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Decreased Oral Colonization of *Streptococcus mutans* During Aging of Sprague-Dawley Rats

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The colonization by streptomycin-resistant *Streptococcus mutans* strains of the teeth of conventional and ex-germfree Sprague-Dawley rats of various ages fed either a high-sucrose or a high-glucose diet was studied. Bacterial colonization occurred with increasingly greater difficulty as the rats became older. This was observed in studies of the implantation of the test organism after oral inoculation with different cell numbers as well as its transmission between infected and uninfected rats. With rats fed sucrose diet, the effect of age could not be demonstrated until they were age 3 months or older; the results from rats fed a glucose diet suggest that changes may already have occurred early after weaning. Changes in susceptibility to colonization during aging manifested themselves as a decrease in the proportions of rats which became infected as well as lower population levels in infected rats. The possible mechanism(s) involved as well as the possible significance of the findings was discussed.

Humans are well known to acquire immunity to many common infections. Changes in susceptibility of humans to infection by oral bacteria during aging may also occur, at present only little information is available. Of interest in this respect are the reports that *Bacteroides melaninogenicus* (2, 3, 19) or spirochetes (2) do not become firmly established until puberty in the gingival crevice, which constitutes their prime oral habitat. In monkeys, the oral transmission of *Lactobacillus salivarius* has been found to occur readily between mother and infant but not between mature animals (4).

Information about the degree of susceptibility of humans to infection by oral bacteria during different periods of life could be of practical significance. It could aid in the development of methods to prevent the oral establishment of suspected dental pathogens or to eradicate them from the mouth. The purpose of the present study was to investigate the influence of the age of rats on the colonization of *Streptococcus mutans* on their teeth. *S. mutans* has been implicated in the etiology of dental caries (14). The colonization of streptomycin-resistant *S. mutans* strains on the teeth of conventional or ex-germfree rats of different ages and fed a high-sucrose or high-glucose diet was determined following their oral inoculation with different cell numbers. The transmission of labeled cells of an *S. mutans* strain from rats preinfected with the test organism to uninfected rats of different ages was also studied.

MATERIALS AND METHODS

Animals. Conventional Sprague-Dawley rats of varying ages were obtained from Charles River Breeding Laboratories, Wilmington, Mass. All animals were housed in screen-bottom, stainless-steel cages without bedding and individually caged unless noted otherwise. Food and drinking water were given ad libitum. Prior to arrival from Charles River Breeding Labs, the animals had been fed a stock diet 4-RF (Country Best, Agway, Inc., Syracuse, N.Y.).

Germfree Sprague-Dawley rats, raised at the Forsyth Dental Center (12), were removed from the isolators and caged in a manner similar to the conventional rats. These rats had been fed diet L-356 (Teklad, Madison, Wis.). Analysis of both diets showed that the fluoride concentration in diet L-356 was 0.9 ppm but about 17 ppm in diet 4-RF.

Bacterial strains. The *S. mutans* strains used were 6715 (serotype d) which is of human origin and resistant to streptomycin (11, 26), OMZ-61 (serotype a) isolated from rats (17, 26), and 65 (serotype d) isolated from human saliva (26). Resistance to streptomycin was induced in strains OMZ-61 and 65 by serial transfer in broth containing increasing concentrations (up to 2,000 μg/ml) of streptomycin (resistance indicated by suffix "R", e.g., OMZ-61-R). Cells for animal inoculation were obtained from 16-h cultures of the strains cultivated in Trypticase salts medium (28) supplemented with 2% D-glucose (Fisher Scientific Co., Fairlawn, N. J.) and incubated anaerobically in Brewer Anaerobic Jars filled with 10% H_2, 10% CO_2, and 80% N_2 at 37°C. Concentrated and diluted cell suspensions were made by using the same medium without added carbohydrate.

Animal inoculation and determination of im-

plantation. Animals were inoculated orally once with 0.2 ml of different cell dosages of the test organism unless noted otherwise. Bacterial colonization was monitored by culturing oral Calgiswab samples on mitis-salivarius agar (Difco, Detroit, Mich.) with 200 μg of streptomycin per ml added (28). At the end of the experiments, the rats were killed by chloroform inhalation and decapitated; all 12 molar teeth were removed with the aid of a sterile forceps and finely ground by means of glass tissue grinders, and samples from undiluted and appropriately diluted suspensions were cultured on duplicate streptomycin-containing mitis-salivarius agar plates as described previously (28).

Prior to the experiments it was found that occasionally animals were supplied which harbored *S. mutans*. Obtaining animals from other areas at Charles River Breeding Labs often only temporarily eliminated this problem. Because prior infection with *S. mutans* may interfere with the establishment of antibiotic-resistant strains of this same organism in hamsters (10, 20), samples were removed with Calgiswabs from the mouths of all rats once or twice prior to inoculation with the test organism and cultured on mitis-salivarius agar and modified mitis-salivarius agar selective for *S. mutans* (15) to determine the presence of this organism. In addition, in experiments with complete litters, the dam and/or some of the litter-mates were sacrificed to determine more accurately the presence of *S. mutans* via culturing of appropriate dilutions of the ground molar teeth on the same media. Bacterial identification was based on the characteristic colonial morphology evaluated with a stereoscopic microscope (×15 magnification). Studies of weanlings fed laboratory chow or a high-sucrose diet showed that rats that were initially considered free of *S. mutans* based on oral swabbing could harbor *S. mutans* populations of up to 10^6 colony-forming units (CFU) 1 to 2 weeks later. Consequently, at the end of the experiments, samples from ground molar teeth of all rats, with the exception of the ex-germfree rats or the rats of the experiment in which transmission of *S. mutans* was studied, were not only cultured on streptomycin-containing mitis-salivarius agar but also on regular mitis-salivarius agar and the medium selective for *S. mutans*.

Experiments with conventional rats fed high-sucrose diet. Male and female rats ranging in age from 27 days to 4 months were used. Upon arrival from Charles River Breeding Labs, all rats were given a basic diet containing the following: vitamin-free casein, 34.7%; brewer's yeast extract, 3.5%; salt mixture W, 5%; and vitamin mixture, 0.8% (28); supplemented with 56% sucrose. This high-sucrose diet was free of fluoride. At 2 or 3 days later, groups of rats, 29 days or 4 months of age, were inoculated with about 10^8, 10^7, or 10^6 CFU of strain 6715 (experiment 1); groups of rats 41 days or 3 months and 20 days of age (experiment 2) were inoculated with about 10^6 or 10^5 CFU; and rats 27 days or 2.5 months of age (experiment 3) were inoculated with about 10^6, 10^5, or 10^4 CFU of the same strain.

The implantation of strain OMZ-61-R was studied in groups of rats 24 days or 4 months of age (experiment 4). All rats were obtained when 20 days old and were fed laboratory chow (Ralston Purina Co., St. Louis, Mo.) containing 21.6 ppm fluoride. Three days prior to inoculation with about 10^6 or 10^5 CFU of the test organism, all animals were switched to the high-sucrose diet.

Groups of rats 20, 26, 28, 33, 40, and 63 days old were also studied (experiment 5). Dams and their litter (12 to 14 days old) were obtained two at a time according to a schedule which insured that all age groups could be inoculated simultaneously. All rats were immediately provided with the basic diet supplemented with 56% glucose (high-glucose diet) and switched to the high-sucrose diet 2 days prior to inoculation with strain 6715. Rats of each of the age groups (20 to 40 days), derived from two litters, were divided into two inoculum groups. One-half of the rats of each litter was assigned to each inoculum group so that both sexes were about equally represented in each group. The 63-day-old rats were the dams of some of the litters, and two inoculum groups were also formed. The cell inocula used were about 10^6 and 10^7 CFU.

All rats studied in each experiment were inoculated with cells obtained from the same culture. All rats were killed 5 weeks after inoculation to determine the establishment of the test organism.

Influence of indigenous *S. mutans* populations on the implantation of strain 6715. Weanling 20-day-old rats and retired breeders over 6 months of age, chosen at random from different litters or breeding areas at Charles River Breeding Labs, were fed the high-glucose diet and repeatedly sampled to determine the presence of indigenous *S. mutans* populations. Groups of young and old animals with or without oral indigenous *S. mutans* were then inoculated with 1.4×10^9 CFU of strain 6715 obtained from the same culture, while they remained on the high-glucose diet during the 36-day experimental period.

Experiments with conventional rats fed high-glucose diet. Groups of rats ranging in age from 17 to 155 days were immediately fed the high-glucose diet upon arrival from Charles River Breeding Labs. After 3 days, rats from each of the age groups (20, 26, 32, 42, and 158 days) were inoculated with 10^8 CFU of strain 6715, using the same culture for all age groups. All rats continued to be fed the high-glucose diet until the end of the 29-day experiment.

A similar experiment was performed with rats 20, 26, 32, and 42 days old when inoculated, using *S. mutans* strain 65-R. The size of the inoculum was 3×10^7 CFU and the experimental period was 23 days. In both experiments, the group of 20-day-old rats contained females and males; the rats in all other age groups were male.

Experiments with ex-germfree rats. Female and male germfree rats ranging in age from 14 days to nearly 5 months were removed from the isolators and immediately fed the high-sucrose diet. Within 1 day after conventionalization, all rats were found to harbor large numbers of bacteria as determined by oral sampling with Calgiswabs and culturing on anaerobically incubated blood agar. Three days

after conventionalization, groups of rats 17 days and 2, 3, or 5 months old (experiment 1) and rats 27 days and 5 months old (experiment 2) were inoculated with various cell inocula of strain 6715. All rats continued to receive the high-sucrose diet and were killed 5 weeks after inoculation to determine the implantation of strain 6715.

In short-term experiments, germfree rats of either sex were conventionalized and immediately fed the high-glucose diet. Rats 22, 62, or 121 days of age (experiment 1) or 24, 70, and 120 days of age (experiment 2) were inoculated with 5.0×10^8 CFU or 1.8×10^7 CFU of strain 6715, respectively, 5 h after conventionalization. Rats of both sexes were about equally represented in each of the different age groups. The rats were killed 4 days (experiment 1) and 14 days (experiment 2) after inoculation to determine the implantation of the test strain.

Transmission between the infected and uninfected conventional rats. Rats 67, 73, and 87 days old and heavily infected with strain 6715 while maintained on the high-sucrose diet served as donors. These rats were obtained from another experiment (experiment 5, see Table 3). They had been fed the high-sucrose diet and had received an inoculum of 1.4×10^7 CFU of strain 6715 when 20, 26, and 40 days of age. Young rats 21 days of age or retired breeders 8 months old were received from Charles River Breeding Labs and fed the high-sucrose diet. After 3 days, donors and the uninfected young and old rats were randomly assigned to different cages. Each cage contained two donors and one recipient. All rats continued to be fed the high-sucrose diet until killed 49 or 77 days after they had been caged together.

RESULTS

Implantation in conventional rats fed high-sucrose diet. The results from experiments in which the establishment of *S. mutans* strain 6715 on the teeth of rats of different age and fed the high-sucrose diet was studied are shown in Table 1. Good implantation, as evaluated after death, occurred in all 29-day-old rats inoculated within the order of 10^8, 10^7, and 10^6 CFU (experiment 1); in contrast only half of the 4-month-old rats inoculated with the highest cell number became infected and the others remained uninfected. Differences in implantation were also observed between 41-day-old animals and rats over 3.5 months of age (experiment 2), but no difference was detected between 27-day-old and 2.5-month-old rats (experiment 3). Age influenced the implantation in males and females (experiments 1 and 2).

Younger and older rats also differed with respect to the cell numbers of the test organism recovered from the teeth (Table 1). The recoveries from the molar teeth of the younger rats

TABLE 1. *Oral establishment of S. mutans strain 6715 in rats of different age fed high-sucrose diet and inoculated with different cell inocula*

Expt.	Age at time of inoculation	Determination		
		3.6×10^{8a}	3.6×10^{7a}	3.6×10^{6a}
1	29 days (M)[b]	4/4[c] (3.5×10^7–7.4×10^7)[d]	4/4 (1.9×10^7–6.0×10^7)	4/4 (3.1×10^7–5.4×10^7)
	4 months (M)	3/6 2.1×10^3[e] 6.0×10^4 2.6×10^7	0/4	0/4
2		2.6×10^{6a}	2.6×10^{5a}	
	41 days (M)	3/3 (2.7×10^7–7.0×10^7)	2/4 (0.3×10^6–1.8×10^6)	
	3 months, 20 days (F)[b]	2/3 2.0×10^4 9.8×10^5	0/3	
3		7.8×10^{5a}	7.8×10^{4a}	7.8×10^{3a}
	27 days (M)	3/4 (2.0×10^7–4.1×10^7)	2/4 (0.2×10^7–3.6×10^7)	0/4
	2.5 months (M)	3/4 2.0×10^3 3.5×10^3 1.2×10^7	1/4 7.9×10^7	0/4

[a] Inoculum in colony-forming units.
[b] M, Male; F, Female.
[c] Number of rats infected/number of rats tested.
[d] Numbers in parentheses indicate range of total recoveries from infected rats (per 12 molar teeth).
[e] Recovery from 12 molar teeth of one rat.

were in the order of 10^7 CFU except in the case of the 41-day-old rats inoculated with the lower cell dosage (experiment 2). In contrast, the levels of recovery from the teeth of the older rats were generally far lower; in only three of the nine infected rats (experiments 1, 2, and 3), the numbers of the test organism reached 10^7 CFU whereas in the other six rats they ranged from 2.0×10^3 to 9.8×10^5 CFU. In experiments with strain 6715 and 21- to 35-day-old rats under identical conditions reported elsewhere (28), the recoveries of the test strain from infected rats also nearly always reached 10^7 CFU. Findings comparable to those with strain 6715 were obtained with *S. mutans* strain OMZ-61-R (Table 2). However, the cell populations of this strain on the teeth were considerably lower than those of strain 6715.

To determine more precisely the age at which changes in the sucrose-fed animals occurred, rats of 20, 26, 28, 33, 40, and 63 days of age were studied (Table 3). No consistent differences in implantation of strain 6715 between the age groups were detected. All rats inoculated with the lower dose, except three animals in the 28- and 33-day age groups, became infected. Rats of the 28- and 33-day age groups, as well as those in the other age groups (data not shown), inoculated with the higher dose all became heavily infected (Table 3). As previously observed (Ta-

TABLE 2. *Oral establishment of S. mutans strain OMZ-61-R in rats of different age fed high-sucrose diet and inoculated with different cell inocula (experiment 4)*

Age at time of Inoculation	Determination	
	3.1×10^6[a]	3.1×10^5[a]
24 days (M)[b]	3/3[c] (0.8×10^6–2.8×10^6)[d]	2/3 (1.1×10^6–7.2×10^6)
4 months (M)	0/3	0/3

[a] Inoculum in colony-forming units.
[b] M, Male.
[c] Number of rats infected/number of rats tested.
[d] Numbers in parentheses indicate range of total recoveries from infected rats (per 12 molar teeth).

TABLE 3. *Oral establishment of S. mutans strain 6715 in rats of different age fed high-sucrose diet and inoculated with different cell inocula (experiment 5)*

Age at time of inoculation (days)	Determination		
	1.4×10^6[a]		1.4×10^7[a]
20	8/8[b] 2.6×10^7[d] (1.4×10^3–7.4×10^7)[f]	1.4×10^3[e] 6.0×10^5	NK[c]
26	7/7 2.4×10^7 (0.5×10^7–3.8×10^7)		NK
28	4/6 1.2×10^7 (8.6×10^2–3.0×10^7)	8.6×10^2	6/6 (1.4×10^7–6.7×10^7)
33	5/6 1.1×10^7 (4.0×10^2–3.8×10^7)	4.0×10^2	6/6 (4.1×10^7–10×10^7)
40	6/6 1.5×10^7 (0.7×10^7–2.4×10^7)		NK
63 (F)[g]	4/4 3.7×10^7 (6.0×10^5–6.3×10^7)	6.0×10^5	NK

[a] Inoculum in colony-forming units.
[b] Number of rats infected/number of rats tested.
[c] NK, Not killed; these rats were used in transmission studies (see Table 8).
[d] Arithmetic mean of total recoveries from each infected rat (per 12 molar teeth).
[e] Recovery from 12 molar teeth of one rat.
[f] Numbers in parentheses indicate range of total recoveries from infected rats (per 12 molar teeth).
[g] F, Female.

ble 1), the recoveries from the teeth were in the order of 10^7 CFU except in the case of five animals in the 20-, 28-, 33-, and 63-day age groups.

In the experiments of which the results are shown in Tables 1, 2, and 3, the suspensions of ground molar teeth were not only cultured on streptomycin-containing mitis-salivarius agar but also on regular mitis-salivarius agar or the medium selective for *S. mutans*. No other *S. mutans* types were detected in the rats in which the test organism failed to establish on the teeth in high numbers (10^6 to 10^7 CFU) or at all.

Influence of indigenous *S. mutans* populations on implantation of strain 6715. As earlier mentioned, indigenous *S. mutans* populations may impair the implantation of antibiotic-resistant *S. mutans* strains in hamsters (10, 20). The results from an experiment shown in Table 4 suggest that a similar interaction may occur in rats. In young or old animals with indigenous *S. mutans* populations and inoculated with about 10^9 CFU of strain 6715, labeled 6715 cells were initially detected as determined by oral swabbing but disappeared later and were not found after sacrifice; at this time these rats harbored substantial indigenous *S. mutans* populations and the mean concentrations for young and older rats were 1.9×10^7 and 6.9×10^6 CFU, respectively. In contrast, the test strain established rapidly in most young rats initially categorized as free of indigenous *S. mutans* populations and reached eventually a mean level of 3×10^6 CFU. In the retired breeders of this group, strain 6715 established initially and could not be detected by oral swabbing 21 days after inoculation. After their death, it was found in much lower concentrations (1.4×10^5 CFU) in only half of the animals, which again indicates the influence of age. Neither in the young nor in the older animals of this group could the presence of indigenous *S. mutans* be detected after they had been killed.

Implantation in conventional rats fed high-glucose diet. In previous experiments with strain 6715 and conventional rats fed the high-sucrose diet, even with inocula close to the minimum infective dose (Table 3), the effect of age could not be detected prior to about the age of 3 months. Because sucrose is more effective than glucose in promoting the colonization of *S. mutans* strain 6715 in rats (28), experiments were also carried out with glucose-fed rats of various ages. Table 5 shows the results from an experiment in which groups of rats 20, 26, 32, 42, and 158 days old were first inoculated with about 10^8 CFU and, since only few of the rats became infected after the first inoculation, 8 days thereafter with about 5×10^8 CFU. It was found that the test organism implanted to a significant extent in four (two females and two males) of the 20-day-old rats, but only in a few of the older rats and then less effectively than in younger animals. Although these differences were relatively small, they could be demonstrated in three or four other experiments with strain 6715 under comparable conditions. In the case of strain 65-R, differences were evident shortly after inoculation with 3×10^7 CFU, both with respect to the proportions of rats infected and the population levels attained, but they were negligible at the end of the experiment. The implantation of this strain in sucrose-fed rats is only slightly better than in glucose-fed rats (26). In none of the rats which remained uninfected with strain 6715 or strain 65-R was indigenous *S. mutans* detected.

Experiments with ex-germfree rats. To eliminate the possibility of interference by indigenous *S. mutans* populations, and to gain

TABLE 4. *Oral establishment of S. mutans strain 6715 in inoculated rats of different age fed high-glucose diet and with or without indigenous S. mutans populations*

Days after inoculation	+ Indigenous *S. mutans*		− Indigenous *S. mutans*	
	Young (38 days)	Retired breeders	Young (34–38 days)	Retired breeders
5	4/8[a], ±[b]	3/9, ±	15/19, ± to +	5/6, ±
12	0/8	1/9, ±	16/19, ± to 3+	5/6, ± to 2+
21	0/8	0/9	16/19, ± to 3+	0/6
36 (killed)	0/8	0/9	17/19	3/6
	1.9×10^7[c]	6.9×10^6	3.0×10^6	1.4×10^5
	(9×10^6–3.6×10^7)[d]	(1.4×10^5–3.2×10^7)	(9×10^1–2.8×10^7)	(6×10^1–4.2×10^5)

[a] Number of rats infected/number of rats tested.

[b] Oral swabbing; recovery from infected rats: ± = <50 CFU; 1+ = 50 to 250 CFU; 2+ = 250 to 500 CFU; 3+ = >500 CFU.

[c] Arithmetic mean of total recoveries from each infected rat (per 12 molar teeth).

[d] Numbers in parentheses indicate range of total recoveries from infected rats (per 12 molar teeth).

TABLE 5. *Oral establishment of S. mutans strains 6715 and 65-R in rats of different age fed high-glucose diet*

Strain	Days after inoculation	Determination				
		20[a]	26[a]	32[a]	42[a]	158[a]
6715	4	2/10[b], ± [c]	0/6	1/6, ±	1/5, ±	0/5
	8 (reinoculation)					
	14	3/8, ± to 3+	0/6	0/6	1/5, ±	0/5
	29 (killed)	4/10	0/6	1/6	1/5	0/5
		1.6×10^{6}[d]		9×10^{2}[e]	6×10^{3}	
		(3.6×10^{5}-40×10^{5})[f]				
65-R	4	9/9, 1+ to 3+	2/5, ±	1/4, ±	4/6, ± to 1+	
	8	9/9, 1+ to 3+	4/5, ±	1/4, ±	4/6, ±	
	23 (killed)	9/9	4/5	3/4	6/6	
		1.0×10^{6}	1.6×10^{5}	2.1×10^{5}	3.9×10^{5}	
		(4.8×10^{4}-2.6×10^{6})	(7.2×10^{4}-2.1×10^{6})	(1.8×10^{3}-4.5×10^{5})	(7.5×10^{3}-1.2×10^{6})	

[a] Age in days at time of first inoculation.
[b] Number of rats infected/number of rats tested.
[c] Oral swabbing; recovery from infected rats; ± = <50 CFU; 1+ = 50 to 250 CFU; 2 + = 250 to 500 CFU; 3+ = >500 CFU.
[d] Arithmetic mean of total recoveries from each infected rat (per 12 molar teeth).
[e] Recovery from 12 molar teeth of one rat.
[f] Numbers in parentheses indicate range of total recoveries from infected rats (per 12 molar teeth).

some further insight with respect to the mechanism(s) responsible for the effect of age, experiments were also performed with ex-germfree rats. The results with sucrose-fed rats inoculated 3 days after their conventionalization are shown in Table 6. The effect of age on implantation was again demonstrable with rats of either sex and about 3 months of age or older and its manifestation depended upon the inoculum size.

Comparable observations were made in short-term experiments with glucose-fed rats conventionalized for only 5 h prior to inoculation (Table 7). With the higher inoculum size (experiment 1), differences in the proportions of inoculated rats of various age were only demonstrable shortly after inoculation; the populations on the teeth of the oldest rats after sacrifice were about 100-fold lower than those on the teeth of the younger rats. In the case of the rats inoculated with the lower inoculum (experiment 2), differences in the proportions of infected rats were present initially as well as after sacrifice and the 120-day-old rats that became infected only harbored relatively low numbers of the test organism.

Transmission between infected and uninfected conventional rats. The transmission of strain 6715 between rats heavily infected with the organism and uninfected young or old rats is shown in Table 8. Transfer of the test organism to the young uninfected rats occurred more rapidly than to the older animals. After 37 days, all young rats and only two of the six older rats were infected. After sacrifice, 49 days after the initiation of the experiment, the recoveries from all young recipient rats, as well as their donors, were in the order of 10^7 CFU. Although the donors of the older rats were infected to a comparable degree, only one of the three older rats killed at that time was infected. After an additional 4 weeks (77 days caged together), two of the three remaining older recipients also had become infected.

DISCUSSION

This study indicates that it is more difficult to infect the mouths of Sprague-Dawley rats with *S. mutans* when the animals become older. Changes in level of bacterial implantation were clearly manifest with conventional sucrose-fed rats or ex-germfree rats fed either sucrose or glucose diet when the animals were 3 or 4 months of age. The experiments with glucose-fed conventional rats and strains 6715 and 65-R suggest that changes in the colonization of *S. mutans* may already occur early after weaning. Whether the early and later changes are due to different processes or represent one process, the effect of which increases with age, is unclear.

The detection of early changes with glucose-fed conventional rats only may be a reflection of the unfavorable and favorable effect of sucrose and the conventional rat flora, respectively, on the sensitivity of the model. Thus, recent studies with *S. mutans* strains 6715 have shown that the minimum infective dose for sucrose-fed rats is about 10^5 CFU, whereas that for glucose-fed rats is in the order of 10^8 to 10^9 CFU (28). Sucrose probably exerts its favorable effect on the colonization of this strain by promoting

firmer initial cell binding to the teeth and also larger cell accumulation (27, 28). Furthermore, it is common experience that germfree rats are easier to infect than conventional animals (16); in the present study, the minimum infective dose for glucose-fed conventional rats was at least 10-fold higher than for glucose-fed germfree rats conventionalized for only a few hours prior to inoculation (Tables 5 and 7).

The differences associated with age not only manifested themselves as differences in the proportions of rats infected with the labeled strains but also as differences in the population levels reached. In contrast, the size of indigenous *S. mutans* in populations of young and old rats were comparable (Table 4). The labeled *S. mutans* populations resulted from one or a few oral inoculations and were determined within a short time period of about 5 weeks or less after inoculation. Prolongation of these experiments conceivably could have led to comparable populations in infected rats of both age groups via gradual spread of the infection from infected oral sites or fecal reservoirs. The indigenous *S. mutans* populations were most likely established at an early age; continuous transfer of

TABLE 6. *Oral establishment of S. mutans strain 6715 in ex-germfree rats of different age fed high-sucrose diet and inoculated with different cell inocula*

Expt	Age at time of Inoculation	Determination					
		3.1×10^{7}[a]	3.1×10^{6}[a]	3.1×10^{5}[a]	3.1×10^{4}[a]		
1[b]	17 days	ND[c]	ND	1.8×10^{8} 7.5×10^{7}	ND		
	2 months	7.5×10^{7}[d]	7.5×10^{7}	3.3×10^{7}	2.0×10^{7}		
	3 months	2.8×10^{7}	1.1×10^{7}	2.2×10^{7}	0		
	5 months	2.5×10^{5}	3.6×10^{7}	0	0		
2		2.3×10^{8}[a]	2.3×10^{7}[a]	2.3×10^{6}[a]	2.3×10^{5}[a]	2.3×10^{4}[a]	2.3×10^{3}[a]
	27 days	ND	ND	2.5×10^{7} (F)[e] 7.5×10^{7} (M)[e]	6.0×10^{7} (F) 6.3×10^{7} (F) 6.6×10^{7} (F) 1.1×10^{8} (M)	3.1 × 10⁷ (F) 0 (M) 0 (M)	0 (F) 0 (F) 0 (M)
	5 months	3.5×10^{7} (F) 9.6×10^{7} (M)	1.8×10^{7} (F) 2.6×10^{7} (M)	4.5×10^{7} (F) 3.4×10^{7} (M) 5.8×10^{7} (M) 0 (F)	0 (F) 0 (M) 0 (M)	ND	ND

[a] Inoculum in colony-forming units.
[b] Rats in experiment 1 undifferentiated with respect to sex.
[c] ND, Not done.
[d] Recovery from 12 molar teeth of one rat.
[e] M, Male; F, female.

TABLE 7. *Oral establishment of S. mutans strain 6715 in ex-germfree rats fed high-glucose diet 5 h after conventionalization*

Expt	Inoculum	Days after inoculation	Determination		
			22[a]	62[a]	121[a]
1	5.0×10^{8}	2	9/9[b]	9/9	5/9
		4 (killed)	9/9	9/9	8/9
			3.5×10^{7}[c]	1.9×10^{7}	2.0×10^{5}
			(1.7×10^{6}–6.9×10^{7})[d]	(3.6×10^{6}–3.2×10^{7})	(2.1×10^{4}–3.2×10^{5})
			24[a]	70[a]	120[a]
2	1.8×10^{7}	2	5/8, ± to 2+[e]	4/7, ± to +	1/6, ±
		3	6/8, ± to 3+	6/7, ± to +	2/6, ± to 2+
		9	5/8, ± to 3+	4/7, ± to +	2/6, 1+
		14 (killed)	7/8	7/7	2/6
			1.9×10^{6}	1.7×10^{6}	1.8×10^{3}
			(7.5×10^{2}–6.3×10^{6})	(2.1×10^{3}–1.1×10^{7})	(1.8×10^{2}–3.6×10^{3})

[a] Age in days at time of inoculation.
[b] Number of rats infected/number of rats tested.
[c] Arithmetic mean of total recoveries from each infected rat (per 12 molar teeth).
[d] Numbers in parentheses indicate range of total recoveries from infected rats (per 12 molar teeth).
[e] Oral swabbing; recovery from infected rats: ± = <50 CFU; 1+ = 50 to 250 CFU; 2+ = 250 to 500 CFU; 3+ = >500 CFU.

TABLE 8. *Transmission of S. mutans strain 6715 from infected rats to uninfected rats of different age fed high-sucrose diet*

Days caged together	Donor or recipient	Determination									
		Recipient (M)[a]: 24 days old				Recipient (M): 8 months old					
		1[b]	2	3	4	1	2	3	4	5	6
0	Donors 1 and 2	3+[c]	3+	3+	3+	3+	3+	3+	3+	3+	3+
	Recipient	−	−	−	−	−	−	−	−	−	−
		Recipients				Recipients					
4		1/4[d], 2+				0/6					
7		2/4, ± to 2+				1/6, 2+					
11		2/4, 2+ to 3+				1/6, 2+					
15		3/4, ± to 3+				2/6, + to 3+					
37		4/4, 3+				2/6, + to 3+					
49 (killed)		4/4				1/3					
	Donors 1 and 2	(2.1×10^7–1.1×10^8)[e]				(2.2×10^7–1.8×10^8)					
	Recipient(s)	(7.2×10^6–1.2×10^8)				1.6×10^7[f]					
77 (killed)						2/3					
	Donors 1 and 2					(2.5×10^7–5.6×10^7)					
	Recipients					9.0×10^6					
						2.5×10^7					

[a] M, Male.
[b] Cage number.
[c] Oral swabbing; recovery from infected rats: ± = <50 CFU; 1+ = 50 to 250 CFU; 2+ = 250 to 500 CFU; 3+ = >500 CFU.
[d] Number of rats infected/number of rats tested.
[e] Numbers in parentheses indicate range of total recoveries from infected rats (per 12 molar teeth).
[f] Recovery from 12 molar teeth of one rat.

high cell numbers in feces via coprophagy to the mouth may have maintained the earlier-established population levels in spite of age-associated adverse conditons.

The differences between young and old rats are most likely due to a change in host susceptibility. No marked differences in food intake were noted; in fact, to be of influence, such differences should be of a very high order. Thus, in the case of rats fed a sucrose diet, separate implantation studies with strain 6715 under identical conditions have shown that its establishment on the teeth of young rats fed either a basic diet (99%) with 1% sucrose or a basic diet (44%) and glucose (55%) with 1% sucrose (28), or fed a sucrose (99%) or sucrose (56%) and glucose (43%) diet supplemented with only 1% basic components (van Houte, unpublished data), is comparable to that in rats fed the customary 56% sucrose diet. On the other hand, in the transmission studies (Table 8) in which bacterial transfer mainly occurs via coprophagy, significant differences in bacterial challenge could have occurred due to individual variation in the amounts of feces consumed.

The actual mechanism responsible for the observed changes with age is not known. In rats infected with *S. mutans*, the organism is present in the mouth and the intestinal canal. Recent studies with orally inoculated conventional rats showed that the minimum infective dose of strain 6715 is comparable for rats with or without access to their feces (28); furthermore, the colonization of this strain on the teeth appears to be required for its presence in feces whereas the levels of 6715 cells in feces of older rats over 3 or 4 months of age that do become infected are similar to those in feces of younger rats (28; G. Huber, J. van Houte, and S. Edelstein, manuscript submitted for publication). These data suggest that the effect of age on the colonization of *S. mutans* on the teeth results from changes in the mouth rather than in the intestinal canal.

With respect to the mouth, it is unlikely that the effect of age can be attributed to a change in tooth morphology, e.g., a decrease in the depth of the fissures which occurs in aging rats (21) in view of the magnitude of the changes observed. Furthermore, it is known that immediately after tooth eruption the enamel surface of the molar teeth of rats is covered by materials such as the reduced enamel organ and connective tissue; this material progressively disappears in about 1 week (18). The first, second, and third molars in Spraque-Dawley rats erupt at the age of 13 to 20 days, 17 to 23 days, and 33 to 41 days, respectively (22). It is possible, there-

fore, that these tissues, which alter the surface to which the labeled *S. mutans* cells must adhere, were responsible for the differences in implantation early after weaning with rats fed a glucose diet; this mechanism cannot account, however, for the difference observed with older rats.

The conventional rats obtained from Charles River Breeding Labs had been provided with a stock diet containing 17 ppm fluoride prior to their use. Thereafter, they had been fed a fluoride-free high-sucrose or glucose diet. Thus, the teeth of rats of different ages had received a different exposure to fluoride. The fluoride level of hydroxyapatite has been reported to influence in vitro the adherence of *S. mutans* (W. B. Clark, T. H. Howell, S. N. Kreitzman, and K. S. Kornman, Int. Ass. Dent. Res., p. 27, 1973). On the other hand, no correlation between the fluoride level of teeth and the colonization of *S. mutans* has been found in hamsters (9) or humans (1; J. van Houte, R. Aasenden, and T. C. Peebles, Int. Ass. Dent. Res., p. 267, 1976). Furthermore, the changes in the colonization of *S. mutans* with conventional rats, fed the high-fluoride stock diet, and the ex-germfree rats, fed diet L-356 with only 0.9 ppm fluoride, occurred at the same age. This would seem to further minimize the role of fluoride in the observed effect of age on bacterial implantation.

As suggested earlier by studies with hamsters (10, 20), and in the present study with rats, the prior presence of *S. mutans* may interfere with the subsequent introduction of other *S. mutans* strains. However, as shown by the studies with ex-germfree rats, the manifestation of the effect of host age on the implantation of labeled *S. mutans* cells did not require the presence of natural *S. mutans* populations. On the other hand, the possibility cannot be excluded that other differences between the oral flora of conventional young and older rats were responsible for the differences in implantation. Thus, the age effect was observed with germfree rats conventionalized for only a few hours and already within a few days after inoculation (Table 7). However, since rats of different age exhibited a different susceptibility for the test organism, they may also have quickly developed an oral flora different with respect to other bacterial species. So far no attempts have been made to study the effect of age with rats maintained in the germfree state. Such experiments are hampered by problems of control of the inoculum size, since even very small numbers of inoculated cells may establish under these conditions and therefore differences between old and young animals may not become demonstrable.

The data obtained do not readily suggest a role of antibody. Antibody specifically directed against *S. mutans* appears not to be required for the manifestation of the effect of age. In the case of the ex-germfree rats challenged within a few hours after conventionalization, little or no antibody may be expected to be present at that time in serum or saliva as suggested by the studies of Ebersole et al. with germfree rats challenged with *Escherichia coli* (8). The influence of cross-reacting antibody induced by dead bacteria in the germfree diet or by other dietary constituents cannot be ruled out at present.

Changes in host susceptibility to infection by *S. mutans* could also be due to changes in the composition of the host secretions such as saliva. Besides aiding in mechanical clearance of oral bacteria, recent studies indicate that certain components of saliva may specifically influence the interactions between different oral bacteria and oral surfaces leading to cell attachment and accumulation (13); these include high-molecular-weight glycoproteins which continually bathe the oral surfaces and which selectively adsorb to the tooth surface leading to the formation of the "acquired pellicle." Of interest, therefore, is the observation that the salivary flow of pilocarpine-stimulated rats is far lower in weanlings than in 4- to 12-month-old rats, whereas the reverse is true for the viscosity of saliva (25). In addition, male and female Sprague-Dawley rats, as used in the study, are bred at Charles River Breeding Labs starting at about the age of 56 to 59 days, respectively. However, sexual maturity is reached earlier and also the serum levels of gonadotropic or sex hormones in this rat strain or in others undergo major changes prior to that time (5, 7). Furthermore, the administration of sex hormones, e.g., estrogens, to rats induces morphological changes in their salivary glands and in the salivary sialic acid content as well as changes in their caries experience (6, 23, 25). Recently, sex hormone administration to humans has been shown to lead to changes in the protein content, sialic acid, fucose or hexosamine content, or the electrolyte concentration of saliva (24). Consequently, the relationship between hormonally induced salivary changes which accompany puberty and bacterial colonization would seem to merit further study.

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