

Factors Affecting Autolysis of *Neisseria gonorrhoeae*¹ (38025)

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Although scattered observations have been made of autolysis of *Neisseria gonorrhoeae* (1, 2), the phenomenon has not been studied in detail, and the prevalence of autolytic strains is unknown. This report demonstrates that glucose- and nitrogen-limited cultures differ in their susceptibility to autolysis.

Materials and Methods. Organisms. Strains of *Neisseria gonorrhoeae* used in these studies were isolated from the urethral exudate of males with gonococcal urethritis. A stable T-4 colony type (3) of strain CS-7 was selected on GC agar (Difco) plates following passage in the yolk sac and allantoic cavity of a chicken embryo. Strains 71H409, 71H411, and 71H661 were obtained from Dr. E. S. Murray (Harvard School of Public Health). All strains were identified as *N. gonorrhoeae* on the basis of cell morphology in Gram stained smears, oxidase reaction, and the ability to ferment only glucose. Stock cultures were suspended in skim milk, frozen in a dry-ice-ethanol mixture and stored at -70°. Working cultures were maintained in a CO₂ incubator (5% CO₂) at 37° in cystine-tryptic agar containing 0.5% glucose. Cultures were transferred at weekly intervals.

Medium. The basal medium unless otherwise indicated, contained the following per liter: proteose peptone No. 3 (Difco), 15 g, K₂HPO₄, 4 g, KH₂PO₄, 1 g, NaCl, 5 g, and soluble starch, 1 g. A growth factor supplement, similar in composition to Iso

Vitalex enrichment (BBL) but lacking glucose, NaHCO₃, 42 mg/l, and an appropriate energy source, 5 g/l, were added after autoclaving.

Inocula and cultural conditions. Inocula were prepared from a liquid culture grown overnight at 37° in a Model G-76 gyrotory water bath shaker (New Brunswick Scientific Co.). Cells were harvested by centrifugation, washed twice in a solution containing 0.85% NaCl and 0.1% cysteine hydrochloride at pH 6.5 (4). The viability of these cells remained constant for up to one hour. A 1% inoculum, which corresponded to approximately 1 × 10⁷ CFU/ml at zero time, was used in all experiments.

Viable counts. For viable counts, aliquots of the culture were diluted in a solution containing 0.85% NaCl and 0.1%

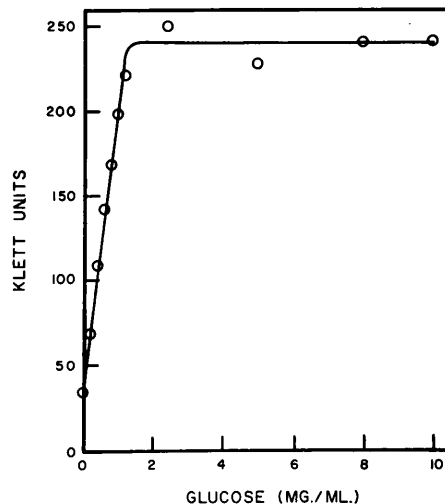


FIG. 1. Effect of glucose concentration on the growth yield of *Neisseria gonorrhoeae* CS-7.

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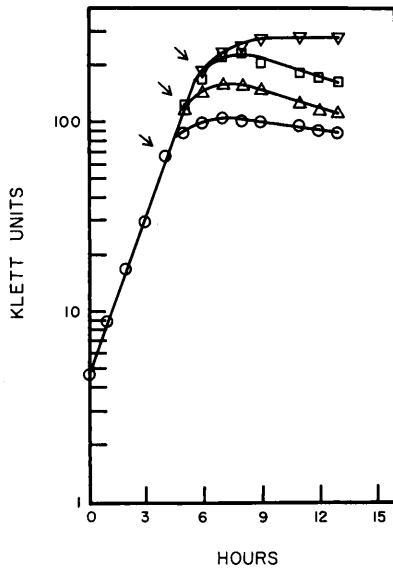


FIG. 2. Relationship between decrease in turbidity and limiting glucose concentrations in broth-grown cultures of *Neisseria gonorrhoeae* CS-7. Arrows indicate time of glucose depletion. Symbols: ○, 0.025% glucose; △, 0.05% glucose; □, 0.10% glucose; ▽, 0.50% glucose.

cysteine hydrochloride (pH 6.5) and plated on GC agar (Difco) plates containing glucose (0.5%) and growth factor supplement. The plates were incubated for 24–48 hr in a CO₂ incubator (5% CO₂) and the colonies counted. Viable counts are

expressed as colony forming units per ml (CFU/ml).

Miscellaneous measurements. Turbidity was measured by Klett-Summerson colorimetry using a No. 54 filter. Glucose uptake was measured by determining the amount of glucose remaining in the medium at the indicated times by the glucostat method (Worthington Biochemical Corp., Freehold, N.J.).

Results. Only glucose, pyruvate, and lactate are utilized efficiently as a source of energy by *N. gonorrhoeae*. The growth of a typical strain CS-7 was directly proportional to the concentration of glucose in the medium up to 1.5 mg/ml (Fig. 1); increasing the concentration of glucose to 10 mg/ml had no appreciable effect upon the growth yield. Similar results were obtained when the basal medium was supplemented with either pyruvate or lactate.

Distinct differences were observed between cultures of strain CS-7 grown with limiting and excess glucose. Figure 2 shows that the turbidity of the cultures decreased after depletion of limiting concentrations of glucose. Microscopic examination indicated extensive cell lysis. A similar decrease in turbidity was not observed in cultures grown with excess glucose (0.50%). There was no correlation between cell lysis and a decrease in pH. End products accumulat-

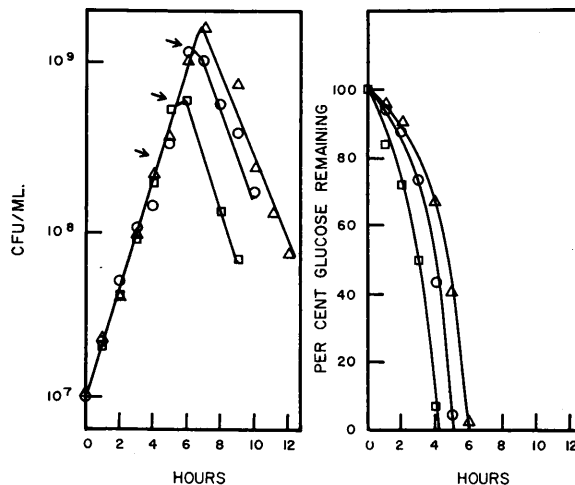


FIG. 3. Effect of limiting glucose on the viability of *Neisseria gonorrhoeae* CS-7. Arrows indicate time of glucose depletion. Symbols: □, 0.025% glucose; ○, 0.05% glucose; △, 0.10% glucose.

TABLE I. Comparison of Viable Counts at Time of Glucose Depletion and at Onset of Autolysis.

Initial glucose concentration	CFU/ml at time of glucose depletion	CFU/ml at onset of autolysis	Generations
0.025%	2.6×10^8	6.0×10^8	2.31
0.050%	5.4×10^8	1.2×10^9	2.22
0.10%	9.7×10^8	1.6×10^9	1.65

ing in the medium during the metabolism of limiting concentrations of glucose were not sufficient to effect a marked change in the pH (7.3–6.9).

An exponential decrease in viability was correlated with the onset of lysis in glucose-limited cultures (Fig. 3). Its initiation was delayed as the glucose concentration increased, but did not begin immediately upon depletion of glucose (as indicated by the arrows). An examination of viable cells at the time of glucose depletion and at the onset of the loss in viability (Table I) revealed that cells underwent approximately one division before the death phase began. This decrease in viability was not limited to strain CS-7 (Table II); the viability of other isolates also decreased markedly in glucose-limited cultures.

We next examined the effect of nitro-

gen limitation on the growth and viability of *N. gonorrhoeae* CS-7. In the presence of excess glucose (0.5%), the growth yield was proportional to the concentration of the nitrogen source up to 0.3% (Fig. 4A). Increasing the concentration above this level had little effect upon the growth yield. Unlike glucose-limited cultures, nitrogen-limited cultures showed no decrease in turbidity or viability for a period of several hours following the cessation of growth (Fig. 4B).

The addition of chloramphenicol (100 $\mu\text{g/ml}$) or rifampicin (2 $\mu\text{g/ml}$) to an exponentially growing culture inhibited any further increase in growth (not shown). However, no decrease in turbidity was observed for a period of several hours. Since the addition of pyruvate, lactate, or glucose to glucose-limited cultures markedly de-

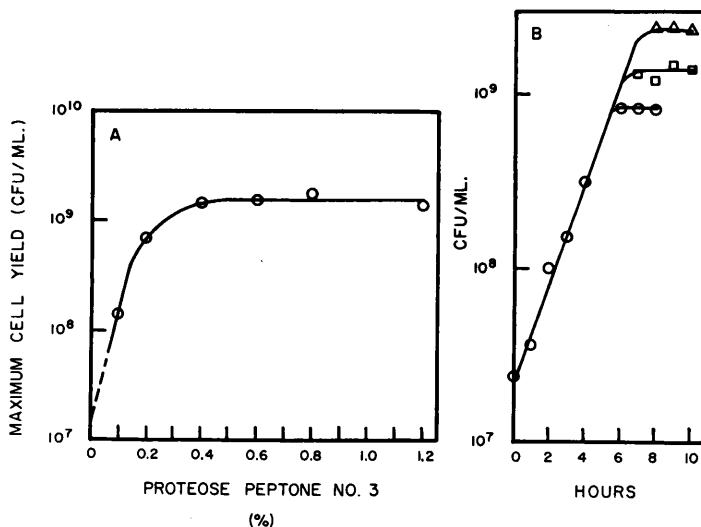


FIG. 4. Effect of the concentration of proteose peptone No. 3 on: (A), Maximum cell yield and, (B) Viability of *Neisseria gonorrhoeae* CS-7. All cultures were grown in the presence of excess glucose (0.5%). Cell yields were determined during the maximum stationary phase of growth. Symbols: \circ , 0.1%; \square , 0.2%; \triangle , 0.4% proteose peptone No. 3.

TABLE II. Effect of Limiting Glucose on the Viability of *Neisseria gonorrhoeae*.^a

Isolate	CFU/ml	
	6 hr	22 hr
CS-7	1.60×10^9	1.40×10^4
71H409	2.22×10^9	2.40×10^6
71H411	2.92×10^9	$\leq 10^1$
71H661	2.82×10^9	2.50×10^2

^a All cultures were grown in basal medium containing 0.1% glucose.

layed the loss of viability, the autolysis of *N. gonorrhoeae* was related to the depletion of its energy source rather than growth limitation or inhibition *per se*. Similar results were obtained with the other strains. Further studies are in progress to elucidate the mechanism(s) involved.

Summary. Glucose-limited cultures of *N. gonorrhoeae* were susceptible to autolysis. The onset of autolysis was not due to a decrease in pH but rather from depletion of the energy source. Cultures limited by nitrogen or by the addition of chloramphenicol or rifampicin were not susceptible to autolysis. The prevalence of autolytic strains appears to be wide spread.

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