Survival of Bacteria from Human Dental Plaque Under Various Transport Conditions

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The effects of transport media, temperature, and anaerobiosis on the survival of bacteria from human supragingival dental plaque were studied. Individual samples were obtained by passing sterile, unwaxed dental floss through the interproximal spaces. The plaque-bearing portion of floss was immediately placed in vials containing reduced transport fluid, viability-preserving microbistatic medium, or reduced salt solution transport fluid. Plaque samples were dispersed by ultrasonic oscillation, serially diluted, and plated in duplicate on MM10-sucrose-blood agar, mitis salivarius bacitracin agar, and Rogosa tomato juice agar. Initial viable counts (time 0) were compared with viable count determinations after 48- and 72-h storage. Quantitative recovery (>30%) of various groups of oral bacteria was accomplished from both reduced transport fluid and viability-preserving microbistatic medium after 48- and 72-h storage. Storage of dental plaque in reduced salt solution proved unsatisfactory for most bacteria (less than 10% survival). Since growth of some bacteria may occur in viability-preserving microbistatic medium and the charcoal present interferes with colony enumeration on low-dilution plates, we found reduced transport fluid to be the most suitable medium for transport and recovery of bacteria from supragingival dental plaque. Subzero storage (-196 and -40°C) did not enhance the survival of bacteria from dental plaque; storage at moderate (5 and 20°C) temperatures gave better recovery of viable bacteria. Survival after anaerobic or aerobic storage was comparable for total colony-forming units; however, anaerobic storage enhanced survival of *Streptococcus mutans* and *Lactobacillus*. Since these organisms are specifically associated with dental caries, anaerobic techniques are preferred for caries activity testing of plaque.

Limited information is available on the survival of selected oral bacteria under various transport conditions. Möller (15) introduced in 1966 the first transport fluid, viability-preserving microbistatic medium (VMG II), designed for storage of oral microbes. He demonstrated VMG II superiority to Stuart medium for preserving the viability of oral streptococci, especially at 24°C. Jordan et al. (9) isolated caries-inducing streptococci from dental plaque stored in VMG II at room temperature. VMG II maintained the viability of these organisms for several days. Syed and Loesche (23) compared survival of bacteria from dental plaque stored at 10°C in reduced transport fluid (RTF) and VMG II. VMG II was superior to RTF for storage of non-disease-associated dental plaque, whereas plaque samples obtained from carious lesions and periodontally involved teeth survived best in RTF. Rundell et al. (20) compared survival of oral streptococci in VMG II and RTF at 10 and 25°C. Survival of oral streptococci was enhanced by storage at 10°C; VMG II and RTF

performed equally well at this temperature. RTF maintained the most constant population of streptococci with 25°C storage, since growth of some organisms occurred in VMG II at this temperature.

Our objective in this study was to ascertain which transport conditions (media, temperature, oxygen tension) would produce optimum survival of potentially cariogenic bacteria from human supragingival dental plaque. In addition to reinvestigating the work of previous studies, we have determined the effects of subzero storage and anaerobic processing of plaque samples on the survival of oral bacteria.

MATERIALS AND METHODS

Three different storage media were studied at 5 and 20°C: RTF (12), VMG II (15), and a reduced salt solution (RSS) modified from PRAS of Holdeman and Moore (7). Table 1 lists the composition percentage of each transport medium. The percentage of survival for each bacterial group was determined in each transport medium. Percentage survival is de-

defined as the viable recovery of colony-forming units (CFU) after 48- and 72-h storage, divided by the CFU obtained on immediate culture, multiplied by 100. The effect of temperature on survival of bacteria from dental plaque was studied in RTF stored at -196 (liquid nitrogen), -40 (dry ice), 5 (artificial ice), and 20°C (ambient). Aerobic processing of dental plaque was compared with anaerobic processing (sonic treatment, dilution, plating, and storage). Incubation in both cases was anaerobic at 37°C for 48 to 72 h. Dental plaque was collected from over 14 individuals of varying age, including members of our laboratory staff and patients from the Pedodontic Clinic at the University of California-San Francisco. Specimens were obtained from noncarious interproximal surfaces between either the first and second mandibular molars or the first and second maxillary molars.

Aerobic sample dilution and storage. Dental plaque was obtained by passing sterile, unwaxed dental floss (John O. Buttler Co., Chicago, Ill.), held in a sterile plastic support (E-Z Floss, Palm Springs, Calif.) through the interproximal spaces. The plaque-bearing portion of floss was cut, with sterile scissors, and transferred directly to screw-cap vials containing 1.0 ml of the appropriate transport medium (RTF, VMG II, or RSS). Plaque samples were then dispersed by ultrasonic oscillation at 20 W for 10 s with a Lab-Line Ultratip Labsonic System equipped with an 1/8-inch (ca. 3.17 mm) titanium microtip (Lab-Line Instruments, Melrose Park, Ill.). Tenfold serial dilutions of the dispersed plaque were prepared in RTF (irrespective of the transport medium), and 0.1-ml aliquots were plated in duplicate on MM10-sucrose-blood (MM10-SB) agar (24) (total CFU and differential *Streptococcus sanguis* counts), mitis salivarius bacitracin (MSB) agar (4) (*Streptococcus mutans* counts), and Rogosa tomato juice agar (19) (*Lactobacillus* counts). The inoculated plates were incubated anaerobically for 48 to 72 h at 37°C in GasPak jars (Baltimore Biological Laboratory, Cockeysville, Md.) that were flushed three times with an 85% N_2 , 10% H_2 , and 5% CO_2 gas

mixture. Colonies were enumerated by examining plates at $\times 10$ magnification with a dissecting microscope (Olympus Instruments, Tokyo, Japan). Only those plates containing between 15 and 200 colonies were counted. Differential counting of *S. sanguis* on MM10-SB agar was accomplished by comparison with known strains. *S. sanguis* forms clear, hard, convex, adherent colonies easily recognizable on MM10-SB agar. MSB is a selective differential medium for *S. mutans*; colony enumeration was performed as described by Gold et al. (4). Rogosa tomato juice agar is highly selective for *Lactobacillus*.

After the original plating procedures, the plaque samples were stored at -196 , -40 , 5 , or 20°C , and new serial dilutions, cultures, and colony counts were performed after 48 and 72 h. After storage, the sample vials were vortexed, but sonic treatment was not repeated before serial dilution and plating.

Anaerobic sample dilution and storage. Transport media, dilution blanks, and plating media were prerduced by storage at room temperature in an anaerobic glove box (Clinical Analysis Products Co., Sunnyvale, Calif.) for 24 h before use. Dental plaque was collected as in the aerobic procedure and placed in prerduced transport medium. Sonic treatment, serial dilution, and plating of samples were performed in the anaerobic glove box (2) containing a mixture of 85% N_2 , 10% H_2 , and 5% CO_2 . Cultures were incubated and counted as in the aerobic procedure. The original plaque samples were stored at either 0 to 5 or 20°C under anaerobic conditions in Bio-Bag Anaerobic Culture Sets (Marion Scientific Corp., Kansas City, Mo.).

Storage temperature. The percentages of survival of bacteria from human dental plaque stored at -196 , -40 , 5 , and 20°C in RTF were compared. Liquid nitrogen (-196°C) storage was accomplished by placing plaque samples in A/S Nunc plastic vials (Almac Cryogenics, Inc., Oakland, Calif.) containing 1.0 ml of RTF. The samples were sonically treated; an aliquot was removed for initial enumeration of bacteria; and samples were then stored in liquid nitrogen in a 4-liter Dewar flask (UC-4, Al-

TABLE 1. Composition percentage of various transport media

RTF	VMG II	RSS
0.045% K_2HPO_4	0.05% tryptose	0.02% CaCl_2
0.045% KH_2PO_4	0.05% thiotone	0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
0.09% NaCl	0.05% cysteine-HCl	0.1% K_2HPO_4
0.09% $(\text{NH}_4)_2\text{SO}_4$	0.05% thioglycolic acid	0.1% KH_2PO_4
0.018% MgSO_4	1.0% bacteriological charcoal	1.0% NaHCO_3
0.038% EDTA	0.0003% phenylmercuric acetate	0.2% NaCl
0.04% Na_2CO_3	0.024% $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	0.02% dithiothreitol
0.02% dithiothreitol	0.042% KCl	
	0.1% NaCl	
	0.01% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	
	1.0% sodium glycerophosphate	
	0.01% agar	
	1.0% gelatin	

mac Cryogenics, Inc.) for 72 h. Dental plaque was stored at -40°C by wrapping sample vials in paper towels and placing them in 8-inch (ca 20.32-cm) square styrofoam boxes (Preferred Plastics, Inc., Putnam, Conn.) containing approximately 1.5 lb (ca. 681 g) of dry ice. Artificial ice (four packages, Royal Super Ice Co., San Leandro, Calif.) was used in the styrofoam containers to maintain 0 to 5°C . For ambient (20°C) storage, samples were packed in the container without any added coolant. Storage temperature was monitored in the styrofoam containers by a YSI model 42SC Tele-Thermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio).

RESULTS

Supragingival dental plaque was stored under conditions simulating those encountered during transit from the dental office to the laboratory. Of the three storage media studied, VMG II and RTF maintained good survival ($>30\%$) of bacteria from dental plaque stored at

5 (Table 2) and 20°C (Table 3). RSS proved unsatisfactory for recovery of viable bacteria from stored dental plaque, the percentage of survival being less than 10% for either storage temperature (Tables 2 and 3).

Table 4 compares percentages of survival of bacteria from plaque stored in RTF at -196 , -40 , 5, and 20°C . Storage at 5 and 20°C produced the greatest recovery of viable bacteria ($>30\%$). Storage at -196°C also gave satisfactory (approximately 20%) recovery of viable bacteria. Storage at -40°C showed poor ($<10\%$) recovery of viable bacteria.

Table 5 lists the results of anaerobically processed plaque samples. Anaerobic storage and plating did not appreciably increase the survival of total bacteria from supragingival plaque samples. The percentage of survival of total CFU from aerobically treated samples (Tables 2 and 3) is quite similar. Anaerobic

TABLE 2. Relative percentage of survival of bacteria from human dental plaque after storage at 0 to 5°C

CFU	Conditions						
	Range ^a (no storage)	48-h ^b storage (%)	No. of samples	Range	72-h storage (%)	No. of samples	Range
RTF							
Total CFU	9.8×10^5 – 4.6×10^7	44 ± 20	8	5.6×10^5 – 1.8×10^7	29 ± 10	9	3.0×10^5 – 1.0×10^7
<i>S. sanguis</i>	2.9×10^5 – 4.7×10^6	33 ± 37	3	1.1×10^4 – 2.6×10^6	26 ± 20	4	8.0×10^3 – 1.8×10^6
<i>S. mutans</i>	2.9×10^2 – 1.5×10^6	48 ± 44	8	0 – 2.1×10^5	34 ± 39	9	0 – 1.1×10^5
<i>Lactobacillus</i>	1.0×10^1 – 2.2×10^2	50 ± 50	3	0 – 3.0×10^5	42 ± 52	3	0 – 2.4×10^2
VMG II							
Total CFU	3.5×10^6 – 7.1×10^7	64 ± 23	6	4.3×10^6 – 3.3×10^7	36 ± 22	7	1.6×10^6 – 1.8×10^7
<i>S. sanguis</i>	2.8×10^5 – 3.8×10^6	84 ± 11	5	2.0×10^5 – 3.4×10^6	57 ± 28	6	2.0×10^5 – 2.4×10^6
<i>S. mutans</i>	5.0×10^1 – 3.9×10^5	84 ± 20	5	4.0×10^1 – 6.3×10^5	59 ± 41	5	0 – 5.3×10^5
<i>Lactobacillus</i>	2.6×10^2 – 1.2×10^6	87 ± 16	3	1.1×10^3 – 1.1×10^6	83 ± 15	3	1.3×10^3 – 8.8×10^5
RSS							
Total CFU	5.1×10^6 – 3.0×10^7	30 ± 17	3	2.1×10^5 – 1.2×10^7	6.6 ± 2.1	3	2.3×10^5 – 2.0×10^6
<i>S. sanguis</i>		NC ^c	—		NC	—	
<i>S. mutans</i>	5.8×10^3 – 3.7×10^5	26 ± 57	4	0 – 3.3×10^5	6.6 ± 12	4	0 – 7.7×10^4
<i>Lactobacillus</i>	1.3×10^4 – 6.0×10^4	96.1	2	1.2×10^4 – 6.8×10^4	40.8	2	7.1×10^3 – 1.6×10^4

^a The range is expressed as the number of colonies determined for all samples at each time period. These counts are given as a reference value for the proportion of various bacteria in supragingival dental plaque.

^b Percentage of survival $\pm$ standard deviation is obtained from colony counts after 48 and 72 h by comparison with colony counts obtained from immediate culture.

^c NC, Not counted.

TABLE 3. Relative percentage of survival of bacteria from human dental plaque after storage at 20°C

CFU	Conditions						
	Range ^a (no storage)	48-h ^b storage (%)	No. of samples	Range	72-h storage (%)	No. of samples	Range
RTF							
Total CFU	3.2 × 10 ⁶ – 5.6 × 10 ⁷	49 ± 19	7	1.4 × 10 ⁶ – 3.1 × 10 ⁷	39 ± 20	7	6.6 × 10 ⁵ – 1.7 × 10 ⁷
<i>S. sanguis</i>	1.6 × 10 ⁴ – 1.3 × 10 ⁷	43 ± 25	6	2.8 × 10 ⁵ – 1.1 × 10 ⁷	32 ± 27	6	2.7 × 10 ⁴ – 9.8 × 10 ⁶
<i>S. mutans</i>	1.7 × 10 ⁴ – 2.8 × 10 ⁷	54 ± 28	7	1.1 × 10 ⁴ – 9.6 × 10 ⁶	49 ± 34	7	1.1 × 10 ⁴ – 4.0 × 10 ⁶
<i>Lactobacillus</i>	2.0 × 10 ¹ – 2.9 × 10 ²	59 ± 29	5	1.0 × 10 ¹ – 2.2 × 10 ²	24 ± 24	5	0–1.3 × 10 ²
VMG II							
Total CFU	5.5 × 10 ⁶ – 2.0 × 10 ⁷	76 ± 11	3	3.6 × 10 ⁶ – 1.5 × 10 ⁷	60 ± 15	3	2.9 × 10 ⁶ – 1.0 × 10 ⁷
<i>S. sanguis</i>	3.3 × 10 ⁵ – 7.4 × 10 ⁶	82 ± 17	3	2.6 × 10 ⁵ – 5.0 × 10 ⁶	51 ± 20	3	2.0 × 10 ⁵ – 2.1 × 10 ⁶
<i>S. mutans</i>	5.9 × 10 ⁴ – 2.9 × 10 ⁶	54 ± 30	3	3.8 × 10 ⁴ – 2.1 × 10 ⁶	41 ± 29	3	2.1 × 10 ⁴ – 1.9 × 10 ⁶
<i>Lactobacillus</i>		100	1	—	20	1	—
RSS							
Total CFU	5.2 × 10 ⁶ – 6.5 × 10 ⁷	5 ± 5	4	3.8 × 10 ⁴ – 1.2 × 10 ⁶	1 ± 2	4	1.2 × 10 ⁴ – 4.3 × 10 ⁵
<i>S. sanguis</i>	1.3 × 10 ⁶ – 1.4 × 10 ⁷	5 ± 8	3	4.0 × 10 ³ – 2.9 × 10 ⁵	1 ± 1	3	0–1.2 × 10 ⁵
<i>S. mutans</i>	5.8 × 10 ³ – 2.3 × 10 ⁵	27 ± 41	4	9.6 × 10 ² – 7.5 × 10 ³	7 ± 14	4	0–2.2 × 10 ³
<i>Lactobacillus</i>	1.4 × 10 ⁴ – 4.0 × 10 ⁴	16	2	0–1.3 × 10 ⁴	1.5	2	0–1.2 × 10 ³

^a The range is expressed as the number of colonies determined for all samples at each time period. These counts are given as a reference value for the proportion of various bacteria in supragingival dental plaque.

^b Percentage of survival ± standard deviation is obtained from colony counts after 48 and 72 h by comparison with colony counts obtained from immediate culture.

TABLE 4. Relative percentage of survival of various groups of bacteria from supragingival dental plaque stored at various temperatures

CFU	Temp (°C)							
	–196		–40		5		20	
	Survival ^a (%)	No. of samples ^b	Survival (%)	No. of samples	Survival (%)	No. of samples	Survival (%)	No. of samples
Total CFU ^b	19 ± 16	7	5.3 ± 7.1	6	29 ± 10	9	39 ± 20	7
<i>S. sanguis</i>	15.7 ± 11.6	5			26 ± 20	4	32 ± 27	6
<i>S. mutans</i>	28.7 ± 19.3	6	3.8 ± 7.4	5	34 ± 39	9	49 ± 34	7
<i>Lactobacillus</i>	47.8 ± 9.8	6	6.3 ± 7.8	3	42 ± 52	3	24 ± 24	5

^a Percentage of survival ± standard deviation is obtained from colony counts after 72 h by comparison with colony counts obtained from immediate culture.

^b All samples stored in RTF.

procedures did enhance survival of *S. mutans* and *Lactobacillus* (Table 5) when compared with aerobic procedures (Tables 2 and 3).

DISCUSSION

Microbiological examination of dental plaque could become an important diagnostic tech-

nique for identifying caries-prone or periodontally involved individuals and for measuring the effectiveness of preventive therapy. Measuring the potentially pathogenic bacteria in dental plaque may require sending samples from the dental office to the laboratory. Accordingly, the efficacy of various transport media

TABLE 5. Relative percentage of survival of bacteria from human dental plaque stored and plated under anaerobic conditions^a

CFU	Conditions						
	Range ^b (no storage)	48-h ^c storage (%)	No. of samples	Range	72-h storage (%)	No. of samples	Range
RTF							
Total CFU	4.8 × 10 ⁶ – 4.9 × 10 ⁷	39 ± 14	6	1.7 × 10 ⁶ – 1.5 × 10 ⁷	25 ± 2	6	1.3 × 10 ⁶ – 1.2 × 10 ⁷
<i>S. sanguis</i>	1.5 × 10 ⁵ – 4.9 × 10 ⁶	39 ± 29	6	7.3 × 10 ⁴ – 5.6 × 10 ⁵	37 ± 40	6	1.5 × 10 ⁴ – 5.6 × 10 ⁵
<i>S. mutans</i>	2.0 × 10 ¹ – 5.1 × 10 ⁴	80 ± 40	4	2.9 × 10 ² – 6.8 × 10 ⁴	73 ± 32	4	1.9 × 10 ³ – 2.9 × 10 ⁴
<i>Lactobacillus</i>	1.0 × 10 ¹ – 6.0 × 10 ⁹	81 ± 22	6	1.0 × 10 ¹ – 4.0 × 10 ⁴	70 ± 25	6	2.0 × 10 ¹ – 4.1 × 10 ⁴
VMG II							
Total CFU	7.0 × 10 ⁶ – 8.8 × 10 ⁷	58 ± 16	6	5.3 × 10 ⁵ – 4.0 × 10 ⁷	26 ± 5	6	1.8 × 10 ⁶ – 2.7 × 10 ⁷
<i>S. sanguis</i>	4.1 × 10 ⁵ – 1.2 × 10 ⁷	62 ± 23	6	4.2 × 10 ⁵ – 6.0 × 10 ⁶	23 ± 15	6	1.6 × 10 ⁵ – 3.1 × 10 ⁶
<i>S. mutans</i>	8.0 × 10 ² – 4.0 × 10 ⁵	43 ± 45	6	0–1.1 × 10 ⁵	44 ± 45	6	0–1.5 × 10 ⁵
<i>Lactobacillus</i>	3.0 × 10 ¹ – 1.0 × 10 ⁶	74 ± 8	4	2.0 × 10 ¹ – 7.3 × 10 ⁵	72 ± 20	4	3.0 × 10 ¹ – 5.2 × 10 ⁵

^a Results are derived from samples stored at either 5 (two samples) or 20°C (four samples) since little difference in survival percentage was evident at either temperature in aerobic studies.

^b The range is expressed as the number of colonies determined for all samples at each time period. These counts are given as a reference value for the proportion of various bacteria in supragingival dental plaque.

^c Percentage of survival ± standard deviation is obtained from colony counts after 48 and 72 h by comparison with colony counts obtained from immediate culture.

and storage conditions are important factors to be studied. An ideal transport medium should be nonselective, nonsupportive, and capable of maintaining viability without promoting growth or appreciably altering the relative proportion of the various organisms initially present in the plaque sample (1). RTF was tested because of favorable experiences by other investigators (12, 20). VMG II was tried because of its common use in qualitative examination of plaque samples for *S. mutans*. RSS was studied because similar salt solutions have been widely used in clinical microbiology for transporting anaerobic samples.

Our results indicate that quantitative recovery (30 to 80%) of *S. sanguis*, *S. mutans*, and *Lactobacillus* from human dental plaque may be accomplished from either VMG II or RTF (Tables 2 and 3) for up to 72 h after collection. Recovery of viable bacteria from dental plaque stored in RSS was quite low (Tables 2 and 3). High pH (9.2) and lack of ethylenediaminetetraacetic acid (EDTA) in RSS medium could explain the low survival of bacteria. EDTA chelates cations involved in bacterial adherence; since RSS contains no EDTA, it is possible that reaggregation of bacteria could have occurred

during storage. However, VMG II contains no EDTA but gave good recovery of bacteria. High pH probably contributed to the poor performance of RSS more than any other factor. We found RTF the most suitable medium for transport and survival of bacteria from human supragingival dental plaque. Numerous researchers have indicated that growth of organisms may occur in VMG II, especially at 25°C (9, 20, 23). Selective growth of bacteria from plaque samples could prove misleading and troublesome. The charcoal present in VMG II interferes with colony enumeration and could mask the growth of contaminants in uninoculated vials.

Subzero storage (Table 4) did not enhance the survival of bacteria from dental plaque. Recovery of viable bacteria (<10%) from samples stored at –40°C was especially poor. Addition of 20% glycerol did not appreciably increase survival of –40°C-stored samples. Our containers were unable to maintain a temperature of –40°C for longer than 56 h. During the remaining 16 h before plating, sample temperature equilibrated to room temperature (20°C). Survival of bacteria stored at –40°C was significantly lower ($P < 0.01$) than at –196, 5, and

20°C. Recovery of viable bacteria (20%) from liquid nitrogen-stored samples (-196°C) was satisfactory and not statistically different ($P < 0.1$) from samples stored at 5 or 20°C (Table 4). Storage of dental plaque at -196, 5, or 20°C produced satisfactory recovery of cariogenic bacteria. These results support the findings of previous studies. Rundell et al. (20) studied the survival of oral streptococci and pooled plaque bacteria in RTF and VMG II at 10 and 25°C. Their results indicated slightly improved survival of plaque flora with cold storage (10°C) in both RTF and VMG II over a 21-day period. However, storage at 25°C gave quite similar results. Studies on survival of bacteria in fecal specimens (3, 16) have also shown moderate temperatures more useful than subzero storage. Ambient storage (20°C) of dental plaque is the most practical for the dental office. It is uncomplicated, inexpensive, and provides reasonable recovery (>30%) of potentially cariogenic bacteria. Storage of samples in liquid nitrogen, although satisfactory, is more expensive and too cumbersome for the dental office. Long-term storage of plaque flora in liquid nitrogen, however, might be useful for research purposes.

Immediate anaerobic processing of plaque has been shown to give from 60 to 70% viable recovery of organisms by comparison to direct smear counts (5, 24). This is significantly higher than when plaque is immediately processed aerobically and incubated anaerobically. In our studies, the range of CFU counts obtained on immediate culture was also higher with continuous anaerobic procedures (RTF, 4.8×10^6 to 4.9×10^7) than with aerobic procedures (RTF, 9.8×10^5 to 4.6×10^7). However, direct comparisons are not possible since no attempt was made to quantitate (e.g., weight, deoxyribonucleic acid, or protein content) individual samples, which could vary in amount from floss sample to floss sample. Comparable survival of total bacteria ($P < 0.2$) was obtained after 72-h storage from aerobically (Tables 2 and 3) and anaerobically (Table 5) manipulated plaque samples. This is not surprising since a majority of bacteria found in supragingival plaque are facultative anaerobes. Obligate anaerobes also may have grown in both cases, since the storage media contained reducing agents (dithiothreitol or cysteine-HCl) and incubation was anaerobic. Anaerobic procedures appeared to enhance the survival of *S. mutans* and *Lactobacillus* in stored plaque samples (Table 5), but comparison with aerobic samples (Tables 2 and 3) showed the difference was not statistically valid ($P < 0.2$ for *S. mu-*

tans; $P < 0.02$ for *Lactobacillus*). Increased recovery of *S. mutans* and *Lactobacillus* may have been due to prereduction of agar plates used in anaerobic procedures, whereas recovery of total CFU and *S. sanguis* remained the same since MM10-SB contains dithiothreitol (reducing agent).

In microbiological examination of plaque, the total number of organisms recovered or surviving is not the most important measurement of odontopathic potential. *S. mutans* is a component of coronal dental plaque, being found essentially in occlusal fissures and approximal sites. *S. mutans* normally averages less than 1% of the total CFU but is found in large numbers in plaque from caries-active persons, and more frequently in plaque overlying carious lesions than in plaque from sound tooth surfaces (6, 8, 11, 13, 22). *S. mutans* is a facultative anaerobe, growing best in carbon dioxide-enriched atmospheres. Although it is not a strict anaerobe, as are some of the gram-negative rods found in subgingival plaque of patients with periodontitis (17, 18), *S. mutans* survived better when stored under anaerobic conditions. Similarly, *Escherichia coli* survives storage in saline better anaerobically than aerobically (1). Anaerobic sonic treatment has also been shown by Manganiello et al. (14) to maintain the viability of supragingival plaque better than aerobic sonic treatment, even when samples were plated aerobically. Because of the low Eh usually prevailing in established dental plaque (10, 21; S. S. Socransky and A. D. Manganiello, J. Dent. Res., Abstr. no. 118, 54:74, 1975), hydrogen peroxide-detoxifying enzyme systems may not be active in facultative anaerobic bacteria. Therefore, we feel that anaerobic processing of plaque samples is the most useful. Anaerobic procedures are very important in analyzing subgingival plaque or in studying periodontal diseases. Anaerobic storage procedures are not the most convenient but could be adapted for use in the dental office.

We believe the best conditions for the analysis of supragingival plaque samples are suspension in RTF, storage at 5 or 20°C, with anaerobic procedures (storage, sonic treatment, dilution, and plating).

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