The Acquisition of *Streptococcus mutans* by Infant Monkeys (*Macaca fascicularis*) and its Relationship to the Initiation of Dental Caries

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(Received 17 December 1981)

The acquisition and transmission of *Streptococcus mutans* by 16 consecutively born infant monkeys (*Macaca fascicularis*) was studied. The 16 infant monkeys were weaned in four groups and caged together to form a commune. Transmission from mother to infant occurred infrequently. *Streptococcus mutans* was isolated from nine of the mothers but only from the dental plaque of two infant monkeys at weaning at which time the predominant streptococci were *S. sanguis* and *S. mitior*. One further animal was colonized by *S. mutans* during the formation of the commune, but only after it was caged with an infant harbouring the same organism. For 46 d after the completion of the commune, the monkeys were fed a starch-based diet during which time *S. mutans* of serotypes *c*, *e* or *h* were isolated from the faeces of all 16 animals and from the dental plaque taken from the developmental groove of the first deciduous molar of 11 animals. Faecal transmission appeared to be an important factor in the spread of *S. mutans* between monkeys in the commune. The monkeys were then fed a caries-promoting high sucrose diet resulting in a rapid increase in the proportion of *S. mutans* in the plaque and in the faeces. *Streptococcus mutans* serotype *e* was more frequently isolated from both plaque and faeces and its predominance may in part be due to the production of a bacteriocin active *in vitro* against *S. mutans* serotype *h* and other species of oral streptococci isolated from monkey dental plaque. The proportion of *S. mutans* in the developmental groove 8 d after the introduction of the high sucrose diet was significantly related to both the caries status of the groove and the total caries score 6 months later. The results suggest that, in this model of human dental caries, *S. mutans* is the major bacterial factor in the initiation of tooth decay.

INTRODUCTION

Streptococcus mutans has been implicated as a major aetiological agent of human dental caries (Clarke, 1924; Gibbons & van Houte, 1975; Loesche & Straffon, 1979). This has been substantiated in monkey models of human tooth decay (Bowen, 1969*a*; Lehner *et al.*, 1975; Schick *et al.*, 1976; Bahn *et al.*, 1977) by the ability of *S. mutans*-based vaccines to reduce the amount of disease. These vaccines are effective whether whole cells (Bowen, 1969*a*; Lehner *et al.*, 1975), cell walls (Bowen *et al.*, 1975) or purified cell wall proteins are used as the immunogen (Lehner *et al.*, 1980; Russell *et al.*, 1982).

Considerable information is available regarding the establishment and persistence of *S. mutans* in human mouths. *Streptococcus mutans* is probably transmitted from mothers to their infants (Rogers, 1975; Berkowitz & Jordan, 1975; Berkowitz *et al.*, 1975); infection from inanimate objects is probably rare (Köhler & Bratthall, 1978). The established human plaque is very resistant to the introduction of extraneous *S. mutans* strains (Krasse *et al.*, 1967; Jordan *et al.*, 1972; Edman *et al.*, 1975; Svanberg & Loesche, 1978) and established *S. mutans* populations are stable over long periods (Rogers, 1975, 1980).

Recently we described the isolation of *S. mutans* serotype *h* (Beighton *et al.*, 1981) from the dental plaque of monkeys and we reported the occurrence of three distinct types of *S. mutans* populations in the plaque of monkeys fed a sucrose-rich diet (Beighton & Hayday, 1982*a, b*; Cohen & Bowen, 1966). The three *S. mutans* populations were dominated by strains of serotype *e*, by serotype *c* and by a mixture of serotypes *c* and *h*. We do not know the factors that influence the emergence of any of these *S. mutans* populations, although once established they remained stable for at least 2 years after the introduction of the sucrose diet (Beighton & Hayday, 1982*b*). Attempts to introduce extraneous *S. mutans* strains into the plaque of rhesus monkeys (Lehner *et al.*, 1975) or macaque monkeys (Beighton & Hayday, 1982*b*) were unsuccessful unless the natural plaque flora is suppressed by antibiotics (Bowen, 1969*b*).

Many of the features of *S. mutans* ecology reported for monkeys are similar to those observed in humans. An area of study that has received little attention in the monkey model has been the natural acquisition of *S. mutans* by infants and the subsequent transmission of the organism between animals. Our previous studies (Beighton & Hayday, 1982*a, b*) have shown that transmission of *S. mutans* between monkeys occurs readily, as almost all monkeys caged together in a group and fed a sucrose-rich diet harbour the same *S. mutans* population. However, it is not known when the transmission occurs. Animals born in the primate colony at Downe provide an opportunity to investigate the two problems of natural acquisition and transmission of *S. mutans*. In this study we have investigated the transmission of *S. mutans* from 16 mothers to their offspring. We have also studied the transmission of *S. mutans* between the infant monkeys when caged together. The longitudinal nature of this investigation has enabled us to relate the proportion of *S. mutans* in the dental plaque to the subsequent formation of carious lesions.

METHODS

Monkeys. Sixteen infant monkeys (*Macaca fascicularis*) born consecutively over a 3 month period (seven males and nine females) and their mothers were used in this study. The mean birth weight of the infant monkeys was 0.34 ± 0.05 kg.

Diets. A starch-based maintenance diet (Beighton *et al.*, 1981) was fed to the mothers and infants from birth until the monkeys were weaned. The infant monkeys continued to be fed this diet until the introduction of a caries-promoting (CPR) diet provided in a rissole form. The CPR diet contained (in 4 l of water): 1920 g white sausage rusk (T. Lucas & Co., Bristol), 720 g textured soya protein (T. Lucas & Co.), 500 g binder (code 463-R.C.S., D.C.A. Industries, Aylesbury, Bucks.), 300 g SA37, a vitamin and trace element supplement (Intervet Laboratories, Cambridge), 1000 g phosphate-free icing sugar (Tate & Lyle, Croydon, Surrey), 500 g skim milk powder (A. Head Ltd., Manchester), 500 ml calcium syrup (Sandoz Products Ltd., Feltham, Middlesex). This mix was shaped into approximately 75 rissoles. Each monkey received one rissole, a fig roll biscuit (W. & W. Jacobs & Co., Liverpool), half an egg, half a banana and four separate feeds of phosphate-free icing sugar daily.

Dental caries. Dental caries on the deciduous teeth were recorded at the introduction of the CPR diet and 3 and 6 months later using the previously reported criteria (Bowen, 1969*b*).

Media. Streptococci from oral samples were cultivated on TYC medium (Lab M), Mitis-Salivarius Agar, MSA (Oxoid) and on the modification of MSA (BMSA) described by Gold *et al.* (1973). BMSA is suitable for the isolation of *S. mutans* other than serotype *a* (Little *et al.*, 1977) and serotype *h* (Beighton *et al.*, 1981) but it is particularly useful when the number of *S. mutans* in plaque samples is low (Colman & Hayday, 1980).

The MSA results will not be presented as they were similar to those obtained using TYC medium. For example a comparison of the proportion of streptococci on these two media as a percentage of the total anaerobic colony count at one sampling showed a correlation of 0.715 ($P < 0.001$; Colquhoun, 1971) with median values of 75.1% for MSA and 82.3% for TYC. Although the isolation rates of *S. mutans* on TYC and MSA were significantly correlated ($r = 0.812$, $P < 0.001$) the median value of *S. mutans* on TYC was significantly greater than on MSA ($T = 27$, $P < 0.05$, Wilcoxon signed rank test; Colquhoun, 1971). On one occasion the isolation rates of *S. mutans* on TYC were compared with that on the non-selective medium and found not to be significantly different ($T = 40$, $P > 0.05$, Wilcoxon signed rank test).

In preliminary experiments faecal samples were cultured on TYC, MSA, BMSA and Columbia agar (Oxoid) supplemented with 50000 units polymyxin B l^{-1} , 20 mg nalidixic acid l^{-1} and 0.8 mg crystal violet l^{-1} (Unsworth,

1980). However, only BMSA was suitable for the isolation of *S. mutans* from faeces as the other streptococcal selective media supported a luxuriant growth of lactobacilli.

Total anaerobic colony counts were performed on an infusion-based medium composed of Brain-Heart Infusion (Oxoid; 37 g l⁻¹), yeast extract powder (Oxoid; 5 g l⁻¹), neutralized liver digest (Oxoid; 5 g l⁻¹), cysteine.HCl (1 g l⁻¹), polyvinylpyrrolidone (BDH; 10 g l⁻¹) and agar (Oxoid; 15 g l⁻¹). After autoclaving at 15 lbf in⁻² (103.5 kPa) for 15 min, 50 ml of lysed horse blood (Oxoid) and filter-sterilized menadione in 70% (v/v) ethanol was added to a final concentration of 0.5 µg ml⁻¹. This non-selective medium was prereduced for 24 h prior to use. After inoculation all media were incubated in anaerobic jars fitted with cold Deoxy catalyst (Engelhard, Cinderford, Glos.) at 37 °C for 2–3 d.

Microbiological sampling procedures. Mucosal surfaces (tongue, cheeks and gums) were sampled by swabbing with alginate swabs. The swabs were dissolved in 2 ml Thioglycollate medium without dextrose or indicator (TG medium, Difco), made up with Calgon-Ringer solution (Oxoid) to facilitate the dissolution of the alginate swabs. The dissolved swabs were then homogenized in glass/Teflon tissue grinders (Johnson *et al.*, 1978) and serially diluted in 10-fold steps in TG medium.

Plaque samples were scraped from selected tooth surfaces using sterile scalpel blades. The plaque samples were transferred to 2 ml TG medium contained in glass/Teflon tissue grinders, homogenized and diluted in 10-fold steps.

Faecal samples were collected into tared sterile bottles and the wet weight of faeces was calculated. The faeces were suspended in TG medium at a final concentration of 10 or 20 mg wet weight ml⁻¹ and dispersed with sterile glass beads as previously described (Walker, 1981). The samples were diluted in 10-fold steps in TG medium.

Identification of streptococci. The number of each colony type growing on the streptococcal selective media was counted and at least two representatives of each colony count were subcultured into 20 ml Todd-Hewitt broth (Oxoid) for identification. The cultures were checked for purity by plating on to horse blood agar (HBA) and each isolate was Gram-stained, tested for catalase activity, the production of acid from mannitol and sorbitol, hydrolysis of aesculin and arginine, formation of dextran and levan from sucrose in broth and for the formation of hydrogen peroxide, all as previously described (Beighton *et al.*, 1981). Using this initial set of tests we were able to identify some of the isolates from the plaque as either *S. sanguis*, *S. mitior*, *S. milleri* or *S. mutans* according to the scheme of Colman (1976). *Streptococcus mutans* strains were serotyped using the antisera and technique described by Colman & Hayday (1980). *Streptococcus mutans* typing serum for serotype *c* was prepared using *S. mutans* GS5, for serotype *b* using *S. mutans* FA1, for serotypes *a*, *d* and *g* *S. mutans* NCTC 10919 and for serotype *e* Lancefield group E strain 3X27. Isolates forming precipitates with the *a*, *d*, *g* typing serum were not susceptible to inhibition by bacitracin so none was considered to be serotype *a* (Shklair & Keene, 1974). It was not possible to distinguish between serotypes *d* and *g* using these sera so subsequently in this report those strains reacting with the serum prepared using NCTC 10919 will be referred to as serotype *d/g*. *Streptococcus mutans* serotype *h* was identified as previously described (Beighton *et al.*, 1981).

Dental plaque samples taken prior to completion of the commune. Within 1–4 d of parturition, a pooled plaque sample was taken from each mother and the oral mucosa of each edentulous infant was swabbed. Plaque samples were also taken from each mother and from the surfaces of all erupted incisor teeth of the infant monkeys when aged between 39 and 72 d. Monkeys were weaned when 112 ± 14 d old, with a mean weight of 0.78 ± 0.14 kg. At weaning each infant monkey had 12 to 14 teeth erupted, including the first deciduous molars. A plaque sample was taken from the incisor teeth and a second sample was taken from the palatal groove on the upper left first deciduous molar tooth (*d*) of each infant monkey (Fig. 1). The plaque present in the palatal developmental groove was sampled as *S. mutans* preferentially colonizes such sites in macaque monkeys (Colman & Hayday, 1980; Beighton *et al.*, 1981). As the upper tooth *d* is the first grooved tooth to erupt it was the tooth of choice for investigation. In studies of the transmission of *S. mutans* from human mothers to their offspring the level and types of *S. mutans* present in the mother's saliva have usually been investigated to ascertain the infectivity of the mother. However, *S. mutans* may not be detected in the saliva if it is present in the plaque only at low levels (Svanberg & Loesche, 1978). For more reliable isolation of all serotypes from the mothers, plaque rather than saliva was examined.

The infant monkeys were caged together in a commune which was formed by weaning monkeys in four groups of two to five and adding them sequentially to the commune. Within 7 to 14 d of each new group being placed in the commune, a plaque sample was taken from the palatal groove of tooth *d* of all monkeys already in the commune. The first group of two monkeys was caged in the commune prior to its completion for 89 d and four samples were taken from each monkey; the second group of six monkeys was caged in the commune for 63 d and three samples were taken; the third group of five monkeys was caged in the commune for 29 d and two samples were taken from each monkey. The commune was completed by the addition of the three monkeys in the fourth group. All the plaque samples and the oral swabbings referred to in this section were plated on to BMSA and TYC.

Dental plaque samples taken after completion of commune. When all sixteen monkeys had been housed in the commune, plaque samples were taken only from the palatal groove of tooth *d*. For clarity the day on which the commune was completed will be referred to as day zero and plaque samples were subsequently taken on days 8,

Fig. 1. Dental plaque samples were taken from the palatal developmental groove (arrow) of the left maxillary first deciduous molar tooth (d). The bar marker represents 0.5 cm.

26, 46, 54, 75, 96, 111, 131 and 147. The samples taken on day 8 were plated on the MSA, BMSA and TYC; all subsequent samples were also plated on to the non-selective medium.

Faecal samples. Faecal samples were taken on days 8, 28, 40, 60, 90, 117 and 150 and were plated on to BMSA.

Enumeration of streptococci. The plaque samples taken from the mothers and the oral swabbings taken from the infant monkeys at birth and the first plaque sample taken from the incisor teeth were examined solely for the presence of *S. mutans*. In all subsequent samples the proportion of each streptococcal species was expressed as a percentage of the total colony count on the non-selective medium. The *S. mutans* counts in the faeces were expressed as the number of c.f.u. (mg wet wt)⁻¹.

Change of diet. The diet was changed to the CPR diet on day 46. The mean weight at the introduction of the CPR diet was 0.94 ± 0.12 kg and on day 150, 1.31 ± 0.19 kg. No attempts were made to implant any extraneous organisms into the plaque of any monkey.

Bacteriocin production by *S. mutans* serotype *e*. As *S. mutans* serotype *e* strains predominated in the plaque of the monkeys fed the CPR diet (see Results) it was decided to investigate whether this domination might be due to the elaboration of a bacteriocin(s) by the serotype *e* strains isolated from the monkeys. The stab culture technique (Fredericq, 1957) was used to detect bacteriocin production. Possible producer strains were stabbed into Diagnostic Sensitivity Test Agar, DSTA (Oxoid) and incubated at 37 °C for 48 h. DSTA was used as this was found to give larger zones of inhibition than any of the other media used in preliminary experiments. The inoculated plates were then overlayed with a 4.5 ml of Trypticase Soya Broth (Difco) made semi-solid by the addition of 0.8% (w/v) agar (Oxoid) to which 0.5 ml of a 24 h culture of the indicator strain in Todd-Hewitt Broth was added. The plates were incubated for a further 24 h at 37 °C and the diameter of the zones of inhibition about the stabs were measured in millimetres.

Representative strains of *S. mutans* serotypes *c*, *e*, *h* and *d/g* isolated from the monkeys were assessed for production of bacteriocins using the *S. mutans* serotypes and other streptococcal species isolated from the monkey plaque as indicator strains.

To test the sensitivity of the bacteriocin produced by serotype *e* strains for inhibition by various enzymes, 0.1 ml of filter-sterilized solutions of catalase (Sigma, 11 000 Sigma units mg⁻¹), trypsin (BDH, from beef pancreas), protease Type XIV (Sigma, from *Streptomyces griseus*, 5.4 units mg⁻¹) and phospholipase C Type 1 (Sigma, from *Clostridium perfringens*, 4.8 units mg⁻¹), each at 1 mg ml⁻¹, were spotted over the regions of DSTA plates known to contain preformed bacteriocin produced by the technique described above. Each solution was allowed to dry into the medium for 1 h and the plates were overlayed with an indicator strain (*S. mitior* FW102) as described above. The plates were incubated for 24 h and the zones of inhibition were measured.

RESULTS

Classification of the streptococci isolated from dental plaque and faeces

Using the initial set of tests it was possible to identify many of the 2544 plaque isolates as *S. mutans*, *S. milleri*, *S. mitior* or *S. sanguis* (Colman, 1976). The remainder of the plaque streptococci were classified into four biotypes on the basis of the initial set of tests. These were considered variants of *S. sanguis* and *S. mitior*. The two *S. sanguis* variants were the same as *S. sanguis* type I (Colman, 1976) except that one did not form extracellular polysaccharide from sucrose and the other did not hydrolyse arginine. The two *S. mitior*

Table 1. *Caries status of mothers and carriage of S. mutans serotypes by mother-infant pairs*

Pair no.	Caries score*	<i>S. mutans</i> in plaque
1	2/32	—
2	1/32	<i>c</i>
3	0/32	<i>c</i>
4	3/32	<i>c</i>
5	1/32	—
6	2/32	—
7	6/32	<i>c, h</i>
8	22/32	—
9	0/32	—
10	0/32	—
11	15/32	<i>c, d/g</i>
12	10/28	<i>e, h, d/g</i>
13	2/32	—
14	0/32	<i>c, h</i>
15	0/32	<i>c</i>
16	11/32	<i>c, h</i>

* Number of carious teeth per total number of teeth present.

—, Indicates no *S. mutans* strains were isolated on BMSA or TYC media.

variants were able to hydrolyse either aesculin or arginine but otherwise they resembled *S. mitior* (Colman, 1976). In this report the proportions of these four variants are not shown separately but are included together with percentages of *S. sanguis* or *S. mitior*.

A total of 948 streptococcal isolates were subcultured from the faecal samples plated on to BMSA and these were identified as *S. mutans* (Colman, 1976; Beighton *et al.*, 1981).

Isolation of S. mutans from mother-infant pairs

The caries score and the *S. mutans* serotypes isolated from the mothers are shown in Table 1. Five mothers were caries-free and *S. mutans* strains were isolated from nine of the 16 mothers' plaque at one or both of the two sampling times. The major *S. mutans* serotype isolated from the mothers was serotype *c* (eight mothers); serotype *h* was isolated from four, strains belonging to serotypes *d/g* were isolated from two and only one mother harboured detectable levels of *S. mutans* serotype *e* in her plaque.

No *S. mutans* strains were isolated from the edentulous infants and only the infant of pair 16 harboured detectable levels of *S. mutans* (serotype *c*) on its incisor teeth at the second sampling. At weaning *S. mutans* serotype *e* was isolated from the palatal groove of tooth d of the infant of pair 12 and serotype *c* was isolated from the same site of the infant of pair 16. The infant of pair 16 also harboured serotype *c* strains on its incisor teeth. The *S. mutans* serotype present on the teeth of these two infant monkeys was also cultivated from their mothers' plaque. However, in the seven other cases where the mothers' plaque harboured detectable *S. mutans*, none was detected in the oral cavity of their offspring. In the remaining seven pairs, where the mothers did not harbour detectable *S. mutans*, none was isolated from their offspring prior to weaning.

Streptococci in dental plaque of monkeys at weaning

The composition of the streptococcal flora isolated from the palatal groove on tooth d and from the incisor teeth is shown in Table 2. In the palatal groove *S. sanguis* formed the major proportion of the streptococcal flora (median = 47.7%) with *S. mitior* forming 17.8% of the streptococcal colony count on TYC medium. From the incisors, *S. mitior* strains formed 69.1% of the streptococci while *S. sanguis* strains comprised only 13.5%. *Streptococcus milleri* was grown from both sites but at a lower frequency of isolation than either *S. sanguis* or *S. mitior*.

Table 2. *Streptococcal flora of plaque collected from the palatal groove of the tooth d and from the incisor teeth of 16 newly weaned monkeys*

The mean $\pm$ s.d. of the total streptococcus colony count on TYC medium, median, range and number (No.) of monkeys from which organism was isolated are shown. Samples from the incisor teeth of two monkeys were contaminated and are not included.

	Palatal groove on tooth d				Incisor			
	Mean $\pm$ s.d.	Median	Range	(No.)	Mean $\pm$ s.d.	Median	Range	(No.)
<i>S. mutans</i>	1.5	<0.01	<0.001-12.6	(2)	0.6	<0.01	<0.005-8.5	(1)
<i>S. sanguis</i>	45.9 $\pm$ 32.8	47.7	<0.5-100	(14)	21.6 $\pm$ 25.8	13.5	<0.5-74.0	(10)
<i>S. mitior</i>	29.0 $\pm$ 30.7	17.8	<0.5-100	(13)	68.1 $\pm$ 29.7	69.1	6.4-100	(14)
<i>S. milleri</i>	16.6 $\pm$ 30.3	<0.5	<0.5-95.6	(7)	9.3 $\pm$ 16.8	<0.5	<0.5-50.0	(5)
Unidentified	7.0	<0.5	<0.5-75.4	(3)	0.4	<0.5	<0.5-5.3	(1)

Streptococcus mutans in plaque of monkeys during the formation of the commune

As the weaned monkeys were added to the commune in four separate groups not all the animals were examined for the presence of *S. mutans* in the plaque on the same number of occasions. Prior to the completion of the commune, serotype *e* strains were isolated from the infant of pair 6 but only after the addition to the commune of the infant of pair 12 (which also harboured serotype *e*). Except for the infants of pairs 12 and 16 no other infant harboured detectable levels of *S. mutans* during the formation of the commune.

Streptococcal flora of dental plaque after commune completion

The streptococcal flora of plaque samples taken from the palatal groove of tooth d after the completion of the commune before and after the introduction of the CPR diet, is shown in Table 3. Due to the skewed distribution of results the median rather than the mean values are tabulated. The change to the CPR diet resulted in an increase in the proportion of total streptococci and *S. mutans*. The proportion of *S. sanguis* as well as the number of monkeys from which *S. sanguis* strains were isolated was greatly reduced following the introduction of the CPR diet. When the CPR diet was introduced (day 46) *S. mutans* serotype *c* strains were isolated from two monkeys, serotype *e* strains from five, serotype *h* from four and serotype *d/g* from two; two monkeys each harboured two different serotypes. On day 147 five monkeys harboured *S. mutans* serotype *c* (range <0.5-65.2), 15 serotype *e* (<0.5-100), three serotype *h* (<0.5-0.6) and three serotype *d/g* (<0.5-0.6). Only the change in the proportion of serotype *e* was significant (Wilcoxon signed rank test, $P < 0.01$; Colquhoun, 1971). The numbers of monkeys harbouring each *S. mutans* serotype at each time of sampling after the completion of the commune is shown in Fig. 2.

Streptococcus mutans in faeces

Between day 8 and day 40 there was a marked increase in the *S. mutans* carriage rate in the faeces (Table 4). All monkeys harboured *S. mutans* in their faeces prior to the introduction of the CPR diet; 10 monkeys harboured serotype *e* strains while four harboured serotype *c* and serotype *h* was isolated from six monkeys on day 40. The concentration of *S. mutans* (all serotypes) also rose from a median value of <10 on day 8 to 5.9×10^2 c.f.u. mg⁻¹ by day 40. The CPR diet increased the level to a relatively constant $1-2 \times 10^5$ c.f.u. mg⁻¹ between days 90 and 150. This increase in *S. mutans* was due entirely to the increase in the concentration of serotype *e* strains. By day 60 and at all subsequent samplings all monkeys harboured serotype *e* strains; on day 150 serotype *h* strains were isolated from only one monkey and no serotype *c* strains were isolated.

Correlation between S. mutans in faeces and plaque

Prior to the introduction of the CPR diet *S. mutans* could be isolated from the faeces but not from the plaque of five monkeys. Similarly, on day 147 *S. mutans* was not isolated from

Table 3. The streptococcal flora of plaque samples taken from the palatal groove on tooth d of a group of 16 monkeys

A starch-based maintenance diet was fed up to day 46 and subsequently the CPR diet was provided. Median values are expressed as a percentage of the total anaerobic colony count. No. is the number of monkeys from which organisms were isolated.

Day		Total streptococci	<i>S. mutans</i>	<i>S. sanguis</i>	<i>S. mitior</i>	<i>S. milleri</i>	Other
26	Median	32.2	<0.001	18.0	<0.5	<0.5	<0.5
	Range	11.4-82.3	<0.001-78.8	1.1-66.1	<0.5-10.0	<0.5-3.0	<0.5-5.8
	No.	16	6	16	5	2	2
46	Median	39.2	<0.05	2.8	<0.5	<0.5	<0.5
	Range	0.6-95.5	<0.001-86.4	<0.5-71.4	<0.5-24.7	-	-
	No.	16	11	11	2	0	0
54	Median	41.9	31.8	3.3	<0.5	<0.5	<0.5
	Range	14.0-100	<0.001-99.3	<0.5-35.3	<0.5-15.8	-	<0.5-5.9
	No.	16	12	10	5	0	1
75	Median	82.3	81.6	<0.5	<0.5	<0.5	<0.5
	Range	10.0-100	<0.001-100	<0.5-25.4	<0.5-34.0	<0.5-6.4	<0.5-16.0
	No.	16	15	7	2	1	1
96	Median	88.4	85.3	<0.5	<0.5	<0.5	<0.5
	Range	23.3-100	1.0-100	<0.5-4.8	<0.5-17.5	<0.5-6.0	-
	No.	16	16	1	1	1	0
111	Median	81.7	81.2	<0.5	<0.5	<0.5	<0.5
	Range	8.5-100	0.01-100	<0.5-8.5	-	<0.5-0.9	-
	No.	16	16	2	0	1	0
131	Median	77.4	77.4	<0.5	<0.5	<0.5	<0.5
	Range	5.3-100	2.9-100	<0.5-2.4	<0.5-1.3	-	-
	No.	16	16	1	1	0	0
147	Median	92.6	92.6	<0.5	<0.5	<0.5	<0.5
	Range	33.3-100	<0.5-100	<0.5-2.0	-	<0.5-32.5	-
	No.	16	15	1	0	1	0

Fig. 2. The frequency of isolation of *S. mutans* serotype c (■) e (●) and h (▲) from the dental plaque of the 16 infant monkeys after the completion of the commune. The CPR diet was introduced on day 46.

the plaque of one monkey but was present in the faeces of all monkeys on day 150. This discrepancy is probably due to the large samples of faeces that were examined for the presence of *S. mutans* compared with the very small (less than 1 mg wet weight) plaque samples obtained from the single developmental groove sampled. The increase in *S. mutans* serotype e in the plaque paralleled the increase apparent in the faecal samples (Fig. 3).

Bacteriocin production by *S. mutans* serotype e

Streptococcus mutans serotype e strains inhibited *S. sanguis*, *S. mitior*, *S. milleri* and *S. mutans* serotype h strains isolated from monkey dental plaque (Table 5). Variable results

Table 4. Median *S. mutans* serotype concentrations (c.f.u. mg⁻¹) in the faeces of infant monkeys after the completion of the commune (day 0) prior to and following the introduction of the CPR diet

Median values are expressed as a percentage of the total anaerobic colony count. No. is the number of monkeys from which organisms were isolated.

Day		Total	<i>c</i>	<i>e</i>	<i>d/g</i>	<i>h</i>
8	Median	<10	10	<10	<10	<10
	Range	<10-2 × 10 ³	10-2 × 10 ³	<10-2.2 × 10 ²	-	<10-10 ²
	No.	4	1	2	0	2
28	Median	<10	<10	<10	<10	<10
	Range	<10-3.2 × 10 ⁴	<10-10	<10-3.2 × 10 ⁴	<10-6 × 10 ²	<10-20
	No.	8	2	4	1	2
40	Median	5.9 × 10 ²	<10	90	<10	<10
	Range	15-5.0 × 10 ⁴	<10-6.3 × 10 ³	<10-5 × 10 ⁴	-	<10-5.6 × 10 ³
	No.	16	4	10	0	6
60	Median	2.2 × 10 ⁴	<10	2.1 × 10 ⁴	<10	<10
	Range	2 × 10 ² -3.5 × 10 ⁵	<10-1.3 × 10 ⁴	2 × 10 ² -3.5 × 10 ⁵	<10-4.8 × 10 ⁴	<10-5 × 10 ⁴
	No.	16	2	16	3	5
90	Median	1.7 × 10 ⁵	<10	1.7 × 10 ⁵	<10	<10
	Range	1.3 × 10 ⁴ -6.2 × 10 ⁵	<10-7 × 10 ⁴	1.3 × 10 ⁴ -6.2 × 10 ⁵	-	<10-7.4 × 10 ⁴
	No.	16	1	16	0	3
117	Median	1.9 × 10 ⁵	<10	1.9 × 10 ⁵	<10	<10
	Range	1.5 × 10 ³ -6.8 × 10 ⁵	-	1.5 × 10 ³ -6.8 × 10 ⁵	-	<10-1.5 × 10 ⁴
	No.	16	0	16	0	1
150	Median	1.2 × 10 ⁵	<10	1.2 × 10 ⁵	<10	<10
	Range	1.1 × 10 ⁴ -4.7 × 10 ⁵	-	8 × 10 ³ -4.7 × 10 ⁵	-	<10-2.7 × 10 ³
	No.	16	0	16	0	1

Fig. 3. Changes in the proportions of *S. mutans* serotype *e* in the dental plaque (■) and faeces (●) of the 16 infant monkeys.

Table 5. Inhibition of streptococci isolated from monkey dental plaque by ten strains of *S. mutans* serotype *e*

Indicator organism	Number tested	Mean diam. of inhibition zone (mm)	Percentage of tests showing inhibition
<i>S. sanguis</i>	17	5.9	99
<i>S. mitior</i>	7	11.0	97
<i>S. milleri</i>	3	10.7	97
<i>S. mutans</i> serotype <i>h</i>	7	4.9	97

Table 6. *Proportion of S. mutans in the palatal groove of tooth d*

The proportion of *S. mutans* (as a percentage of the total anaerobic colony count) in the palatal groove of tooth d was determined for each of 16 monkeys 8 d after the change to the CPR diet; the total caries score 3 and 6 months after the diet change and the caries status of the palatal groove 6 months after the diet change are also shown.

	Percent <i>S. mutans</i>	Total caries score		State of palatal groove on tooth d at 6 months
		3 months	6 months	
Group A	92.9	2	16	carious
	99.3	2	20	carious
	94.1	1	11	carious
	55.0	2	13	carious
	62.5	2	15	carious
	72.0	2	16	carious
	16.2	1	16	carious
	67.8	2	11	carious
	46.4	3	5	carious
	12.5	0	11	carious
Group B	9.0	0	0	not carious
	0.004	1	4	not carious
	<0.001	0	5	carious
	<0.001	0	1	not carious
	0.003	0	2	not carious
	0.001	0	0	not carious

were obtained with serotype *c* strains as indicators with small zones of inhibition (3 mm in diameter) sometimes being observed; these zones were not reproducible. The few serotype *d/g* strains isolated were inhibited to the same extent as serotype *h* strains by serotype *e* strains. Serotype *e* strains were not self-inhibitory. The bacteriocin activity of serotype *e* was not inhibited by catalase, trypsin, phospholipase C or *Streptomyces griseus* protease.

Serotypes *c* and *h* strains did not inhibit serotype *e* strains and serotype *h* strains could not be shown to be antagonistic to any other streptococcal isolates. Serotype *c* strains were infrequently found to inhibit some serotype *h* strains and some isolates of other oral streptococci.

Dental caries scores

The mean number of carious lesions per monkey 3 months after the introduction of the CPR diet was 1.1 ± 1.0 which after 6 months had increased to 9.1 ± 6.4 lesions per animal. In order to assess the relationship between the initial level of *S. mutans* and the number of carious lesions which subsequently developed, the monkeys were divided into two groups according to the concentration of *S. mutans* in the plaque sample taken from tooth d 8 d after the change to the CPR diet. Group A (11 monkeys) harboured large numbers of *S. mutans* in their plaque while the five monkeys in group B harboured very low numbers of *S. mutans* (Table 6). On day 147 (14 weeks after the change to the CPR diet) the median percentage of *S. mutans* in the grooves of the monkeys in group A (95.2; range 33.0–100) was not significantly different (Wilcoxon two sample test; $T = 20$; $P > 0.1$) from that of group B (77.8; range <0.5–89.4). After 3 and 6 months the mean number of carious lesions per monkey was significantly higher in group A than in group B (3 months $t = 3.02$; $P < 0.01$ and 6 months $t = 3.71$; $P < 0.01$).

As the plaque samples were taken from the groove on tooth d it was possible to relate the early *S. mutans* concentration with the caries status of the groove 6 months after the diet change. In group A, 10 of 11 grooves became carious after 6 months while in group B only 1 of 5 grooves became carious. This difference was significant ($P = 0.013$, Fisher's Exact Test; Colquhoun, 1971).

DISCUSSION

Edentulous infant monkeys did not harbour *S. mutans* in their oral cavities, which is consistent with the findings of human studies in which *S. mutans* is rarely isolated in the absence of erupted teeth (Carlsson *et al.*, 1975; Berkowitz *et al.*, 1975, 1980; Catalanotto *et al.*, 1975; Stiles *et al.*, 1976). There was a limited transmission from mother to infant during the period of breast feeding. A direct comparison between the degree of *S. mutans* infection in human and monkey infants is not possible because of the more rapid development of the monkeys. However, these results indicate that, as in humans (Berkowitz *et al.*, 1980), *S. mutans* is not usually detected in the mouth until the later stages of incisor emergence.

The rapid transmission of *S. mutans* between infant monkeys was probably due to the caging conditions and to their social habits. The cages in which the monkeys are housed have aluminium mesh floors (Coid, 1967) to which faecal material often adheres so that when food is picked up from the floors it will be contaminated. The fur of the animals is also often contaminated with faecal material and this may be picked off and eaten during grooming activities. The high level of *S. mutans* in faeces would therefore facilitate its transmission between monkeys. The extent of the salivary transmission of *S. mutans* is not known; however given the large numbers of *S. mutans* in faeces and the grooming habits of monkeys it would seem that the importance of salivary transmission is negligible. In contrast, saliva is thought to be the main vehicle for the transmission of *S. mutans* between humans (Berkowitz *et al.*, 1975; Carlsson *et al.*, 1975; Köhler & Bratthall, 1978; Rogers, 1980).

As expected, the introduction of the sucrose-rich diet resulted in an increase in the proportion of *S. mutans* in the plaque in accord with previous studies of this species (Cornick & Bowen, 1972; Colman & Hayday, 1980; Beighton & Hayday, 1982*b*) and of rhesus monkeys (Caldwell *et al.*, 1977). The proportion of *S. mutans* in the plaque of monkeys fed the CPR diet is much higher than that found in humans (Socransky & Manganiello, 1971). The demonstration of an accompanying rise in the proportion of *S. mutans* in the faeces has not been reported previously. *Streptococcus mutans* has been isolated from human (Kilian *et al.*, 1971; Stiles *et al.*, 1976; Hamada *et al.*, 1980; Unsworth, 1980) and monkey (Walker, 1981; Walker *et al.*, 1982) faeces but no attempts have been made to relate the numbers of *S. mutans* to the dietary sucrose concentration. Our results indicate that the addition of sucrose to the diet can have a profound effect on the numbers of *S. mutans* in the faeces: these were high suggesting that, even though seeding from the oral cavity might be needed to maintain its presence there, *S. mutans* is able to proliferate, especially when the monkeys were fed the sucrose-rich diet. As in humans (Hamada *et al.*, 1980) the same predominant *S. mutans* serotype was usually isolated from both the plaque and faeces of individual monkeys. *Streptococcus mutans* serotype *h* does not grow well on BMSA (Beighton *et al.*, 1981) so the proportion of this serotype in the faeces may be underestimated.

Previous studies (Beighton & Hayday, 1982*a, b*) have identified three types of *S. mutans* populations in the plaque of monkeys fed a similar sucrose rich diet (Cohen & Bowen, 1966). In the present group of monkeys an *S. mutans* serotype *e* population predominated in all but one of the 16 monkeys. The ability of serotype *e* to elaborate bacteriocins that have an antagonistic effect on most of the oral streptococcal species tested might partly explain the dominance of serotype *e* strains. The role bacteriocins play in the ecology of the dental plaque has been investigated both *in vitro* and *in vivo* by other workers. A bacteriocin-producing *S. mutans* strain selectively killed sensitive *S. sanguis* in artificial plaque formed on glass rods (Weerkamp *et al.*, 1977). Van der Hoeven & Rogers (1979) found that bacteriocin-producing strains of *S. mutans* more readily colonized the dental plaque of specific pathogen-free rats and suggested that bacteriocin production might be an important property in the successful invasion of plaque by *S. mutans*. Their observations are in accord with our suggestion that the dominance of serotype *e* strains may be due partly to its capacity to produce bacteriocins. However, bacteriocin-mediated inhibition does not appear to be the explanation for the apparent inability of serotype *c* strains to compete successfully with serotype *e* strains *in vivo*.

In most cases serotype *c* strains were not isolated from the serotype *e* dominated plaque suggesting that additional and unknown factors are responsible for the successful domination of serotype *e* strains.

In a previous report from this Unit (Colman & Hayday, 1980) plaque samples were taken from the lingual development grooves of the upper second deciduous molar tooth of each of 22 monkeys at the time of the introduction of a caries-promoting diet, and 6 and 12 months later. The majority of these monkeys harboured *S. mutans* serotype *c* and 11 sites became carious while the remainder were caries-free. No carious lesion occurred in the absence of *S. mutans*; however, the presence of *S. mutans* was not always associated with the development of a lesion. The proportions of *S. mutans* in the carious and non-carious grooves were not different 6 and 12 months after the introduction of the sucrose-containing diet. In the present study there was no difference in the proportion of *S. mutans* in the carious and non-carious grooves 14 weeks after the introduction of the CPR diet. However, the rate with which the proportion of *S. mutans* increased in response to the sucrose in the diet was related to the number of lesions subsequently developing. In monkeys harbouring a plaque with high numbers of *S. mutans* 8 d after the diet change, the mean caries score was greater and the site sampled was more prone to caries than in monkeys with initially low numbers of *S. mutans*.

We are grateful to our colleagues at the Dental Research Unit for their expert assistance during the course of this investigation.

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