# THE SO-CALLED REDUCED OXYGEN TENSION FOR GROWING THE MENINGOCOCCUS

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Received for publication, April 5, 1919

The following series of experiments was planned to determine the rôle that carbon dioxide plays in the cultivation of the meningococcus. Although they have the limitation of dealing only with two strains, they emphasize the importance of the use of this gas in culturing the meningococcus within certain reactions of the media. The possibility was considered that definite knowledge as to the exact effect of this gas on such cultures might lead to some application of the principle involved to the treatment of meningococcus infections.

Wherry and Oliver (1916) found that thousands of colonies of the gonococcus could be produced by growth at partial oxygen tension when control aerobic tubes remained sterile or showed only a few colonies. In accordance with this Cohen and Markle (1916) made aerobic, partial tension and anaerobic cultures from a case of cerebrospinal meningitis on a medium composed of equal parts of the basic sodium phosphate agar described by Martin (1911), and sterile pleuritic fluid. The pyrogallic acid method was used for the anaerobic cultures, and for the partial tension the culture was connected to a freshly inoculated agar slant of *B. subtilis* by means of a rubber tube. Two colonies appeared on the aerobic tubes, whereas hundreds appeared on the partial tension tubes. The anaerobic cultures remained sterile. In a later article Cohen (1918) again reports favorably on this method and draws the conclusion that the meningococcus is a micro-aerophil, and that the growth ordinarily obtained by aerobic methods consists only of the small minority of meningococci that are resistant to full oxygen tension. Accordingly

Cohen and Fleming (1918) have worked out the optimum amount of air to be replaced by carbon dioxide to culture the meningococcus. Their method has been in use for about six months in the laboratory of the Base Hospital at Camp Jackson. It consists in placing 5 grams of sodium carbonate in a beaker in a 10 liter museum jar containing the cultures either in tubes or on plates. A solution of 5 cc. of sulphuric acid in 35 cc. of water is poured into the beaker and as soon as the violent reaction begins to subside, the cover is clamped down. A tin pail with a close fitting cover has subsequently been used with equally good results. The carbon dioxide thus generated will replace about 10 per cent of the air.

St. John (1919), in a recent paper, concludes that the only advantage in growing the meningococcus in connection with *B. subtilis* and in a partial tension of carbon dioxide comes from the increased moisture in the atmosphere and on the surface of the media under these conditions. He partly replaced the air in Novy jars by means of hydrogen so that the oxygen content varied between 15.6 per cent and 1.2 per cent. He also reduced the oxygen tension by means of *B. subtilis* to amounts varying between 15.3 per cent and 9.5 per cent of atmospheric tension, and reduced the air pressure by means of a vacuum in various degrees. He compared growths obtained under these conditions with that on plates in an atmosphere of air, as well as on plates in a sealed chamber containing moisture. His conclusions are that the moist chamber gives as good or better growth than the other methods. He states that growth is inhibited by carbon dioxide when more than 50 per cent of the air is replaced, but does not say anything as to the effect of the amount recommended by Cohen and Fleming.

It is no doubt true that a moist atmosphere is very beneficial for the growth of the meningococcus, as it probably is in the case of most organisms. But the results of the experiments reported below are in accord with the findings of Cohen and Fleming that the replacement of air by carbon dioxide has a very stimulating effect on the growth of the meningococcus under certain conditions. It did not seem, however, that the

effect could be due to reduced oxygen tension as they claim. The atmosphere contains nearly 21 per cent of oxygen. Replacing 10 per cent of the air with carbon dioxide reduces this figure to between 18 and 19 per cent. The resulting change in pressure is scarcely appreciable as it is within the variations of barometric pressure. But 10 per cent of carbon dioxide in the air is many times the normal figure and carbon dioxide is by no means an inert gas.

In seeking for explanations two other possibilities seemed worthy of consideration in accounting for the effect of carbon dioxide on the growth of meningococcus. It could not be disregarded that the gas might be one of the nutrients necessary for this organism. Such a case would not be without parallel as Nathanson (Loeb, 1917), as well as Winogradsky, have shown. Wherry and Ervin (1918) state that if carbon dioxide is removed from the atmosphere of a culture of the tubercle bacillus, growth is inhibited. Chapin (1918) claims that carbon dioxide has a very beneficial effect on the growth of the gonococcus other than its influence on the reaction of the media in as much as it stimulates growth even in acid reaction. But he does not give the hydrogen ion concentration of the acid reaction used by him. On the other hand the effect of this gas on the reaction of the media seemed the most plausible explanation. The importance of this phase of nutrient media is just beginning to be realized. Cohen and Clark (1918) have recently shown that even in the case of the more common bacteria, *B. bulgaricus*, *B. coli*, *B. aerogenes*, *B. proteus*, *B. dysenteriae*, (Flexner and Shiga), the position of the optimal zone varies enough to prohibit the use of a common reaction.

Let us consider what happens in a slightly alkaline media under a given pressure of carbon dioxide. The gas will be absorbed until the free alkali is converted into sodium bicarbonate. After this reaction has gone to completion the gas will still be absorbed to form carbonic acid, the final concentration depending upon the pressure. A buffer system thus results like that which is so effective in maintaining the constancy of the reaction of the blood stream and other biological fluids, where

the carbon dioxide tension is maintained by the respiratory system. Considerable variation in the pressure does not materially affect the reaction, because appreciable variation in the ratio, $H_2CO_3$: $NaHCO_3$, produces but little change in the hydrogen ion concentration. The result then, of an atmosphere containing a certain tension of carbon dioxide, on media of varying degrees of alkalinity is first to reduce all to the same reaction, and then maintain it by a very delicately adjusted buffer system; for as soon as acid substances are produced by the growing organism, an equivalent amount of carbon dioxide is evolved and the reaction remains unchanged.

In the following experiments 2.5 per cent slant agar tubes made with meat infusion and containing 1 per cent Bacto-peptone and 4 per cent defibrinated human blood were used. The hydrogen ion concentration was determined by comparison with standard solutions of known hydrogen ion concentration. For the titration a "comparator" block was used to hold the tubes as represented in the following diagram.

| | | | |
|---|---|---|---|
| | *Media* | *Water* | *Media* |
| Second row | 4 | 5 | 6 |
| | *Standard* | *Media* | *Standard* |
| First row | 1 | 2 | 3 |

Into the tubes numbered 2, 4, and 6 was put 5 cc. of the media and this was diluted to 15 cc. Into the tubes 1 and 3 was put 5 cc. of the standard solution, also diluted to 15 cc. Tube 5 contained distilled water. At 1 was placed the standard solution with which the media were to be compared. For 3 one tube was prepared of the standard above and one of the standard below that in 1. These two were interchanged as a guide to get a closer comparison between the media and the standard in 1. Into tubes 1, 2 and 3 were put equal amounts of the particular indicator best adapted to the range desired as recommended by Clark and Lubs (1917). Other variable contents of the media will be noted in the different experiments as they are described. For incubation all tubes were placed in 10 liter museum jars which had moisture in the bottom as well as a tumbler containing water. Vaseline was used to make the

covers fit closely on these jars but no attempt was made to clamp them down to maintain a pressure. To produce an atmosphere containing approximately 10 per cent carbon dioxide, 5 grams of sodium carbonate were placed in a tumbler in the jar and a solution of 5 cc. of sulphuric acid in 35 cc. of water poured over it, the cover being placed on the jar as soon as the reaction began to subside. This will be designated the $CO_2$-jar. By air-jar is meant a similar jar with similar moisture conditions but without the carbon dioxide.

A strain of meningococcus was first used which was obtained from the blood culture of a case of meningococcus septicemia. When this culture was twenty-four hours old it was transplanted to brain media (ground beef brain with enough meat infusion broth added to cover the brain after being autoclaved) and incubated without carbon dioxide. By propagating the organism in this medium throughout the experiments, a uniform inoculating material was used and the organism did not become accustomed to an atmosphere of carbon dioxide as the experiments progressed. To inoculate the tubes, 0.2 cc. of the supernatant liquid of a brain media culture was emulsified with 5 cc. of broth and into each tube was introduced a uniform drop of this emulsion. This drop, with the water of condensation in the butt of the agar slant, gave an abundance of liquid to flood the entire slant by properly tipping it, which resulted in a uniform seeding and gave a uniformly moist medium. A system of + signs is used to designate the degree or heaviness of growth. One + means the lightest growth obtained in that particular experiment in which it occurs. Five + is the heaviest growth recorded and in that case the individual colonies were 0.4 cm. in diameter. Two + is a satisfactory growth whereas three + and four + both represent a heavy growth.

*Experiment I. February 1, 1919.* When the first brain media culture was twenty-four hours old it was used to inoculate six blood agar slants, containing 1 per cent glucose, of each of the given reactions. Three of the tubes of each reaction were incubated in the air-jar and the other three in the $CO_2$-jar. The results after eighteen hours are given in table 1.

TABLE 1

| | REACTION OF MEDIA | | | | | |
|---|---|---|---|---|---|---|
| | pH = 7.6 | pH = 7.8 | pH = 8.0 | pH = 8.2 | pH = 8.4 | pH = 8.6 |
| Growth in air-jar..... | 0 | 0 | 0 | 0 | 0 | 0 |
| Growth in $Co_2$-jar..... | +++ | +++ | +++ | ++++ | +++ | + |

This experiment was repeated the next day when the inoculating material was forty-eight hours old with the same results.

*Experiment II. February 3, 1919.* This experiment is a repetition of experiment I with the exceptions that the inoculating culture was seventy-two hours old, and that all the slants, six for each reaction, were set up in the $CO_2$-jar with 10 per cent carbon dioxide a day previous to inoculation. After inoculation three tubes of each reaction were incubated in the $CO_2$-jar and three in the air-jar. The results after twenty-hours are tabulated in table 2.

TABLE 2

| | REACTION OF MEDIA | | | | | |
|---|---|---|---|---|---|---|
| | pH = 7.6 | pH = 7.8 | pH = 8.0 | pH = 8.2 | pH = 8.4 | pH = 8.6 |
| Growth in air-jar..... | ++ | +* | 0 | 0 | 0 | 0 |
| Growth in $CO_2$-jar..... | +++ | +++ | +++ | +++ | +++ | ++ |

*Only one of the three tubes showed any growth.

In these two experiments the media were the same. It will be noticed that the only conditions under which growth was obtained in an atmosphere of air was for the reaction pH = 7.6 and 7.8 when these tubes had first been kept in a $CO_2$-jar for a day previous to inoculation. But the growth with these reactions in the air-jar was distinctly inferior to that in similar tubes incubated in the $CO_2$-jar. Evidently enough carbon dioxide was absorbed the day previous to inoculation to induce some growth by partially adjusting the reaction. This experiment was repeated twice later and in both cases similar results were obtained. From the laws of the solubility of gases it follows that as soon as the tubes are removed from the $CO_2$-jar they begin to lose the gas absorbed by the media in excess of that which combines with the free alkali, and thus the buffer effect of the carbon dioxide is in part lost. This explains why a less

favorable growth resulted in air even though the media had been in the $CO_2$-jar on the day previous to inoculation.

*Experiment III. February 7, 1919.* Six blood agar slants with a reaction of pH = 8.2 and containing 1 per cent glucose, and six of the same lot of media but lacking the glucose were inoculated with a twenty-four hour brain media culture. Three of the glucose and three of the glucose-free tubes were incubated in the air-jar and three in the $CO_2$-jar. After twenty hours incubation all the tubes in the $CO_2$-jar had a heavy growth of meningococcus, no difference being noticeable between the glucose and the glucose-free media. In the air-jar no growth was obtained and none developed upon twenty-four hours subsequent incubation in the $CO_2$-jar. Evidently the carbohydrate cannot supply the carbon dioxide or act as a substitute for it under the given conditions, an assumption that might be made if the gas were being used in the metabolism of this organism.

*Experiment IV. February 11, 1919.* An emulsion of freshly precipitated and washed calcium carbonate was made in the inoculating material, and twelve glucose tubes with a reaction of pH = 8.2 were then inoculated with it. Six of these were incubated in the air-jar and six in the $CO_2$-jar. At the end of twenty hours the tubes in the $CO_2$-jar all had a very satisfactory growth of meningococcus whereas the tubes in the air-jar remained sterile. Apparently the insoluble calcium carbonate cannot bring about the satisfactory condition that is produced by the carbon dioxide. This is what would be expected if it is a matter of adjusting the initial reaction.

*Experiment V. February 11, 1919.* Twelve glucose blood agar slants with a reaction of pH = 8.2 were inoculated in the usual manner. Six of these were incubated in the $CO_2$-jar and six in a similar jar in which 10 per cent of the atmosphere was replaced by nitrogen gas. This was generated from sodium nitrite and ammonium chloride, washed by passage through a solution of sulphuric acid and one of sodium hydroxide and finally by shaking with a solution of sodium hydroxide. After twenty hours incubation the tubes in the $CO_2$-jar had a good growth while the tubes in the $N_2$-jar showed no growth. This is evidence that the carbon dioxide does not produce its effect by diminishing the oxygen tension, with the reaction of media here used. In an experiment given below it will be shown that with more acid reactions, i.e., the optimum for air growths, this gas has an inhibiting effect.

*Experiment VI. February 13, 1919.* An attempt was made to incorporate the carbon dioxide in the media by means of sodium bicarbonate. In each of six bottles was put 100 cc. of glucose-free agar with a reaction of pH = 7.6. After sterilization 2 cc., 4 cc., 6 cc., 8 cc., and 10 cc. of twice normal sodium bicarbonate was added respectively to five of these bottles, the sixth being used for a control. The addition of 2 cc. of twice normal sodium bicarbonate to 100 cc. of this agar gave it a reaction of pH = 7.9. The reaction of the other lots was not determined. Six tubes of each of these lots of media, with human blood, were inoculated in the given way, three incubated in the air-jar and three in the $CO_2$-jar. After twenty hours all the tubes in the $CO_2$-jar had a luxuriant growth whereas in the air-jar only the control tubes, with no sodium bicarbonate, showed any growth and this was scanty. The blood in the tubes containing 6 cc. or more of sodium bicarbonate per 100 cc. of media became very much decolorized upon incubation in the air-jar but retained its bright color in the $CO_2$-jar where the high alkalinity was neutralized by the carbon dioxide. This fact, together with the results of the growth, is evidence that the organism could not tolerate the more alkaline media produced by the addition of the sodium bicarbonate unless this was neutralized by the carbon dioxide. The experiment was repeated the next day and similar results obtained.

*Experiment VII. February 19, 1919.* Upon seeing the results of experiment VI, it was decided to make a series of lots of media with a very wide range of reaction. Eight different reactions were made from a lot of agar with a reaction of pH = 7.2 as represented in table 3.

TABLE 3

| AMOUNT PER BOTTLE | N/1 HCl ADDED | pH | N/1 NaOH ADDED |
|---|---|---|---|
| cc. | cc. | | cc. |
| 250 | 6.0 | 4.7 | 0 |
| 250 | 4.0 | 5.3 | 0 |
| 250 | 2.25 | 6.0 | 0 |
| 250 | 1.5 | 6.7 | 0 |
| 250 | 0 | 7.2 | 0 |
| 250 | 0 | 7.8 | 1.0 |
| 250 | 0 | 8.4 | 4.5 |
| 250 | 0 | 9.0 | 8.0 |

For the two most alkaline reactions there was considerable flocculation during sterilization and a resultant decrease in alkalinity. Consequently a portion of the normal solution of sodium hydroxide was

added after sterilization to bring back the desired reaction. Six tubes of each reaction were inoculated with a four day old brain medium culture of the meningococcus. Three were incubated in the air-jar and three in the $CO_2$-jar. The results are tabulated in table 4.

TABLE 4

| | REACTION OF MEDIA | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | pH = 4.7 | pH = 5.3 | pH = 6.0 | pH = 6.7 | pH = 7.2 | pH = 7.8 | pH = 8.4 | pH = 9.0 |
| Growth in air-jar, 24 hours... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Growth in $CO_2$-jar, 24 hours.. | 0 | 0 | 0 | 0 | ++ | ++ | ++ | ++ |
| Growth in air-jar, 48 hours... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Growth in $CO_2$-jar, 48 hours.. | 0 | 0 | 0 | ++ | ++++ | ++++ | ++++ | ++++ |

This experiment was repeated the next day, using the same culture for inoculation, which was then five days old, and similar results were obtained.

*Experiment VIII. February 21, 1919.* In this experiment six tubes of each of the reactions used in the previous experiment were inoculated with a twenty-four hour brain medium culture which was made from the five day old brain medium culture used in the previous experiment. Three tubes of each reaction were incubated in the air-jar and three in the $CO_2$-jar. The results are tabulated in table 5.

TABLE 5

| | REACTION OF MEDIA | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | pH = 4.7 | pH = 5.3 | pH = 6.0 | pH = 6.7 | pH = 7.2 | pH = 7.8 | pH = 8.4 | pH = 9.0 |
| Growth in air-jar, 24 hours..... | 0 | 0 | 0 | +++ | ++ | + | 0 | 0 |
| Growth in $CO_2$-jar, 24 hours..... | 0 | 0 | 0 | + | ++++ | +++++ | +++++ | +++++ |

It will be noticed that the growth in the air-jar for the reaction pH = 6.7 was decidedly better than that for the same reaction in the $CO_2$-jar; but for the reaction pH = 7.2 the reverse is true, and for the reaction pH = 7.8 the growth is much heavier in the $CO_2$-jar than in the air-jar. The results of this experiment, together with the previous ones, may be interpreted as follows. This organism is very susceptible

to the reaction of the medium. The optimum reaction is found in a medium in which the pH = 7.0 or thereabout. The tension of the carbon dioxide used tends to bring all reactions more alkaline than this down to this figure. As the organism grows it produces acids which displace an equivalent amount of carbon dioxide and therefore do not greatly increase the acidity. This also explains a phenomenon noticed throughout these experiments, i.e., the first growth appears at the upper end of the slant, where the medium is thin and therefore effected more quickly by the carbon dioxide; but the final growth is heaviest on the lower end of the slant. In the $CO_2$-jar the carbon dioxide apparently increases the acidity of an initial reaction of pH = 6.7 enough to exert an inhibiting effect. This serves as additional evidence that this gas exerts its effect entirely by adjusting the reaction of the medium. At any rate any other effect is overshadowed in this experiment. The fact that frequently growths were obtained in the $CO_2$-jar with the less viable inoculations, as will be shown in the experiments given below, when no growth was obtained in the air-jar with any of the reactions used may find its explanation in the closer adjustment of the reaction of the medium to the optimum by the carbon dioxide than was secured by titration.

*Experiment IX. February 23, 1919.* Six tubes each of glucose and of glucose-free blood agar, reaction of pH = 8.0, were inoculated in the given manner and half of each lot incubated in the air-jar and half in the $CO_2$-jar. In this case both jars were thoroughly dried. To replace 10 per cent of the atmosphere in the $CO_2$-jar one liter of carbon dioxide was run into the jar by means of a rubber tube under a tuft of cotton in the bottom to prevent too rapid diffusion and escape of the gas. The gas was measured by replacement with water. A similar tuft of dry cotton was placed in the bottom of the air-jar. At the end of twenty hours all the tubes in the $CO_2$-jar, both glucose and glucose-free had a luxuriant growth whereas in the air-jar only one tube showed any growth and it was very scanty. Here again no difference was noticeable in the growth on the glucose and the glucose-free media in the $CO_2$-jar. This experiment is evidence that the effect of the carbon dioxide is not a moisture effect.

In the last three experiments a number of the tubes were contaminated by a Gram positive bacillus, probably *B. subtilis*. This contamination was only in the butt of the tube and did not obscure the growth of meningococcus in the upper part of

the slant. Moreover, for every different reaction used, at least one of the three tubes was not contaminated. No difference in the density of the growth, or other evidence of symbiotic effect, could be noticed in the contaminated and the uncontaminated tubes of like reaction and otherwise similar conditions.

*Experiment X. February 27, 1919.* Another attempt was made to incorporate the carbon dioxide in the medium by means of sodium bicarbonate, beginning with a more acid reaction. Agar was made up to a reaction of pH 6.5, and 100 cc. put into each of six bottles. The pH after sterilization was 6.8. One bottle was used as a control, and to the other five was added respectively, just before pouring the slants at a temperature of about 45°C., 0.2, 0.4, 0.8, 1.5, 2 grams of sodium bicarbonate. Even the addition of 0.2 gram gave a slight evolution of gas at this reaction and the alkalinity therefore increased to a pH of about 7.2. Six tubes of each of the different lots of media just described, containing the given amount of defibrinated human blood, were inoculated as before with a three day old brain media culture, and six with a twenty-four hour culture. The results after eighteen hours incubation are given in table 6.

TABLE 6

| | GRAMS OF $NaHCO_3$ PER 100 CC. MEDIA | | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.2 | 0.4 | 0.8 | 1.5 | 2.0 |
| *Results from three day old culture* | | | | | | |
| Growth in air-jar | 0 | 0 | 0 | 0 | 0 | 0 |
| Growth in $CO_2$-jar | 0 | + | +++ | +++ | 0 | 0 |
| *Results from one day old culture* | | | | | | |
| Growth in air-jar | +++ | 0 | 0 | 0 | 0 | 0 |
| Growth in $CO_2$-jar | + | ++++ | ++++ | +++ | 0 | 0 |

The results of this experiment bear out the statement above that vigorous organisms grow in an atmosphere of air within a narrow range of reaction; the acidity of the optimum reaction in air is increased by the carbon dioxide used sufficiently to inhibit the growth; with more alkaline reactions this gas produces a condition which is much more favorable and will give growth with the less viable organisms which will no longer grow under the conditions used without the carbon dioxide.

*Experiment XI. March 1, 1919.* The various reactions of media described in table 3, in which the contamination occurred, were again inoculated, six tubes of each reaction with a five day old culture, six with a three day old culture, and six with a one day old culture. The results after twenty hours incubation are given in table 7.

TABLE 7

| | REACTION OF MEDIA | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | pH = 4.7 | pH = 5.3 | pH = 6.0 | pH = 6.7 | pH = 7.2 | pH = 7.8 | pH = 8.4 | pH = 9.0 |
| *Results from five day old culture* | | | | | | | | |
| Growth in air-jar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Growth in $CO_2$-jar | 0 | 0 | 0 | 0 | + | ++ | +++ | + |
| *Results from three day old culture* | | | | | | | | |
| Growth in air-jar | 0 | 0 | + | +++ | ++ | + | 0 | 0 |
| Growth in $CO_2$-jar | 0 | 0 | 0 | + | ++++ | ++++ | +++ | 0 |
| *Results from one day old culture* | | | | | | | | |
| Growth in air-jar | 0 | 0 | + | +++ | ++ | + | 0 | 0 |
| Growth in $CO_2$-jar | 0 | 0 | 0 | ++ | +++ | ++++ | ++++ | +++ |

It will be noticed that in the above experiment this strain of meningococcus shows an inclination to grow within a wider range of reaction than in earlier experiments. Even the three day old culture gave a very satisfactory growth in air although not as abundant as in the $CO_2$-jar. The impression that one received from working with this organism was that in the later experiments it gave a more vigorous growth than in the earlier ones. This was probably due to its becoming accustomed to the artificial media. However the growth obtained above with the one day old inoculating material was distinctly more luxuriant than was obtained with the three day old material, a fact that could not be registered with the system of + signs.

When the above experiments were completed another strain of meningococcus was isolated in the laboratory from a case of cerebrospinal meningitis. Several experiments, similar to those described with the varying reactions of the media, were performed with it and this strain showed the same tendency, in as

striking a manner, to give better growth with the alkaline media in the $CO_2$-jar than was obtained in air with the most favorable reaction.

## CONCLUSIONS

Experiments were conducted with two strains of the meningococcus. The optimum reaction for twenty hour growths on 4 per cent defibrinated human blood agar lies between a pH of 6.7 and 7.4 when incubated in an atmosphere of air. Much better growths can be obtained by making the media with a pH lying between 7.6 and 8.4 and incubating in an atmosphere in which 10 per cent of the air is displaced by carbon dioxide. Frequently the less viable inoculations failed to grow in an atmosphere of air on any reaction of the media used, while a good growth was obtained if 10 per cent of the air in the atmosphere was replaced by carbon dioxide. This was explained by the closer adjustment of the reaction of the media to the optimum by means of the carbon dioxide and by the buffer effect of the equilibrium set up between the gaseous carbon dioxide and the carbonic acid and sodium bicarbonate in the medium. As the organism grows it produces acids which displace an equivalent amount of dissolved carbon dioxide and therefore the acidity is not greatly increased. It is possible that the principles here involved may be applied equally well to the growing of other delicate organisms. By making media with a pH of 7.8 to 8.0 and incubating the meningococcus in a partial atmosphere of carbon dioxide the same media may be used for this organism which are used for the pneumococcus and the streptococcus.[1]

[1] Since the above article was written a paper has appeared by Doctor Gates in the Journal of Experimental Medicine for April vol. xxix, Pp. 325, in which Doctor Gates has similarly shown that the effect of carbon dioxide on the growth of meningococcus is really due to its effect on the reaction on the medium. His experiments do not, however, bring out the fact that at times a more vigorous growth is obtained if the alkalinity of the medium is reduced to the optimum by carbon dioxide rather than by a mineral acid. Theoretically this is no more than would be expected, since there is an equilibrium between the carbon dioxide in the atmosphere and that in the solution.

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