ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1972, p. 433-440 Vol. 1, No. 5
Printed in U.S.A.
Copyright © 1972 American Society for Microbiology

Effect of Several Components of Anaerobic Incubation on Antibiotic Susceptibility Test Results

JON E. ROSENBLATT AND FRITZ SCHOENKNECHT

Infectious Disease Section, Wadsworth General Hospital (Veterans Administration); UCLA School of Medicine, Los Angeles, California 90073; and Clinical Microbiology Laboratory, University of Washington, Seattle, Washington 98105

Received for publication 9 December 1971

The factors influencing the in vitro activity of antibiotics during anaerobic incubation were studied by the disc method with a facultative organism, *Escherichia coli*. We observed the effects of incubation aerobically, anaerobically (Torbal jars), in a CO_2 incubator, and aerobically and anaerobically with all CO_2 removed. We also monitored *p*H changes during incubation and observed the effect of two different initial agar *p*H values (7.4 and 8.3). With aminoglycosides, zones were larger at *p*H 8.3 and, in each agar *p*H group, zones were decreased by incubation with increased CO_2 (anaerobically and CO_2 incubator). A fall in agar *p*H took place during the first 5 to 7 hr of incubation when increased CO_2 was present. Decreased aminoglycoside zones in the presence of increased CO_2 were due to fall in agar *p*H. Erythromycin showed the same zone size changes as the aminoglycosides. Chloramphenicol zones were somewhat smaller at the lower medium *p*H. Zones around tetracycline discs were largest after incubation anaerobically. Further aerobic (or CO_2) incubation of plates after anaerobic incubation resulted in large "zones of relative inhibition" around the aminoglycoside discs. This suggests that these antibiotics had become more active after exposure to aerobic conditions. Our studies indicate that antibiotic susceptibility test results can be significantly altered by several components of anaerobic incubation including changes in agar *p*H and CO_2 concentration as well as anaerobiosis per se.

The recent development of more effective methods for the isolation and identification of anaerobic bacteria has produced a heightened interest in these microorganisms. Increased isolation of anaerobes in the clinical laboratory should accentuate the need for simple and accurate antibiotic susceptibility tests for anaerobic conditions. There is no standard method for doing these tests. As an initial approach toward the development of such a standardized method, we decided to study the effects of anaerobic incubation on antibiotic disc zones of inhibition.

In order to determine which components of the anaerobic environment were responsible for changes in zone sizes, we examined the effect of various conditions of incubation. A facultative organism, *Escherichia coli*, was incubated on blood Mueller-Hinton agar plates with nine different antibiotic discs and under five different conditions of incubation. These were: aerobic; anaerobic with 4 to 5% CO_2 present; in a CO_2 incubator with 5 to 7% CO_2 present; and aerobic and anaerobic both with all CO_2 absorbed out of the system. Zones of inhibition and agar *p*H changes were then measured. These studies were performed at initial medium *p*H 7.4 and then duplicated at *p*H 8.3 to observe the effect of *p*H alone on zone sizes. In addition, we measured zones after initial incubation anaerobically and then again after subsequent aerobic incubation to evaluate the effect of anaerobiosis per se.

MATERIALS AND METHODS

Mueller-Hinton agar containing 5% defibrinated sheep blood was used throughout the study except in the experiment measuring *p*H changes due to temperature; here Mueller-Hinton agar without blood was used. A single laboratory standard strain of *E. coli* (ATCC 25922; University of Washington clinical laboratory strain used for quality control of "Kirby-Bauer" method of antibiotic disc susceptibility testing) was the test organism.

Studies on the effect of different incubation environments on disc zone sizes were performed with 100-mm diameter plastic petri dishes containing 20 ml of medium. A 2-ml amount of a 10^{-4} dilution of an 18-hr broth culture of *E. coli* was "flooded" onto the plates, and the excess was aspirated off with a Pasteur pipette.

We used the "high content" discs (BBL) recommended in the Kirby-Bauer method of antibiotic susceptibility testing (2). Zone size readings were done in quadruplicate in experiments run at medium *p*H 7.4. Eight readings were made in another set of experiments at initial *p*H 8.3. Vernier calipers were used to measure the zones. Incubation was carried out at 35 to 37 C over 18 to 24 hr. The following incubation atmospheres were studied: aerobic by exposing the plates to room air; anaerobic using Torbal anaerobic jars (methylene blue used as an indicator of anaerobiosis) from which all air was evacuated and replaced with a gas mixture containing 5% CO_2, 10% H_2, and 85% N_2; CO_2 incubation by using an incubator with CO_2 flowing, providing a concentration of 5 to 7% CO_2; and aerobic and anaerobic conditions with no CO_2 present. We removed the CO_2 by evacuating all air from Torbal jars and replaced the vacuum by passing either room air or the anaerobic gas mixture through a tube containing CO_2 absorbant (Malacosorb, Mallinckrodt Chemical Works). A plate of CO_2 absorbant was also included in each jar. No CO_2 was detectable in the jar atmosphere (Radiometer-Kopenhagen physiologic gas analyzer) after this procedure.

Measurements of media *p*H changes were made during the different incubation conditions. Figure 1 shows the apparatus for support of a glass combination electrode with a semimicro bulb (Sargent 5-30070-10) which was immersed below the surface of the agar. Thirty-five to 40 ml of medium was dispensed in each plate to obtain a suitable thickness. The support apparatus was placed in an anaerobic jar, and the electrode lead wire led out of the jar through Plasticine lid sealant.

Plates for *p*H measurement studies were inoculated by streaking in six different directions a swab dipped in an overnight growth of *E. coli* which had been diluted in saline to a density comparable to a barium sulfate standard (McFarland 0.5; reference 2). A 1-cm diameter unstreaked zone was left in the center of each plate for immersion of the electrode bulb which was sterilized by rinsing with a phenolic disinfectant (Staphene) and sterile distilled water.

The effect of temperature changes alone on medium *p*H was also studied by using similar techniques. Uninoculated plates were incubated aerobically.

In an additional experiment, susceptibility plates were incubated initially anaerobically for a period of 20 hr. Zone sizes were measured and then the plates were reincubated for a further 20 hr, either anaerobically, aerobically, or in CO_2. After this reincubation, zones of inhibition were again measured.

Determinations of *E. coli* growth were made in the various experiments by measuring colony diameters in the third streak area on streaked plates with the use of a dissecting microscope with a calibrated eyepiece micrometer. A mean diameter was calculated from measurements of five different colonies.

RESULTS

pH changes in media during incubation. Figure 2 demonstrates the changes in Mueller-Hinton agar *p*H which occur with changes in temperature through a range of 80 to 20 C. Similar changes were seen when blood-agar base (BBL) was used. *p*H values ranged from a low of 6.7 at 80 C to a high of 7.3 at 15 C. A change in temperature from approximately 20 to 37 C was associated with a decrease of between 0.10 and 0.15 *p*H units.

The *p*H changes in inoculated blood Mueller-Hinton agar plates incubated in a variety of conditions are shown in Fig. 3. Each graphic line represents the results of duplicate runs. During the first 1 to 2 hr of incubation under all conditions, there is a small decrease in *p*H of 0.1 to 0.2 units. This can be related to a change from room temperature to 37 C. Under aerobic conditions, both with and without removal of all CO_2 from the atmosphere, there is little further change until approximately 6 or 7 hr, when the *p*H begins to rise and reaches values above 8.0 at the end of 20 hr of incubation. This late rise is probably due to microbial growth and metabolism, especially the deamination of amino acids, since peptones or hydrolysates of casein are the principal nutrients in Mueller-Hinton agar.

Incubation in 5 to 7% CO_2 and anaerobically (ANA) with 4 to 5% CO_2 produces a continual

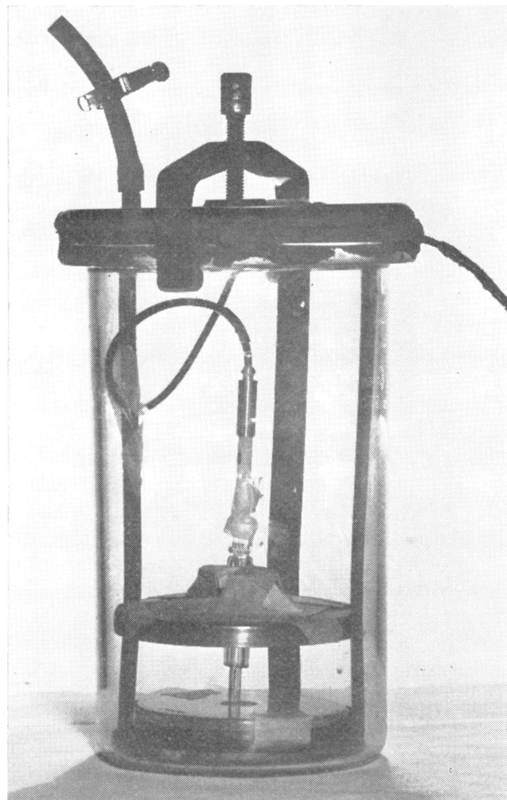

FIG. 1. *Apparatus used for measuring medium pH during different conditions of incubation.*

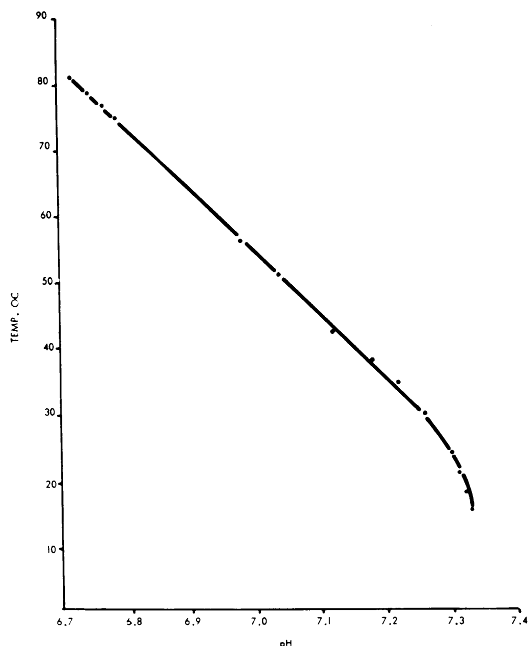

FIG. 2. *pH changes in Mueller-Hinton agar related to changes in medium temperature.*

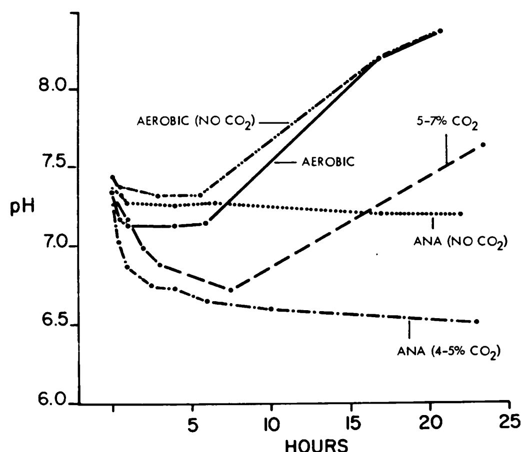

FIG. 3. *pH changes in blood Mueller-Hinton agar inoculated with E. coli during different conditions of incubation.*

fall in medium *p*H over the first 6 to 7 hr. At this time, the *p*H in the 5 to 7% CO_2 environment begins to rise, and at the end of incubation it is above the initial value. Again, this late rise in *p*H is probably due to the metabolic effects of *E. coli* growth which is good in 5 to 7% CO_2. On the other hand, the *p*H in the anaerobic (ANA) environment continues a slight downward trend and shows no terminal rise. *E. coli* growth anaerobically is considerably poorer than in CO_2 (*see below*). In addition, *E. coli* metabolism of sugars anaerobically produces more acid than does aerobic metabolism. This type of growth and metabolism combined with the effect of 4 to 5% CO_2 in the atmosphere results in the maintenance of a low medium *p*H.

Under anaerobic conditions with the CO_2 absorbed out of the system (ANA-NO CO_2), there is neither an initial fall in medium *p*H nor a rise during the latter part of incubation. This can be explained by a lack of atmospheric CO_2. There is, therefore, no initial *p*H fall and insufficient microbial growth and metabolism for terminal alkalinization of the media.

Incubation of uninoculated plates under these different conditions produced the same early medium *p*H changes as are shown in Fig. 3. However, the terminal *p*H increases which were seen during incubation in CO_2 and aerobically do not occur in the uninoculated plates. This confirms the suggestion that these latter *p*H changes are due to the metabolic activity accompanying microbial growth.

E. coli colony sizes. Since differences in *E. coli* growth rates under different incubation conditions could affect the zone sizes, we compared growth by measuring colony size on streaked plates (Table 1). Smaller colonies were found at the higher *p*H (except after CO_2 incubation). The generation time for *E. coli* is somewhat longer

TABLE 1. *Escherichia coli colony sizes (diameter in millimeters) after incubation under different conditions and with different initial medium pH values*

Incubation atmosphere	*p*H 7.4[a]	*p*H 8.3[b]
Aerobic	2.84 ± .07[c]	2.09 ± .08
5-7% CO_2	2.41 ± .09	2.52 ± .12
Anaerobic (4-5% CO_2)	1.83 ± .12	1.73 ± .15
Anaerobic (no CO_2)	2.24 ± .07	1.72 ± .05
Aerobic (no CO_2)	2.93 ± .10	2.30 ± 0.18

[a] Each figure in this column represents the mean of five separate colony diameters (mm) in third streak of streaked plates.

[b] Each figure in this column represents the mean of 10 separate colony diameters (mm) in third streak of streaked plates.

[c] Standard deviation.

at pH 8.3 than at 7.4 (30 min versus 25 min; reference 9). Growth also was poorer after incubation anaerobically.

It is possible that poor growth at pH 8.3 and anaerobically may have contributed to the establishment of large zones of inhibition. This might apply to the aminoglycosides and erythromycin which had larger zones at pH 8.3. Anaerobically, zones were actually smaller or unchanged when compared to aerobic conditions, so that poor *E. coli* growth is not a factor here.

In the absence of excess CO_2, chloramphenicol had larger zones at the higher pH. Also, tetracycline zones were larger at pH 8.3 after aerobic incubation. Again, poor growth of *E. coli* might have contributed to the development of these large zones.

E. coli colony size was not affected by incuba-

TABLE 2. *Aminoglycoside disc zones of inhibition[a] after incubation under different conditions and with different initial medium pH values*

Incubation atmosphere	Gentamicin		Streptomycin		Kanamycin	
	pH 7.4[b]	pH 8.3[c]	pH 7.4[b]	pH 8.3[c]	pH 7.4[b]	pH 8.3[c]
Aerobic	20.5 ± 0.5[d]	25.1 ± 0.9	15.5 ± 0.5	19.2 ± 0.8	20.0 ± 0.0	22.9 ± 0.6
5-7% CO_2	18.3 ± 0.5	20.4 ± 0.8	12.3 ± 0.5	15.7 ± 0.5	17.0 ± 0.8	19.3 ± 0.7
Anaerobic (4-5% CO_2)	18.0 ± 0.0	18.9 ± 0.6	14.5 ± 0.5	15.9 ± 1.0	18.0 ± 0.0	18.3 ± 1.0
Anaerobic (no CO_2)	21.0 ± 0.0	23.7 ± 0.5	16.0 ± 0.0	18.7 ± 1.3	21.0 ± 0.5	20.9 ± 1.1
Aerobic (no CO_2)	21.3 ± 0.5	29.3 ± 0.7	16.8 ± 0.5	21.1 ± 0.8	20.5 ± 0.5	25.9 ± 0.8

[a] Zone diameter (mm).
[b] Each figure in this column represents the mean of four separate results.
[c] Each figure in this column represents the mean of eight separate results.
[d] Standard deviation.

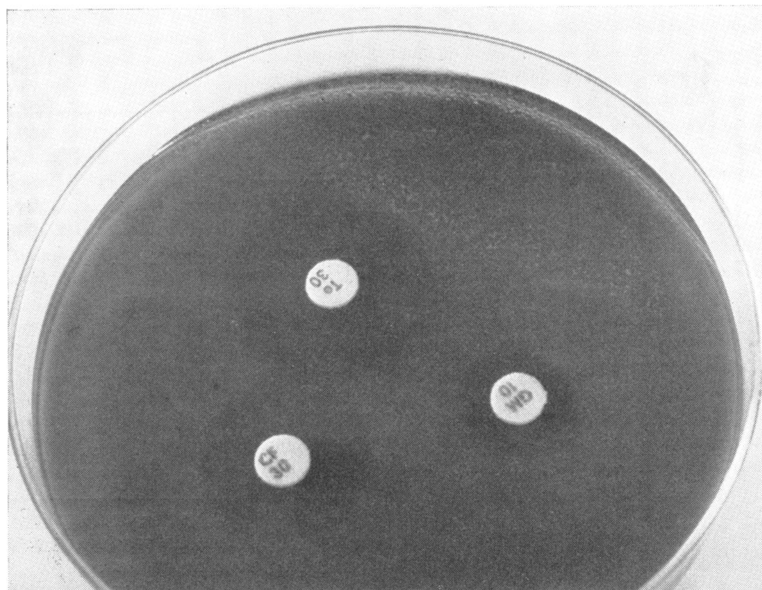

FIG. 4. *Antibiotic disc zones of inhibition after anaerobic incubation (Te, tetracycline; CF, cephalothin; GM, gentamicin).*

tion in excess CO_2. Therefore, variable *E. coli* growth rates due to CO_2 would not have been a factor in final zone sizes.

Antibiotic disc zone sizes. Table 2 shows that the aminoglycoside antibiotics, gentamicin, streptomycin, and kanamycin, have decreased zone sizes in the presence of excess CO_2, that is, after incubation in the CO_2 incubator and under anaerobic conditions with CO_2 included. Zone sizes, in general, were larger at higher medium *p*H. The largest zone sizes were seen under aerobic conditions with all CO_2 removed. Aminoglycoside zones under anaerobic conditions with no CO_2 present were similar to those under aerobic conditions and were larger than those for anaerobic conditions with 4 to 5% CO_2 included. This indicates that excess CO_2 results in smaller aminoglycoside zones of inhibition and suggests that this effect is mediated through a decrease in medium *p*H during the first 7 hr of incubation.

As has been mentioned, zones sizes under aerobic conditions were similar to those obtained under anaerobic conditions with no CO_2. This implies that the 4 to 5% CO_2 in the anaerobic environment is responsible for the smaller aminoglycoside zones observed rather than any other factor in the anaerobic atmosphere or "anaerobiosis per se." However, Fig. 4 and 5 demonstrate a phenomenon which provides an alternative explanation. Susceptibility plates were first incubated anaerobically with CO_2 included, and then they were reincubated either aerobically or in a CO_2 incubator. Figure 4 shows that relatively small zones of inhibition appeared around the aminoglycoside (here gentamicin) discs after anaerobic incubation. However, on reincubation (Fig. 5), improved luxuriant growth of the organism occurred over most of the plate, but a much larger "zone of relative inhibition" (inhibition of new growth) was observed around the aminoglycoside disc. (No actual zone sizes are given since the zone edges were indistinct and difficult to measure accurately.) This phenomenon was observed both on reincubation in room air and in 5 to 7% CO_2, indicating that the increased aminoglycoside activity on reincubation was not simply due to a rapid increase in *p*H. The new zones did not appear when reincubation was carried out anaerobically.

Erythromycin zone sizes were in general larger at the high initial *p*H (Table 3). In addition, zones were smallest under those incubation conditions in which excess CO_2 was present. Zones of inhibition under aerobic conditions and anaerobically with no CO_2 present were similar. The largest zone was obtained after aerobic incubation

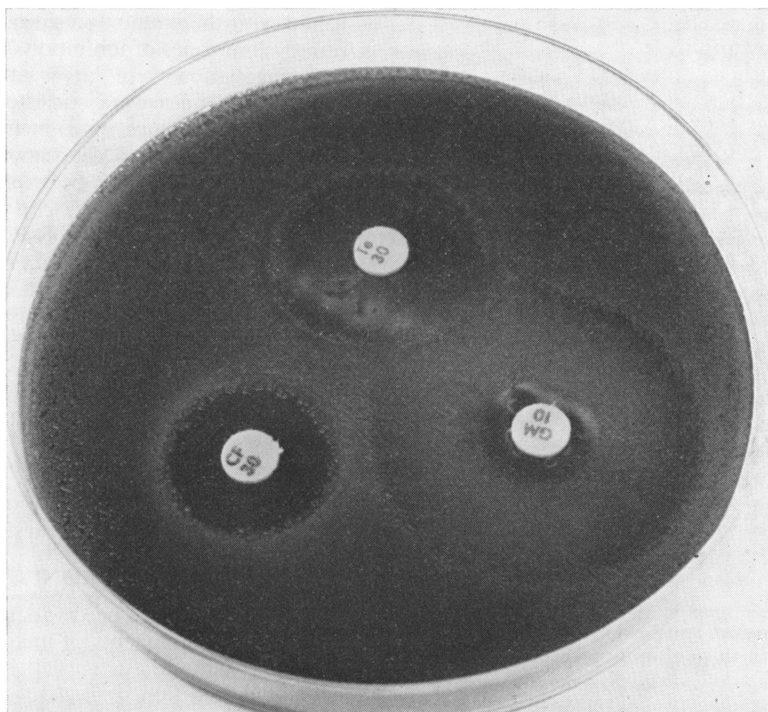

FIG. 5. *Same plate as shown in Fig. 4 after reincubation aerobically. New zone of "relative inhibition" of new growth has appeared around the gentamicin (GM) disc (CF, cephalothin; Te, tetracycline).*

with all CO_2 removed. These observations are similar to those described already for the aminoglycosides, and the same suggestions about the effect of CO_2 and medium pH can be applied.

TABLE 3. *Erythromycin disc zones of inhibition[a] after incubation under different conditions and with different initial medium pH values*

Incubation atmosphere	Erythromycin	
	pH 7.4[b]	pH 8.3[c]
Aerobic	8.3 ± 0.5[d]	15.3 ± 1.0
5-7% CO_2	6.0 ± 0.0	9.2 ± 0.3
Anaerobic (4-5% CO_2)	6.0 ± 0.0	6.0 ± 0.0
Anaerobic (no CO_2)	6.0 ± 0.0	15.8 ± 1.8
Aerobic (no CO_2)	8.3 ± 0.5	17.4 ± 0.5

[a] Zone diameters (mm); 6 mm indicates no zone since this is the diameter of the disc.
[b] Each figure in this column represents the mean of four separate results.
[c] Each figure in this column represents the mean of eight separate results.
[d] Standard deviation.

TABLE 4. *Tetracycline disc zones of inhibition[a] after incubation under different conditions and with different initial medium pH values*

Incubation atmosphere	Tetracycline	
	pH 7.4[b]	pH 8.3[c]
Aerobic	17.0 ± 0.8[d]	19.8 ± 0.6
5-7% CO_2	22.0 ± 0.0	22.3 ± 2.0
Anaerobic (4-5% CO_2)	21.8 ± 1.3	22.9 ± 1.2
Anaerobic (no CO_2)	19.5 ± 0.6	17.7 ± 2.1
Aerobic (no CO_2)	17.3 ± 1.3	20.2 ± 0.9

[a] Zone diameters (mm).
[b] Each figure in this column represents the mean of four separate results.
[c] Each figure in this column represents the mean of eight separate results.
[d] Standard deviation.

TABLE 5. *Chloramphenicol disc zones of inhibition[a] after incubation under different conditions and with different initial medium pH values*

Incubation atmosphere	Chloramphenicol	
	pH 7.4[b]	pH 8.3[c]
Aerobic	22.8 ± 1.0[d]	25.2 ± 0.7
5-7% CO_2	22.3 ± 0.5	23.4 ± 0.9
Anaerobic (4-5% CO_2)	20.5 ± 0.5	21.7 ± 1.2
Anaerobic (no CO_2)	21.3 ± 1.0	24.9 ± 1.6
Aerobic (no CO_2)	22.5 ± 0.5	27.9 ± 1.0

[a] Zone diameters (mm).
[b] Each figure in this column represents the mean of four separate results.
[c] Each figure in this column represents the mean of eight separate results.
[d] Standard deviation.

However, the development of a large "zone of relative inhibition" after aerobic reincubation of anaerobic plates did not occur with erythromycin as it had with the aminoglycosides.

Tetracycline zones of inhibition (Table 4) after aerobic incubation were larger at medium pH 8.3. However, zones were consistently larger in both media pH groups after incubation in the presence of excess CO_2. These data are somewhat conflicting, but they may be explained by the fact that aerobic growth of *E. coli* was poorer at medium pH 8.3 than at 7.4 (*see above*), and this allowed the development of larger zones at the higher pH. Thus, in situations where *E. coli* growth is similar, excess CO_2 increases tetracycline zones, presumably by decreasing medium pH.

Chloramphenicol had larger zones at pH 8.3, at least when no CO_2 was present (Table 5). At pH 8.3, excess CO_2 results in smaller zones by lowering medium pH.

No consistent zone size changes, relative to the parameters discussed above, were seen when ampicillin and cephalothin were tested.

DISCUSSION

An illustration of the need to study the effects of the various components of anaerobic incubation on the results of susceptibility tests appears in a recent publication by Ingham et al. (7). The authors noted a marked discrepancy between the minimal inhibitory concentrations of erythro-

mycin and lincomycin for *Bacteroides fragilis* as determined in their laboratory (0.15 and 0.55 μg/ml) and those reported by Finegold et al. (> 1.0 and 6.2 μg/ml). The differences were found to be due to different anaerobic incubation techniques. Ingham et al. used an anaerobic atmosphere containing hydrogen gas only, whereas Finegold et al. used hydrogen and 10% CO_2. Ingham concluded that the 10% CO_2 produced a more acid medium which decreased the activity of erythromycin and lincomycin, resulting in a much greater minimal inhibitory concentration. An alternate interpretation is that the 10% CO_2 enhanced *B. fragilis* growth to such an extent that greater concentrations of antibiotic were required to inhibit the organism. The authors do point out, however, that anaerobic incubation on an acid medium resulted in susceptibility patterns similar to those of anaerobic growth in excess CO_2. This would imply that any possible *B. fragilis* growth-enhancing effect of CO_2 was mediated through a lowering of medium *p*H. There are no specific data on the effect of CO_2 on *B. fragilis* growth, although general observations in our laboratory indicate that CO_2 is definitely stimulatory, resulting in larger colonies and a heavier "lawn" of growth. The role of *p*H, if any, in this CO_2 effect is unknown. Moreover, it has not been demonstrated that such CO_2-related enhanced growth has any effect on the results of susceptibility tests.

Our studies have correlated CO_2 concentrations in the incubation atmosphere with *p*H changes in the medium. The effect of *p*H on the antimicrobial activity of a number of antibiotics has been demonstrated previously (1, 4-6). Aminoglycosides and erythromycin (and apparently lincomycin, *see above*) are more active in an alkaline medium and tetracycline is more active in an acid medium. Penicillin, ampicillin, and cephalothin are not significantly affected by different medium *p*H values. We have confirmed these observations and demonstrated their importance in disc diffusion susceptibility testing. Furthermore, we have observed smaller chloramphenicol zones with a decrease in medium *p*H. Previously chloramphenicol activity was not thought to be affected by *p*H changes.

Growth of *E. coli* is relatively poor at high initial *p*H values of medium. The large aminoglycoside, erythromycin, and chloramphenicol zones of inhibition ("enhanced antibiotic activity") observed at *p*H 8.3, therefore, may have been caused, at least in part, by poor *E. coli* growth. Excess CO_2 in the incubation environment did not affect *E. coli* growth and therefore would not have influenced zones of inhibition in this way.

We have indicated that anaerobiosis per se produced small aminoglycoside zones of inhibition, independent of any CO_2 effect. Kogut et al. (8) found that streptomycin was less active under anaerobic conditions. Alternatively, since the aminoglycosides inhibit bacterial protein synthesis, they may be less effective when growth and metabolism is poor. Another explanation for the phenomenon we observed is that the prolonged *E. coli* lag phase under anaerobic conditions allowed time for much more antibiotic to diffuse from the disc before the critical time (*see below*) expired. Once aerobic conditions were established, the organism grew much more vigorously outside the wider zone of antibiotic diffusion, but inside it the organism is confronted with a concentration of antibiotic sufficient to prevent any further growth.

This observation, that susceptibility plates initially incubated anaerobically show new zones of relative inhibition after subsequent aerobic incubation, seems to be at odds with Cooper's theory of critical time (3). This theory is one of the basic concepts of disc diffusion susceptibility testing. It states that there is a certain time required for the inoculum to reach a critical density, after which it will grow in concentrations of the antibiotic many times the minimal inhibitory concentration of the organism. The critical time would correspond to the time during which the zone edges are formed and is usually given as the first 2 to 6 hr of incubation. The formation of new zones on our plates after 20 hr of incubation does not appear consistent with the critical time theory. However, this theory was originally applied to rapidly growing organisms aerobically incubated. Under anaerobic conditions, growth of *E. coli* is poor and its "critical time" may not be reached even after 20 hr. Likewise, the growth of strict anaerobes is slow and their critical time is much longer than the usually given figure of 2 to 6 hr for rapidly growing organisms. In Cooper's experiments, the critical time was equal to the lag phase plus four times the generation time of the organism. If this formula were applied to the anaerobes *B. fragilis* and *B. melaninogenicus*, even under optimal growth conditions (10), the critical time would be 26 to 27 hr. Viewed in this way, our results are compatible with the theory of "critical time."

Finally, it must be pointed out that these studies cannot be directly related to disc diffusion susceptibility testing of anaerobes, since we utilized a facultative organism, *E. coli*, whose metabolism is different under different conditions of incubation. Moreover, the effects of the components of anaerobic incubation may differ for individual bacterial species. However, our observations do point out some of the problems inherent in antibiotic susceptibility testing under anaerobic con-

ditions and indicate the need for a standardized method which will take into consideration these problems.

ACKNOWLEDGMENT

We gratefully acknowledge the advice and criticisms of John C. Sherris in the preparation of this manuscript.

LITERATURE CITED

1. Abraham, E. P., and E. S. Duthie. 1946. Effect of *p*H of the medium on activity of streptomycin and penicillin and other chemotherapeutic substances. Lancet 1:455–459.
2. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disc method. Amer. J. Clin. Pathol. 45:493–496.
3. Cooper, K. E. 1952. Note on the theory of inhibition zones. J. Gen. Microbiol. 7:13–14.
4. Ericsson, H. M., and J. C. Sherris. 1971. The influence of variations of atmospheric conditions on the results of sensitivity tests, p. 34. *In* Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. Sec. B 1971, Suppl. 217.
5. Garrod, L. P., and F. O'Grady. 1971. Antibiotic and chemotherapy, 3rd ed. The Williams & Wilkins Co., Baltimore.
6. Haight, T. H., and M. Finland. 1952. The antibacterial action of erythromycin. Proc. Soc. Exp. Biol. 81:175–183.
7. Ingham, H. R., J. B. Selkon, A. A. Codd, and J. H. Hale. 1970. The effect of carbon dioxide on the sensitivity of *Bacteroides fragilis* to certain antibiotics *in vitro*. J. Clin. Pathol. 23:254–258.
8. Kogut, M., J. W. Lightbown, and P. Isaacson. 1965. Streptomycin action and anaerobiosis. J. Gen. Microbiol. 39:155–164.
9. Munro, A. L. S. 1970. Measurement and control of *p*H, p. 44. *In* Methods in microbiology, vol. 2. Academic Press Inc., London.
10. Wahren, A., and T. Holme. 1969. Growth of *Bacterioidaceae* in stirred fermentors. Appl. Microbiol. 18:235–239.