Effects of Bicarbonate on Growth of Neisseria gonorrhoeae: Replacement of Gaseous CO₂ Atmosphere

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Received for publication 23 September 1974

The effect of NaHCO₃ on the growth of Neisseria gonorrhoeae cultures was studied in a liquid and a semisolid growth medium. With a broth culture, NaHCO₃ (0.009 M) greatly reduced the lag phase and also increased the total growth. The same concentration of bicarbonate supported rapid growth when added to the semisolid medium if the plates were individually incubated in sealed plastic bags. In a container with a large air space, a higher concentration of NaHCO₃ was necessary to support growth. The assimilation of ¹⁴C-labeled NaHCO₃ by growing cultures was also investigated.

Most strains of Neisseria gonorrhoeae require an atmosphere of 2 to 10% carbon dioxide for growth, especially on primary isolation. James-Holmquest et al. (1) studied the effect of various atmospheric conditions on growth and reported that after 24 h cultures grown in a candle jar (3 to 4% CO₂) had the greatest number and size of colonies, but after 48 h growth in an incubator containing 10% CO₂ equaled that obtained with the candle jar.

Martin et al. (3) pointed out some of the disadvantages of the candle jar and devised a replacement system with a citric acid-sodium bicarbonate tablet for generating CO₂ in a sealed plastic bag. Trials with clinical samples revealed that the plastic bag method was equally as effective as the candle jar.

The present study demonstrates that the gaseous CO₂ requirement of N. gonorrhoeae can be replaced by the addition of NaHCO₃ directly to the growth medium. Preliminary studies concerning the uptake of radioactive NaHCO₃ by growing cultures of N. gonorrhoeae are also presented.

MATERIALS AND METHODS

Cultures. Primary cultures on Transgrow agar (Texas State Laboratory) were obtained from clinical specimens from the City-County Health Department, Lubbock, Texas (all strains were confirmed as N. gonorrhoeae by Gram stain, positive oxidase reaction, and fermentation of glucose). These cells were transferred to GC medium containing hemoglobin, V-C-N, and IsoVitalex and incubated for 24 h at 35 C. Different cultures were used for each growth experiment. No comparisons of the HCO₃⁻ requirements were made for different strains.

Growth media. The basic growth medium contained the following (grams or milliliters per liter of deionized water): polypeptone (Baltimore Biological Laboratories), 15; glucose, 2; KH₂PO₄, 1; Na₂HPO₄, 3; NaCl g; gelatin, 10; IsoVitalex (BBL), 10 ml; and V-C-N (Grand Island Biological Co.), 5 ml. IsoVitalex, V-C-N, and NaHCO₃ were added aseptically after the basic medium was autoclaved. The final pH was 7.0.

GC agar base medium (BBL) containing IsoVitalex (10 ml/liter) and V-C-N (5 ml/liter) was used as the basic medium for the growth studies on semisolid media.

Growth studies. Cell suspensions were prepared in the broth growth medium and treated in a Vortex-Genie (model K-550-G) to disperse any clumps of cells. The cell suspension (3 to 5 ml) was inoculated into 250-ml nethelo culture flasks (Bellco Glass Inc.) containing 100 ml of prewarmed (35 C) broth medium. The flasks were rubber stoppered, and the cultures were incubated at 35 C on a controlled environmental incubator shaker at 200 rpm (New Brunswick Scientific, model G25). Growth was measured turbidimetrically with a Klett-Summerson colorimeter (model 900-3) with a number 42 filter.

For the growth studies utilizing semisolid media, 0.1 ml of the appropriate cell suspension was inoculated on previously prepared agar plates containing varying amounts of NaHCO₃. The organisms were then dispensed evenly over the surface with a bent glass rod, and the cultures were incubated in either a closed, 2-liter polycarbonate plastic jar with a lid and clamp or in an individual polyethylene bag (6.25 by 5.5 inches [15.9 by 13.9 cm]) (Union Carbide Corp.) secured and taped to insure a tight seal. All the cultures were incubated at 35 C.

¹⁴C-labeled NaHCO₃ uptake studies. ¹⁴C-labeled NaHCO₃ (2 x 10⁷ counts/min, 59.7 mCi/mmol) (New England Nuclear) was added with carrier NaHCO₃ to a final concentration of 0.009 M in the basic growth medium. The cells were harvested, after being incubated at 35 C for 24 h, and washed three times with normal saline solution until the solution was free of radioactivity. The cells were then fractionated by the
methods of Roberta et al., (4). Individual fractions were added to a xylene-based scintillation solution (Aquasol, LSC solution, New England Nuclear) and counted in a Beckman LS-150 liquid scintillation counter.

RESULTS AND DISCUSSION

Figure 1 shows the effect of supplemental NaHCO₃ on the growth of N. gonorrhoeae in a broth culture. The culture grown in the medium containing NaHCO₃ showed a much shorter lag phase and also greater total growth than the culture grown in air without NaHCO₃. Excellent growth after a prolonged lag phase was obtained in the growth medium without supplemental NaHCO₃ or gaseous CO₂.

Somewhat similar results were reported by La Scola and Young (2) using a defined minimal medium. In their growth system, the gonococcal cultures grew in air, if the growth medium had been incubated overnight at 37°C with an atmosphere of 8 to 10% CO₂. However, some strains had a very long lag phase, indicating a possible requirement for a higher concentration of CO₂.

It is of special interest that the concentration of NaHCO₃ found to greatly stimulate early growth of N. gonorrhoeae is in the range of NaHCO₃ concentration (0.012 M) required for the growth of virulent cells of Yersinia pestis in agitated broth cultures at 37°C (5).

With semisolid medium, the concentration of supplemental NaHCO₃ required to support growth of N. gonorrhoeae cultures varied greatly with the size of the container (Table 1). When the plates were incubated in a container with a large air space, the concentration of NaHCO₃ had to be increased to 0.072 M to obtain growth. However, when each individual plate was incubated separately in a tightly sealed plastic bag to reduce the loss of CO₂ to the atmosphere, excellent growth of N. gonorrhoeae was obtained when the NaHCO₃ concentration was 0.009 to 0.09 M. Although, the results are not shown, GC medium containing 0.009 M NaHCO₃ also supported the growth of N. gonorrhoeae slant cultures in screw-cap test tubes or in 8-oz (0.59-liter) prescription bottles. Although only a limited number of clinical samples (40 to 50) have been studied, every strain tested has shown rapid growth (12 to 24 h) when NaHCO₃ was added to the growth medium.

This growth system has several obvious advantages over culture methods using the traditional CO₂ atmosphere. It requires less incubator space, the concentration of bicarbonate can be regulated much easier than gaseous CO₂ and individual plates can be examined without disturbing the atmosphere of other incubating cultures. Further studies will be necessary to determine the effect of NaHCO₃ on colony type and to determine if a NaHCO₃-containing medium can be used in genetic studies or in a transport system.

Growing cultures of N. gonorrhoeae actively assimilate NaHCO₃ from the medium (Table 2). Approximately 10% of the ¹⁴C-labeled NaHCO₃ was incorporated into organic cellular material. The radioactivity was found in every fraction of the cells, with the highest activity being associated with the nucleic acid fraction (hot trichloroacetic acid fraction) and the cellular protein.

![Diagram](http://aem.asm.org/) on March 21, 2020 by guest

**Figure 1.** Effect of NaHCO₃ on growth of N. gonorrhoeae in a broth culture. Symbols: x, with NaHCO₃; O, no addition.
TABLE 2. Incorporation of $^{14}$C-labeled NaHCO$_3$ by growing cultures of N. gonorrhoeae

| Cell fraction                          | Total counts/ min |
|---------------------------------------|-------------------|
| Whole cells                           | $2.20 \times 10^6$|
| Cold trichloroacetic acid soluble      | $1.00 \times 10^6$|
| Alcohol soluble                       | $2.60 \times 10^6$|
| Alcohol-ether soluble                  | $1.13 \times 10^6$|
| Hot trichloroacetic acid soluble       | $1.20 \times 10^6$|
| Protein                               | $8.00 \times 10^6$|

Studies are in progress to identify the radioactive compounds and to characterize the enzyme(s) responsible for the carbon dioxide fixation reactions.

ACKNOWLEDGMENTS

This research was supported by state organized research funds, The Institute for Environmental Chemistry, and Office of the Dean, Graduate School, Texas Tech University.

We wish to thank Dan Dennison, Laboratory Director of the City-County Health Department Laboratory, Lubbock, Texas, for his technical assistance in primary isolation and confirmation of all strains of N. gonorrhoeae.

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ERRATUM

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Volume 29, no. 4. Change “NaHO₃” to “NaHCO₃” in the following places: p. 469, line 8 (abstract) and col. 2, lines 7 and 8 from bottom; p. 470, col. 2, line 6 from bottom; and p. 471, legend to Table 2.

Page 469, col. 2, line 3: Change “NaCl g” to “NaCl, 5…”

Page 470, legend to Fig. 1, last line: Change the symbol “Ο” to “●.”