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Growth of Several Cariogenic Strains of Oral Streptococci in a Chemically Defined Medium

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A chemically defined medium in which *Streptococcus mutans* strains AHT, BHT, GS-5, JC-2, Ingbritt, At6T, At9T, 6715, and OMZ-176 and *Streptococcus salivarius* strain HHT grew rapidly to high turbidities was formulated. Maximal turbidities of each strain were observed after 8 to 12 h of aerobic growth. The subsequent transfer of exponentially growing cells into fresh medium resulted in growth at the same rate without lag. Growth of these strains occurred with rates at least one-half of those observed in an organic medium, such as Todd-Hewitt broth. *S. mutans* strains FA-1 and OMZ-61 grew at relatively slow rates in the defined medium, but more rapid growth to higher turbidities of both strains was obtained when sodium bicarbonate was added to the medium. *Streptococcus sanguis* strain OMZ-9 and another group H streptococcus (strain 72x46) grew rapidly in the defined medium after the addition of sodium carbonate. The presence of carbonate or bicarbonate yielded higher turbidities of all the other strains, and the growth rates of several of the strains tested were also increased.

The in vivo growth and nutrition of cariogenic streptococci bears considerable significance in the cariogenic process. Dental plaque principally results from bacterial growth and retention of microorganisms, including streptococci, on smooth tooth surfaces (12, 15). Physiological characteristics, such as acid production and cell wall thickening, have been shown to be influenced by growth and nutrition in other streptococci and lactobacilli (14, 20, 21). In addition to thickening of cell walls (24) and lactic acid metabolism (13), other physiological properties, such as production of dextran and levan from sucrose (11, 12, 22) and intracellular polysaccharide synthesis (25), are also directly or indirectly related to growth, nutrition, and ultrastructure of cariogenic streptococci. Therefore, to study growth and nutrition and the subsequent effects on the physiology of these microorganisms, it is preferable to grow them in a chemically defined medium.

There have been relatively few studies of oral streptococci grown in chemically defined media. These studies have generally involved variations of growth components and incubation times, and quantitation of results on the basis of bacterial turbidities without determining growth rates (4-8, 16). Carlsson (4-7) obtained good growth yields of many streptococci for numerous transfers, but incubated cultures for 120 h. Lawson (16), on the other hand, grew cultures for 18 to 20 h, but had to use large inocula and perform several transfers before obtaining good growth yields. In the above-cited studies, growth rates were not determined, and chemically defined media differed. The present study was undertaken to obtain a chemically defined medium in which cariogenic and other oral streptococci could grow as rapidly as they do in an organic medium such as Todd-Hewitt broth when incubated either aerobically or anaerobically.

MATERIALS AND METHODS

The human cariogenic strains used in this study included *S. mutans* AHT and BHT, which were obtained from A. S. Bleiweis, University of Florida, Gainesville; *S. mutans* GS-5 and 6715, which were obtained from S. Weiss and A. Gaffar, Colgate-Palmolive Co., Piscataway, N.J.; and *S. mutans* OMZ-176 and *S. sanguis* OMZ-9, which were obtained from B. Guggenheim, University of Zurich, Switzerland. *S. salivarius* strain HHT (obtained from A. S. Bleiweis) was also isolated from humans but was demonstrated to be less cariogenic in animals than the *S. mutans* strains (12). *S. mutans* FA-1 (obtained from A. S. Bleiweis) and OMZ-61 (obtained from B. Guggenheim) were cariogenic strains isolated from rats (10, 12). Cariogenic *S. mutans* strains At6T, At9T, and IB (Ingbritt) were obtained from J. Van Houte, Forsyth Dental Center, Boston, Mass., whereas *S. mutans* JC-2 was obtained from J. Carlsson, University of Umeå, Sweden, and strain 72x46, a Lancefield group H streptococcus, was obtained

from R. M. Cole, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

Growth media. Todd-Hewitt broth supplemented with 2% D-glucose (THG) was heat-sterilized by autoclaving for 15 min at 121 C and stored at 4 C. All streptococcal strains were subcultured weekly on Todd-Hewitt agar or blood agar. Plates were routinely incubated for 24 h at 37 C in a candle jar with 10% carbon dioxide and stored at 4 C between subcultures.

All chemically defined media used in this study basically consisted of 2% glucose, amino acids, vitamins, phosphate buffer, salts, and nitrogenous bases (Table 1). During the course of the development of the final medium, two modified versions of the *Streptococcus faecalis* medium (19, 23) were utilized. Medium M1 (Table 1, column 3) differed from *S. faecalis* medium in that it contained 0.02 M instead of 0.3 M phosphate. Medium MC (Table 1, column 4) is medium M1 to which 225 μg of sodium citrate per ml was added. The third chemically defined medium used (FMC; Table 1, column 5) was based on medium MC and contained twice the vitamin concentration of the other media and a phosphate concentration of 0.043 M. In preliminary experiments, the *Lactobacillus acidophilus* medium was also used because it contained ingredients not present in *S. faecalis* medium and was lower in phosphate content (9). The compositions of all chemically defined media are shown in Table 1. All chemicals were obtained from either General Biochemical Co., Laboratory Park, Chagrin Falls, Ohio, or J. T. Baker Chemical Co., Phillipsburg, N.J.

During the course of these studies, defined media were supplemented with L-cysteine, sodium carbonate, and sodium bicarbonate. Freshly prepared solutions were routinely sterilized by filtration through an 0.45-μm membrane in a Millipore Swinnex no. 25 filter and were added immediately before inoculation of cultures.

Growth measurements. Growth of cultures incubated at 37.8 ± 0.1 C in a water bath was measured by recording the absorbance in a Coleman model 14 spectrophotometer set at a wavelength of 675 nm. In every growth experiment, a minimum of 6 ml of media was aseptically pipetted into heat-sterilized, calibrated culture tubes (18 by 150 mm) with Bellco stainless-steel tops. The net observed optical density readings of cultures were multiplied by 1,000 and then converted to adjusted optical density (AOD) units (19, 23). After conversion to AOD units, readings agree with Beer's law and are proportional to bacterial mass.

The turbidities of *S. mutans* strains FA-1, GS-5, and OMZ-176 and *S. salivarius* strain HHT after 24 h of growth were quantitated by dry weight determinations. Cell suspensions (50 ml of washed, stationary-

TABLE 1. *Composition of chemically defined media*[a]

Components (per ml)	*L. acidophilus* medium (μg)[b]	*S. faecalis* medium	M1	MC	FMC
KH_2PO_4	440	+	+	+	+
K_2HPO_4	300	+	+	+	+
Na_2HPO_4	—	26.65 mg	2.10 mg	2.10 mg	3.15 mg
NaH_2PO_4	—	16.45 mg	–	–	2.05 mg
Sodium citrate	225	–	–	+	+
Riboflavin	0.2	+	+	+	0.4 μg
Biotin	0.005	+	+	+	0.01 μg
Folic acid	0.05	+	+	+	0.1 μg
Pantothenate	0.4	+	+	+	0.8 μg
p-Aminobenzoic acid	0.05	+	+	+	0.1 μg
Thiamine	0.2	+	+	+	0.4 μg
Nicotinamide	1.0	+	+	+	2.0 μg
Pyridoxamine	0.4	+	+	+	0.8 μg
Asparagine	5.0	–	–	–	–
Thymine	4.0	–	–	–	–
Spermidine	5.0	–	–	–	–
Deoxyguanosine	8.0	–	–	–	–
Adenylic acid	16.0	–	–	–	–
Cytidylic acid	50.0	–	–	–	–
Tween 80	1.0	–	–	–	–

[a] All media also contained the following components per ml: 20 mg of D-glucose, 6 mg of sodium acetate, 600 μg of $(NH_4)_2SO_4$, 35 μg of adenine, 27 μg of guanine, 30 μg of uracil, 200 μg of $MgSO_4$, 10 μg of NaCl, 10 μg of $FeSO_4$, 10 μg of $MnSo_4$, 5 μg of glutamine, 300 μg of L-glutamic acid, and 110 μg of L-lysine, L-aspartate, L-isoleucine, L-leucine, L-methionine, L-serine, L-phenylalanine, L-threonine, and L-valine were present at a concentration of 100 μg/ml, whereas DL-alanine, L-arginine, L-cystine, L-histidine, glycine, L-hydroxyproline, L-proline, L-tryptophan, and L-tyrosine were present at 200 μg/ml.

[b] *L. acidophilus* medium was adjusted to pH 7.0 with KOH; all other media were adjusted to pH 6.5 ± 0.1 with NaOH. Media were filter-sterilized through an 0.45-μm membrane filter. The sterilized media were poured aseptically into sterile flasks and stored at 4 C for no longer than 5 weeks. Tween 80 is shown in milligrams.

phase cultures) were dried over H_2SO_4 and P_2O_5 in a desiccator evacuated to a pressure of 0.5 mm of Hg. One AOD unit was found to be equivalent to 0.39 μg of cellular dry weight per ml.

Growth studies. Cells from colonies on blood agar or Todd-Hewitt agar were inoculated in a culture tube containing 6 to 10 ml of either THG or chemically defined media. The culture was incubated overnight for 15 to 20 h in the water bath. The turbidities obtained by these overnight cultures varied from 1,200 to 3,000 AOD units. Approximately 0.1 to 0.2 ml of these cells was used as an inoculum for 6 to 10 ml of media in a second tube, yielding an initial AOD between 20 to 80. The culture was then incubated and growth was followed turbidimetrically with readings every 30 min. Prior to each growth measurement, cultures were vigorously agitated with a Vortex mixer. After the reading, the cultures were re-incubated in the water bath.

The time required for the turbidity of a culture to double during exponential growth (T_D) was graphically estimated in minutes from plots of AOD readings versus time. Doubling times during exponential growth were routinely confirmed by inoculating 1 ml of an exponential-phase culture into a second tube containing 9 ml of fresh, prewarmed media. Growth in the second tube was followed turbidimetrically and, if necessary, a further 1:10 dilution of this culture was made.

RESULTS

Initial experiments. Initial experiments were performed with the nine strains of oral streptococci listed in Table 2. All nine strains failed to grow in *S. faecalis* medium but grew to variable and visible turbidities in *L. acidophilus* medium after incubation for 30 h. Reducing the phosphate buffer concentration of *S. faecalis* medium from 0.3 to 0.02 M permitted growth of seven of the nine strains (Table 2). For five strains (AHT, GS-5, 6715, OMZ-176, and HHT), growth was relatively rapid and turbidity measurements after 23 h approached those observed in THG. Two strains, BHT and OMZ-9, grew poorly and growth was not transferable. The addition of L-cysteine (100 μg/ml) slightly increased the growth yield of strains BHT and OMZ-9 but did not permit subculture of these two strains. L-Cysteine addition affected neither the growth rate nor final turbidity of strains OMZ-61 and FA-1.

Effect of citrate on growth. Two strains of *S. mutans*, FA-1 and BHT, grew to higher turbidities in *L. acidophilus* medium than they did in M1. Addition of individual components of *L. acidophilus* medium to M1 showed that supplementation of M1 with sodium citrate (225 μg/ml) resulted in growth of both strains to high turbidities after overnight incubation. In medium M1 plus citrate (medium MC), strain BHT grew at a T_D of 61 min after a short lag (Fig. 1), and an AOD of 2,000 was obtained after 23 h of growth. Subculture of an exponentially growing culture of BHT into fresh, prewarmed MC medium resulted in continued exponential growth at the same T_D (61 min). Citrate addition improved growth of strain FA-1 (to a T_D of 120 min and 1,800 AOD at 23 h) but did not affect the growth rates or turbidities at 23 h of the other strains listed in Table 2.

TABLE 2. *Growth of streptococci in M1 medium and in THG*

Microorganism	Chemically defined M1 medium		THG broth	
	T_D (min)	Turbidity at 23 h (AOD)	T_D (min)	Turbidity at 23 h (AOD)
S. mutans strains				
AHT	64	2,400	47	2,400
OMZ-61	120	1,300	35	2,000
FA-1	140	1,400	45	1,600
BHT	175[a]	300[a]	44	2,300
GS-5	78	2,100	48	2,200
6715	66	1,800	45	2,000
OMZ-176	52	2,400	38	2,200
S. sanguis strain				
OMZ-9	140[a]	400[a]	38	1,600
S. salivaris strain				
HHT	59	1,200	43	1,800

[a] Growth of these strains was not transferable and the indicated T_D do not represent true exponential doubling times.

Effect of sodium bicarbonate and sodium carbonate on growth of S. mutans strains FA-1 and OMZ-61 and S. sanguis OMZ-9. The addition of sodium bicarbonate (0.024 M) to medium MC greatly decreased the doubling time (to a T_D of 80 min) and increased the growth yield (to 2,200 AOD at 7 h) of *S. mutans* FA-1 (Fig. 2A). In MC plus HCO_3^-, subculture of exponential-phase cells of strain FA-1 into fresh, prewarmed medium resulted in continued growth at the same rate. Similarly with strain OMZ-61, sodium bicarbonate decreased the doubling time from a T_D of 120 to a T_D of 67 min and increased growth yield from 1,300 AOD at 23 h to 2,700 AOD at 8 h. For strains FA-1 and OMZ-61, sodium carbonate failed to duplicate the effect of sodium bicarbonate. In contrast, with *S. sanguis* OMZ-9, sodium carbonate (0.019 M) increased the rate of growth (to a T_D of 50 min) and growth yield (to 2,900 AOD at 7 h) whereas sodium bicarbonate had little effect on growth (Fig. 2B).

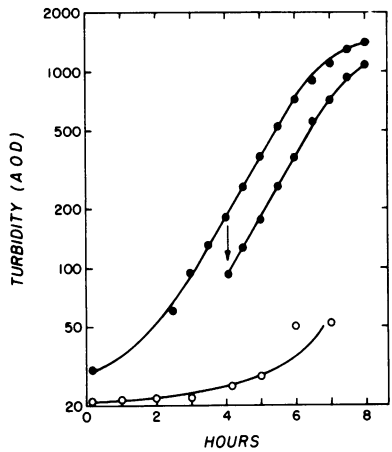

FIG. 1. *Growth of Streptococcus mutans strain BHT in M1 medium in the presence and absence of sodium citrate. An overnight culture, grown in M1 medium containing 225 μg of sodium citrate per ml, was inoculated (0.1 ml) into 7 ml of M1 medium containing the same concentration of citrate (●) and a second tube containing the same medium but lacking citrate (○). Subculture (1:2) of the culture growing exponentially in the medium containing citrate (arrow) into fresh, prewarmed medium (containing citrate) yielded continued exponential growth at the same rate.*

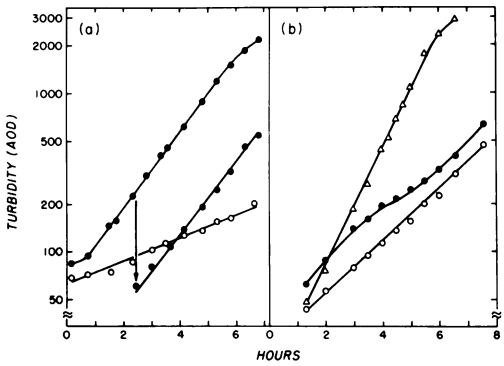

FIG. 2. *Growth of Streptococcus mutans strain FA-1 and Streptococcus sanguis OMZ-9 in MC medium in the presence and absence of sodium carbonate or sodium bicarbonate. (a) An overnight culture of strain FA-1 grown in MC medium (2,200 AOD) was inoculated (0.2 ml) into 7 ml of MC medium (○) and into MC medium containing 0.024 M sodium bicarbonate (●). Dilution (1:4) of the culture growing exponentially in MC plus bicarbonate (arrow) yielded continued exponential growth at the same rate. (b) An overnight culture of S. sanguis strain OMZ-9 grown in MC medium (2,900 AOD) plus sodium carbonate (0.019 M) was inoculated (0.1 ml) into 7 ml of MC medium (○), MC medium containing 0.019 M carbonate (△), and into MC medium containing 0.019 M sodium bicarbonate (●).*

Effect of increased phosphate concentration. To minimize the growth-inhibiting effects of acid production and thereby increase cellular turbidities reached at the end of exponential growth, the effect of increasing the phosphate content, and therefore the buffering capacity of the medium, was studied.

With strain AHT (Fig. 3), sodium phosphate concentrations of 0.11 M or higher resulted in exponential growth at a slower rate. At phosphate concentrations of 0.11 M and higher, longer growth lags were observed. In 0.11 and 0.14 M phosphate, high culture densities (over 3,000 AOD) were reached at 23 h, but little growth (370 AOD at 23 h) was obtained in 0.18 M phosphate. Rapid exponential growth to a high culture density was observed in MC medium containing 0.08 M phosphate or less.

Studies of the effect of sodium phosphate concentration on the growth of nine strains are summarized in Table 3. In MC containing 0.043 M (or 0.055 M) phosphate, culture turbidities reached by all strains tested were approximately as high as those reached in THG (Table 3). Growth of strains AHT, GS-5, 6715, OMZ-176, and HHT was more rapid in MC containing 0.043 M phosphate than in MC containing the 0.02 M concentration. For the remaining strains the T_D was the same in both media (Table 3). A further increase in phosphate concentration to 0.055 M increased the

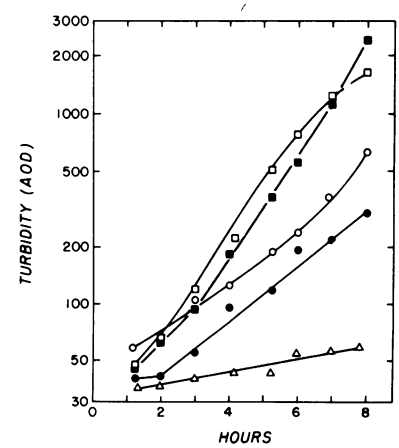

FIG. 3. *Growth of Streptococcus mutans strain AHT in MC medium containing various concentrations of phosphate buffer, pH 7.0. An overnight culture grown in MC medium (2,500 AOD) was inoculated (0.1 ml) into 7 ml of MC medium containing 0.02 M (□), 0.08 M (■), 0.1 M (○), 0.14 M (●), and 0.18 M (△) phosphate. The phosphate concentrations were obtained by adding an appropriate volume of a sterile 4.2 M sodium phosphate, pH 7.0, to double-strength medium containing both potassium and sodium phosphates.*

TABLE 3. *Growth of oral streptococci in media with higher phosphate concentrations*

Microorganism	Phosphate concn in chemically defined MC medium						THG broth	
	0.02 M		0.043 M		0.055 M			
	AOD[a]	T_D (min)[b]	AOD[a]	T_D (min)[b]	AOD[a]	T_D (min)[b]	AOD[a]	T_D (min)[b]
S. mutans strain								
AHT	2,500	64	2,800	54	3,600	48	2,400	47
OMZ-61	1,300	120	1,600	110	NT[c]		2,000	35
FA-1	1,800	120	2,000	120	2,300	140	1,600	45
BHT	1,400	61	2,300	63	3,200	64	2,300	44
GS-5	2,100	78	2,600	65	3,500	63	2,200	48
6715	1,800	70	2,300	56	3,200	55	2,000	45
OMZ-176	2,500	53	2,800	50	3,500	45	2,200	38
S. sanguis strain								
OMZ-9	NG[c]	NG	NG	NG	NG	NG	1,600	38
S. salivarius strain								
HHT	1,200	59	2,200	50	2,400	53	1,800	43

[a] Turbidities after 23 h of growth.
[b] Doubling times during exponential growth.
[c] NT, Not tested; NG, no transferable growth.

T_D of two strains (FA-1 and HHT). Since our intent was to design a medium which would provide rapid exponential growth to high turbidities of a wide variety of oral streptococci, all further experiments were carried out in MC containing 0.043 M phosphate (medium FMC). The addition of sodium carbonate, or in the case of strains FA-1 and OMZ-61 sodium bicarbonate, to FMC in all cases resulted in turbidities at 23 h substantially higher than those observed in THG, or in the same medium without carbonate or bicarbonate (Table 4). Also, carbonate addition resulted in growth of strains OMZ-9 and 72x46, and faster exponential growth of strains GS-5, JC-2, At6T, At9T, 6715, and OMZ-9, whereas bicarbonate addition had a similar effect for strains FA-1 and OMZ-61.

Growth of 14 strains of oral streptococci in FMC. Except for the two Lancefield group H streptococci, all of the strains tested grew relatively rapidly to high culture densities in FMC (Table 4). All of these strains grew with the same growth characteristics after five or more subcultures in this medium (1:50 or 1:100 dilution for each subculture). The addition of sodium carbonate, or in the case of two strains sodium bicarbonate, resulted in growth of all the strains tested to culture densities at 23 h substantially higher than those obtained in THG, and in growth rates comparable to those obtained in THG.

In all of the experiments described above cultures were routinely grown in 6- to 10-ml volumes in culture tubes (18 by 150 mm) with frequent mixing before taking turbidity measurements. Since these organisms are thought to grow better under anaerobic conditions (or at least at reduced oxygen tensions), some aspects of the effect of degree of aerobiosis were examined. With all strains, little or no effect of surface area-to-volume ratio on (i) lag before growth, (ii) rate of exponential growth, or (iii) culture turbidity at 23 h was observed when exponentially growing inocula were used. For example, inoculation of an exponential-phase culture into 10 ml of medium in a tube (18 by 150 mm) (surface area approximately 2 cm^2) or into a 300-ml side-arm flask (surface area approximately 53 cm^2) resulted in growth at the same rate and both cultures reached the same culture turbidity. Similarly, exponential-phase cultures inoculated into as little as 0.5 ml in a tube (18 by 150 mm) grew as rapidly and to the same densities as 10-ml cultures in the same type of tube.

In contrast, when overnight stationary-phase cells were used as inocula, longer lag periods, slower exponential growth rates, and lower turbidities after 23 h were routinely observed when the surface area-to-volume ratio was increased. Although rate of growth of cultures grown under anaerobic conditions was not investigated, it was observed that cultures grown anaerobically (10% H_2, 5% CO_2, 85% N_2) in FMC plus carbonate resulted in the same culture densities at 20 to 23 h as aerobically grown cultures.

TABLE 4. *Growth of oral streptococci in FMC*

Microorganism	Serotypes[a]	FMC		FMC with Na_2CO_3		THG	
		T_D (min)	AOD at 23 h	T_D (min)	AOD at 23 h	T_D (min)	AOD at 23 h
S. mutans strain							
AHT	*a*	54	2,800	56	3,800	47	2,400
OMZ-61	*a*	110	1,600	67[b]	3,000[b]	35	2,000
FA-1	*b*	120	2,000	83[b]	2,800[b]	45	1,600
BHT	*b*	63	2,300	66	3,400	44	2,300
GS-5	*c*	65	2,600	53	3,800	45	2,200
IB	*c*	63	2,800	63	3,600	47	2,400
JC-2	*c*	68	2,500	58	3,600	48	2,900
At6T	*c*	80	2,000	72	3,400	52	2,400
At9T	*c*	85	2,200	72	3,000	52	2,400
6715	*d*	56	2,300	50	3,600	45	2,000
OMZ-176	*d*	50	2,800	50	3,700	38	2,200
S. salivarius strain							
HHT		50	2,200	50	2,500	43	1,800
S. sanguis strain							
OMZ-9	Lancefield group H	NG[c]	NG[c]	46	3,400	33	1,600
72x46	Lancefield group H	NG[c]	NG[c]	58	3,000	35	2,000

[a] Serological groupings of *S. mutans* according to Brathall (20–22).

[b] These strains grew at the rates and AODs indicated in the presence of 0.014 M sodium bicarbonate. The final concentration of sodium bicarbonate supplemented to FMC was 0.019 M.

[c] NG, No growth.

DISCUSSION

A chemically defined medium (FMC) which supports rapid, luxuriant, and transferable growth of 14 oral streptococcal strains was developed (Table 1). Growth of 10 cariogenic strains, *S. mutans* AHT, BHT, GS-5, IB, At6T, At9T, JC-2, 6715, and OMZ-176, and *S. salivarius* HHT in FMC medium was comparable to their growth in an organic medium, Todd-Hewitt broth (Table 4). The two group H streptococci tested, strain 72x46 and *S. sanguis* OMZ-9, grew at relatively faster rates and to higher turbidities in FMC supplemented with sodium carbonate. All of the strains tested grew in FMC containing carbonate or bicarbonate at rates which were at least one-half of their growth rates in the organic medium (Table 4). For all of the strains examined, the T_D ranged from 46 to 83 min in FMC plus $CO_3^=$ compared with a T_D range of 35 to 52 min in THG. Subculture of exponentially growing cells to fresh FMC always resulted in growth at the same rate without a detectable lag.

In FMC plus carbonate (or bicarbonate), all strains grew to higher turbidities after incubation for 20 to 23 h than they did in either FMC or THG (Table 4). Although the effect of carbonate on increasing growth yields could be explained on the basis of an increase in buffer capacity of the medium, it seems much more likely that, as in other heterotrophic bacteria (26), the increased growth rate of several strains of *S. mutans* is due to an active role for CO_2 fixation in the metabolism of this species. Recently, Repaske et al. (18) showed that gaseous CO_2 was required for growth of a Lancefield group H streptococcus. Similarly in our studies, two Lancefield group H streptococci failed to grow significantly in the absence of carbonate. Additionally, Carlsson (6) observed that strains of *S. salivarius* grew aerobically only in a medium supplemented with seven amino acids or in the presence of a source of CO_2 such as bicarbonate or urea. In our experiments, we made no attempt to exclude CO_2, some of which was probably retained in media or inocula (17), but still were able to observe growth stimulation by added carbonate. The normal equilibrium among $CO_3^= \rightleftarrows HCO_3^- \rightleftarrows CO_2$ should result in an adequate supply of CO_2 from either $CO_3^=$ or HCO_3^-. Whereas most strains grew equally well in the presence of $CO_3^=$ or HCO_3^-, strains FA-1 and OMZ-61 preferred bicarbonate (Fig. 2A),

whereas strain OMZ-9 preferred carbonate (Fig. 2B). At present, we have no explanation for these observations.

We are also unable to offer an explanation for the effect of citrate on growth of *S. mutans* strains BHT and FA-1. Streptococci are considered to have a fermentative type of metabolism and to lack at least some of the enzymes of the citric acid cycle. Although citrate may be utilized for biosynthesis, its role could be merely in chelation of cations.

In the chemically defined media used by Carlsson (4, 5, 7), most strains grew only when incubations were anaerobic. Carlsson (4) observed that the addition of phosphate and acetate to the concentrations present in his chemically defined medium inhibited aerobic growth of *S. sanguis* strains of his group 1A in brain heart infusion broth. Using aerobic incubations in FMC plus carbonate (or bicarbonate), we obtained culture densities in 7 to 12 h similar to those obtained by Carlsson (5) after incubation for 120 h.

These studies have provided us with a chemically defined medium which can be used for studies of nutrition, physiology, and macromolecular synthesis with a wide variety of strains of oral streptococci, grown under both aerobic and anaerobic conditions.

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