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Effects of Bicarbonate on Growth of *Neisseria gonorrhoeae*: Replacement of Gaseous CO_2 Atmosphere

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The effect of $NaHCO_3$ on the growth of *Neisseria gonorrhoeae* cultures was studied in a liquid and a semisolid growth medium. With a broth culture, $NaHCO_3$ (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 $NaHCO_3$ was necessary to support growth. The assimilation of ^{14}C-labeled $NaHO_3$ 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% CO_2) had the greatest number and size of colonies, but after 48 h growth in an incubator containing 10% CO_2 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 CO_2 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 CO_2 requirement of *N. gonorrhoeae* can be replaced by the addition of $NaHCO_3$ directly to the growth medium. Preliminary studies concerning the uptake of radioactive $NaHCO_3$ 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 HCO_3 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; KH_2PO_4, 1; Na_2HPO_4, 3; NaCl g; gelatin, 10; IsoVitalex (BBL), 10 ml; and V-C-N (Grand Island Biological Co.), 5 ml. IsoVitalex, V-C-N, and $NaHCO_3$ 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 nephelo 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 $NaHCO_3$. The organisms were then dispersed 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.

^{14}C-labeled $NaHO_3$ uptake studies. ^{14}C-labeled $NaHO_3$ (2×10^7 counts/min, 59.7 mCi/mmol) (New England Nuclear) was added with carrier $NaHCO_3$ 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 Roberts 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 $NaHCO_3$ on the growth of *N. gonorrhoeae* in a broth culture. The culture grown in the medium containing $NaHCO_3$ showed a much shorter lag phase and also greater total growth than the culture grown in air without $NaHCO_3$. Excellent growth after a prolonged lag phase was obtained in the growth medium without supplemental $NaHCO_3$ or gaseous CO_2.

Somewhat similar results were reported by La Scolea 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% CO_2. However, some strains had a very long lag phase, indicating a possible requirement for a higher concentration of CO_2.

It is of special interest that the concentration of $NaHCO_3$ found to greatly stimulate early growth of *N. gonorrhoeae* is in the range of $NaHCO_3$ 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 $NaHCO_3$ 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 $NaHCO_3$ 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 CO_2 to the atmosphere, excellent growth of *N. gonorrhoeae* was obtained when the $NaHCO_3$ concentration was 0.009 to 0.09 M. Although, the results are not shown, GC medium containing 0.009 M $NaHCO_3$ 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 $NaHCO_3$ was added to the growth medium.

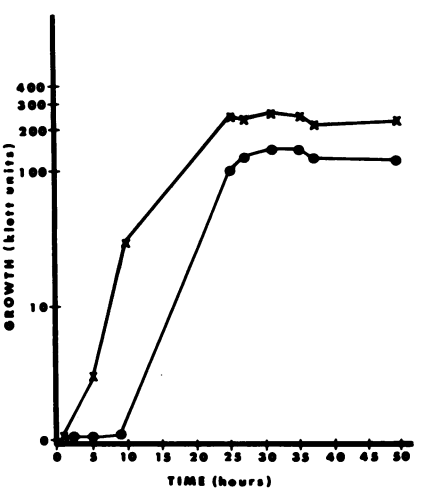

FIG. 1. *Effect of $NaHCO_3$ on growth of N. gonorrhoeae in a broth culture. Symbols: ×, with $NaHCO_3$; O, no addition.*

TABLE 1. *Effect of varying concentrations of $NaHCO_3$ on the growth of N. gonorrhoeae on GC medium*

$NaHCO_3$ concn (M)	Method of incubation	
	Closed 2-liter container	Individual plastic bag
0.00	–[a]	–
0.00 (Candle jar)	+[b]	NA[c]
0.005	–	+
0.009	–	+
0.018	–	+
0.036	–	+
0.054	–	+
0.072	+	+
0.090	+	+

[a] –, No growth in 48 h.
[b] +, Growth in 24 h at least comparable in colony number and size to that in a candle jar.
[c] NA, Not applicable.

This growth system has several obvious advantages over culture methods using the traditional CO_2 atmosphere. It requires less incubator space, the concentration of bicarbonate can be regulated much easier than gaseous CO_2 and individual plates can be examined without disturbing the atmosphere of other incubating cultures. Further studies will be necessary to determine the effect of $NaHCO_3$ on colony type and to determine if a $NaHCO_3$-containing medium can be used in genetic studies or in a transport system.

Growing cultures of *N. gonorrhoeae* actively assimilate $NaHCO_3$ from the medium (Table 2). Approximately 10% of the ^{14}C-labeled $NaHO_3$ 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.

TABLE 2. *Incorporation of ^{14}C-labeled $NaHO_3$ by growing cultures of N. gonorrhoeae*

Cell fraction	Total counts/min
Whole cells	2.20×10^6
Cold trichloroacetic acid soluble	1.00×10^5
Alcohol soluble	2.60×10^5
Alcohol-ether soluble	1.13×10^5
Hot trichloroacetic acid soluble	1.20×10^6
Protein	8.00×10^5

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

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