	4.45
B. distasonis	11	2.36
B. ovatus	25	0.713
B. fragilis	29	0.628

Data taken from Moore and Holdeman (53).

when all metabolism has ceased. Others use the failure to divide as the criterion for cell death (24). Hence, to some these two terms may have the same meaning.

Smith (73) used a temporal definition. Bacteriostatic was defined as an effect "in which there is an inhibition of growth, which in time results in failure of the organism to multiply, that is, inhibited growth in time equals death." Bactericidal was defined as an effect, which "when initiated almost invariably results in cell death." The end result in both of these cases is death, the main distinction being one of time. Bacteriostatic drugs are considered to take a relatively long time to act, and if the process if interrupted, some bacteria may survive and begin multiplication again. A difficulty here is that what constitutes a significant time has never been defined. In addition, all bacteriostatic drugs might not require the same amount of time to cause cell death, thus making the distinction between bactericidal and bacteriostatic that much less understood.

Perhaps the best definition of bactericidal has been given by Rolinson et al. (67). A bactericidal drug was defined as an agent which prevented cells from resuming growth and division when it was no longer present. That is, a bactericidal drug acts by causing a loss of viability, while a bacteriostatic drug's effects are reversible. The strength of this definition lies in its strict empiricism. Various parameters need not be defined, and whether a cell is viable or not can easily and accurately be determined.

1. Methods used to determine antibiotic effects on bacteria.

There are four basic techniques used to determine the susceptibility of bacteria to antimicrobial agents. These include 1) disk diffusion, 2) broth disk, 3) agar dilution, and 4) broth dilution tests (89). Dilution methods are used to determine the minimal inhibitory concentration (MIC) of a drug. The MIC is defined as the least concentration of antibiotic which inhibits bacteria from growing. Dilution methods are the only susceptibility tests which can be used to determine the minimal bactericidal concentration (MBC) of a drug. The MBC is defined as the least concentration of antibiotic which results in a loss of viability when the antibiotic is removed.

Agar dilution techniques consist of incorporating serial two-fold dilutions of antibiotic into an agar medium. A fixed number of cells (usually 1×10^5) is inoculated onto the media within a defined area. After an incubation period the MIC is read as the least amount of antibiotic which results in no growth, a barely visible haze, or less than three colonies (89). An inoculum replicating apparatus is often used which permits the simultaneous testing of up to 36 organisms per agar plate (75). MBCs can be determined by using velvet pads for replica plating onto an antibiotic-free medium (34). The MBC is read as the least amount of antibiotic which prevents significant growth after subculture. What constitutes significant growth varies from author to author. Ingham et al. (34) considered the presence of 20 or more colonies as significant. On the other hand Wüst (96) considered the presence of more than one colony as significant.

Broth dilution tests are similar to agar dilution methods except that a liquid medium is used as opposed to a solid one. Several broth dilution tests for susceptibility testing of anaerobes have been described (74, 76, 91). To date there is no accepted standardized method. The described methods have in common that serial two-fold dilutions of antibiotic are added to a liquid media and inoculated with a fixed, known number of cells. Following incubation the MIC is read as the least amount of antibiotic which results in no growth. No growth is defined as a lack of visible turbidity. MBCs are determined by subculturing tubes showing no growth onto antibiotic-free agar. A fixed volume of broth, usually 0.001 ml or 0.01 ml is used for subculturing. The MBC is usually read as the least amount of antibiotic needed to decrease the viability of the original inoculum by 99 or 99.9% (39).

2. Interpretation of MIC and MBC data.

It would appear that the MBC of a bactericidal drug would be equal to its MIC. However, some antibiotics which are considered as bactericidal (penicillin for example), when administered in low doses may produce a static effect (MIC less than MBC). Likewise, antibiotics which are characterized as bacteriostatic (tetracycline and chloramphenicol for example), when administered in high doses may produce a bactericidal effect (73). In this respect, all antibiotics can be considered as bactericidal. It is generally agreed that the MBC to MIC ratio of a drug whose main mechanism of action results in cell death (bactericidal) be no greater than four (13, 85).

Many investigators prefer to avoid the question of whether a drug acts in a bactericidal or bacteriostatic manner. Instead a drug is considered as bactericidal to an organism when the MBC falls below the achievable blood level of the drug (34, 97). This type of interpretation is of benefit in clinical situations where the only point of interest is whether a drug will kill bacteria at a concentration which can be reached in the blood, regardless of whether the MIC of the organism is one dilution or ten dilutions below the MBC.

Thus the terms MIC and MBC are very artificial. The environment in which the MIC or MBC of a drug is determined is one of fixed experimental conditions, some of which may be far removed from those encountered in the body. For instance, in in vitro tests an inoculum of 1×10^5 cells/ml is usually used whereas in the body many infected sites contain on the order of 1×10^{10} cells/ml. Reports by investigators indicate that as higher initial inocula are used in broth dilution tests, corresponding MICs will also increase (39, 50, 82, 95). In addition, cells in in vitro tests are logarithmically growing, whereas in the body, if this occurs, the rates of growth are probably slower. In vitro susceptibility data indicate the sensitivity of an organism under specified conditions. To be of value in in vivo situations MIC and/or MBC data must be compared with the in vivo efficacy of a drug. Such comparisons can be used to determine whether an organism with a given MIC and/or MBC will be amenable to therapy with a given drug.

Therapy of Bacteroides fragilis Infections

Anaerobes tend to form closed-space infections, usually in the form of an abscess. The primary mode of treatment is surgical drainage coupled with resection of necrotic tissue. Appropriate antibiotics should be employed since once drainage has been established, sepsis and even extension of the abscess may occur (27, 43). The overall mortality of B. fragilis infections is 60% (25, 27) in cases where inappropriate antibiotics are employed and 15% when an effective drug is used (27).

Clinically, whether a drug exerts a bactericidal or bacteriostatic effect is often not important since bactericidal drugs are not required in the majority of infections (73). However, in cases of bacterial endocarditis (21, 54, 55, 73), septicemia (73), cerebral abscess and intraabdominal sepsis (9, 34) the use of a bactericidal drug is essential or highly recommended for successful treatment of the infection. B. fragilis has been found in cases of each of the aforementioned infections. Bodner et al. (8) were among the first to realize the need for antibiotics with bactericidal action against B. fragilis. The search for such agents is hindered by the fact that B. fragilis is perhaps the most resistant of all anaerobes to commonly employed antibiotics. Currently the drugs of choice in the treatment of B. fragilis infection are clindamycin, chloramphenicol, and metronidazole (26, 27, 43, 73).

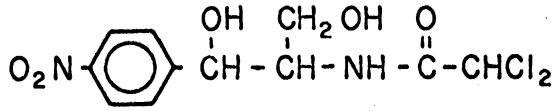
1. In Vitro Findings

There is a wealth of information available on the in vitro sensitivity of B. fragilis to clindamycin, chloramphenicol, and metronidazole. A summary of the major literature on these drugs follows. In addition, tetracycline and cefoxitin will also be considered.

Clindamycin: Clindamycin is a chemically modified form of its parent compound lincomycin (Fig. 1). Lincomycin binds to the 50S subunit of ribosomes. Inhibition of peptide bond formation occurs, and results in the suppression of protein synthesis (26). The lincomycin-ribosome linkage formed is reversible (50). Clindamycin is believed to act in the same manner. Clindamycin has several advantages over lincomycin. It is more readily and completely absorbed from the gastrointestinal tract, clindamycin blood levels are twice as high as lincomycin for a given dose (26), and its activity against anaerobes is four times greater than that of lincomycin (40, 49, 97). Clindamycin, like lincomycin, is active against gram-positive cocci and gram-positive rods. Nearly all gram-negative aerobic bacteria are resistant. A notable exception is that gram-negative anaerobes are generally very susceptible to clindamycin.

Peak serum levels of 6 to 15 mcg/ml can be obtained depending on the mode of administration (41).

Shortly after its introduction, McGehee et al. (50) determined the MIC and MBC of clindamycin for six strains of Staphylococcus aureus. The MBCs of five of these strains were the same or only twice the



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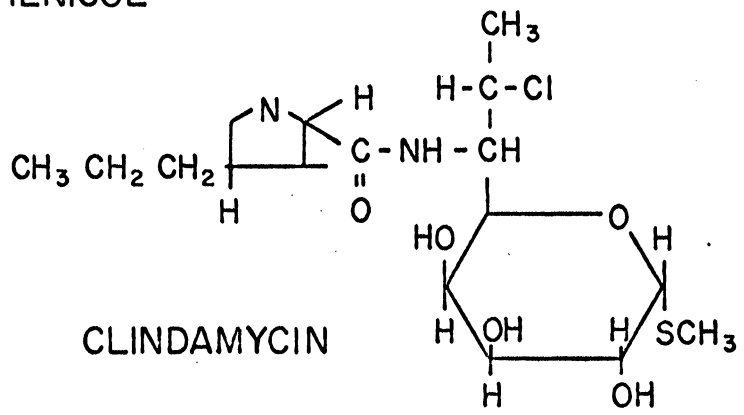

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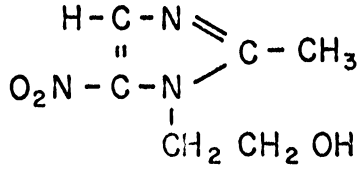

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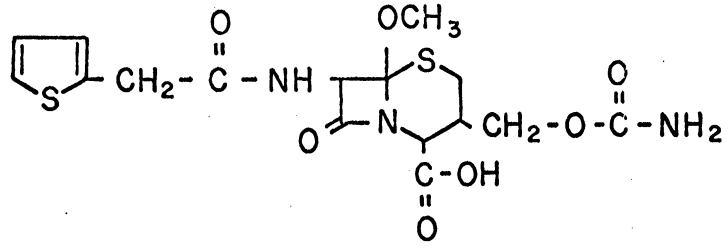

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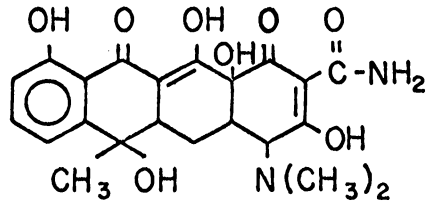

TETRACYCLINE

Figure 1. Antibiotic structures.

MIC. They concluded that in general clindamycin can be considered as a bactericidal agent. In a similar study Modde (51) concluded that clindamycin was bactericidal for streptococci, staphylococci, and pneumococci. In addition the MBC of clindamycin for these organisms were far below obtainable blood levels. Wagneravá et al. (82) also concluded that clindamycin was bactericidal to a strain of S. aureus. In addition the authors noted that a 6 to 8 h exposure to the drug was necessary before a consistent MBC could be determined.

There have been numerous reports giving the MIC of clindamycin to strains of B. fragilis (7, 37, 40, 49, 54, 69, 78, 97). Kislak (40) reported that 19 strains from the B. fragilis group were inhibited with 3.1 mcg/ml or less of clindamycin. The median MIC was 0.19 mcg/ml. Sutter and Finegold (78) determined the susceptibilities of 76 strains from the B. fragilis group to clindamycin. All strains were inhibited by 8 mcg/ml or less of clindamycin. These investigators noted that despite the widespread use of this drug, resistance among previously susceptible anaerobes did not appear to be a problem.

Blazevic (7) studied the antibiotic susceptibilities of subspecies of B. fragilis. She reported that B. fragilis ss. fragilis (B. fragilis) was slightly more sensitive to clindamycin than the remaining members of the group. All 39 strains of B. fragilis tested had MICs of 0.4 mcg/ml or less. Of 32 B. fragilis ss. thetaiotaomicron (B. thetaiotaomicron) strains, one required 12.5 mcg/ml, one required 6.3 mcg/ml for inhibition of growth, and the remaining strains were inhibited by 3.1 mcg/ml or less. Blazevic considered

this difference in MIC levels to be nonsignificant, and concluded that one could not predict the susceptibility of a strain by identifying it to the subspecies level. In a similar study Jones and Fuchs (37) reported comparable results. Of 44 B. fragilis ss. thetaiotaomicron (B. thetaiotaomicron) strains tested only 34% were inhibited by 0.5 mcg/ml or less of clindamycin. Of the remaining subspecies (species) of the B. fragilis group 75-97% were inhibited by 0.5 mcg/ml or less of clindamycin. The authors' only conclusion was that the mean MIC of clindamycin was higher for B. thetaiotaomicron than for other species in the group. Santoro et al. (69) have also studied the susceptibilities of the B. fragilis group to clindamycin. The MICs of clindamycin for 29 clinical isolates of the B. fragilis group were determined and statistically treated. It was noted that no appreciable difference in the MICs of the strains to clindamycin existed ($P > 0.05$, chi-square test), even though the data were comparable to those of the previous investigators.

Several studies have been undertaken in which the MIC and MBC of clindamycin to B. fragilis have been determined (13, 34, 49, 54, 55, 69, 97). Martin et al. (49) reported the MICs and MBCs of clindamycin to 25 strains of the B. fragilis group. MICs were determined by a broth dilution test. MBCs were determined by subculturing 0.05 ml onto antibiotic-free brain heart infusion (BHI) agar. The MBC was defined as the lowest concentration of antibiotic from which the subculture showed no growth. The MBC for clindamycin generally exceeded the MIC by at least 16 fold, although no difference was detected for some of the

strains. It was concluded that clindamycin was inconsistently bactericidal against B. fragilis. Nastro and Finegold (54) in a similar study determined the MIC and MBC of clindamycin to 19 strains of the B. fragilis group. MBCs were determined by subculturing 0.001 ml from MIC tubes. The authors reported that if fewer than 10 colonies were used as the criterion for the MBC, then clindamycin was bactericidal against 11 of the 19 strains. If zero colonies were used as the MBC clindamycin showed bactericidal activity against only a few strains. They concluded that the bactericidal activity of this drug was irregular. Zabransky et al. (97) studied the activity of clindamycin on 50 strains of the B. fragilis group. A broth dilution test was used, and MBCs were determined by subculturing 0.01 ml and defined as the lowest drug concentration which resulted in no growth. The authors reported that the MBCs were four to eight fold higher than corresponding MICs. It was noted that 88% of the strains tested were killed at a concentration of 8 mcg/ml, a clinically achievable level. The authors concluded that clinically clindamycin was bactericidal against B. fragilis, and cited this as an advantage of the drug.

Chloramphenicol: Chloramphenicol (Fig. 1) is effective against a wide range of gram-positive and gram-negative bacteria. This was the first broad spectrum antibiotic to be used clinically. In addition chloramphenicol was the first antibiotic to be synthesized by organic methods, and today is the only antibiotic which is industrially produced by chemical synthesis (28).

Chloramphenicol is considered to act chiefly as a bacteriostatic agent (26). The mechanism of action involves the binding of the drug to the 50S subunit of the bacterial ribosome. The end result is the inhibition of peptidyl transferase which forms peptide bonds between growing polypeptide chains on ribosomes and incoming amino acids (24). Although not structurally related to clindamycin, chloramphenicol is believed to bind at or near the binding site of the former drug, since the binding of one of these drugs may inhibit the binding of the other (26).

Peak blood levels of 10 to 20 mcg/ml (26, 41) can be obtained 30 min after the administration of a conventional dose. The half-life of chloramphenicol in the serum is 1.6 to 3.3 h. Therapeutic levels may still be detected 6 to 8 h after administration of a dose (41).

The sensitivity of the B. fragilis group to chloramphenicol has been reported by a variety of investigators (7, 34, 37, 40, 78, 91). Kislak (40) determined the MIC of chloramphenicol to 40 clinical isolates to this drug. Using an agar dilution technique the median MIC was 3.1 mcg/ml. Ten percent of the strains required 12.5 mcg/ml to inhibit growth. In a similar study, Sutter and Finegold (78) reported on the susceptibilities of 76 strains from the B. fragilis group. One isolate had an MIC of 32 mcg/ml and the remainder were inhibited by 16 mcg/ml or less.

Ingham et al. (34) determined the MIC and MBC of chloramphenicol to 31 strains of the B. fragilis group to chloramphenicol with an agar dilution technique. Replica plating was used to determine MBCs, and MBCs

were defined as the lowest concentration of antibiotic necessary to result in fewer than 20 colonies after subculture. MBCs were two to four times higher than the corresponding MICs. It was concluded that chloramphenicol was bactericidal to B. fragilis.

Martin et al. (49) in their study of 25 strains belonging to the B. fragilis group found that the MBCs of chloramphenicol were eight or more fold higher than the MICs. The MBCs of the majority of these strains were above achievable blood levels. The authors concluded that chloramphenicol was inconsistently bactericidal for B. fragilis. These results were in disagreement to those of Ingham et al. (34). Martin et al. (49) attributed this conflict of data to procedural differences and to differences in the interpretation of the MBC.

In the previously mentioned study by Zabransky et al. (97) the MICs and MBCs of chloramphenicol to 50 isolates of the B. fragilis group were determined. The MBCs of chloramphenicol were eight or more fold higher than corresponding MICs. They concluded that chloramphenicol was bacteriostatic.

Metronidazole: Metronidazole (Fig. 1) was originally introduced as a highly active trichamonacide (66, 93). Clinical activity against bacteria was first reported as a result of a chance observation by Shinn (71) and Shinn et al. (72). A patient with vaginal trichamoniasis also had chronic Vincent's stomatitis. After one week of therapy a double cure was reported.

As a result of this observation studies were made on the activity of metronidazole against a variety of bacteria (15, 64). Prince

et al. (64) reported that metronidazole was active against anaerobic bacteria and virtually without effect on facultative and aerobic bacteria. In addition it was shown that facultative bacteria, when grown anaerobically, still retained their resistance to the drug. Today it is believed that metronidazole is active only against anaerobic bacteria (35, 66, 93).

Peak serum levels obtained with conventional dosages of metronidazole are usually 8 to 10 mcg/ml (44, 77). However, dosages used in the treatment of trichamoniasis yield peak blood levels of 50 mcg/ml (88). In a study of high dose metronidazole therapy it was reported that serum levels of up to 200 mcg/ml could reliably be obtained (16).

Several studies have been conducted on the in vitro sensitivity of the B. fragilis group to this drug (12, 69, 78, 95, 96). Chow et al. (12) determined the susceptibilities of 358 clinical anaerobic isolates. Of 41 isolates of the B. fragilis group 36 were inhibited by 6.25 mcg/ml or less. Wüst (96) studied 39 strains belonging to the B. fragilis group. He reported the MIC of metronidazole for all strains was equal to or less than 3.1 mcg/ml. In general, all other in vitro studies have agreed with these findings.

Metronidazole exerts a bactericidal effect on susceptible organisms. Nastro and Finegold (54) in their study of 19 strains of the B. fragilis group reported that metronidazole consistently demonstrated bactericidal activity. When zero colonies were used as the end point for MBC determination after subculturing 0.001 ml, they found that the MIC and MBC were usually identical or at most a two-

fold dilution apart. Virtually the same results were obtained when less than ten colonies were used as the MBC end point. These results have been confirmed by several other investigators (18, 38, 39, 65, 88, 96).

Ralph and Kirby (65) reported on the extremely rapid bactericidal action of metronidazole. With an inoculum of 10^6 cells/ml in supplemented Brucella broth, they found that the maximum bactericidal effect occurred over a period of one hour or less. During this time the number of viable B. fragilis cells in the inoculum decreased to 10 to 100 cells/ml. Similar results were obtained with Clostridium perfringens. It was noted that metronidazole showed little or no activity during the initial 8 h lag phase of B. fragilis or the 4 h lag phase of C. perfringens.

The mechanism of action of metronidazole has not been proven. Metronidazole has been shown to inhibit H_2 production in clostridial-type pyruvate phosphoroclastic reactions (18, 19, 57). O'Brien and Morris (57) made a detailed study of purified C. pasteurianum hydrogenase. They showed that hydrogenase itself was not physically inhibited by metronidazole, but that metronidazole could act as an e^- acceptor when the reverse reaction was assayed ($H_2 \rightarrow 2e^- + 2H^+$). In addition it was shown that extracts of C. acetobutylicum depleted of ferredoxin (Fd) failed to reduce metronidazole. This effect could be reversed by the addition of Fd to the reaction mixture.

It is generally agreed that in the reduction process the nitro group of metronidazole is reduced (36, 62). Following this reduction the activated metronidazole binds to DNA (36), in particular to

guanosine and cytosine residues (42). After this step several modes of action have been reported or postulated. Ings et al. (36) in studies involving Trichomonas vaginalis reported that once reduced, metronidazole forms a complex with DNA. The end result is the inhibition of nucleic acid synthesis. They hypothesized that once the metronidazole-DNA complex is formed, DNA can no longer act as an effective primer for DNA or RNA polymerase. Plants and Edwards (62) studied the effects of metronidazole on actively growing C. bifermentans cells. They found that following addition of the drug DNA synthesis immediately ceased, followed 30 min later by the degradation of any preexisting cellular DNA. These same investigators maintain that their data differ from those of Ings et al. (36) either because of methodology differences or because a bacterial species was used in the one study as opposed to a protozoan in the other. Recently LaRusso et al. (42) studied the effects of metronidazole on various physical parameters of DNA such as melting point, viscosity, and sedimentation rate. They found that when mammalian, bacterial, or phage DNA was exposed to artificially reduced metronidazole none of the indicated parameters were affected. This indicates that if preexisting cellular DNA is broken down, metronidazole is not the direct cause but instead may stimulate or activate various DNA degrading enzymes within the cell.

Whatever the exact mode of action of metronidazole, it is apparent that as metronidazole acts against bacteria it is consumed in the process. Ings et al. (36) studied the fate of radioactive metronida-

zole in T. vaginalis. It was shown that metronidazole was readily accumulated by cells and that none of the radioactive products recovered corresponded to the parent drug. These researchers went on to say that the complete metabolism of metronidazole would effectively maintain a concentration gradient for the continued diffusion of metronidazole into the cell.

Miscellaneous drugs: Tetracycline (Fig. 1) was once considered the drug of choice in the treatment of B. fragilis infection. However, in the last few years numerous reports have appeared in the literature citing the emergence of resistant strains (7, 11, 49, 54, 78, 79, 97). At present approximately 64% (78) of clinical isolates are resistant to the achievable blood levels of 4 to 5 mcg/ml (41).

Tetracyclines have a wide range of activity, being effective against both gram-positive and gram-negative organisms. The drug binds to the 30S subunit of bacterial ribosomes and inhibits the enzymatic binding of aminoacyl-tRNA to the acceptor site located on the ribosome (4, 41).

Tetracycline is considered to be mainly a bacteriostatic agent (4), but is frequently bactericidal in high concentrations (26, 73). Ingham et al. (34) in their study of the sensitivity of B. fragilis to antibiotics reported that tetracycline was irregularly bactericidal to 17 isolates from this group. MBCs were eight or more times higher than MICs. Nastro and Finegold (54) observed essentially the same effects. These authors did note that tetracycline appeared to be bactericidal to 2 of 19 sensitive strains from the B. fragilis group.

Data obtained by Martin et al. (49) also indicated that occasionally tetracycline was bactericidal to B. fragilis.

Although no longer considered the drug of choice in the treatment of B. fragilis infection, therapy with this drug is indicated when susceptibility data are available to confirm a strain as sensitive.

Ninety percent of the isolates of the B. fragilis group are resistant to penicillin (27). This resistance has been attributed to the presence of a beta-lactamase in B. fragilis (58). Recently cefoxitin (Fig. 1), a semisynthetic cephamycin has been introduced. This beta-lactam antibiotic has been reported to be resistant to the action of beta-lactamase (14, 61). In vitro susceptibility data (3, 78) have shown that 85 to 89% of recent B. fragilis isolates are susceptible to the action of this antibiotic (MIC $\leq$ 32 mcg/ml). Darland and Birnbaum (14) have attributed this increased susceptibility to the resistance of cefoxitin to the beta-lactamase of B. fragilis. However, other factors may be involved. Weinrich and Del Bene (86) studied the beta-lactamase substrate requirements of ten B. fragilis strains. Of eight beta-lactamase producing strains seven possessed low but detectable activity against cefoxitin.

2. In Vivo Findings

In vivo findings can be considered to fall into two main categories. These include the results of clinical findings and the results of antimicrobial therapy of experimental models.

Clinical studies with clindamycin have proven the drug to be of high value in the treatment of anaerobic infections (5, 17, 20, 22, 27,

45). Bartlett et al. (5) used clindamycin to treat 14 patients with anaerobic infections. All of the patients responded well to the drug, and a cure was achieved in 13 of the cases. Fass et al. (20) treated 19 patients with serious anaerobic infections with clindamycin. In all but one of the 19 patients a clinical cure was achieved. Douglas and Kislak (17) used clindamycin to treat eight patients with B. fragilis bacteremia. Seven of these patients showed a prompt and favorable clinical and bacteriological response to treatment.

Proof of the efficacy of chloramphenicol in anaerobic infections is largely testimonial (43), but it is generally held that the clinical effectiveness of this drug is well established (22, 23, 27). Restrictions on the use of clinical trials is largely due to the fear of haematologic complications encountered with this drug. Goodman and Gilman (26) indicate the use of chloramphenicol only when sensitivity tests indicate it as the most active antimicrobial agent. This is in conflict to Finegold (22) who considers chloramphenicol as the drug of choice in serious anaerobic infection, and particularly in central nervous system infections which are not yet defined bacteriologically.

In the treatment of anaerobic infections metronidazole has been shown to be an extremely promising agent (25, 66, 70, 80, 81, 94). Talley et al. (80) were the first to evaluate metronidazole therapy of Bacteroides infection. In this study three patients with deep seated infections were successfully treated with metronidazole. Talley et al. (81) in a subsequent trial treated ten patients suffering from anaerobic infection. Six patients were cured, three showed a good

response but circumstances required a change to another drug, and one patient did not respond to therapy. Recently Giamarellan et al. (25) treated 48 patients with metronidazole. These patients represented a variety of serious anaerobic infections. Following therapy, 38 patients were completely cured, nine showed a satisfactory response, and three were considered as treatment failures.

Experimental models have only recently been described for pure B. fragilis infection which enable large scale investigations to be made. Such models are of value since they can be used to test experimental drugs, the effect of antibiotics on bacteria in in vivo environments, and pathogenic mechanisms.

The earliest experimental models involving B. fragilis involved mixed infections. In 1974, Hill et al. (31) described liver abscess production by nonsporing-forming anaerobic bacteria in mice. Bacterial inocula were grown in supplemented fluid thioglycollate media and mixed in equal volumes with sterile mucin. Mice were injected intraperitoneally with 0.5 ml of inoculum. When individual strains of B. fragilis were tested abscesses developed in 25 to 50% of the mice. When B. fragilis was mixed with Fusobacterium nucleatum abscesses developed in 50 to 99% of the mice depending on the B. fragilis strain used. When B. fragiliswas mixed with F. necrophorum abscesses developed in 75 to 100% of the mice.

In this same year Wilkins and Smith (92) described an experimental F. necrophorum infection in mice. Cultures were grown in chopped meat carbohydrate broth (CMC) and washed in anaerobic di-

lution fluid (32). Mice were injected subcutaneously or intraperitoneally with 0.1 ml of the washed cells. The infection was lethal to mice. This model was subsequently adapted by Hackman and Wilkins (29) so that a mixed F. necrophorum and B. fragilis infection could be obtained. Cultures were grown and prepared as described in the original model except that B. fragilis cells were not washed. Both cultures were mixed and 0.1 ml were injected subcutaneously into mice. When B. fragilis was injected alone no infection was observed. When mixed with F. necrophorum, B. fragilis grew rapidly to a concentration of 10^{10} cells/g abscess material. This model was used to determine the effect of B. fragilis on penicillin therapy. The pure F. necrophorum infection responded to penicillin therapy, whereas the mixed infection did not. This protection phenomenon appeared to be associated mainly with penicillinase production by B. fragilis. In a later study, Hackman and Wilkins (30) compared cefoxitin and cephalothin therapy using this same mixed model. Cefoxitin, a beta-lactamase resistant cephamycin, was determined to be more efficacious than cephalothin. The cefoxitin mean effective dosage (ED_{50}) was 263 mg/kg, whereas the ED_{50} for cephalothin was greater than 2000 mg/kg. These data indicated that beta-lactamase production by B. fragilis may play an important part in the protection of F. necrophorum from penicillin and cephalothin in vivo.

In 1974 Weinstein et al. (87) reported on an experimental intra-abdominal abscess model for rats. Rat cecal contents were mixed with barium sulfate. Gelatin capsules containing 0.5 ml of this suspension

were surgically implanted into the pelvic region of rats. When the cecal contents of meat fed rats were used as inocula a biphasic disease resulted. The initial phase was characterized by a 43% mortality rate. Autopsies revealed the cause of death to be peritonitis. Of the animals surviving this first phase 100% developed discrete intra-abdominal abscesses. When colonic contents from grain fed rats were used there was an initial 80% mortality followed by abscess development in 78% of survivors. This type of progressive biphasic disease simulates the disease as seen in humans after bowel contamination of the peritoneal cavity. In a later study the quantitative bacteriology of infected animals was determined (60). Inocula were derived from meat fed animals. In the peritonitis stage Escherichia coli (mean concentration of 10^6/ml) enterococci (10^5) and B. fragilis (10^6) were always present. Major isolates from abscess material of survivors were B. fragilis ($10^{8.7}$), Fusobacterium ($10^{8.6}$), E. coli ($10^{7.8}$) and enterococci ($10^{5.7}$). Rank order analysis of data indicated that during the first phase of the disease aerobes outnumbered anaerobes and the opposite was seen during the second phase of the disease. Onderdonk et al. (59) studied this infection using individual strains of E. coli, enterococci, B. fragilis, and F. varium. Strains were studied singly and in combination. Microorganisms were mixed with sterile rat colonic contents and barium sulfate. Gelatin capsules were implanted into rats as described in the original model. Mortality was only observed in rats inoculated with E. coli either alone or in combination. Abscesses developed in 94% of animals

receiving a combination of an anaerobe and a facultative organism. Any other combination failed to produce abscesses.

Louie et al. (47) used the original model (87) to determine the efficacy of cephalothin, cefazolin, cefoxitin, cefamanadole, clindamycin and gentamicin therapy. Antimicrobial therapy was begun 4 h after surgical implantation and continued every 8 h for 10 days. Dosages were adjusted to give peak levels similar to those recommended for humans. Results were determined by mortality rates and the incidence of intra-abdominal abscesses. A 37% mortality occurred in controls and 100% of survivors developed abscesses. Therapy with each of the cephalosporins or gentamicin resulted in mortality rates of 0 to 13%. No significant difference was noted between any of the drugs. Clindamycin was ineffective in reducing mortality. These data are consistent with the view that coliforms are responsible for acute mortality in this model. Of the cephalosporins, cefoxitin was the most effective in reducing the number of abscesses (7% abscess rate), cefamanodole and cephalothin were intermediate (35 and 38% respectively) and, cefazolin was the least effective (50%). With gentamicin therapy an abscess rate of 98% was reported. The most efficacious drug tested was clindamycin (5% abscess rate). These data are very consistent with the susceptibility patterns of aerobes and anaerobes in vitro.

McConville et al. (J. H. McConville, M. J. Snyder, F. M. Calia, and R. B. Hornick. Abstr. Inter. Conf. Antimicrob. Agents Chemother. 1976, A449) developed a model for B. fragilis infection which was very

similar to the mixed model described by Onderdonk et al. (59). Intra-abdominal abscesses were initiated in mice by intraperitoneal injection of a sterile fecal suspension (5% wt/vol) and B. fragilis cells.

Walker and Wilkins (83) recently described a model for the initiation of pure B. fragilis infection in mice. B. fragilis was grown in chopped meat carbohydrate semi-solid agar medium (0.25% agar wt/vol). Mice were injected subcutaneously and abscesses were visible within 1 week. These abscesses persisted for at least 2 months. The major advantages of this model over previous models are its simplicity and the ability to study a large number of animals in one experiment.

Using this model Walker et al. (84) tested the efficacies of several antibiotics against B. fragilis. Therapy was begun by administering antibiotics 4 h after bacterial challenge and every 8 h thereafter for 5 days. Antibiotic dosages were adjusted to give peak blood levels similar to those recommended for use in humans. Efficacies were determined by observing mice for the presence or absence of abscesses at 7 and 10 days after challenge. Clindamycin protected 74% of the mice from infection at day 7 and this protection persisted at day 10. Chloramphenicol protected 43% of the mice at day 7, but by the 10th day this level had decreased to 23%. Since therapy ended on day 5, this increase in the number of abscesses was attributed to the bacteriostatic nature of chloramphenicol. The strain used in this study was tetracycline sensitive (MIC = 0.5 mcg/ml). Therapy with tetracycline protected 77% of the mice both at day 7 and day 10. These data reflect very well the clinical findings of clindamycin and

tetracycline. The chloramphenicol results appear in conflict to the view of this antibiotic as a drug of choice in the treatment of anaerobic infections. A possible explanation for this discrepancy could be that blood levels in the mouse fell below the MIC of the test organism (4 mcg/ml) after 3 h while in humans therapeutic levels may still be detected 6 to 8 h after administration (41).

In a subsequent study Walker (C. B. Walker, Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg. 1977) determined the efficacies of new or experimental drugs. Dramatic results were seen with metronidazole. All of the mice were protected from infection both at day 7 and day 10. These results parallel clinical findings with metronidazole very well. Therapy with cefoxitin resulted in the protection of only 38 to 39% of the mice at the 7th and 10th days. These results are not easily explained as the MIC of the test organisms was 4 mcg/ml and blood levels of cefoxitin remained above this value for at least 8 h.

Walker (C. B. Walker, Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg. 1977) has studied the concentration of antibiotic in the blood versus the amount in abscess material from mice infected with B. fragilis. A single dose of antibiotic was administered to mice to give blood levels similar to those recommended for use in humans. Blood and abscess samples were collected and the concentration of active antibiotic determined by microbiological assay. They found that tetracycline was present in the blood at twice the concentration seen in abscess material during the first hour. During the

remaining 7 h of observation abscess levels were twice the blood levels. Equal amounts of metronidazole were detected in abscesses and blood over an 8 h period. Clindamycin produced striking results. During the first 1 to 2 h abscess levels were at least twice the corresponding blood levels. Abscess levels were 8 to 10 times higher by the 4th h and by the 8th h abscess levels of clindamycin had decreased to 4 to 8 times the corresponding levels found in the blood.

Statement of Purpose

The purpose of this study was to 1) determine which antibiotics in current usage are bactericidal to *B. fragilis in vitro*, and 2) determine whether such antibiotics also display bactericidal activity in an experimental infection.

MATERIALS AND METHODS

Organisms. Sixteen strains of Bacteroides fragilis and six strains of Bacteroides thetaiotaomicron were used. Sources and strain designations are listed in Table 2. All Bacteroides strains were identified by Holdeman and Moore according to published criteria (32) unless otherwise noted. Escherichia coli strain K-12 was obtained from the Biology Department at the Virginia Polytechnic Institute and State University. Stock cultures were maintained at room temperature (25 C) in chopped meat broth (32) and transferred monthly.

In experiments with animals lyophilized B. fragilis 9032 culture samples were used. Before such experiments were begun aliquots of 0.1 to 0.2 ml of an 18-h culture in chopped meat broth (32) were placed in sterile ampoules, frozen in liquid N_2, then lyophilized. Cultures used in each animal experiment were begun by opening an ampoule into chopped meat broth (32). This method prevented any effects of repeated subculture on strain pathogenicity.

Anaerobic techiques. Unless otherwise noted all cultural manipulations were made under O_2-free CO_2 as described in the VPI Anaerobe Laboratory Manual (32). Where indicated, a glove box similar to the one described by Aranki and Freter (2) was used. The atmosphere in the chamber was 85% N_2, 10% H_2, and 5% CO_2.

Media. Chopped meat broth (CM), chopped meat carbohydrate broth (CMC), supplemented brain heart infusion broth (BHI-S), peptone yeast-extract glucose broth (PYG), and anaerobic dilution fluid were prepared prereduced and anaerobically sterilized (PRAS) as described

Table 2. Strain designations and sources of organisms used in this study

Bacteroides fragilis		*Bacteroides thetaiotaomicron*	
VPI No.	Source	VPI No.	Source
9032	Blood	5482	Normal Flora (intestine)
4912	Blood	0633-1	Peritoneal Cavity
3625	Uterus	0911-A1	Normal Flora (intestine)
8429	Blood	3089	Blood
8708[a]	Clinical Sample[b]	7330-1	Stab Wound
9132	Clinical Sample	3443	Normal Flora (intestine)
9309	Abdominal Wound		
7310	Blood		
7428	Wound Aspiration		
4948	Recto-Peritoneal Abscess		
9836A	Appendectomy		
F351[c]	Wound		
F363	Wound		
F342	Blood		
F364	Abdominal Abscess		
F336	Clinical Sample		

[a]This strain was animal passed once through a mouse abscess and reisolated before used in this study (C. B. Walker, Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg, 1977).

[b]All such strains were from unknown anatomical sites.

[c]All F strains were obtained from Sharon Hansen, Veteran's Administration Hospital, Baltimore, Md. These strains were identified according to the methods of Holdeman and Moore (32).

in the VPI Anaerobe Laboratory Manual (32). Aerobic BHI-S was prepared without resazurin and dispensed in test tubes fitted with metal caps.

PRAS Schaedler broth (BBL, Cockeysville, MD.) was prepared by weighing ingredients into a 2 liter flask; resazurin (0.0001% wt/vol) was added and the solution brought to a boil; when the resazurin became colorless (reduced) the flask was immediately brought into the anaerobic glove box. The broth was dispensed into anaerobe tubes (32) in 5 ml aliquots and stoppered with no. 1 black rubber stoppers. The tubes were autoclaved in a press (32) at 120 C (15 psi) for 15 min, cooled and stored at room temperature (25 C) until used.

Wilkins and Chalgren (90) have recently described an agar medium they recommend for susceptibility testing of anaerobes. I used a modified PRAS broth form of the medium for this study. Ingredients are listed in Table 3. All components except Tween 80, cysteine, hemin and vitamin K were put in a 2 liter flask. Tween 80 and resazurin were added from stock solutions of 2.5% and 0.025% (wt/vol) respectively. Hemin and vitamin K were also added from a stock solution (32). After mixing the pH of the medium was 7.0 to 7.2. The broth was brought to a boil and, after the resazurin had reduced, cysteine was added. The medium was immediately brought into the anaerobic glove box, dispensed, sterilized, and stored as described for Schaedler broth.

Cooked meat (Difco, Detroit, Mich.) semi-solid medium (Difco CM-SS) was prepared by adding cooked meat to a flask, resazurin (0.0001% wt/vol) was added; agar (0.25% wt/vol; Difco) and the solution

Table 3. Formulation of a modified PRAS broth form of Wilkins-Chalgren media

Component	% Concentration (wt/vol)	Source
Trypticase	1.0	BBL
Gelysate	1.0	BBL
Yeast Extract	0.5	Difco
Glucose	0.3	Fisher
Arginine HCl	0.5	Sigma
Na Pyruvate	0.5	Sigma
Tween 80	0.025	Difco
Na Carbonate	0.1	Fisher
Resazurin	0.0001	Difco
Cysteine	0.05	Sigma
Hemin	0.0005	Sigma
Vitamin K	0.00005	Sigma
Distilled Water	976 ml	

brought to a boil. Where indicated agarose (Sigma Chemical Co., St. Louis, Mo.) was used instead of agar. After the resazurin had reduced a N_2 atmosphere was maintained by sparging the flask with O_2-free N_2. Broth without meat particles was dispensed into tubes in 10 ml aliquots, and autoclaved in a press.

Modified Wilkins-Chalgren, Schaedler, and PYG semi-solid media were prepared by adding sterile agar (from a 2.5% wt/vol solution; Difco) to obtain a concentration of 0.25% wt/vol, CMC semi-solid medium was prepared by adding agar (0.25% wt/vol; Difco) to meat-free CMC prior to sterilization. In some experiments with CMC semi-solid media different agar concentrations were used. These media were prepared by weighing out appropriate concentrations of agar (Difco) into sterile, meat-free CMC. The media were re-autoclaved.

Brain heart infusion agar (BHIA; Difco) was supplemented with 0.5% (wt/vol) yeast extract (Difco), and hemin and vitamin K (32). BHIA was mixed, sterilized and dispensed in an Agar-Matic (New Brunswick Scientific Co., New Brunswick, N.J.). Plates were dried for 30 min at 37 C and stored at room temperature in the air. Plates not used by the end of 1 week were discarded.

Antimicrobial assay medium was prepared as described by Sabbath and Toftegard (68). This medium consisted of brain heart infusion (Difco), agarose (1% wt/vol; Sigma), defibrinated sheep blood (8% vol/vol; The Brown Laboratory, Topeka, Kansas), and indicator organism (2% vol/vol of a 4 to 5 h BHI-S culture). Plates were prepared aerobically the day before use and stored at 5 C.

Antibiotics. The antibiotics used and the sources were: penicillin G, potassium salt (1,595 U/mg) and tetracycline-hydrochloride, Sigma; chloramphenicol-sodium succinate, and chloramphenicol-hydrochloride, Parke-Davis, Detroit, Mich.; clindamycin-phosphate, and clindamycin-hydrochloride (860 mcg/mg), The Upjohn Co., Kalamazoo, Mich.; metronidazole, Searle Laboratories, Chicago, Ill.; and cefoxitin, sodium salt, (949 mcg/mg) Merck Institute, Rahway, N.J.

Antibiotics prepared from the same lot each were used in all studies. Drugs were dissolved in distilled water with the exception of chloramphenicol-hydrochloride which was dissolved in 28.8% ethanol (vol/vol), and metronidazole which was dissolved in 0.4 N HCl. Chloramphenicol-hydrochloride and clindamycin-hydrochloride were used in all *in vitro* susceptibility studies. Chloramphenicol-succinate and clindamycin-phosphate were used for administration to animals.

Plate counts. The number of viable bacteria in various experiments was determined with the droplet plating method of Neblett (56). Appropriate dilutions of sample were prepared and 10 µl were applied to BHIA plates. An automatic pipeting device (Oxford Laboratories Inc., Foster City, Ca.) was used to deliver this and all other 10 µl samples used in this study. Plates were incubated in the glove box at 37 C until colonies became apparent (24 to 48 h).

MIC and MBC determinations. A broth dilution technique similar to the one described by Wilkins *et al.* (91) was used in minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) studies. Serial two-fold dilutions of antibiotic were prepared

fresh for each experiment in screw-cap tubes. Antibiotic concentrations in these tubes were 500x the concentrations needed in MIC tests. Ten microliters of each antibiotic dilution were added to 5 ml of broth. Tubes were inverted several times to insure equal distribution of antibiotic. Antibiotics were added to media just prior to inoculation. BHI-S was used in all susceptibility tests except in cases where different media were being tested.

Inocula for the MIC tests were prepared by adding one drop of stock culture from a Pasteur pipet to a tube containing 5 ml of BHI-S broth. After 18 h incubation at 37 C these cultures were diluted in the same medium to one-half the McFarland no. 1 turbidity standard (6). MIC tubes and control tubes containing no antibiotic were inoculated with 10 µl of this diluted culture. This inoculum size resulted in approximately 5×10^5 cells/ml as determined by plate counts of control tubes.

MICs were defined as the least amount of antibiotic which resulted in no growth after 24 h incubation at 37 C. In some experiments MICs were also read at 48 h.

MBCs were determined by subculturing duplicate 10 µl samples from tubes with no growth at 24 h onto BHIA plates. Several droplets were aerobically added to each plate and allowed to dry in place without spreading. The total concentration of antibiotic calculated to be present per ml (based on a minimum of 10 ml BHIA/plate) was never above the MIC of the organism. Plates were incubated at 37 C in the anaerobic glove box until control samples showed good growth

(24 to 48 h). MBCs were defined as the least concentration of antibiotic at which subcultures yielded 5 or less colonies (based on an average of two samples). Thus, at the MBC, 99.9% or more of the original inoculum had been killed. MBCs were also determined at 48 h in some experiments.

Since prior to anaerobic incubation the MBC subculture samples were exposed to air, we investigated whether this bench top method affected cell viability. An experiment was performed where subcultures were also prepared in the glove box and therefore never exposed to air.

B. fragilis VPI 9032 was used as an indicator organism in susceptibility tests. The MIC and MBC of this strain had been determined a number of times under the different conditions used in this study. When the MIC and MBC for this strain agreed with previous findings, results for other strains in a given experiment were considered reliable. Occasional repeats with other strains proved this method to be accurate.

The MIC and MBC of E. coli was also determined in an aerobic environment. In these experiments the same procedures for susceptibility testing were used except aerobic BHI and aerobic conditions were used in all phases of the experiments.

Death rates. An MIC test was prepared in BHI broth and at various times samples (0.1 ml) were taken from appropriate tubes for viable count determinations. The original inoculum contained approxi-

mately 5 x 10^5 cells/ml, so a decrease to 5 x 10^2 cells/ml indicated a 99.9% kill rate.

Effect of inoculum size on MICs and MBCs. In these experiments an MIC test was prepared in BHI-S broth. Tubes were inoculated by adding either 0.01 ml of 10^{-1} and 10^{-2} dilutions (in anaerobic dilution fluid) or 1.0, 0.1, 0.01 of an 18 h BHI-S culture. In cases where 1.0 or 0.1 ml were added to tubes an equivalent amount was removed from tubes prior to inoculation or addition of antibiotic. Viable cell counts were determined with positive control tubes to accurately determine the number of cells in the original inoculum. MICs and MBCs were determined as described under MIC and MBC determinations.

Animals. The animals used and the sources were: random bred male Swiss white mice, strain ICR, 18 to 20 g, white male rats, strain Sprague-Dawley, 150 to 170 g, Flow Laboratories, Dublin, Va.; random bred, male, Swiss white mice, strain CD-1, 18 to 20 g, Charles River Laboraoties, Wilmington, Mass.; and brown, inbred male mice, strain CBA-J, 18 to 20 g, Jackson Laboratories, Barharbor, Maine.

Abscess initiation. A pure B. fragilis infection was obtained in mice by using the model described by Walker and Wilkins (83). The bacterial inoculum was prepared by opening an ampoule containing a lyophilized sample of B. fragilis strain 9032 into a CM broth tube. In experiments where different B. fragilis strains were used, CM broth tubes were inoculated with one drop of stock culture. Following overnight incubation at 37 C, 0.1 ml was inoculated into a

10 ml tube of the indicated semi-solid medium. These cultures were incubated for 18 to 20 h at 37 C, then anaerobically transferred to serum bottles, sealed with a rubber septum, and capped. Syringes were flushed several times with O_2-free CO_2, then filled from the serum bottles. Mice were injected subcutaneously with 0.5 ml near the right side of the animal's groin. Rats were injected at the same site with 1.5 ml. As described in the original model (83) abscesses were visible as enlarged areas at the point of injection after approximately 4 to 5 days. Abscesses were filled with a viscous pus containing only B. fragilis as determined by subculture of abscess material.

Antibiotic injection. All antibiotics except metronidazole were dissolved in distilled water. Metronidazole was suspended in propylene glycol (Sigma). All antibiotics were administered in 0.1 ml amounts. Metronidazole and tetracycline were administered by oral gavage, and the remaining antibiotics were administered intraperitoneally on the animal's left side. Antibiotic dosages were adjusted to approximate peak blood levels recommended for use in humans.

Determination of antibiotic levels. Antibiotic concentrations in blood and abscess material were determined by microbiological assay as described by Sabbath and Toftegard (68). The strains of Clostridium perfringens used and their sensitivities to the antibiotics they were used to assay for are given in Table 4. With this method, samples of antibiotic-containing material are placed on antibiotic assay media. As the clostridia grow, hemolysins are

Table 4. Susceptibilities of strains of C. perfringens used in antibiotic assays

Strain	Antibiotic	MIC (mcg/ml)
ATCC 13124	Clindamycin	0.05
VPI 8050A	Metronidazole	1.6
	Cefoxitin	0.8
SAL 249[a]	Chloramphenicol	3.1
SAL 19	Tetracycline	0.05

[a]SAL strains were obtained from F. Tally, Tufts Medical Center, Boston, Mass.

elaborated which result in the lysis of the red blood cells (RBC) in the medium, which in turn results in a clearing of the plate. At areas of the plate where antibiotic concentrations are too high to permit growth, no hemolysis of the blood occurs. As a result, a region of intact RBCs occur around the point where antibiotic was added to the plate. The size of this region is proportional to the concentration of antibiotic applied.

Abscesses were allowed to develop in mice for 5 to 6 days. A single dose of antibiotic was given and blood and pus samples were taken at the indicated times. For each antibiotic tested, 5 mice were used at each sampling time. Blood was obtained by tail bleeding and collected with an automatic pipeter (Oxford). Immediately following this bleeding, mice were killed by cervical dislocation and pus samples from abscesses collected. Glass pipets (0.1 ml) were used to collect pus samples. Ten microliters of blood or pus were placed on 6.35 mm paper disks (Schleicher and Schwell, No. 740-E). Disks were placed on the assay plates within 10 min and incubated at 37 C in the anaerobic glove box. Plates were examined following hemolysis of the blood (3 to 5 h). Quantitation of the amount of antibiotic present was determined by comparing results with standard curves constructed by experimental regression on a computer (Wang 2200, Wang Laboratories, Inc., Tewksbury, Mass.). The program used was exponential regression where the x and y data points are fitted to the curve described by $y = Ae^{Bx}$. Clindamycin-hydrochloride and chloramphenicol-hydrochloride were used for standard curves since clindamycin-phos-

phate and chloramphenicol-succinate are inactive against bacteria until the respective phosphate or succinate moieties are removed by mammalian enzymes. Defibrinated sheep blood (The Brown Laboratory) was used in standard curve construction since previous investigators have found no significant difference when mouse blood or sheep blood is used (84).

Quantitation of cells present in abscess material. At the same time that blood and abscess material were collected for antibiotic determinations viable cell counts were also done on pus. A 0.1 ml sample of pus was added to a 9.9 ml anaerobic dilution fluid blank and vigorously shaken by hand to obtain an even suspension. Appropriate dilutions were made in this same fluid and the number of viable cells present was determined as described for plate counts.

RESULTS

Susceptibility of Bacteroides fragilis to six antibiotics. The minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of tetracycline, chloramphenicol, clindamycin, metronidazole, penicillin and cefoxitin were determined for 16 strains of Bacteroides fragilis. In this thesis, I have defined a drug as bactericidal if the MBC to MIC ratio is four or less.

The MICs and MBCs of tetracycline for 16 strains of B. fragilis are listed in Table 5. For sensitive strains (MIC < 6 mcg/ml), all MICs were 0.2 mcg/ml. Corresponding MBCs ranged from 0.8 to 3.1 mcg/ml. The majority of these strains had MBC to MIC ratios of 8 or 16. Two strains (9836A and F342) each had a ratio of four. Thus, for these two strains, tetracycline would be considered bactericidal; however, MBCs for all the sensitive strains were within the achievable blood levels of 4 to 5 mcg/ml (41).

Of the 16 strains, 9 were resistant to tetracycline (MIC > 6 mcg/ml). MICs for these strains varied from 6.3 to 25 mcg/ml. Corresponding MBCs ranged from 12.5 to 50 mcg/ml. Each resistant strain had an MBC to MIC ratio of four or less. Thus, for all resistant strains used in this study tetracycline was bactericidal.

The results obtained with chloramphenicol are shown in Table 5. All 16 strains had MICs of 3.1 mcg/ml. MBCs ranged from 3.1 to 12.5 mcg/ml. All strains had MBC to MIC ratios of four or less indicating that chloramphenicol was bactericidal to all of these

Table 5. MICs and MBCs of tetracycline and chloramphenicol for *B. fragilis*

	Tetracycline (mcg/ml)			Chloramphenicol (mcg/ml)		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
9032	0.2	1.6	8	3.1	6.3	2
4912	0.2	3.1	16	3.1	12.5	4
3625	0.2	1.6	8	3.1	12.5	4
8429	0.2	3.1	16	3.1	12.5	4
8708	12.5	25	2	3.1	6.3	2
9132	6.3	12.5	2	3.1	6.3	2
9309	25	50	2	3.1	6.3	2
7310	12.5	50	4	3.1	6.3	2
7428	12.5	12.5	1	3.1	12.5	4
4948	12.5	50	4	3.1	12.5	4
9836A	0.2	0.8	4	3.1	3.1	1
F351	12.5	25	2	3.1	6.3	2
F363	0.2	3.1	16	3.1	6.3	2
F342	0.2	0.8	4	3.1	6.3	2
F364	12.5	25	2	3.1	6.3	2
F336	12.5	25	2	3.1	6.3	2

[a]MBC to MIC ratio

strains. In addition, all MBCs were within the clinically achievable blood levels of 10 to 20 mcg/ml (26, 41).

Listed in Table 6 are the MICs and MBCs of clindamycin for B. fragilis. All strains were extremely sensitive to this drug. MICs varied from 0.05 to 0.4 mcg/ml. The majority of strains were inhibited by 0.05 mcg/ml of the drug. Only one strain (7310) was not inhibited by at least 0.1 mcg/ml. MBCs ranged from 0.1 to 1.6 mcg/ml. One strain (7310) had an MBC of 1.6 mcg/ml, another strain (4948) had an MBC of 0.4 mcg/ml, and the remaining strains had MBCs of 0.2 mcg/ml or less. All MBC to MIC ratios were equal to or less than four. Thus, clindamycin was bactericidal against all strains tested. In addition the MBCs were all below concentrations of clindamycin easily achievable in the blood.

MIC and MBC results for metronidazole are shown in Table 6. With the exception of four strains (9032, 4912, 4948, and F363) MICs were either 0.2 or 0.4 mcg/ml. These four strains had MICs of 0.8 mcg/ml. MBCs ranged from 0.2 to 12.5 mcg/ml for all strains. Two strains had an MBC to MIC ratio of eight, two strains had ratios of four and the remaining strains had ratios of two or one. Therefore, for all but two strains used in this study, metronidazole was bactericidal.

While testing metronidazole, I frequently observed that an MBC subculture at a given concentration would not grow, while a subculture from a tube with a higher concentration of drug would grow. For instance strain 9032 would sometimes show growth from a 12.5 mcg/ml

Table 6. MICs and MBCs of clindamycin and metronidazole for *B. fragilis*

	Clindamycin (mcg/ml)			Metronidazole (mcg/ml)		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
9032	0.1	0.2	2	0.8	1.6	2
4912	0.05	0.1	2	0.8	12.5	8
3625	0.1	0.2	2	0.4	0.8	2
8429	0.05	0.1	2	0.4	0.4	1
8708	0.05	0.1	2	0.4	0.8	2
9132	0.05	0.2	4	0.2	0.4	2
9309	0.05	0.2	4	0.4	0.4	1
7310	0.4	1.6	4	0.2	0.2	1
7428	0.1	0.2	2	0.4	0.8	2
4948	0.1	0.4	4	0.8	1.6	2
9836A	0.1	0.1	1	0.4	0.8	2
F351	0.05	0.1	2	0.4	0.4	1
F363	0.05	0.1	2	0.8	3.1	4
F342	0.05	0.2	4	0.4	0.8	2
F364	0.05	0.2	4	0.4	1.6	4
F336	0.05	0.1	2	0.2	1.6	8

[a]MBC to MIC ratio

subculture but not from a 6.3 mcg/ml subculture. This was not due to growth of resistant mutants since colonies picked and cultured from the 12.5 mcg/ml subculture had the same MICs and MBCs as the original culture.

The sensitivity of B. fragilis to penicillin is shown in Table 7. MICs ranged from 8 to 32 mcg/ml. One strain (8708) was extremely resistant to this drug with an MIC of 256 mcg/ml. Penicillin was bactericidal to all strains of B. fragilis.

The MICs and MBCs of cefoxitin for B. fragilis are given in Table 7. Cefoxitin, like penicillin, is a beta-lactam antibiotic that interferes with cell wall synthesis and as such is expected to exert a bactericidal effect on bacteria. In general, MICs of this drug were higher than corresponding MICs of penicillin. Half of the strains tested had MICs of 16 mcg/ml, while the remaining strains had MICs of 32 mcg/ml. Strain 8708, which was highly resistant to penicillin (MIC = 256 mcg/ml), was considerably more sensitive to cefoxitin (MIC = 32 mcg/ml). MBCs for all strains were from 16 to 64 mcg/ml. As with penicillin, nearly all MBC to MIC ratios were equal to or less than two. Thus, like penicillin, cefoxitin was bactericidal against all strains tested.

Rate of killing of B. fragilis by antibiotics. To determine the rate of killing of bacteria by antibiotics viable cell numbers were determined over a 24-h period at selected concentrations of antibiotic. Two strains of B. fragilis were tested with chloramphenicol, clindamy-

Table 7. MICs and MBCs of pencillin and cefoxitin for B. fragilis

	Penicillin (mcg/ml)			Cefoxitin (mcg/ml)		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
9032	32	32	1	32	64	2
4912	32	64	2	16	32	2
3625	16	32	2	32	32	1
8429	16	32	2	32	32	1
8708	256	512	2	32	32	1
9132	16	32	2	16	32	2
9309	8	16	2	16	64	4
7310	8	8	1	32	64	2
7428	8	16	2	16	16	1
4948	32	64	2	32	32	1
9836A	2	4	2	32	64	2
F351	16	32	2	16	16	1
F363	16	64	4	16	32	2
F342	16	16	1	16	32	2
F364	8	16	2	16	32	2
F336	8	16	2	32	32	1

[a]MBC to MIC ratio

cin, metronidazole and cefoxitin. Only one strain was tested with tetracycline.

At concentrations above the MIC, metronidazole rapidly killed B. fragilis (Fig. 2). Similar data were obtained with strains 9032 and 8708. The MIC in these experiments for both strains was 0.4 mcg/ml, and the MBC was 1.6 mcg/ml and 0.8 mcg/ml respectively. When 16 times the MIC (6.25 mcg/ml) was tested the number of viable bacteria decreased within 1 h from approximately 5 x 10^5/ml to less than 100/ml for both strains. At 0.8 mcg/ml (two times the MIC) the rate of killing was much slower. Thus, the higher the concentration of metronidazole the more rapid the killing effect. With strain 9032 the number of viable bacteria at 0.8 mcg/ml decreased to less than 100 cells/ml at 12 h. However, by 24 h the number of viable bacteria had increased to 1500/ml.

Fig. 3 illustrates the results obtained with cefoxitin. In these experiments the MIC for strain 9032 was 32 mcg/ml, and 16 mcg/ml for strain 8708 and MBCs were 64 and 32 mcg/ml respectively. The bactericidal action of this antibiotic also was evident. That is, even at concentrations which were four times or less the MIC all cells died (greater than 99.9% of the initial inoculum).

The rates of death obtained with clindamycin are shown in Fig. 4. In these experiments the MIC for strains 9032 and 8708 was 0.05 mcg/ml, and the MBC was 0.2 and 0.1 mcg/ml respectively. With strain 9032 all four antibiotic concentrations tested produced an initial decrease in cell viability during the first 8 h of the experi-

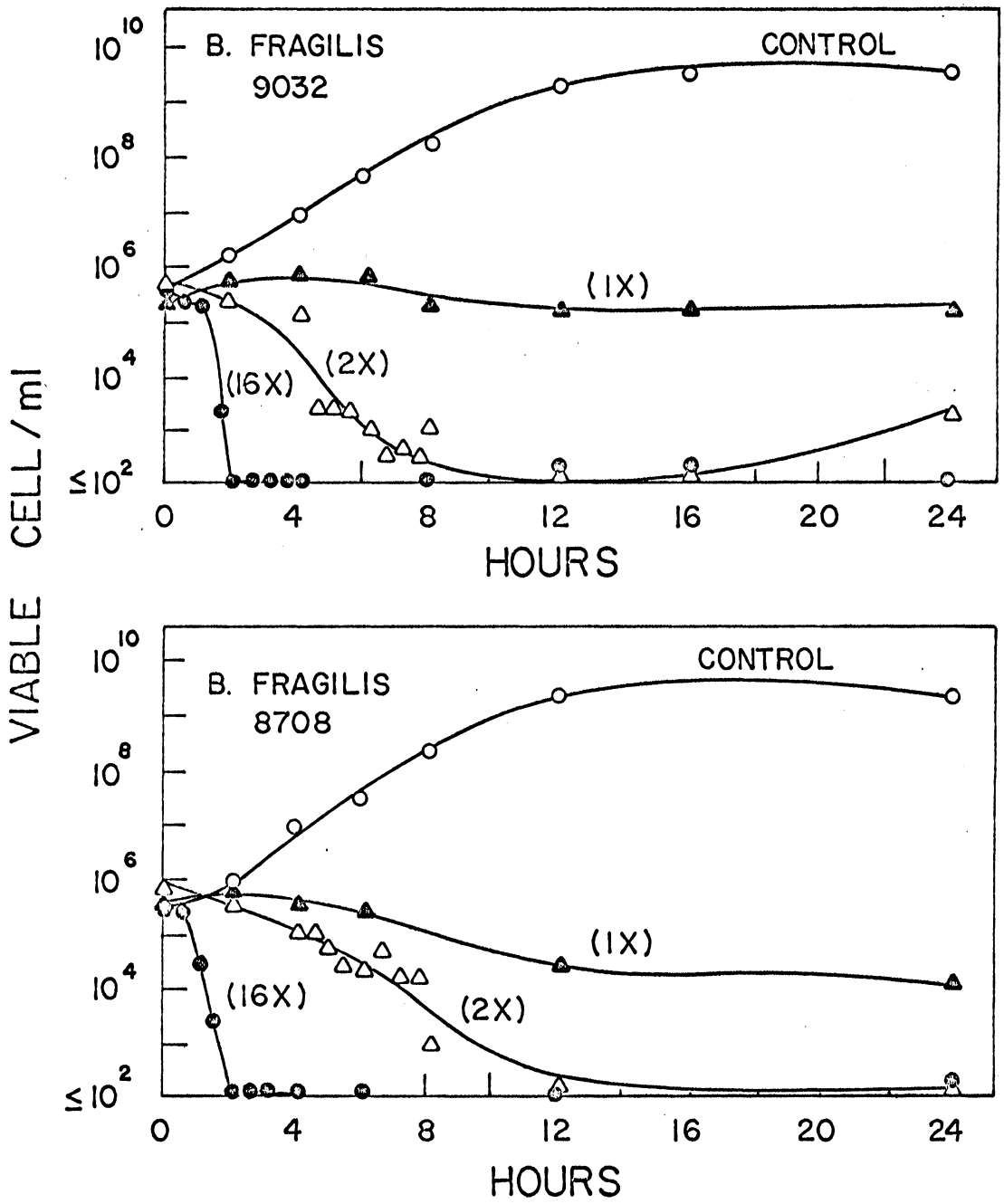

Figure 2. Effect of metronidazole on the viability of 2 strains of B. fragilis. Viable cell numbers in tubes from an MIC test were determined at the indicated times. Numbers in parenthesis are multiples of the MIC. Symbols: (▲) 0.4 mcg/ml tube; (△) 0.8 mcg/ml tube; (◉) 6.3 mcg/ml tube; (○) control tube containing no antibiotic.

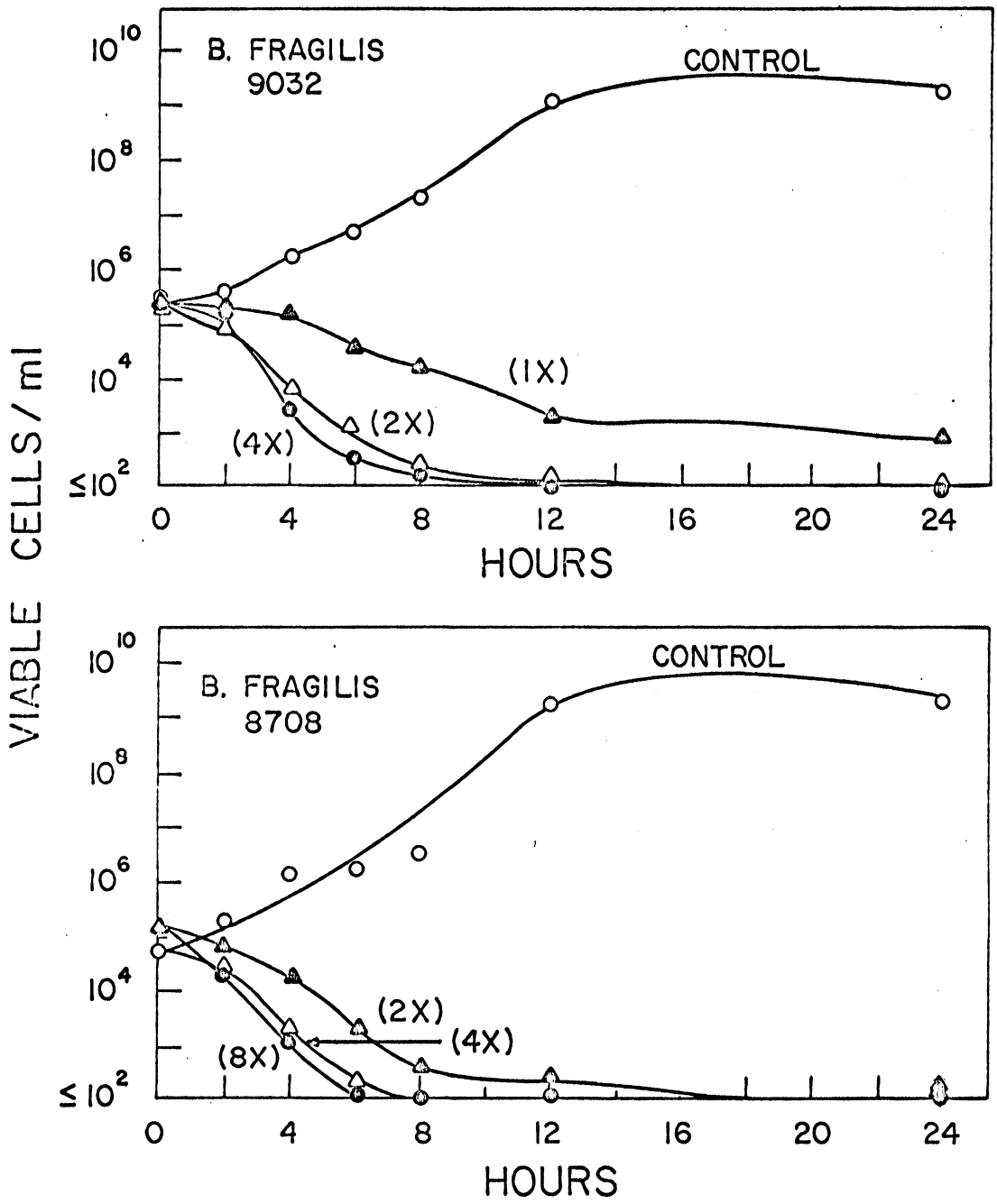

Figure 3. Effect of cefoxitin on the viability of 2 strains of B. fragilis. Viable cell numbers in tubes from an MIC test were determined at the indicated times. Numbers in parenthesis are multiples of the MIC. Symbols: (▲) 32 mcg/ml tube; (△) 64 mcg/ml tube; (●) 128 mcg/ml tube; (○) control tube containing no antibiotic.

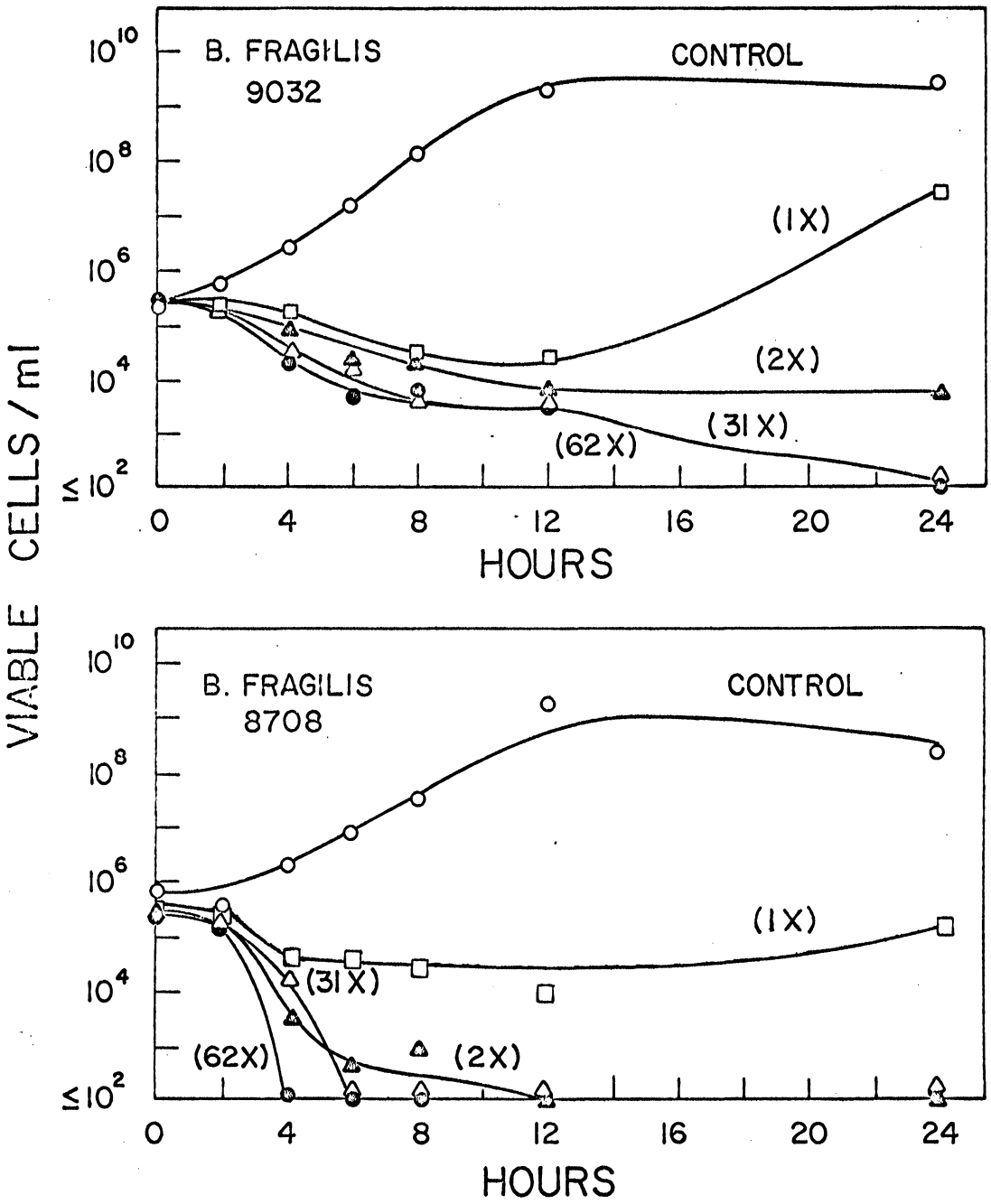

Figure 4. Effect of clindamycin on the viability of 2 strains of B. fragilis. Viable cell numbers in tubes from an MIC test were determined at the indicated times. Numbers in parenthesis are multiples of the MIC. Symbols: (□) 0.05 mcg/ml tube; (▲) 0.1 mcg/ml tube; (△) 1.6 mcg/ml tube; (●) 3.1 mcg/ml; (○) control tube containing no antibiotic.

ment. At the MIC of this strain the number of viable cells remained the same from 8 to 12 h, then increased by approximately 1000 fold to 2×10^7 cells/ml by the 24th h. At double the MIC (0.1 mcg/ml) the number of viable cells remained essentially constant from 8 to 24 h. Concentrations of 1.6 and 3.1 mcg/ml (31 and 62 times the MIC respectively) were also tested since these concentrations are readily achievable in the blood (41). With these concentrations of clindamycin viable cell numbers continued to decline after 8 h and were below 100/ml after 24 h. With strain 8708 (Fig. 4) results were similar except that cells were killed at a slightly faster rate.

Chloramphenicol death rates are shown in Fig. 5. The MICs for strains 9032 and 8708 were both 3.1 mcg/ml, and the MBCs were 12.5 and 6.3 mcg/ml respectively. At the MIC of this drug for both strains the number of viable bacteria changed very little during the 24 h observation period. At four and eight times the MIC (12.5 and 25 mcg/ml) the number of viable bacteria decreased to less than 100/ml over a period of 22 h.

Since strain 8708 is resistant to tetracycline, only strain 9032 was used when testing this drug. The results are given in Fig. 6. Strain 9032 had an MIC to tetracycline of 0.2 mcg/ml, and an MBC of 0.8 mcg/ml. At the MIC of this strain there was an increase in the number of cells between 12 and 24 h. At twice the MIC (0.4 mcg/ml) the number of viable bacteria remained essentially constant over the 24 h observation period. At the MBC, cells began dying after 4 h and

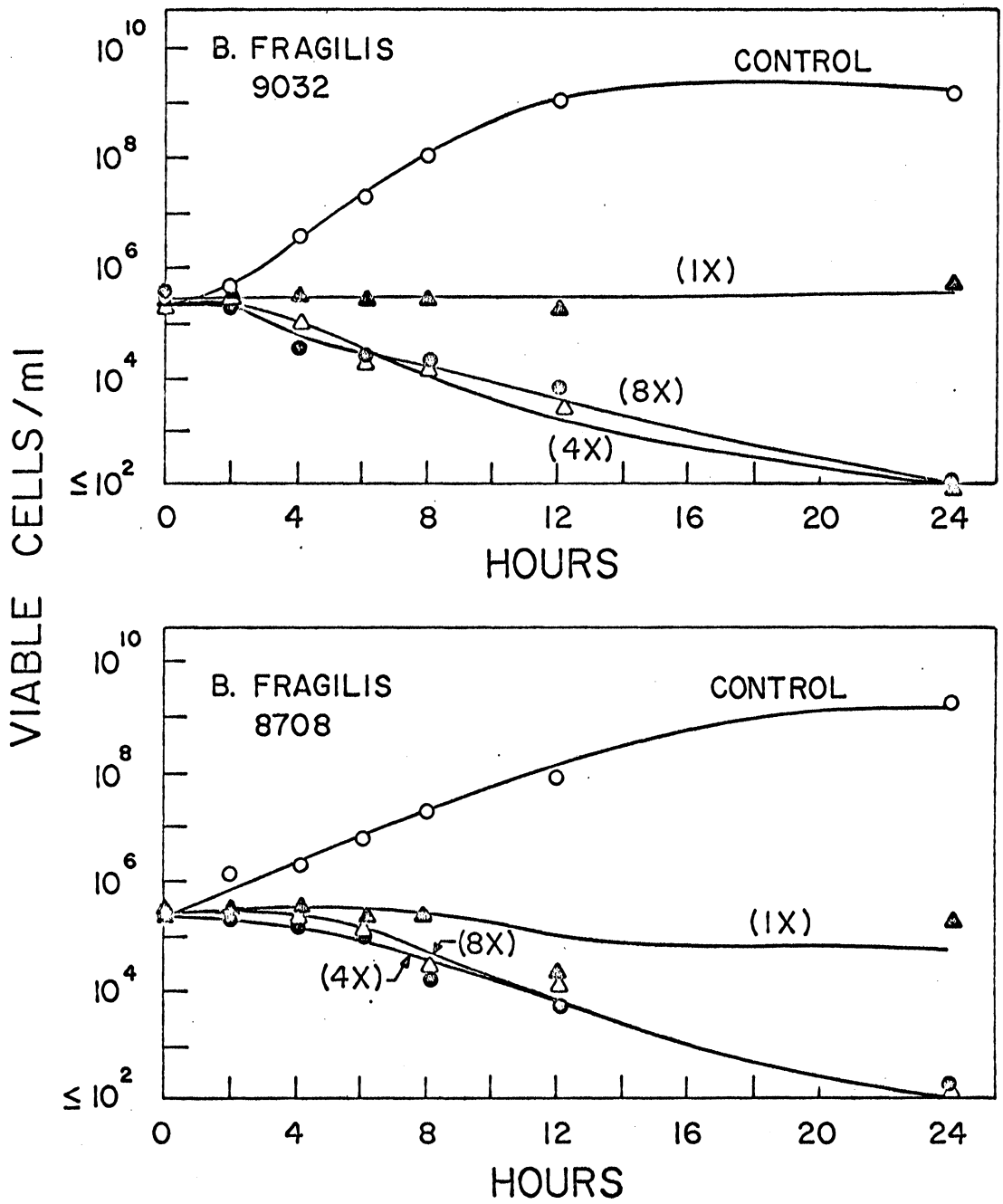

Figure 5. Effect of chloramphenicol on the viability of 2 strains of B. fragilis. Viable cell numbers in tubes from an MIC test were determined at the indicated times. Numbers in parenthesis are multiples of the MIC. Symbols: (▲) 3.1 mcg/ml tube; (△) 12.5 mcg/ml tube; (●) 25 mcg/ml tube; (○) control tube containing no antibiotic.

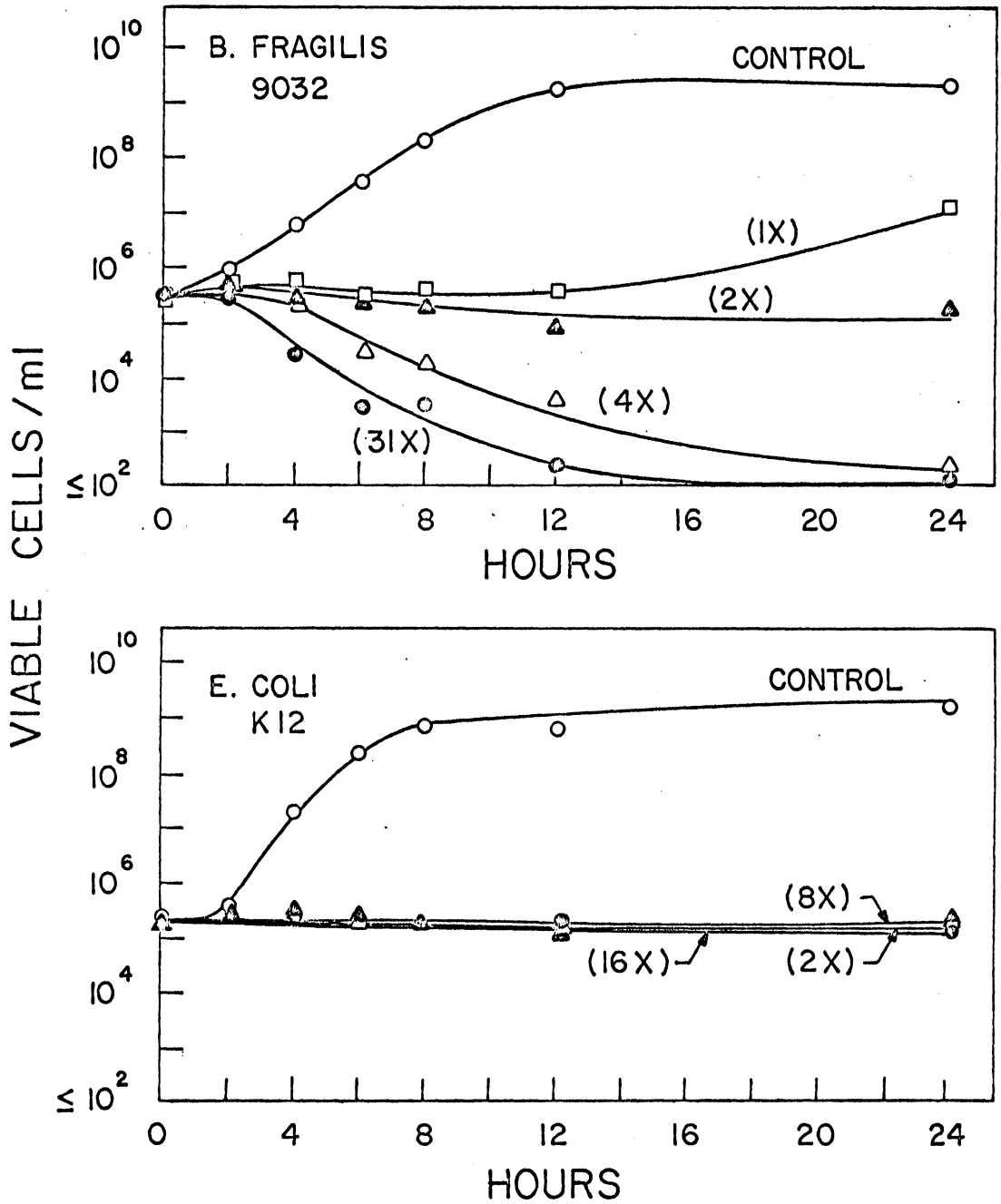

Figure 6. Effect of tetracycline on the viability of B. fragilis and E. coli. Viable cell numbers in tubes from an MIC test were determined at the indicated times. Numbers in parenthesis are multiples of the MIC. Symbols: B. fragilis, (□) 0.2 mcg/ml tube; (▲) 0.4 mcg/ml tube; (△) 0.8 mcg/ml tube; (◎) 6.3 mcg/ml tube; E. coli, (△) 6.3 mcg/ml tube; (▲) 25 mcg/ml tube; (●) 50 mcg/ml tube. Open circles represent control tubes containing no antibiotic.

by 24 h less than 100 cells/ml were present. Loss of cell viability occurred more rapidly at 31 times the MIC (6.3 mcg/ml).

MICs and MBCs of antibiotics to Escherichia coli. Classically, tetracycline and chloramphenicol are regarded as bacteriostatic, and beta-lactam antibiotics such as penicillin and cefoxitin are considered bactericidal to Escherichia coli. Since tetracycline and chloramphenicol did not behave in this manner with B. fragilis, MICs and MBCs of tetracycline, chloramphenicol, penicillin and cefoxitin were determined for E. coli K-12. Results are shown in Table 8. MBC to MIC ratios for tetracycline and chloramphenicol were 32 and >64 respectively, thus confirming the expected bacteriostatic action of these drugs against E. coli. MBC to MIC ratios for penicillin and cefoxitin were one and two respectively, thus confirming the expected bactericidal nature of these drugs to E. coli. As anticipated, MICs of clindamycin and metronidazole were extremely high (400 and >400 mcg/ml respectively) since this organism is resistant to both drugs. MBCs for both drugs were higher than the concentrations used in these experiments.

Effect of aerobic or anaerobic incubation on the MIC and MBC of E. coli. These experiments were done to determine whether the oxidation-reduction potential of the media and/or the atmosphere used in these susceptibility tests affected MIC and MBC results. The MIC and MBC of tetracycline and chloramphenicol for E. coli were determined both with aerobic media incubated aerobically, and with PRAS media incubated anaerobically. Results are shown in Table 9. With both

Table 8. MICs and MBCs of various antibiotics for E. coli K-12

Antibiotic	MIC	MBC	MBC/MIC[a]
Tetracycline	3.1[b]	100[b]	32
Chloramphenicol	3.1	>200	>64
Clindamycin	400	>800	>2
Metronidazole	>400	>400	-
Penicillin	64	64	1
Cefoxitin	8	16	2

[a]MBC to MIC ratio
[b]mcg/ml

Table 9. Comparison of aerobic versus anaerobic incubation on the sensitivity of E. coli K-12 to tetracycline or chloramphenicol

	Aerobic		Anaerobic	
Antibiotic	MIC	MBC	MIC	MBC
Tetracycline	3.1[a]	100	3.1	100
Chloramphenicol	6.3	25	6.3	25

[a]All results in mcg/ml

drugs, the MICs and MBCs remained the same for each testing condition used.

Rate of killing of E. coli by antibiotics. Death rates were determined for E. coli using tetracycline, chloramphenicol, and cefoxitin. The bacteriostatic action of tetracycline is shown in Fig. 6. The MIC in this experiment was 3.1 mcg/ml, and the MBC was greater than 50 mcg/ml. At 2, 8 and 16 times the MIC of the organism, viable cell numbers neither increased or decreased. Fig. 7 illustrates the results obtained with chloramphenicol, another drug which is considered to be bacteriostatic. The MIC in this experiment was 3.1 mcg/ml, and the MBC was greater than 50 mcg/ml. When levels of 4, 8 and 16 times the MIC were tested the results were practically identical to those of tetracycline. That is, a bacteriostatic effect was observed. Fig. 7 also depicts the results obtained with cefoxitin, a bactericidal beta-lactam antibiotic. In this experiment the MIC was 16 mcg/ml, and the MBC was 32 mcg/ml. At two and eight times the MIC viable cell numbers decreased to less than 100/ml by 24 h. Thus, as expected, a bactericidal effect was observed. At the MIC for this organism cell numbers decreased 100 fold during the first 12 h of the experiment. This was followed by an increase to 1×10^7 cells/ml between the 12th and 24th h. This was probably the result of antibiotic breakdown which enabled surviving cells to resume normal growth.

Effect of media on the MIC and MBC of B. fragilis. To determine whether MIC and MBC results for tetracycline and chloramphenicol were related to the type of media used, the following experiments were done.

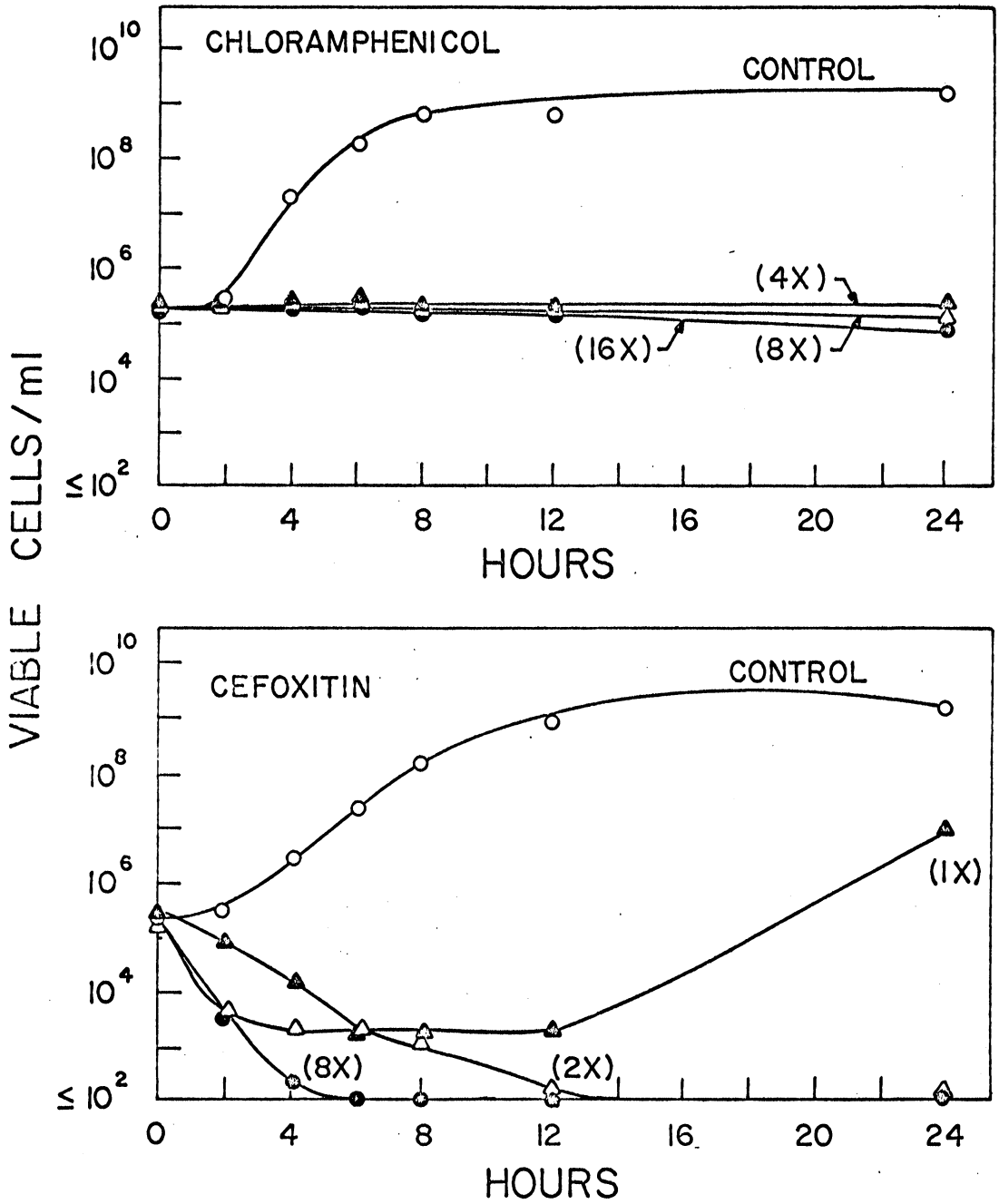

Figure 7. Effect of chloramphenicol and cefoxitin on the viability of E. coli. Viable cell numbers in tubes from an MIC test were determined at the indicated times. Numbers in parenthesis are multiples of the MIC. Symbols: Chloramphenicol, (▲) 12.5 mcg/ml tube; (△) 25 mcg/ml tube; (●) 50 mcg/ml tube; Cefoxitin, (▲) 16 mcg/ml tube; (△) 32 mcg/ml tube; (●) 128 mcg/ml tube. Open circles represent control tubes containing no antibiotic.

MICs and MBCs of both tetracycline and chloramphenicol were determined for three strains of B. fragilis in the following media: modified Wilkins-Chalgren broth, Schaedler broth and BHI-S. Results are shown in Table 10. Data obtained with Schaedler broth and modified Wilkins-Chalgren broth were comparable to results in BHI-S. In all cases, results never varied by more than 2 two fold dilutions, a margin of error which is considered acceptable in MIC testing.

Effect of anaerobic subculture conditions on MBC determinations with B. fragilis. These experiments were conducted to determine whether exposure to O_2 of MBC subculture samples affected the MBCs. Therefore, in addition to the bench top method which was routinely used to determine MBCs, a glove box method was also used. This latter method prevented any contact of MBC subculture samples with O_2. The MBCs of both tetracycline and chloramphenicol were determined for two strains of B. fragilis. Results are given in Table 11. The same results were obtained when either the glove box or bench top method was used.

Effect of length of incubation time on the MICs and MBCs for B. fragilis. Many investigators prefer to read susceptibility data after a 48 h incubation period. Therefore, I determined MICs and MBCs at 24 and 48 h for B. fragilis. The activity of tetracycline on 11 strains of B. fragilis was tested and results are shown in Table 12. In general the prolonged incubation resulted in a one or two dilution increase in MICs, and a similar decrease in MBCs. The effect of these changes was that the MBC to MIC ratios at 48 h for all strains was equal to or less than four. Thus, when incubated

Table 10. Effect of different media on the sensitivities of 3 *B. fragilis* strains to tetracycline

	Schaedler		W-C[a]		BHI-S[b]	
Strain	MIC	MBC	MIC	MBC	MIC	MBC
Tetracycline:						
9032	0.4[c]	1.6	0.4	0.8	0.2	1.6
F363	0.4	0.8	0.4	3.1	0.2	3.1
8708	12.5	12.5	12.5	25	12.5	25
Chloramphenicol:						
9032	1.6	6.3	3.1	12.5	3.1	6.3
F363	3.1	6.3	3.1	12.5	3.1	6.3
8708	3.1	6.3	3.1	12.5	3.1	6.3

[a]Modified Wilkins-Chalgren broth

[b]Supplemented Brain-Heart Infusion broth

[c]All results in mcg/ml

Table 11. Comparison of bench top and glove box methods for determination of MBCs of tetracycline and chloramphenicol for 2 strains of B. fragilis

Antibiotic	Strain	MBC (mcg/ml)	
		Bench top[a]	Glove box[b]
Tetracycline	9032	0.8	0.8
	8708	25	25
Chloramphenicol	9032	6.3	6.3
	8708	6.3	6.3

[a]MBC subcultures were exposed to air

[b]MBC subcultures were never exposed to air

Table 12. Effect of incubation time on the MIC and MBC of tetracycline for B. fragilis

	24 h			48 h		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
9032	0.1[b]	0.8[b]	8	0.2	0.8	4
4912	0.2	3.1	16	0.4	0.8	2
3625	0.2	1.6	8	0.8	0.8	1
8429	0.2	3.1	16	0.4	0.8	2
8708	6.3	25	4	25	25	1
9132	6.3	12.5	2	12.5	25	2
9309	25	50	2	50	50	1
7310	12.5	50	4	50	50	1
7428	12.5	12.5	1	25	25	1
4948	12.5	50	4	25	50	2
9836A	0.2	0.8	4	0.2	0.8	4

[a]MBC to MIC ratio

[b]mcg/ml

48 h tetracycline would be considered bactericidal to all strains in this particular experiment, as opposed to only a few strains when incubated 24 h (Table 5).

The MICs of chloramphenicol at 24 h were 3.1 mcg/ml (Table 13). After 48 h incubation the MICs increased for four strains to 6.3 mcg/ml. MBCs for three of the strains decreased by one dilution during the prolonged incubation, while the MBC for one strain (9836A) increased by one dilution. MBC to MIC ratios at 24 h were equal to or less than four. By 48 h this value had decreased to two. Therefore, on extended incubation the bactericidal action of chloramphenicol against B. fragilis also was more obvious.

Two strains of B. fragilis (9032 and 8708) were used to determine MICs and MBCs of clindamycin, metronidazole and cefoxitin (Table 14). In all cases the MBC to MIC ratios of each drug for each strain either decreased or stayed the same. These data indicate that if incubated 48 h instead of 24 h, the only effect on MIC and MBC results will be a more pronounced bactericidal activity.

Effect of inoculum size on the MIC and MBC of antibiotics. The affects of various inocula sizes on the MIC and MBC of tetracycline, chloramphenicol, clindamycin, metronidazole and cefoxitin were determined for B. fragilis. This was done since actual areas of infection contain more cells/ml than the inoculum used in MIC tests, and because the size of the inoculum is known to affect MIC and MBC results (39, 50, 82, 95). The inocula for the MIC tests ranged from 10^5 and 10^9 cells/ml. Results are shown in Table 15. Data for tetracycline, chlor-

Table 13. Effect of incubation time on the MIC and MBC of chloramphenicol for B. fragilis

	24 h			48 h		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
9032	3.1[b]	6.3[b]	2	3.1	6.3	2
4912	3.1	12.5	4	6.3	12.5	2
3625	3.1	12.5	4	6.3	12.5	2
8429	3.1	12.5	4	6.3	6.3	1
8708	3.1	6.3	2	3.1	6.3	2
9132	3.1	6.3	2	3.1	6.3	2
9309	3.1	6.3	2	6.3	6.3	1
7310	3.1	6.3	2	3.1	6.3	2
7428	3.1	12.5	4	3.1	6.3	2
4948	3.1	12.5	4	3.1	6.3	2
9836A	3.1	3.1	1	3.1	6.3	2

[a]MBC to MIC ratio

[b]mcg/ml

Table 14. Effect of incubation time on the MIC and MBC of clindamycin, metronidazole and cefoxitin for 2 strains of B. fragilis

	24 h			48 h		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
Clindamycin:						
9032	0.1[b]	0.2[b]	2	0.1	0.2	2
8708	0.05	0.1	2	0.1	0.1	1
Metronidazole:						
9032	0.8	1.6	2	3.1	3.1	1
8708	0.4	0.8	2	1.6	3.1	2
Cefoxitin:						
9032	32	32	1	64	64	1
8708	32	32	1	32	32	1

[a]MBC to MIC ratio

[b]mcg/ml

Table 15. Effect of inoculum size on the MIC and MBC of various antibiotics for B. fragilis 9032

	Tetracycline		Chloramphenicol		Clindamycin		Metronidazole		Cefoxitin	
Cells/ml	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
10^9	1.6[a]	25	25	50	0.1	1.6	>50	>50	64	512
10^8	0.8	6.3	12.5	50	0.1	0.4	12.5	12.5	64	256
10^7	0.4	6.3	6.3	6.3	0.1	0.8	6.3	6.3	32	64
10^6	0.2	1.6	3.1	6.3	0.1	0.2	3.1	3.1	32	32
10^5	0.1	0.8	3.1	6.3	0.1	0.2	0.8	1.6	32	32

[a]All results in mcg/ml

amphenicol and metronidazole were similar. As the inocula increased, MICs and corresponding MBCs also increased. Clindamycin and cefoxitin results differed, as the inocula increased MICs remained essentially the same, while the MBCs increased. These data indicate that the antibiotic susceptibility (MIC and/or MBC) of B. fragilis is dependent on how many cells are present when the antibiotic is introduced.

Susceptibility of B. thetaiotaomicron to six antibiotics. B. thetaiotaomicron is isolated about half as often as B. fragilis in clinical specimens and as such is considered to be a clinically important anaerobe; therefore, the same antibiotics used in the B. fragilis study were used to determine MICs and MBCs for six strains of B. thetaiotaomicron. In most cases the results obtained in this study were very similar to those obtained with B. fragilis. This is shown in Tables 16 through 18. Tetracycline was bactericidal to two of the six strains (Table 16). Chloramphenicol was bactericidal to only four of the six strains (Table 16). This is in contrast to results obtained with B. fragilis where chloramphenicol was bactericidal to all strains tested (Table 5).

MICs of clindamycin (Table 17) were slightly higher for B. thetaiotaomicron than for B. fragilis (Table 6). As with B. fragilis, clindamycin was bactericidal to all strains of B. thetaiotaomicron tested. Results with metronidazole (Table 17) differed from those of B. fragilis (Table 6). Metronidazole was bactericidal against all six B. thetaiotaomicron strains, while with B. fragilis, it was bactericidal against 14 of 16 strains.

Table 16. MICs and MBCs of tetracycline and chloramphenicol for *B. thetaiotaomicron*

	Tetracycline (mcg/ml)			Chloramphenicol (mcg/ml)		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
5482	0.4	3.1	8	3.1	25	8
0633-1	0.4	1.6	4	3.1	6.3	2
0911-A1	0.1	1.6	16	3.1	6.3	2
3089	0.4	3.1	8	6.3	12.5	2
7330-1	0.2	0.8	4	3.1	25	8
3443	12.5	25	2	3.1	12.5	4

[a]MBC to MIC ratio

Table 17. MICs and MBCs of clindamycin and metronidazole for B. thetaiotaomicron

	Clindamycin (mcg/ml)			Metronidazole (mcg/ml)		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
5482	3.1	3.1	1	0.8	3.1	4
0633-1	0.8	1.6	2	0.8	3.1	4
0911-A1	0.2	0.8	4	0.4	0.8	2
3089	1.6	6.3	4	0.8	1.6	2
7330-1	0.1	0.2	2	0.4	1.6	4
3443	0.2	0.8	4	0.4	1.6	4

[a]MBC to MIC ratio

B. thetaiotaomicron had about the same sensitivity to both penicillin and cefoxitin (Table 18). This is in contrast to results obtained with B. fragilis where MICs of penicillin were slightly lower than MICs of cefoxitin. All but two strains of B. thetaiotaomicron had MICs of 16 or 32 mcg/ml to both drugs. As expected, penicillin and cefoxitin demonstrated bactericidal activity against the six strains of B. thetaiotaomicron.

Experiments with animals. In experiments with animals my goal was to determine whether the antibiotics which had been found to be bactericidal in vitro were also bactericidal in vivo. To accomplish this I planned to use the murine model of Walker and Wilkins (83) for the initiation of a pure B. fragilis infection in mice. In these studies, antibiotics were to be administered to mice with well developed abscesses and the effect of these antibiotics on viable cell numbers in abscess material (pus) was to be measured. However, within the last year we have been unable to reproduce this experimental infection exactly as described in the original publication. Originally, abscesses persisted for at least two months and contained on the order of 0.5 to 1.0 ml of pus. However, in this study I found that abscesses formed and drained through the skin 4 or 5 days following bacterial challenge. Any abscesses which remained after this time contained little pus and were too small to work with.

Attempts to reestablish B. fragilis infection in mice. I made several attempts to reestablish the infection as originally described. Parameters such as the strain of B. fragilis, strain of mice, types of

Table 18. MICs and MBCs of penicillin and cefoxitin for B. thetaiotaomicron

	Penicillin (mcg/ml)			Cefoxitin (mcg/ml)		
Strain	MIC	MBC	MBC/MIC[a]	MIC	MBC	MBC/MIC
5482	32	32	1	32	64	2
0633-1	16	16	1	32	32	1
0911-A1	16	16	1	4	8	2
3089	32	32	1	32	64	2
7330-1	32	32	1	16	32	2
3443	8	16	2	32	32	1

[a]MBC to MIC ratio

media, concentration of agar, and ages of cultures were investigated.

Table 19 presents the results obtained when 10 strains of B. fragilis were tested for the ability to produce abscesses that would not spontaneously drain. After 5 days, more than 50% of the formed abscesses had drained in mice injected with most strains. By day 10 few mice (regardless of the strain used) had intact abscesses present. Abscesses which had not drained at this time were small (less than 0.5 mm in diameter) and contained little pus.

Walker and Wilkins (83) had found that 18- to 20-h cultures produced the highest incidence of abscesses in mice. To determine whether this parameter had changed, the effect of culture age on abscess formation and drainage were determined (Table 20). B. fragilis was grown in 200 ml of CMC-SS medium. At the indicated times samples (0.5 ml) were injected into mice. At day 5, 80 to 100% of the mice had abscesses. This was independent of the culture age. Several abscesses had drained by day 5, and by day 10 only 20 to 50% of abscesses which had formed were still intact. Those that were still present were small and contained little pus. Thus, the age of the culture did not appear to be responsible for drainage of the abscess. However, it is apparent that the optimum culture age for abscess formation had also changed.

Table 21 gives the results obtained when different animals or different animal strains were used. B. fragilis 9032 was grown in CMC-SS media and three strains of mice were injected subcutaneously with 0.5 ml. With ICR mice, 100% of the animals developed abscesses by day 3, and only one of the 8 had drained. However, by day 7 only 25%

Table 19. Abscess production in ICR mice by various strains of *B. fragilis*

Strain	No. Abscesses/No. Mice Injected		
	Day 5[a]	Day 7	Day 10
9032	4/8(4)[b]	2/8(6)	0/8(8)
8708	7/10(2)	6/10(3)	2/10(7)
8429	3/5(2)	3/5(2)	1/5(4)
7428	2/5(3)	0/5(5)	0/5(5)
7310	2/5(3)	0/5(5)	0/5(5)
9836A	4/5	2/5(2)	2/5(2)
4912	2/5(3)	1/5(4)	0/5(4)
4948	3/5(1)	4/5(1)	2/5(2)
9309	5/5	2/5(2)	1/5(4)
3625	4/5	4/5(1)	3/5(2)

[a]Number of days following bacterial challenge

[b]Numbers in parenthesis indicate the number of abscesses that have drained

Table 20. Effect of culture age on abscess production in ICR mice by *B. fragilis* 9032

Culture Age (h)	No. Abscesses/No. Mice Injected	
	Day 5[a]	Day 10
15	6/8(2)[b]	2/8(6)
18	7/10(2)	2/10(8)
21	8/10	5/10(5)
24	9/10(1)	3/10(6)
27	8/10(1)	4/10(5)

[a]Number of days following bacterial challenge

[b]Numbers in parenthesis indicate the number of abscesses that have drained

Table 21. Effect of animal or animal strain on abscess production by B. fragilis 9032

Animal	Animal Strain	No. Abscesses/No. Animals Injected			
		Day 3[a]	Day 4	Day 7	Day 8
Mouse	ICR	7/8(1)	5/8(3)	2/8(6)[b]	-
	CD-1	-	20/20	6/20(13)	-
	CBA-J	-	22/22	10/22(12)	-
Rat	Sprague-Dawley	3/3	-	-	3/3

[a]Number of days following bacterial challenge

[b]Numbers in parenthesis indicate the number of abscesses that have drained

[c]Results were not recorded on these days

of these mice had intact abscesses. At day 4 all CD-1 mice had abscesses, but by day 7 only 35% remained intact. Results similar to these were also seen with CBA-J mice. In general, all abscesses which were still intact 7 days after injection in each of the animal strains were small and contained less than 0.1 ml of pus. Many abscesses did not contain pus.

Sprague-Dawley rats were tested by injecting them subcutaneously with 1.5 ml of a culture of 9032 in CMC-SS. All the animals developed abscesses, none of which had drained by day 8. When examined at day 3, abscesses contained approximately 0.3 to 0.5 ml of a viscous pus. By day 14, (data not shown) all abscesses had regressed in size so that on visual examination only a small lump was present. Drainage of abscesses through the skin was never observed in rats. However, attempts to reproduce these results were unsuccessful. In subsequent experiments rats produced a visible swelling at the site of injection, but the abscesses were small and contained what appeared to be a slightly viscous mixture of pus and blood.

The incidence of abscess formation and drainage was also determined with B. fragilis grown in various media. I also compared agar with agarose to determine whether the type of solidifying agent would have affected results. Results are shown in Table 22. Nearly all mice injected with cultures grown in Difco CM with either agar or agarose developed abscesses by the 3rd day and by day 7 drainage was apparent. These same results were observed in mice injected from CMC-SS or Wilkins-Chalgren-SS cultures, however about half of the

Table 22. Effect of various semi-solid media on abscess production in ICR mice by B. fragilis 9032

Medium	No. Abscesses/No. Mice Injected	
	Day 3[a]	Day 7
CMC-SS	4/8	0/8(7)[b]
W-C-SS[c]	5/12	1/12(9)
Schaedler-SS	3/12	0/12(3)
PYG-SS	0/6	0/6
Difco CM-SS (Agar)	3/4	1/4(2)
Difco CM-SS (Agarose)	4/4	2/4(2)

[a]Number of days following bacterial challenge

[b]Numbers in parenthesis indicate the number of abscesses that have drained

[c]Modified Wilkins-Chalgren semi-solid medium

abscesses did not form as rapidly as did the previous two cultures. It is interesting to note that few or no abscesses developed in mice injected with Schaedler-SS or PYG-SS cultures. Three of 12 mice injected with the cultures grown in Schaedler broth developed abscesses by day 3, however all of these had drained by the 7th day. No abscesses were produced in mice injected with PYG-SS cultures.

Since Walker and Wilkins (83) had found that the presence of agar is critical in this model I determined the effect of agar concentration on abscess development. Results are shown in Table 23. The formation of abscesses was dependent on the concentration of agar. After 3 days, abscesses were evident in 5 of 5 mice when 0.5% agar (wt/vol) was used, but only in 3 of 5 with 0.25% agar, and 1 of 5 with 0.1% agar. By day 7 one half of the abscesses in mice injected from 0.5% agar cultures had drained. The lower agar concentrations showed a higher incidence of abscess formation at this time and no drained abscesses were observed. However, within another five days, the abscesses did drain.

Effect of clindamycin on the viability of B. fragilis in vivo. In what was to be a preliminary experiment, CBA-J mice with 5 day old abscesses were used to determine the effect of clindamycin on the viability of B. fragilis in vivo. Mice were given a single dose of clindamycin-phosphate and blood and pus samples were taken at indicated times. These samples were assayed for levels of clindamycin. However, the abscesses were so small and contained so little pus, it was not possible to obtain 0.1 ml samples for viable cell determina-

Table 23. Effect of agar concentration on abscess production and drainage in CBA-J mice by B. fragilis 9032

% Agar Conc.[a](wt/vol)	No. Abscesses/No. Mice Injected	
	Day 3[b]	Day 7
0.5	5/5	2/4(2)[c,d]
0.25	3/5	3/4[d]
0.1	1/5	3/5

[a]The medium used was CMC

[b]Number of days following bacterial challenge

[c]Numbers in parenthesis indicate the number of abscesses that have drained

[d]Between days 3 and 7 one mouse with an intact abscess was sacrificed so the number of mice injected has decreased by one

tions. Instead, 0.01 ml of pus was collected and added to 9.9 ml anaerobic dilution fluid blank.

Results are given in Table 24. The blood levels obtained were those which are generally recommended for use in humans. Clindamycin concentrations in pus were two to six times higher than corresponding blood levels except those observed at 1 h. Few mice could be sampled for viable cell numbers since it was difficult to obtain abscess material after the first 0.01 ml sample was withdrawn for the determination of the clindamycin concentration. The data obtained indicate that clindamycin had little or no effect on viable cell numbers in these abscesses. Whether the two-fold decrease observed at 2 h as compared to 1 h is significant is not known. Other antibiotics were not tested because samples for use in viable cell count determinations could not reliably be obtained.

Table 24. Concentrations of clindamycin in blood and abscess pus and the effect on the viability of B. fragilis 9032 in mice.

	Clindamycin Conc. (mcg/ml)[a]			
Time (h)	Blood	Pus	Viable Cells/ml Pus	
1	5.6 ± 1.6[b]	3.0 ± 1.1	3.0×10^{10}	(1)[c]
2	1.7 ± 0.4	6.2 ± 2.8	1.5×10^{10}	(2)
4	0.4 ± 0.0	2.4 ± 1.0	-	
6	<0.4	1.7 ± 0.6	-	
8	0.6 ± 0.05	1.3 ± 0.05	2.7×10^{10}	(3)

[a]CBA-J mice with 5 day abscesses were given one 150 mg/kg intraperitoneal injection of clindamycin-phosphate. Each concentration given represents an average of 5 mice, except the 4 and 8 h blood levels where the results of 4 mice are averaged

[b]Standard deviation

[c]Numbers in parenthesis indicate the number of mice from which it was possible to obtain samples to give the indicated values

DISCUSSION

Infection with Bacteroides fragilis is cause for concern since few antibiotics are effective against this anaerobe. Of particular concern are diseases such as bacterial endocarditis where treatment with a bactericidal drug is especially desirable. When B. fragilis is identified as the causative agent even treated bacterial endocarditis has a mortality of 46% (55). The three drugs of choice in the treatment of B. fragilis infections are clindamycin, chloramphenicol and metronidazole. Of these three, only metronidazole is considered to be bactericidal.

It has been reported that tetracycline is irregularly bactericidal to members of the B. fragilis group (34, 54). The results obtained in this thesis are in agreement with this. However, many investigators (49, 54, 55, 97) have reported that MBCs of tetracycline for the B. fragilis group (mainly B. fragilis and B. thetaiotaomicron) are greater than 6 mcg/ml and frequently greater than 50 or 100 mcg/ml. In addition some have reported MBCs to be 64 fold or greater than corresponding MICs (55, 97). I found that MBCs were 1 to 16 times higher than corresponding MICs. This is in agreement with Martin et al. (49). In addition, for all sensitive strains tested, MBCs were within achievable blood levels of 4 to 5 mcg/ml (41); the average MBC was 2 mcg/ml. It is interesting to note that based on MBC to MIC ratios, tetracycline was bactericidal to all clinically resistant strains of B. fragilis and B. thetaiotaomicron.

Martin et al. (49) reported that chloramphenicol was inconsistently bactericidal, while Zabransky et al. (97) reported that chloramphenicol was simply bacteriostatic. In addition, the MBCs of most of the strains in these two studies were reported to be well above the achievable peak blood levels of 10 to 20 mcg/ml (26, 41). I found that chloramphenicol was bactericidal to all 16 strains of B. fragilis tested, and bactericidal to four of six B. thetaiotaomicron strains. The MBCs of all strains were within the achievable peak blood levels of the drug, except for those B. thetaiotaomicron strains where chloramphenicol was bacteriostatic. It should be noted that the MBCs of chloramphenicol for the majority of these strains would probably be above blood levels 1 or 2 h after the administration of a given dose. The results obtained in this study are in agreement with those of Ingham et al. (34), however it is difficult to interpret their MBC data since an agar dilution technique was used, so they could not determine the percent of the inoculum that was killed.

When clindamycin was originally introduced, several investigators determined the MICs and MBCs for a variety of facultative organisms and concluded that the drug was bactericidal (50, 51, 82). However, when others determined the MICs and MBCs of clindamycin for the B. fragilis group it was concluded that the drug was bacteriostatic (49, 54, 65, 80). Martin et al. (49) reported that MBCs of clindamycin for isolates of the B. fragilis group generally exceeded MICs by at least 16 fold. In addition, they reported that the MBCs for the majority of strains were well above the achievable blood levels of 6 to 15 mcg/ml (41). I

found that clindamycin was bactericidal to all 16 strains of B. fragilis and B. thetaiotaomicron I tested. In addition, MBCs were well below achievable blood levels. These data are similar to those of Zabransky et al. (97). Although they reported MBCs to be four to eight fold higher than MICs, 88% of the B. fragilis group isolates had MBCs lower than 8 mcg/ml.

The MICs of clindamycin for B. thetaiotaomicron were slightly higher than for strains of B. fragilis. This is similar to the observations of others (7, 37, 69). With B. fragilis the majority of clindamycin MICs were 0.05 or 0.1 mcg/ml. Of six B. thetaiotaomicron strains, one had an MIC to clindamycin of 3.1 mcg/ml, another 1.6 mcg/ml, and one had an MIC of 0.8 mcg/ml. As reported by Blazevic (7) these differences are probably not enough to aid in identification schemes, nor in predicting the susceptibility of B. thetaiotaomicron, however, the higher MICs of clindamycin for these strains could be of importance in selecting appropriate antimicrobial therapy.

In this study metronidazole was bactericidal to nearly all strains tested. In most cases MICs and MBCs were the same, or one and occasionally two dilutions apart. All MBCs (except B. fragilis 4912) were below the easily attainable blood levels of 8 to 10 mcg/ml (44, 77). These results are nearly identical to those obtained by others (54, 88).

As expected, cefoxitin was bactericidal to all B. fragilis and B. thetaiotaomicron strains tested. However, most MICs for this drug were at or one dilution below the susceptibility break point of

32 mcg/ml. Thus the *in vitro* data obtained in this study does not indicate that cefoxitin is a particularly efficacious bactericidal drug against *B. fragilis*, especially since most MBCs were at or above 32 mcg/ml.

Viable count data confirmed the MIC and MBC findings obtained in this thesis. I found that *B. fragilis* was killed (greater than 99.9% reduction of the inoculum) within 24 h when exposed to clinically achievable levels of metronidazole, clindamycin, tetracycline or chloramphenicol. The only exception was found with cefoxitin. When *B. fragilis* 9032 was exposed to 32 mcg/ml, 1×10^3 cells/ml remained after 24 h.

When the inocula sizes in MIC tests were varied two types of effects were observed. Either MICs and MBCs both increased or only MBCs increased. With tetracycline, chloramphenicol, and metronidazole, MICs and MBCs increased as the inoculum size was increased. However, the MICs and MBCs changed at different rates for tetracycline. As a result, bactericidal activity was not as pronounced with this drug when an inoculum of 10^9 cells/ml was used as opposed to 10^5 cells/ml. Under the same conditions the MBCs of clindamycin and cefoxitin also increased while the MICs remained essentially the same. Trends of these natures have previously been reported for metronidazole (39, 95) and clindamycin (50, 82). It is not known whether this reflects that a given bacterium requires a certain number of antibiotic molecules for growth inhibition or death to occur, that sensitivity to an antibiotic varies with varying growth rate or that

a combination of these and/or other factors occur. However, these data do point out the importance of inoculum size in susceptibility testing. At low inoculum concentrations, not only are MICs lower, but bactericidal activity is more pronounced for certain drugs since MBC to MIC ratios are lower. In addition, since the concentration of bacteria in infections are often several times higher than inocula sizes used in MIC testing, these data show that the amount of antibiotic needed to inhibit an organism *in vitro* may be quite different from the amount needed to inhibit the same organism *in vivo*.

A number of conclusions can be drawn from the data obtained in these *in vitro* studies. Of the drugs of choice used in the therapy of *B. fragilis* infections, clindamycin along with metronidazole can be considered as bactericidal antibiotics to both *B. fragilis* and *B. thetaiotaomicron*. In addition, chloramphenicol is also bactericidal to *B. fragilis*, however, the drug may be bacteriostatic to a few strains of *B. thetaiotaomicron*. Tetracycline may be considered to be bactericidal to only a few strains of these *Bacteroides* species; i.e. tetracycline is irregularly bactericidal. Most importantly these studies have shown that at achievable blood levels of clindamycin, tetracycline, and metronidazole, these drugs kill *B. fragilis* and *B. thetaiotaomicron*. Although chloramphenicol can also cause death at relatively low MBC levels (as compared to MIC levels), the concentrations which are required may only be maintained in the body a short time after a given dose. The reasons for the discrepancies between my study and others is not known. In many cases a different

media was used, but experiments here show that media did not significantly affect results. Procedural differences cannot be held accountable since approximately the same inocula sizes were used. MICs were defined on the basis of observable turbidity; MBCs were defined as a 99 or 99.9% reduction in inoculum. Forty-eight hour incubations of MIC tests resulted in an even more pronounced bactericidal activity. In addition, tetracycline and chloramphenicol were bacteriostatic when tested against E. coli, thus confirming their expected activity on this organism.

The in vivo experiments planned for this thesis could not be carried out because of problems with the experimental model. Attempts were made to reestablish this infection in mice by examining parameters such as the strain of B. fragilis, strain of mice, types of media, concentration of agar, and ages of cultures. All attempts were unsuccessful. When rats were used, well developed abscesses formed at first, but when actual experiments were begun abscesses failed to develop.

One preliminary experiment with mice was done using clindamycin as the antimicrobial agent. I used blood levels of clindamycin recommended for human use, but found that viable cell numbers in abscess material remained at just over 10^{10} cells/ml over an 8 h period. These results were unexpected because the MIC and MBC for this strain were 0.05 and 0.1 mcg/ml respectively, and over the observation period the abscess levels of clindamycin did not go below 1.3 mcg/ml. Based on death rate data (Fig. 4), 1.3 mcg/ml clindamy-

cin should decrease cell numbers by at least 50 fold within 8 h. This, however, did not occur.

Varying inocula experiments indicated that, at the number of cells present in the abscesses, the MIC for this strain would have been about 0.1 mcg/ml. The MBC would likely have been between 1.6 and 3.1 mcg/ml. It can be assumed that this MBC (of at least 1.6 mcg/ml) of clindamycin was maintained over the 8 h observation period, since at no time were abscess levels of the drug below 1.3 mcg/ml. Death rate data (Fig. 4) indicate that near the MBC for this strain cell numbers should decrease by 10 fold by 8 h. This did not occur as shown in Table 24. It is important to note that in death rate experiments an inoculum of 5×10^5 cells/ml was used, and in abscesses a larger number of cells are present. It has been shown that with metronidazole, higher inocula decrease the bactericidal rate of the drug (F. P. Talley, N. M. Sullivan, and S. L. Gorbach, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, A29). Essentially this same conclusion can be reached with data obtained in this thesis. With all drugs tested in death rate experiments, lower drug concentrations resulted in slower death rates. Thus, as in the study of Talley et al., increasing the inocula concentration to drug concentration ratios results in slower death rates. Therefore it would seem reasonable to conclude that no change in cell numbers were observed in abscesses because death rates were much slower than in vitro experiments indicated. As a result, longer observation times (and additional doses of clindamycin to maintain MBC levels) should be

necessary before a decrease in cell numbers can be observed in these experiments. Since strain 8708 was killed much faster than strain 9032 (Fig. 4) it would be expected that in vivo, cell numbers in abscesses induced with strain 8708 would decrease faster than in abscesses induced by strain 9032.

Slower death rates might also explain why surgical drainage is so often important in treating anaerobic infections. If only bactericidal drugs are relied upon for treatment, the amount of time needed to significantly decrease cell numbers within an abscess might be so long that extensive damage to the body could occur during this time. With surgical drainage, cell numbers are brought down to manageable levels so that even bacteriostatic drugs, in conjunction with body defenses, are adequate to resolve the infection.

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BACTERIOSTATIC AND BACTERICIDAL EFFECTS OF ANTIBIOTICS ON *BACTEROIDES*

by

Benjamin James Veltri

(ABSTRACT)

The bacteriostatic or bactericidal action of metronidazole, clindamycin, tetracycline, chloramphenicol, cefoxitin and penicillin were determined against sixteen *Bacteroides fragilis* strains and six *B. thetaiotaomicron* strains. Drugs were considered bactericidal when minimal bactericidal concentration (MBC) to minimal inhibitory concentration (MIC) ratios were less than or equal to four. As expected, metronidazole, cefoxitin and penicillin were bactericidal. However, clindamycin and chloramphenicol, which other investigators have considered as bacteriostatic antibiotics, were bactericidal. Tetracycline was irregularly bactericidal. When tested against *Escherichia coli*, tetracycline and chloramphenicol were bacteriostatic as reported by others. The MBCs of metronidazole, clindamycin and tetracycline were well within the clinically achievable levels of each drug. Death rate data confirmed these findings. With tetracycline, chloramphenicol and metronidazole MICs and MBCs increased as inocula sizes increased, however, with clindamycin and cefoxitin only MBCs increased. Clindamycin was administered to mice which had pure *B. fragilis* abscesses. Over 8 h cell numbers in the pus remained relatively constant even though, during this same period, the concentration of clindamycin in the pus was above the MBC of this drug for the strain used.