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Effect of pH on the Growth and Glucose Metabolism of *Neisseria gonorrhoeae*

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This study examined the effect of pH on the metabolism of glucose by *Neisseria gonorrhoeae*. Radiorespirometric studies revealed that cells growing at pH 7.2 or 8.0 metabolized glucose primarily (ca. 80%) via the Entner-Doudoroff pathway. The remainder of the glucose was metabolized via the pentose phosphate pathway (ca. 20%). The tricarboxylic acid cycle was not active during glucose catabolism at either pH 7.2 or 8.0, and acetate accumulated in the medium. Cells growing at pH 6.0 had markedly increased pentose phosphate pathway activity (ca. 50%) and a functioning tricarboxylic acid cycle. The alteration in pathways was not due to differences in growth rate, but to the pH of the medium. Chemical fractionation of labeled cells and total hexose analyses revealed that growth pH markedly affected the composition of the gonococcus.

Gonococci are obligate human pathogens which can produce asymptomatic or symptomatic infections at many sites. Undoubtedly, there are both organism- and host-related factors which can influence the course of an infection. Each site of colonization and infection represents an environment in which the available nutrients, pH, and competing microorganisms may differ. There are few characteristics of bacteria which are so directly and so markedly affected by the environment as its chemical composition. Cell composition is a direct consequence of metabolic reactions. Therefore, it is likely that the metabolism and, ultimately, the composition of gonococci may vary depending upon the environmental conditions.

Catlin and others (3, 4, 12, 13) have shown that the majority of gonococcal strains are auxotrophic; that is, they require one or more nutritional factors that are not necessarily required by other gonococci. In spite of their numerous nutritional requirements, *Neisseria gonorrhoeae* can survive in a number of environments within the human host, suggesting that sufficient quantities of all required growth factors are present.

It has been demonstrated (3, 4, 12, 13) that the particular growth requirements of a strain of *N. gonorrhoeae* may vary with respect to amino acids, purines, pyrimidines, or vitamins. All strains require an energy source for growth. The nature of this energy source is restricted; only glucose, pyruvate, or lactate are efficiently utilized (16). The study of the metabolism of these energy sources is important since they supply the energy and carbon skeletons needed for the biosynthesis of many important macromolecules. The purpose of this study was to investigate the effect of environmental pH on glucose metabolism during the growth of *N. gonorrhoeae*.

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MATERIALS AND METHODS

Organism. *N. gonorrhoeae* CS-7 was used in most of these studies. A stable T-4 colony type (11) was selected on GC agar (Difco Laboratories, Detroit, Mich.) as previously described (15). Strains JW-31, 71H409, and 72H874 have been described previously (15, 17). *N. gonorrhoeae* FA-19 was obtained from P. F. Sparling (University of North Carolina, Chapel Hill); strain 5713 was obtained from K. Holmes (U.S. Public Health Service Hospital, Seattle, Wash.). The maintenance and the diagnostic criteria for identification of *N. gonorrhoeae* have been described previously (15).

Medium and growth conditions. The basal medium (LGCB) contained the following, per liter of distilled water: proteose peptone no. 3 (Difco), 15 g; K_2HPO_4, 4 g; KH_2PO_4, 1 g; NaCl, 5 g; and soluble starch, 1 g. The final pH of the medium was 7.2. In some experiments, the medium was buffered with 50 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES) adjusted to the desired pH. A growth factor supplement, identical in composition to IsoVitaleX enrichment (Baltimore Biological Laboratory, Cockeysville, Md.), $NaHCO_3$ (420 mg/liter), and glucose (5 g/liter) were added after autoclaving.

Frozen stock cultures were prepared by the method of La Scolea and Young (12) and used for inocula as previously described (7). All liquid cultures were incubated at 37°C (unless otherwise indicated) in a

gyratory shaker (New Brunswick Scientific Co., New Brunswick, N.J.). Turbidity was measured by Klett-Summerson colorimetry, filter no. 54 (540 nm).

Chemicals and radioisotopes. All reagents were purchased from Sigma Chemical Co., St. Louis, Mo. The following isotopes were obtained from New England Nuclear Corp., Boston, Mass.: [1-^{14}C]glucose (specific activity 80 mCi/mmol), [2-^{14}C]glucose (specific activity 4 mCi/mmol), [3-^{14}C]glucose (specific activity 10 mCi/mmol), [3,4-^{14}C]glucose (specific activity 13.7 mCi/mmol), [6-^{14}C]glucose (specific activity 50 mCi/mmol), sodium [1-^{14}C]acetate (specific activity 2.2 mCi/mmol), and sodium [2-^{14}C]acetate (specific activity 2.0 mCi/mmol).

Radiorespirometry and cell fractionations. Pathways of glucose metabolism were determined by radiorespirometry as previously described (17). Acetate utilization was examined radiorespirometrically as described by Hebeler and Morse (7). All constituents were fractionated chemically in screwcapped centrifuge tubes by the procedure described by Morse et al. (17). Radioactivity of samples was measured in a liquid scintillation spectrometer after the addition of 15 ml of scintillation fluid [0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-[2-(5-phenyloxazolyl)] benzene in toluene].

Total hexose determination. Strains of gonococci were grown for 18 h on GC agar (Difco) plates buffered at pH 6.3, 7.2, and 8.0 with 30 mM HEPES. Cells were harvested by suspending the growth in sterile distilled water. They were then washed by centrifugation and resuspended in distilled water. Total hexose was determined by the anthrone reaction (10). Dry weights were determined as previously described (17).

RESULTS

Effect of pH on growth and glucose catabolism in *N. gonorrhoeae* CS-7. Generation times were determined for *N. gonorrhoeae* CS-7 grown in LGCB glucose medium buffered over a pH range of 6.0 to 8.0 with 50 mM HEPES. The results (Fig. 1) demonstrate that the most rapid generation times occurred between pH 7.0 and 7.5. No significant changes in the pH of the medium occurred during the exponential growth phase.

The effect of pH on glucose catabolism was determined by radiorespirometry. The kinetics of the catabolism of specifically labeled glucose by growing cells of *N. gonorrhoeae* CS-7 in LGCB glucose medium buffered with 50 mM HEPES at pH 6.0 and 8.0 are shown in Fig. 2 and 3. The differential rates of [^{14}C]CO_2 evolution varied with the pH of growth. In cells grown at pH 6.0, the differential rates were C1 $\gg$ C4 > C3 > C6 > C2, while in cells grown at pH 8.0 the rates were C1 > C4 $\gg$ C6 > C2 > C3. Significantly more CO_2 was produced from glucose during its catabolism at pH 6.0. The differential rates of [^{14}C]CO_2 evolution observed when cells were grown in LGCB buffered with 50 mM

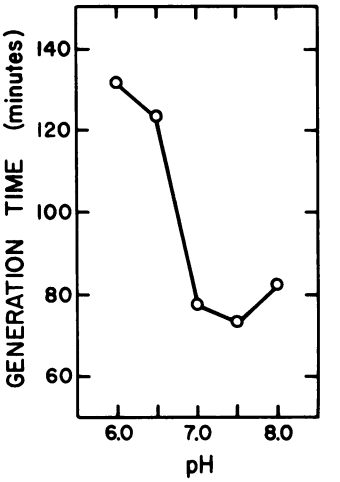

FIG. 1. *Effect of pH on the growth of N. gonorrhoeae CS-7.*

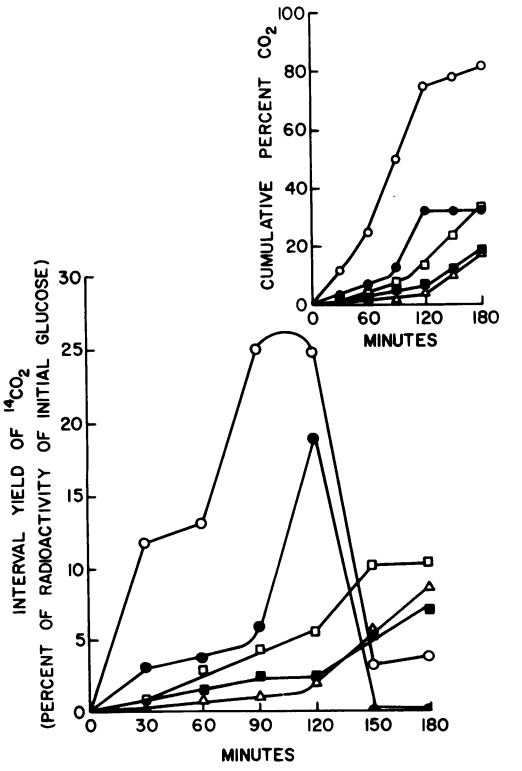

FIG. 2. *Radiorespirometric pattern for the utilization of glucose during growth of N. gonorrhoeae CS-7 at pH 6.0. Flasks contained 69.4 μmol of substrate and 20 mg (dry wt) of cells. Symbols: ○, [1-^{14}C]glucose; □, [2-^{14}C]glucose; △, [3-^{14}C]glucose; ●, [4-^{14}C]glucose (determined by calculation); ■, [6-^{14}C]glucose.*

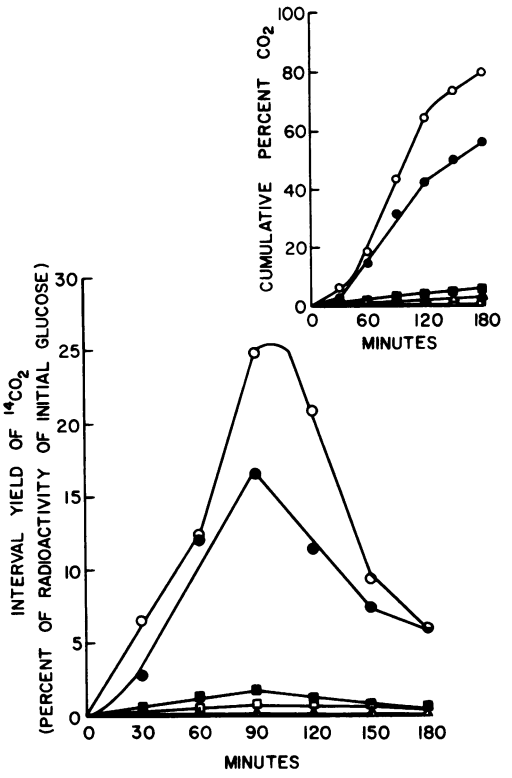

FIG. 3. *Radiorespirometric pattern for the utilization of glucose during growth of N. gonorrhoeae CS-7 at pH 8.0. Flasks contained 69.4 μmol of substrate and 20 mg (dry wt) of cells. Symbols: ○, [1-^{14}C]glucose; □, [2-^{14}C]glucose; △, [3-^{14}C]glucose; ●, [4-^{14}C]glucose (determined by calculation); ■, [6-^{14}C]glucose.*

HEPES at pH 8.0 were very similar to those observed when strain CS-7 was grown in LGCB medium at pH 7.2 (17). In addition, no significant differences in the differential rates were observed between cells grown in LGCB medium buffered with HEPES at pH 8.0 (Fig. 3) or pH 7.2 (data not shown). The inventory of ^{14}C in these experiments is shown in Table 1. The total recoveries were within acceptable limits.

The differences observed in the catabolism of glucose by cells grown at pH 6.0 and 8.0 may be due to differences in the growth rates (Fig. 1) rather than the pH of the medium. To differentiate between these two factors, cells were grown in LGCB glucose medium containing 50 mM HEPES (pH 7.2) and incubated at temperatures ranging from 25 to 40°C. The generation times for *N. gonorrhoeae* CS-7 grown at these temperatures were calculated and are shown in Fig. 4. The shortest generation time was observed between 37 and 38°C; generation times increased rapidly at both lower and higher temperatures.

TABLE 1. *Utilization of [^{14}C]glucose by growing cells of N. gonorrhoeae CS-7*

Substrate	Radioactive inventory[a]			Total ^{14}C recovery (%)
	Respiratory CO_2 (%)	Cells (%)	Medium (%)	
HEPES, pH 6.0, 37°C				
[1-^{14}C]glucose	82	8	11	103
[2-^{14}C]glucose	34	33	34	106
[3-^{14}C]glucose	18	36	45	108
[3,4-^{14}C]glucose	22	42	36	88
[4-^{14}C]glucose[b]	31	48	26	
[6-^{14}C]glucose	19	46	36	92
HEPES, pH 8.0, 37°C				
[1-^{14}C]glucose	80	5	16	97
[2-^{14}C]glucose	3	15	81	90
[3-^{14}C]glucose	1	13	85	87
[3,4-^{14}C]glucose	28	17	54	90
[4-^{14}C]glucose[b]	56	21	23	
[6-^{14}C]glucose	6	24	71	90
HEPES, pH 7.2, 28°C				
[1-^{14}C]glucose	71	3	26	96
[2-^{14}C]glucose	4	11	85	93
[3-^{14}C]glucose	1	11	88	94
[3,4-^{14}C]glucose	25	13	63	90
[4-^{14}C]glucose[b]	49	15	38	
[6-^{14}C]glucose	7	18	75	93

[a] Determined at end of experiment.

[b] Determined by the calculation: 2 × [3,4-^{14}C] − [3-^{14}C] = [4-^{14}C].

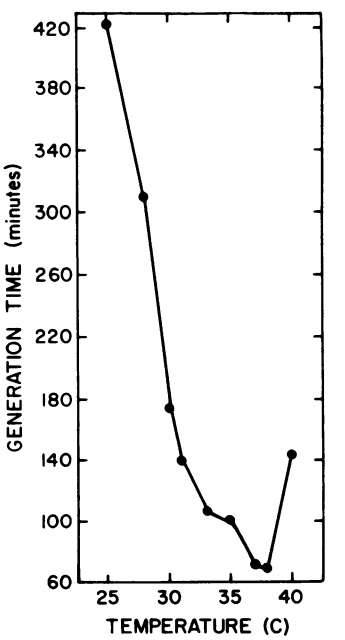

FIG. 4. *Effect of temperature on the growth of N. gonorrhoeae CS-7.*

Glucose catabolism was evaluated radiorespirometrically in cells growing at 28°C (generation time, 310 min) in HEPES-buffered LGCB medium, pH 7.2. The rate of glucose catabolism, as estimated by the rate of $[^{14}C]CO_2$ production from [1-^{14}C]glucose, was slower at pH 7.2 (28°C) (Fig. 5) than at either pH 8.0 (37°C) (Fig. 3) or pH 6.0 (37°C) (Fig. 2). Nevertheless, the differential rates of $[^{14}C]CO_2$ evolution (C1 > C4 ≫ C6 > C2 > C3) at 28°C were similar to those observed at pH 8.0 and 37°C (generation time, 82 min). Therefore, differences in the generation time per se were not responsible for the differences in glucose catabolism observed during growth of *N. gonorrhoeae* CS-7 at pH 6.0 and 8.0.

With prior knowledge of the inherent difficulties involved in estimating concurrent pathways (9), we used the CO_2 yield data (Fig. 2, 3, and 5) and the equations developed by Wang (21) to calculate the relative pathway participation during glucose catabolism by growing cells of *N. gonorrhoeae* CS-7. The results (Table 2) indicate that the pH of growth altered the relative pathway participation. The Entner-Doudoroff (ED) pathway was the major catabolic route (76 to 84% of the glucose) during growth at pH 8.0 (37°C), pH 7.2 (37°C), and at pH 7.2 (28°C); the pentose phosphate (PP) pathway was used to a lesser extent (16 to 24%). Cells grown at pH 6.0 (37°C) utilized the ED and PP pathways to an equal extent.

Effect of pH on glucose incorporation. *N. gonorrhoeae* CS-7 incorporated more than twice as much glucose carbon when grown at pH 6.0 as when grown at pH 8.0 (Table 1). The distribution of the glucose carbon as determined by the chemical fractionation of cells grown at pH 6.0 and 8.0 in the presence of specifically labeled [^{14}C]glucose (Table 3) confirmed the shift in metabolic pathways observed by radiorespirometry. In the ED pathway, carbons 1, 2, and 3 are converted directly to pyruvate and subsequently to acetyl-coenzyme A. The increased incorporation of carbons 2 and 3 into the lipid-containing cell fractions (ethanol soluble plus ethanol-ether soluble fractions) of gonococci grown at pH 8.0 reflects the increased ED pathway activity. The increased amount of carbon 1 incorporated into the nucleic acid cell fraction (hot trichloroacetic acid-soluble cell fraction) of gonococci grown at pH 8.0 may be due to the predominance of $[^{14}C]CO_2$ produced from carbon 1 of glucose, coupled with CO_2 fixation reactions to produce intermediates used for pyrimidine biosynthesis. At pH 6.0, carbons 2, 3, and 6 of glucose were incorporated into the protein-containing cell fraction (papain soluble) to a greater extent than in cells grown at pH 8.0. This difference may reflect the increased activity of the tricarboxylic acid cycle in cells grown at pH 6.0. This possibility will be examined further in a later section.

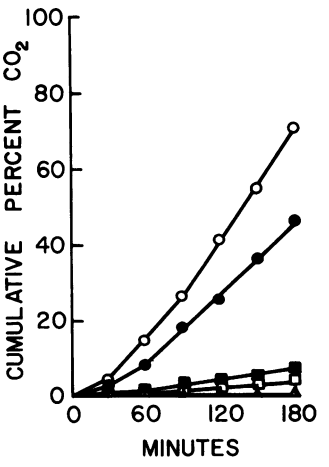

FIG. 5. *Radiorespirometric pattern for the utilization of glucose during growth of N. gonorrhoeae CS-7 at pH 7.2 and 28°C. Flasks contained 69.4 μmol of substrate and 20 mg (dry wt) of cells. Symbols;* ○, *[1-^{14}C]-glucose;* □, *[2-^{14}C]glucose;* △, *[3-^{14}C]glucose;* ●, *[4-^{14}C]glucose (determined by calculation);* ■, *[6-^{14}C]glucose.*

TABLE 2. *Effect of pH and temperature on the pathways of glucose catabolism in N. gonorrhoeae CS-7*

Growth conditions	Participation (%)	
	ED pathway	PP pathway
LGCB, pH 6.0, 37°C[a]	50	50
LGCB, pH 8.0, 37°C[a]	76	24
LGCB, pH 7.2, 28°C[a]	76	24
LGCB, pH 7.2, 37°C[b]	84	16

[a] Medium buffered with 50 mM HEPES at indicated pH.
[b] Data from reference 17.

Effect of pH on total hexose content. The total hexose content of six strains of *N. gonorrhoeae* grown on agar plates at pH 6.3, 7.2, and 8.0 was determined. Some strains were unable to grow at pH 6.0; however, all strains examined were able to grow at pH 6.3. The results (Table 4) show that the total hexose content of all strains was increased by growth at pH 6.3. The average increase for the six strains examined was 56%.

Effect of pH on the metabolism of acetate. Previous studies (17) determined that acetate was the only non-gaseous end product of glucose metabolism produced in any significant concen-

TABLE 3. *Incorporation of specifically labeled [^{14}C]glucose during growth of N. gonorrhoeae CS-7 at pH 6.0 and 8.0*[a]

Cell fraction	Labeled glucose carbon					
	C1 (%)	C2 (%)	C3 (%)	C3,4 (%)	C4[b] (%)	C6 (%)
pH 6.0						
Whole cells	100.0	100.0	100.0	100.0	100.0	100.0
Cold trichloroacetic acid soluble	10.6	6.9	7.9	7.6	7.3	8.4
Ethanol soluble	13.4	37.0	38.5	21.1	17.7	27.7
Ethanol-ether soluble	0.6	1.8	2.0	1.0	0	1.5
Hot trichloroacetic acid soluble	22.7	17.5	14.6	28.8	43.0	28.8
Papain soluble	22.3	17.8	14.2	13.0	11.8	14.0
Residue	30.3	19.0	24.9	21.5	18.1	19.6
Recovery (%)	104.0	109.0	107.0	110.0		111.0
pH 8.0						
Whole cells	100.0	100.0	100.0	100.0	100.0	100.0
Cold trichloroactic acid soluble	4.1	3.7	5.2	4.2	3.2	4.1
Ethanol soluble	7.8	47.7	47.5	31.5	15.5	30.4
Ethanol-ether soluble	1.1	1.9	2.3	1.9	1.5	1.6
Hot trichloroacetic acid soluble	37.4	22.9	20.6	32.9	45.2	34.9
Papain soluble	17.3	6.9	6.0	8.4	10.8	8.4
Residue	32.5	17.8	18.3	21.1	23.9	20.6
Recovery (%)	106.0	93.0	93.0	96.0		97.0

[a] An exponential phase culture (Klett = 90) was harvested by centrifugation and resuspended (0.8 to 0.9 mg [dry wt] per ml) in fresh HEPES-buffered medium and added to a radiorespirometer vessel (25.0 ml/flask). After equilibration at 37°C for 10 min, unlabeled glucose (69 μmol) and the specifically labeled [^{14}C]glucose were added. Cells were harvested and fractionated as described.

[b] Determined by the calculation: 2 × [3,4-^{14}C] − [3-^{14}C] = [4-^{14}C].

TABLE 4. *Effect of growth pH on the hexose content of N. gonorrhoeae*[a]

Strain	Hexose (μg/mg dry wt)		
	pH 6.3	pH 7.2	pH 8.0
CS-7	29.6 (1.46)[b]	20.4 (1.00)	18.5 (0.91)
JW-31	29.4 (1.45)	20.3 (1.00)	21.5 (1.06)
71H409	30.2 (1.30)	23.3 (1.00)	24.3 (1.04)
72H874	20.0 (1.42)	14.1 (1.00)	16.6 (1.18)
FA-19	48.7 (2.52)	19.3 (1.00)	22.7 (1.18)
5713	30.4 (1.19)	25.6 (1.00)	25.0 (0.98)
Average (six strains)	31.4 (1.56)	20.5 (1.00)	21.4 (1.06)

[a] Cells were grown for 18 h on GC agar buffered to the desired pH with 30 mM HEPES. Cells were harvested by suspending the growth in distilled water. Total hexose and dry weight were determined as described in the text. Values represent the average of three experiments. The ranges of the hexose content were ± 10% of the average values.

[b] Numbers in parenthesis represent the amount of hexose relative to that of cells grown at pH 7.2.

tration during exponential growth. Most of the acetate excreted into the growth medium contained carbons 2 and 3 and 5 and 6 of glucose. Additional studies (7) showed that the acetate produced under these conditions was not oxidized until either the glucose was depleted or the culture entered the stationary phase of growth.

The absence of concomitant peaks of [^{14}C]CO_2 containing carbons 2 and 3 (Fig. 3) of glucose suggested that acetate was not oxidized during the dissimilation of glucose at pH 8.0. A different pattern was observed during the dissimilation of glucose at pH 6.0 (Fig. 2). Production of [^{14}C]-CO_2 from carbons 2, 3 (C2 > C3), and 6 of glucose during the active dissimilation of glucose suggested that acetate was being oxidized, albeit at a slow rate, via the tricarboxylic acid cycle.

The oxidation of acetate by *N. gonorrhoeae* CS-7 grown at either pH 6.0 or 8.0 was examined in the presence and absence of exogenous glucose. Cells grown at pH 8.0 (Fig. 6) did not appreciably oxidize acetate until after the glucose was depleted (t = 90 min). When similar cells were suspended in HEPES-buffered medium (pH 8.0) without glucose, acetate oxidation occurred earlier, but not to a great extent. A different pattern of acetate oxidation was observed in cells grown at pH 6.0. The pattern of acetate oxidation in the presence or absence of exogenous glucose was essentially identical. There was substantially more acetate oxidized by cells grown at pH 6.0 than by cells grown at pH 8.0 (Fig. 6; Table 5). The ratio of [^{14}C]CO_2 production from the C1 and C2 carbons of acetate was about 2.4, suggesting a functional tricarboxylic acid cycle. The incorporation of ace-

tate carbon by cells grown at pH 6.0 was three to four times greater than by cells grown at pH 8.0 (Table 5).

Chemical fractionation of cells labeled during growth at pH 6.0 and 8.0 in the presence of [^{14}C]acetate (Table 6) revealed a marked effect of growth pH on the incorporation of acetate carbon. No significant difference was observed in the incorporation of [1-^{14}C]- or [2-^{14}C]acetate, which suggested that the molecule was assimilated as a unit. At pH 8.0, the majority of the acetate carbon (>81%) was incorporated into the lipid-containing cell fractions (ethanol plus ethanol-ether soluble fractions). About twice as much acetate carbon was incorporated at pH 8.0 in the presence of glucose (Table 5) as in its absence. At pH 6.0, the amount of acetate incorporated was similar in the presence or absence of glucose (Table 5). Acetate carbon was incorporated into all cell fractions (Table 6). Significantly less acetate carbon was incorporated at pH 6.0 into the lipid-containing cell fractions and more was incorporated into the residue fraction which is rich in cell wall material.

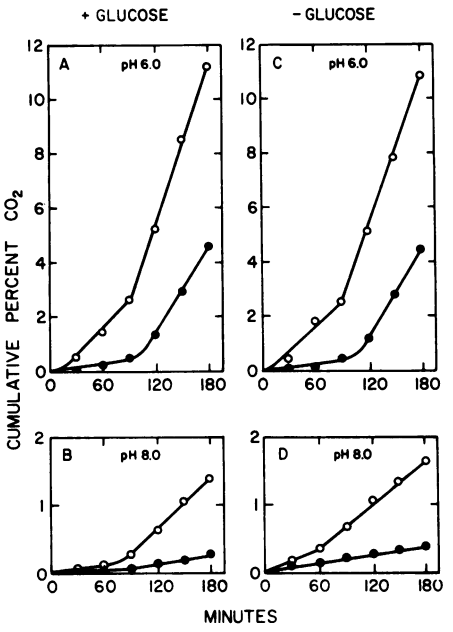

FIG. 6. *Effect of glucose and pH on the utilization of labeled acetate by N. gonorrhoeae CS-7. Symbols: ○, [1-^{14}C]acetate; ●, [2-^{14}C]acetate.*

In an attempt to determine whether the effect of pH on acetate metabolism resulted from a coarse or fine control of the activity of the tricarboxylic acid cycle, cells were grown to midlogarithmic phase in HEPES-buffered LGCB glucose media at pH 6.0 and 8.0. Cells grown at pH 6.0 were harvested by centrifugation and resuspended in fresh medium (pH 8.0) containing 69.4 μmol of glucose and 100 μg of chloramphenicol per ml. Cells grown at pH 8.0 were harvested and resuspended in a similar medium at pH 6.0. If the tricarboxylic acid cycle was regulated by a direct or indirect effect of pH on the activity of a key enzyme, then cells grown at pH 8.0 would oxidize acetate when shifted to pH 6.0, while cells grown at pH 6.0 would not oxidize acetate when shifted to pH 8.0. The addition of chloramphenicol should distinguish between direct and indirect effects of pH on enzyme synthesis. Radiorespirometry (data not shown) indicated that <1% of the added acetate was oxidized at either pH, suggesting that both enzyme induction (pH 8.0-grown cells shifted to pH 6.0) and regulation of enzyme activity (pH 6.0-grown cells shifted to pH 8.0) are involved. Further investigations on the regulation of the tricarboxylic acid cycle in *N. gonorrhoeae* are in progress.

DISCUSSION

The pH of the environment is an important

TABLE 5. *Utilization of [^{14}C]acetate by growing cells of N. gonorrhoeae CS-7*

Substrate	Glucose (69 μmol)	Radioactive inventory[a]			Total ^{14}C recovery (%)
		Respiratory CO_2 (%)	Cells (%)	Medium (%)	
HEPES, pH 6.0, 37°C					
[1-^{14}C]acetate	+	11.3	11.1	78	100
[2-^{14}C]acetate	+	4.5	14.6	81	99
[1-^{14}C]acetate	−	10.7	12.8	77	98
[2-^{14}C]acetate	−	4.4	14.5	81	95
HEPES, pH 8.0, 37°C					
[1-^{14}C]acetate	+	1.3	4.0	95	99
[2-^{14}C]acetate	+	0.2	4.0	96	101
[1-^{14}C]acetate	−	1.4	2.4	96	99
[2-^{14}C]acetate	−	0.4	2.4	97	101

[a] Determined at end of experiment.

TABLE 6. *Effect of pH and glucose on the incorporation of [1-^{14}C]- and [2-^{14}C]acetate by N. gonorrhoeae CS-7*[a]

Cell fraction	Incorporation			
	+Glucose		−Glucose	
	[1-^{14}C]acetate (%)	[2-^{14}C]acetate (%)	[1-^{14}C]acetate (%)	[2-^{14}C]acetate (%)
pH 6.0				
Whole cells	100.0	100.0	100.0	100.0
Cold trichloroacetic acid soluble	5.7	6.8	4.8	6.3
Ethanol soluble	68.3	62.8	66.3	61.0
Ethanol-ether soluble	1.7	2.0	2.3	1.6
Hot trichloroacetic acid soluble	2.7	5.0	3.6	8.2
Papain soluble	4.0	5.0	4.9	4.5
Residue	17.5	18.3	18.1	18.4
Recovery (%)	93.1	97.2	96.7	96.0
pH 8.0				
Whole cells	100.0	100.0	100.0	100.0
Cold trichloroacetic acid soluble	8.7	8.3	15.0	8.3
Ethanol soluble	76.8	73.4	82.1	89.4
Ethanol-ether soluble	5.0	7.6	<1.0	<1.0
Hot trichloroacetic acid soluble	1.6	<1.0	<1.0	<1.0
Papain soluble	2.4	5.3	<1.0	<1.0
Residue	5.4	5.4	2.9	2.3
Recovery (%)	104.0	106.0	96.5	100.4

[a] An exponential phase culture (Klett = 90) was harvested by centrifugation and resuspended (0.8 to 0.9 mg [dry wt] per ml) in fresh HEPES-buffered medium with or without glucose (69.4 μmol). [1-^{14}C]- or [2-^{14}C]-acetate (ca. 1.0 μCi/ml) was added after equilibration at 37°C for 10 min. Cells were harvested and fractionated as described in the text after 3 h of incubation.

parameter in bacterial growth. Early investigators (5, 20) ascertained that gonococci had a pH optimum of 7.3 but could grow over a pH range of 5.8 to 8.3. Similar results were obtained in the present study. Brooks and Hedén (2) determined the growth yields of *N. gonorrhoeae* in batch cultures at constant pH values between 5.8 and 7.4. In a complex medium containing proteose peptone no. 3 and glucose, the greatest cell yield (1.5 g [dry wt]/liter) was obtained at a growth pH of 6.4. This pH value is considerably less than the pH optimum for growth.

Previous studies (8, 14, 17) demonstrated that growing cells of *N. gonorrhoeae* utilize glucose by strictly aerobic mechanisms involving the ED and PP pathways. Acetate, which is excreted into the medium, is the only non-gaseous end product detected (17). Little variation between strains was observed in the relative participation of the ED (ca. 80%) and PP (ca. 20%) pathways. The chemical composition of the medium had little effect upon the dissimilation of glucose (16). However, the prior growth history of the cells was important. Cells initially grown on pyruvate exhibited a significant increase in the participation of the PP pathway (ca. 32%) during the catabolism of glucose (16).

The effect of pH on glucose catabolism was determined by radiorespirometry. Cells grown at pH 6.0 catabolized 50% of the glucose via the ED pathway and the remainder via the PP pathway. These cells also had an active tricarboxylic acid cycle, as evidenced by the concomitant production of CO_2 from carbons 2, 3, and 6 of glucose coupled with their ability to oxidize exogenous acetate. The involvement of the tricarboxylic acid cycle would increase the amount of energy which could be obtained from glucose. The data show that cells growing at pH 6.0 incorporate about twice as much glucose carbon as cells growing at pH 8.0. Cells grown at pH 7.2 or 8.0 catabolize glucose primarily via the ED pathway (ca. 80%) and do not have an active tricarboxylic acid cycle.

Differences in growth rates cannot explain the effect of pH on glucose metabolism in *N. gonorrhoeae*. There are probably several mechanisms by which pH can regulate the activities of the ED and PP pathways. Intracellular pH values may be influenced by the external H^+ concentration (18). A decrease in the internal pH may directly or indirectly increase the activity of 6-phosphogluconate dehydrogenase (EC 1.1.1.44). Alternatively, the activities of the specific ED pathway enzymes, 6-phosphogluconate dehydratase (EC 4.2.1.12) and 6-phospho-2-keto-3-deoxy-gluconate aldolase (EC 4.1.2.14) may be affected.

The factors which regulate the activity of the tricarboxylic acid cycle in *N. gonorrhoeae* are largely unknown. Recently, we have obtained evidence suggesting that citrate synthase (EC 4.1.3.7) is the enzyme which regulates the activity of the tricarboxylic acid cycle in *N. gonorrhoeae* (16). This enzyme is inhibited by reduced nicotinamide adenine dinucleotide phosphate, ATP, and reduced nicotinamide adenine dinucleotide. Glucose-grown cells of *N. gonorrhoeae* have all the tricarboxylic acid cycle enzymes present, although some are present at levels that cannot be measured by spectrophotometric assays (7). The shift in the relative activities of the ED and PP pathways may alter the intracellular levels of reduced nicotinamide adenine dinucleotide phosphate, ATP, and reduced nicotinamide adenine dinucleotide so as to relieve the inhibition of citrate synthase.

Cells grown at pH 6.3 contain more total hexose than cells grown at either pH 7.2 or 8.0. This observation can be explained, in part, by the observed differences in the metabolism of glucose. At pH 7.2 or 8.0, gonococci have a rapid generation time, accumulate acetate in the medium and therefore must utilize a larger percentage of the glucose as a source of energy. At acid pH values, gonococci grow more slowly, have concomitant tricarboxylic acid cycle activity, and have the potential for obtaining more energy from each molecule of glucose. Under these conditions, more glucose carbon would be available for biosynthesis. The identity of the hexose-containing cellular component(s) which increase during growth at pH 6.3 is not known; however, several candidates exist. Hebeler, Morse, Wong, and Young (*In* G. F. Brooks et al., ed., *Immunobiology of Neisseria gonorrhoeae*, in press) reported that the peptidoglycan content of gonococci increased during growth at pH 6.0. An increase in the incorporation of acetate during growth at pH 6.0 into the cell wall-containing fraction was observed in this study (Table 6). An increase in the amount or nature of the lipopolysaccharide or capsule cannot be ruled out at this time. Richardson and Sadoff (19) reported that capsule production was most apparent when gonococci were cultivated on agar medium in the presence of a strain of microaerophilic "viridans streptococci." Viridans streptococci excrete lactic and acetic acids into the medium when grown on a glucose-containing agar medium (6). The accumulation of these compounds can produce a marked decrease in the pH of the medium (6). Preliminary results (data not shown) using a nigrosin-safranin capsule stain (1) suggest that some laboratory strains of *N. gonorrhoeae* exhibit an increased capsule size when grown on solid medium at pH 6.3.

The changes in metabolism which occur in cells growing at pH 6.0 may ultimately have survival value for the organism. In vivo the gonococci compete with the host's cells, and possibly with other microorganisms, for limiting concentrations of available energy sources. The ability to obtain more energy from each substrate molecule would be a definite advantage. In addition, the presence of an active tricarboxylic acid cycle may increase the number of compounds which can be utilized as sources of energy.

Cells grown or placed in an environment with an acid pH may be more stable and less prone to autolysis since peptidoglycan hydrolysis (22) and phospholipid hydrolysis (unpublished data) are markedly reduced. Peptidoglycan hydrolysis (22) and phospholipid hydrolysis are optimal at pH 8.5. This may contribute to the susceptibility of gonococci to autolysis when grown at alkaline pH values (2).

We have demonstrated that an environmental parameter, pH, can markedly alter cellular metabolic processes. These processes ultimately determine the chemical composition of the cell. Further studies on the effect of pH on the metabolism and composition of gonococci are in progress.

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