

THE INFECTIOUS NATURE OF DENTAL CARIES AND
MUTANS STREPTOCOCCI IN AN AUSTRALIAN RURAL SCHOOL COMMUNITY

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SUMMARY

This treatise is presented in two principal parts, a review of the literature and a report of a field study. A third part, the "Closure", includes the Conclusion and a Summary of the work.

The first part of this treatise reviews evidence supporting the description of dental caries as an infectious disease. In particular the role of *Streptococcus mutans* and the mutans streptococci group, is explored and considered. That dental caries is an infectious disease and that in the absence of microorganisms the dental caries process cannot occur is beyond doubt; evidence to support the aetiological role of *S. mutans* and the mutans group of streptococci is very strong.

An analysis of the infectious nature of dental caries and the description of the natural history of dental caries as an infectious disease, has made possible the development of a "medical model" appropriate for prevention and treatment of dental caries as an alternative to the "surgical model", for the treatment of the disease. The "surgical model" essentially relies on removal of diseased tissue and where possible, its replacement. The "surgical model" has been practised for centuries using progressively refined restorative and surgical techniques. The "medical model" suggested relies on interception of the disease process prior to the occurrence of irreversible damage to teeth or dentition.

It is anticipated the "medical model" for the treatment of dental caries will complement the "surgical model" and will become the principal form of treatment of this disease.

There has been no attempt to evaluate or review the contribution of molecular genetics to the microbiology associated with dental caries. The science of molecular biology applied to bacteria related to dental caries is in its infancy. Molecular biologists with researchers in other associated fields for instance bacterial physiology, carbohydrate chemistry and epidemiology are likely to be in a position to make considerable contributions to further the established understanding of dental caries and its causative micro-organisms. Already the molecular mechanisms involved with sugar transport, extracellular polysaccharide formation and surface adhesives are being explored. Notwithstanding the contribution to the control of dental caries which will almost certainly be made using molecular techniques, the principles of prevention of the disease using a "medical model" have been established.

The second part two is concerned with a study made at a small rural community in South Eastern New South Wales. The study was directed to controlling dental caries in primary school-children attending schools in the town of Boorowa. As well as establishing a regular programme of supervised self-application of fluorides at the schools the study included an evaluation of the dental caries experience of the children over the 18 years the study was conducted. In addition the prevalence of *S. mutans* in the children taking part in the program was established. Changes in the dental caries experience of the children at the schools in Boorowa and changes in the prevalence of *S. mutans* in their plaque considered as a group or a community of children aged 5 to 8 years, are reported in the second part of this study.

The relationship between the decline in *S. mutans* in the Boorowa community of school-children and the dental caries

experienced supports the concept that mutans streptococci and in this study *S. mutans* in particular, are agents able to initiate dental caries.

The third part of this treatise, the "Closure", includes a general summary and conclusions. The Closure includes general comments arising from both parts of the treatise

There are also five appendices which contains additional information although not all this information was obtained as part of the study at Boorowa. Quite a lot of the appended information is from sources outside this study. The information in the appendices is nevertheless relevant and may be of assistance in understanding aspects of the discussions set out.

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THE INFECTIOUS NATURE OF DENTAL CARIES AND
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1. AIM

The aim of this treatise is to determine under field conditions the effect of self applied topical fluoride on the prevalence of *Streptococcus mutans* in dental plaque of children age 5 to 8 years, in a rural, non-fluoridated community* and further, to determine whether a relationship between dental caries and *Streptococcus mutans* prevalence in dental plaque could be demonstrated,

1.1. Preliminary comment

This treatise will review the literature and other evidence related to the infectious nature of dental caries and is also to consider the evidence which has led to the development of a "medical model" for treatment of dental caries as an alternative to the widely practised "surgical model".

In particular the role of *Streptococcus mutans* and the group of streptococci collectively referred to as mutans streptococci, is to be reviewed both in relation to the initiation of dental caries and as an infectious agent in the transmission and establishment of this disease.

Additionally, this treatise will document the incidence of *S. mutans* in the dental plaque and of the schoolchildren at Boorowa, a rural town and community on the South West Slopes of New South Wales, and will record their dental caries experience over the years 1970 to 1986. This period corresponds not only with a dramatic decline in dental caries experience of schoolchildren throughout Australia but a similar, although slightly earlier decline, in dental caries experience of the schoolchildren at Boorowa.

* Community water < 0.3 parts per million fluoride

The opportunity to record the incidence of *Streptococcus mutans* in the dental plaque of almost all of the children in a rural community during a period of substantial caries reduction is unlikely to occur again.

1.2. Structure of treatise

For clarity, ease of understanding and presentation the information in this treatise will be presented in two parts.

Part 1 will review the literature relevant to the relationship between *S. mutans* or more recently mutans streptococci, in dental plaque to dental caries and will present evidence that dental caries is a consequence following the establishment of mutans streptococci in dental plaque and that dental caries can be considered to be the product of an infectious process.

Part 2 will present details and results of the field study of children age 5 to 8 years, undertaken at the two schools in Boorowa over the years 1970 to 1986. Details of these studies provided data of the incidence of *S. mutans* in the dental plaque of the children at Boorowa as well as their dental caries experience.

Part 3 consists of a summary of, and conclusions from these studies and as it applies to both Parts 1 and 2 is presented at the close of the treatise.

2. INTRODUCTION

2.1. DENTAL CARIES

Dental caries, the dissolution of the hard structure of the tooth resulting in cavitation, has been present as long as human society has existed. Guido Majno (1975) reviewing the history of medicine, refers to the skull of Rhodesian Man (30,000 to 40,000 years old) with worn and decayed teeth allowing bacteria to penetrate the bone.

The cause of dental caries was not appreciated until the late 19th century when Willoughby Miller (1887, 1890a, 1890b) proposed his chemico-parasitic theory of dental caries and demonstrated the presence of invasive acidogenic and aciduric oral bacteria in carious lesions. The infectious nature of dental caries was not completely and clearly established until the late 1960's, the history of the infectious nature of this disease is reviewed in detail in Section 3 of this treatise.

2.2. *STREPTOCOCCUS MUTANS* STUDIES AT BOOROWA

Attention was directed to the likely causal role of *Streptococcus mutans* from the late 1960's. Woods (1971a) proposed the possibility of using *S. mutans* isolated from dental plaque as an indicator of dental caries susceptibility.

A long term study of *S. mutans* in the dental plaque of primary schoolchildren at Boorowa, a small rural community in south west New South Wales, was initiated in 1970 and continued until 1986 with examinations in 1972, 1978, 1984, and 1986. In addition to studies of *S. mutans* and dental plaque the schoolchildren were examined for dental caries and some

information obtained by questionnaire on whether or not the children had been given fluoride supplements. The community water supply at Boorowa has a fluoride concentration of less than 0.03 part per million*.

Since 1977 schoolchildren in Australia have experienced a very substantial reduction in dental caries (Commonwealth Department of Health 1987). Information from the School Dental Service and from studies conducted at Tamworth (National Health and Medical Research Council 1991) reflect a similar trend and reduction of dental caries. The decline in dental caries was clearly demonstrated commencing in 1970 to 1986 in the schoolchildren at Boorowa.

2.3. *STREPTOCOCCUS MUTANS* AND DENTAL CARIES SUSCEPTIBILITY

Used as an indicator of future caries activity, *S. mutans* in dental plaque has been shown (Newbrun 1989) to demonstrate the best correlation for low caries activity groups. More recently (Roeters, van der Hoeven, Burgersdijk and Schaeken, 1995) demonstrated a highly significant correlation between *S. mutans* and the clinical caries score for children two to five years of age. Studies by Thibodeau and O'Sullivan (1995) have provided evidence to support the view that salivary mutans streptococci may be useful in predicting the dental caries risk in the deciduous dentition of some populations.

* The fluoride concentration of the reticulated water at Boorowa was tested from time to time by the author using a fluoride ion electrode. The fluoride concentration varies slightly up to a maximum concentration of 0.03 parts per million.

Grindefjord, Dahlof, Nilsson and Modeer (1995) in a Swedish study examined a number of potential predictive factors for dental caries in 1-year-old children and have concluded the presence of mutans streptococci with other factors such as an immigrant background, the mother's level of education and the consumption of sugar-containing beverages, had a significant correlation with caries developed at the age of three and a half years.

Continuing studies involving the assessment of a number of factors which could be used to predict dental caries in children up to the age of two and a half years. Grindefjord, Dahlof, Nilsson and Modeer (1996) confirmed the significance of the role of mutans streptococci as predictors. Other predictors which were also significant were immigrant background, mother's education and sugar consumption; these factors appeared to act synergistically in the development of dental caries.

The absence of *S. mutans* has been shown to be associated with absence of dental caries (Ikeda et al 1973,)*.

* The role of bacteria and their relevance to dental caries is reviewed (Section 3) and is presented as it was with this background that the studies at Boorowa were undertaken.

PART 1
EVIDENCE SUPPORTING THE CONCEPT OF DENTAL CARIES AS AN
INFECTIOUS DISEASE

3. A REVIEW OF THE INFECTIOUS NATURE OF DENTAL CARIES

3.1. EARLY STUDIES

Reviewing the history of infections in man, Majno (1975) refers to the earliest reliable record of bacterial infection in man, preserved in the dental caries of *Australopithecus*. Majno based his statement on dental caries present in *Australopithecus* teeth some two million years old. Further, Majno continues, "this full-blown picture of dental infection is painfully clear in the famous skull of Rhodesian man, 30 to 40,000 years old. Its teeth worn or decayed, allowed bacteria to penetrate into the bone, which is honeycombed with abscesses".

Certainly dental caries was an established disease in primitive societies long before a broader civilization overtook their life style. In addition, individuals in these societies suffered from severe secondary infections, involving and eroding the bone supporting and adjacent to the infected teeth, a sequel to the destruction of their teeth by dental caries. One can only speculate on the effect of these infections on the quality of life enjoyed by these early societies and the longevity of individuals as well as the average life span of the community.

Formal and more recent investigation of the bacterial aetiology of dental caries commenced in the nineteenth century with an understanding of the role of bacteria in infectious disease. Jacob Henle in 1840 (Burnett and Scherp 1968) set out what were at that time theoretical requirements to determine whether a microorganism bears a causative relationship to a disease. Some 40 years later Henle's pupil, Robert Koch,

restated these criteria which became known as "Koch's postulates". These postulates have provided the standard used to this day to test the relationship between infectious diseases and their aetiological agents.

Translated and paraphrased the postulates of Henle and Koch are (Burnett and Scherp 1968):

- 1. The organism must be found in all cases of the disease and its distribution must be consonant with the lesions observed.*
- 2. The organism must be isolated in pure culture and cultivated through a number of generations and subcultures outside the body of the host.*
- 3. The pure culture so isolated must reproduce the typical disease when it is injected (introduced) into a suitable host.*

Koch's postulates have provided the foundation for a rational approach to the understanding and control of infectious disease. Their application and relevance to dental caries will be explored later in the light of experimental evidence.

The principle of Henle and Koch did not allow for the event where several organisms could cause a very similar disease, with similar symptoms and characteristics, nor did the principles allow for asymptomatic carriers of disease causing micro-organisms. Nevertheless the criteria they established, subject to these limitations, remain sound. It should be understood these principles were developed when the study of infectious diseases was in its infancy; complex aspects of infectious diseases had not at that time been considered.

The research of Koch and Pasteur in the late nineteenth century influenced the nature of research into all infectious diseases and remains a strong influence in the philosophy of the control and prevention of these diseases.

Following their successful description of the aetiology of anthrax and tuberculosis, attempts were made to apply the principles developed and established by Koch and Pasteur to other infectious diseases thereby gaining an understanding of the nature of these diseases.

Observations of Miller and Underwood (1881) that micro-organisms are always present in carious lesions but not in sound tooth substance, and referred to by Stephan (1948) when reviewing factors associated with development of caries, in the first scientific report of an association between bacteria and dental caries.

It was in this climate that W.D. Miller, an American dentist and researcher, undertook his studies into the nature and aetiology of dental caries. In a series of experiments Miller (1887) demonstrated that saliva could bring about the fermentation of starch and sugar, and could produce acid. These experiments were also described in Miller's treatise, *The Micro-organisms of the Human Mouth* (Miller 1890a), and he concluded, "The agent giving rise to acid fermentation in the juices of the human mouth exists in the form of living organisms".

Miller (1890a) went on to examine the action of specific oral bacteria on carbohydrates. Of 22 oral bacteria examined, 16 brought about an acid reaction when cultured in "beef-extract-peptone-sugar solutions". Miller established that the acid produced was mostly lactic acid with traces of formic and butyric acids.

Using *in-vitro* techniques Miller (1887) continued his investigations and placed sections of sound extracted bicuspid tooth in a solution of beef extract with 0.2 per cent sugar.

This culture was inoculated with *Bacterium acidi lactici* which he had isolated from carious dentine. He also prepared a similar control culture from which he omitted the cane sugar.

After one week the thinner sections of tooth in the culture with sugar were softened, at the end of two weeks all but the thicker sections were decalcified and when the decalcified sections of tooth were examined, bacteria* had penetrated many of the tubules to a considerable depth. After three weeks the invasion appeared more extensive and Miller described it as "a typical case of caries". Acid was only produced in the culture which contained sugar. In the culture that had no sugar the tooth section remained unchanged.

Certainly, Miller's research established that oral bacteria could cause demineralization of tooth which appeared similar to caries. Miller described the characteristics of a bacterium that could cause caries of dentine and which could "without the presence of oxygen produce acid from sugar". Because of its ability to survive in the acid environment of Miller's experiment this organism agreed morphologically with *Bacterium acidi lactici* which he referred to as being "nearly related to if not identical with the fungus (bacteria*) of sour milk..." (Miller 1887).

Miller's attempt to establish the aetiology of dental caries was directed to understanding the processes which brought about decay in a dentition. Even though it was understood at the time, for instance by G.V. Black (1887), who stated that caries always begins at the tooth surface, Miller was unable to investigate the process of decay in enamel and referred only briefly to enamel caries. He described the form of enamel caries but made no attempt to explain its aetiology.

* Miller uses the word "fungi". From his illustrations and text he clearly means bacteria and I have substituted this description for clarity (RGW).

In effect Miller's research produced a working hypothesis to explain the cause of dental caries. It demonstrated that oral bacteria could produce acid capable of causing caries, but to do so they needed a source of carbohydrate. In the absence of carbohydrate these bacteria could not produce acid. Miller's investigations were however orientated towards an explanation of dental caries of dentine rather than enamel.

In his work Miller presented a strong case to support the role of bacteria in dental caries, although he was unable to prove a causal relationship. The chemico-parasitic theory of the cause of dental caries which he expressed, established the pattern for caries research and provided a clinical hypothesis for its prevention for more than 60 years.

3.2. LACTOBACILLUS AND DENTAL CARIES

Even though Miller suggested a species of *Lactobacillus* might be associated with caries of dentine most of his investigations were conducted on saliva, a source of many types of bacteria. Miller's later publication (Miller 1890b) places much less emphasis on specific bacteria and in fact lists six different kinds of bacteria*, five described as bacilli and one as a very large coccus.

Reviewing advances in understanding the mechanism and control of dental caries in 1947, Stephan (1948) considered the following observations to be of importance.

"1. The histological observations of Miller and Underwood in 1881 that micro-organisms are always present in carious lesions, but not in normally intact tooth substance.

"2. The clinical and microscopic observations of J. Leon Williams in 1897 and later by G.V. Black, that dense masses of micro-organisms (so called plaques) grow on 'caries susceptible' surfaces of teeth.

* Miller, quoting from a publication of Vignal and Galippe; Note sur les micro-organismes de la carie dentaire, (L'Odontologie Mars, 1889)

"3. The laboratory observations of W.D. Miller in 1891 that calcified tooth substance incubated in saliva-food mixtures is attacked only when carbohydrate foods are used and acid fermentation takes place.

"4. The bacteriological observations of Kligler in 1915, that certain types of micro-organisms (i.e. lactobacilli) grow frequently on the teeth of individuals with active caries, but infrequently on the teeth of individuals who do not have active caries.

"5. Recent epidemiological observations by Dean and also laboratory and animal experiment data by other investigators which show that an inverse relationship exists between fluoride and dental caries."

The work of Kligler in 1915, also referred to by Stephan (1948), which associated active dental caries with the presence of lactobacilli started a line of investigation which continued for nearly 50 years. A number of laboratory tests were developed based on detecting lactobacilli in saliva in an attempt to predict caries susceptibility.

The development of selective media by Rodriguez (1930) and the quantitative method for salivary *Lactobacillus* counts described by Hadley* (1933), paved the way for studies and tests to assess the reliability of the presence of lactobacilli in saliva as a means to predict caries activity.

A simplified test was described by Snyder in 1940. Snyder's test** employed solid media in tubes or bottles which was melted

* Hadley's technique employed a petri dish of tomato agar, acidified with lactic acid, to a pH of 5.0. A suitable dilution of stimulated saliva was spread over the surface of the media on which *Lactobacillus* colonies have a characteristic appearance after 72 hours incubation at 37°C.

** Snyder's technique employed a dextrose-agar media with the acidity adjusted to pH 4.7 to 5.0. The rate of acid production when a sample of stimulated saliva (0.1-0.2ml) was added to the melted media was determined during several days incubation, by a colour change of the indicator bromocresol green. A positive result in 24 hours was correlated with "marked" caries activity, in 48 hours "moderate", 72 hours "slight", and no colour change "negative" caries activity.

to allow the introduction of a sample of stimulated saliva. Because of the initial acidity of the media the test measured the acid production of aciduric organisms in saliva. The results of this test showed a close correlation with salivary *Lactobacillus* counts.

Davies, King and Collins (1959) reported on the relationship between salivary *Lactobacillus* counts, Snyder tests and the subsequent incidence of caries. Examining 584 patients they reported a close correlation between Snyder test results and salivary *Lactobacillus* counts. It was concluded from their results that either test could be used to predict whether or not a patient would develop a greater number or fewer new lesions in the following 12 months than the average anticipated on the basis of age.

The results of Davies et al, (1959) did not show lactobacilli in the saliva of all patients experiencing active caries. Analysis of their results shows that the absence of lactobacilli could be correlated with a lower incidence of caries than those in the study in whom lactobacilli were detected. The absence of lactobacilli however, could not be correlated with the absence of caries. For instance in children 3 to 13 years old those with a zero *Lactobacillus* count experienced a mean increment of newly decayed surfaces of 1.98 over a seven to twelve month period. This represents a substantial amount of caries activity. It should be appreciated that Davies and his co-authors (1959) made no claim that the presence or absence of salivary *Lactobacillus* were related to the presence or absence of caries, only that changes in anticipated new caries according to age would be likely to occur.

Goldsworthy and Spies (1958) examined the relationship between established caries and *Lactobacillus* counts. They examined 82 children aged from five to fourteen years, living in a rural institution*. Because the children were living in institutional circumstances they must be considered a unique group in the community. These researchers observed a significant correlation between the presence of salivary lactobacilli and the presence of untreated, possibly active, dental caries. Twenty five of the 82 children examined were caries free and had *Lactobacillus* present; caries was present in nine of the 82 children in whom lactobacilli were not found.

Reviewing caries susceptibility tests (Lilienthal and Reid 1959), an agreement between *Lactobacillus* counts and Snyder test results was found.

From these reports (Davies, King and Collins 1959; Lilienthal and King 1959; Goldsworthy and Spies 1958) it can be appreciated that although the presence of lactobacilli in saliva may be correlated with dental caries where groups of children are examined, the presence or absence of lactobacilli is not necessarily a reliable indicator of susceptibility for individuals. Further, variations in numbers of lactobacilli in saliva reflect variations in the anticipated degree of caries but there is no evidence that the presence or absence of salivary lactobacilli can be correlated with the presence or absence of dental caries.

* Hopewood House, located at Bowral in the Southern Tablelands of New South Wales.

An exhaustive evaluation of laboratory tests for the estimation of dental caries activity, (Snyder, Porter, Claycombe and Sims, 1962) reported on five laboratory tests including *Lactobacillus* counts and the Snyder Test. To appraise these tests these authors assessed the caries experienced over varying periods by describing an index, "Relative Increment to Decay". This index was based on the number of caries free surfaces at the beginning of the examination that developed caries by the end of the study.

The study by Snyder et al, (Snyder et al. 1962) involved the examination of 361 children for varying periods, from eighteen months to three years. It was concluded it was not possible to use the caries susceptibility test which they described to estimate caries susceptibility for individuals; the test results could however, reflect caries activity of groups.

In an extensive study over a period of eleven years which involved 2991 children (Hill and Blayney 1965), a correlation between untreated carious lesions and *Lactobacillus* counts was demonstrated. Children with fewer untreated lesions had a lower *Lactobacillus* count than those with a greater number of untreated lesions. Although *Lactobacillus* counts were a reliable index of dental caries attacking a large population it was concluded that the size of the *Lactobacillus* count was influenced by the number of untreated carious lesions. In discussion Hill and Blayney (1965) stated that the study did not permit the appraisal of the role of lactobacilli in the aetiology of dental caries. The implication arising from the work of Hill and Blayney (1965) is that although lactobacilli are associated with developing carious lesions there is no evidence of any aetiological role.

In 1971 data from *Lactobacillus* counts of 44 patients, whose increment of dental caries was measured over a period of from 11 to 13 months, was presented (Woods 1971a). In this

study, 23 of the patients experienced no new caries during the period surveyed, ten of these 23 patients, however, had lactobacilli present in their saliva. Of the 21 patients who experienced some caries, in 12 only was it possible to demonstrate lactobacilli in saliva, in the other nine no lactobacilli were found.

It has not been possible to establish a relationship between salivary *Lactobacillus* counts and future dental caries on the basis of information from the study (Woods 1971a); this was consistent with similar observations reported earlier (Goldsworthy and Spies 1958, Davies et al. 1959, Lilienthal and Reid 1959, Snyder et al. 1962 and Hill and Blayney 1965).

Although the hypothesis, linking lactobacilli with the aetiology of dental caries, developed early this century had been tested by a number of authors for approximately 50 years, in the final analysis no such relationship could be established.

It could be argued that *Lactobacillus* counts performed on stimulated saliva do not reflect the type of bacteria on the tooth surface in dental plaque. Notwithstanding this observation, there are too many individuals in the studies cited in the literature in whom dental caries is present but in whom it is not possible to demonstrate lactobacilli. On the basis of the research cited it is not possible to demonstrate an aetiological role for lactobacilli in dental caries.

* Because the caries increment of patients with differing numbers of teeth and some with a mixed dentition was reported, the caries increment was expressed as new lesions per year per 100 teeth.

3.3. THE ROLE OF ACIDURIC BACTERIA AND DENTAL CARIES

Although the aetiological role of lactobacilli in dental caries has been questioned there appears to be a relationship between lactobacilli and dental caries although not an aetiological relationship. Species of lactobacilli are able to survive and multiply in an acid environment. Bergey's Manual (Breed, Murray and Smith 1957) refers to growth in an acid environment as a characteristic of *Lactobacillus acidophilus*. Burnett, Scherp and Schuster (1976, p31) in their textbook refer to the ability of *Lactobacillus* to grow in an acid environment with a pH as low as 5.4, a characteristic common to many species of oral lactobacilli.

It seems reasonable to expect that an established lesion would provide an acid environment which might exclude most other than aciduric species of bacteria. Accordingly one would expect to find aciduric organisms in established carious lesions.

Information on the role of aciduric bacteria in dental caries was provided from a laboratory model using 29 Sprague-Dawley rats established to test the anticaries effect of di-sodium phosphate as a dietary supplement (Woods 1967). In the course of this study samples of the flora from the buccal sulcus and lateral aspect of the molar teeth were taken at the beginning of the experiment and then approximately every three or four weeks. (See Table 1) Samples were analysed to determine the rate of acid production, mean generation time, ability to ferment a number of sugars and ability to grow in an acid environment, pH5.0*. In addition a number of bacteria which were isolated at the various stages of the study were identified**.

Some of the findings of the study (Woods 1967) have been presented. In addition to these results, but as yet not published, aciduric streptococci were isolated from one cage in the ninth week of the study and with one exception from the remaining cages in the twelfth and fifteenth weeks. Some rats in each cage developed caries during the period of the study. A summary showing the dates and times of appearance of aciduric bacteria in these studies is presented in Table 1.

-
- * These aciduric bacteria were isolated by first introducing a quilt of the suspended flora sample to a one per cent glucose broth adjusted to a pH of 5.0 by the addition of N/10 lactic acid. Bacteria which grew in these broths were subcultured and identified.
 - ** Other micro-organisms isolated were *Streptococcus faecalis*, *Streptococcus durans*, several species of *Proteus* and species of *Candida*. These are consistent with the reports of Michalek and McGee (1977).

The aciduric bacteria recovered from the acid cultures were identified as species of *Streptococcus lactis* (Breed and Murray and Smith 1957 p525)*. They were detected only at a late stage of the study when it was likely that caries, at least in the enamel, would have been initiated**.

The late appearance of aciduric bacteria in this study is consistent with the concept of establishment and invasion of established dental caries lesions by aciduric bacteria. The invasion of of aciduric bacteria may occur in some cases quite early in very early lesions.

The requirement to encourage invasion and establishment of aciduric bacteria could be the acid environment of the developing dental caries lesion. It should be appreciated that to survive in such an acid environment of the developing lesion, bacteria need to be aciduric.

The late appearance of aciduric bacteria in the studies (Woods 1967) is consistent with the perception that their appearance may have been as secondary, opportunistic invaders of the carious lesions.

* These streptococci were capable of growth at pH 5.0; in 4.0 per cent NaCl broth but not in 6.5 percent NaCl broth. Their limiting pH in 1.0 per cent glucose broth was 4.4 to 4.0. On blood agar they grew in small round white colonies 0.8 mm to 1.0 mm in diameter, they were also haemolytic.

** Changes in the flora noted at the time of the second flora assay (3/8/65) about one week after the introduction of the experimental diets showed a close correlation to the amount of caries subsequently developed. This could indicate that the bacterial changes associated with the development of caries commenced in this case at least five weeks before the aciduric organisms were detected.

Table 1 Summary of study of flora of 29 Sprague-Dawley rats which developed dental caries

Cage	Dates of flora samples (1965)						Mean caries score per cage
	13/07	03/08	23/08	14/09	12/10	09/11	
1*	-	-	-	+	+	+	13.8
2*	-	-	-	-	+	+	10.0
3**	-	-	-	-	+	-	9.0
4**	-	-	-	-	-	-	11.6
5***	-	-	-	-	+	+	7.6
6***	-	-	-	-	+	+	9.0

Litter history:

Born 7-13/6/65
 Weaned 13/7/65
 Introduced to experimental diets 26/7/65
 Sacrificed and caries scored 9/11/69

- + Aciduric streptococci isolated (*Streptococcus lactis*)
- No aciduric streptococci isolated
- * Cages 1 & 2 Introduced to Diet 2000, unsupplemented from 26/7/65. Five female rats in each cage
- ** Cages 3 & 4 Introduced to Diet 2000 from 26/7/65, supplement of 2 percent $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ added from 10/8/65.
- *** Cages 5 and 6 Diet 2000, with 2 percent $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ added from 26/7/65

Aciduric bacteria were isolated after 13 weeks following the commencement of the study in cages 2, 3, 5 and 6, and after nine weeks in cage 1. Aciduric bacteria were not isolated from cage 4 during the 16 weeks of the study. There does not appear to be a relationship between the time of appearance or the appearance of aciduric bacteria in the study and dental caries developed.

The concept that aciduric bacteria become established during the caries process is supported by these findings. By 12 to 14 weeks from the commencement of the study a favourable environment for establishment of aciduric bacteria is likely to have been established in the developing lesion.

Possibly because laboratory techniques enabling the ready identification of lactobacilli have long been available, the role of lactobacilli in the dental caries process has been studied at length. From the evidence presented it is reasonable to conclude that lactobacilli are part of an aciduric flora associated with a developing carious lesions after initiation of the lesion. Lactobacilli appear unlikely to be involved in the initiation of dental caries, their role appears to be that of a secondary invader in an established microbial environment.

The role of lactobacilli was further clarified by Ikeda, Sandham and Bradley (1973) who examined the bacteria associated with developing carious lesions in 12 negro children aged between seven and nine years. These authors reported on the prevalence of *S. mutans** and lactobacilli at various sites on lower first permanent molars. The children lived in an area where water supplies contained a negligible amount of fluoride.

The prevalence of *S. mutans* and lactobacilli were determined from plaque samples taken from pits and fissures,

* The role of *S. mutans* in establishing dental caries will be considered in Section 4.

proximal surfaces and buccal surfaces of initially caries free lower first molars. Determinations and examinations for caries were made each three months for a period of a year.

Eight of the twelve children studied developed pit and fissure lesions. All eight had substantial numbers of *S. mutans* present ranging from 36 to 100 percent of the total streptococci present at that site three months prior to the development of a lesion. In seven of the eight children *S. mutans* accounted for from 6 to 88 percent of the streptococci six months prior to the appearance of a lesion.

By contrast lactobacilli were isolated from carious pit and fissure sites where they represented from 0.4 to 4.5 percent of the total bacteria present in only four of the eight cases; this was at the same time that the carious lesion was first noted.

Of the four proximal lesions studied *S. mutans* accounted for 3 or 4 percent of the total streptococci three months prior to a lesion appearing. *S. mutans* was not isolated from any of the proximal sites at the beginning of the study. Once more, by contrast *Lactobacillus* appeared only occasionally* prior to the establishment of any lesion accounting for less than 0.07 percent of the total bacteria. In one case, however, they accounted for 37.7 percent of the bacteria from the proximal sites three months after the lesion was noted.

From these data (Ikeda et al 1973) it was concluded that the development of a carious lesion was associated with a history of the presence of *S. mutans* at the site. None of the lesions studied occurred in the absence of such a history. "Caries frequently occurred in the absence of lactobacilli, but not in the absence of *Strep. mutans*" (Ikeda et al 1973).

* The same remarks could be applied to other gnotobiotic techniques when the natural flora is suppressed by antibiotics to assist the introduction and establishment of a species being tested.

It was considered that a relationship with lactobacilli and the establishment of a carious lesion was unlikely because of the low numbers of lactobacilli present and also, from four of the twelve surfaces where caries occurred no lactobacilli were isolated at any time during the study. Further it was concluded that lactobacilli contributed very little to the initiation of caries but, because they are invariably present within the lesion often accounting for up to 90 percent of the cultivable bacteria, it is likely that they were involved in the progression of the initiated carious lesion (Ikeda et al 1973).

Although it was demonstrated by Fitzgerald, Jordan and Archard (1966) that caries could be produced by mono-infection of rats with *Lactobacillus acidophilus*, the type of caries produced was restricted to fissures. The study demonstrated the cariogenic potential of a species of *Lactobacillus*. The authors expressed reservations about experiments using germ-free animals as they are not comparable with a natural situation where any potentially cariogenic organism would have to establish itself in ecological competition with the natural flora in sufficient numbers to cause the disease*.

It is tempting to consider that, on the evidence of the germ-free studies by Fitzgerald, Jordan and Archard (1966), there is a case that at least one species of *Lactobacillus* is able to cause dental caries. This conclusion is not warranted for the following reasons. First, as pointed out by the authors, the circumstances of development of flora in a germ-free animal are likely to be different from the situation where a cariogenic flora has to be established in competition with an established flora to initiate the disease. Second, the carious lesions

* These remarks could also apply to other gnotobiotic studies employing antibiotic suppression of natural flora to assist the establishment of an introduced species.

produced in the germ-free rats were atypical in that they were restricted to pits and fissures.

The *Lactobacillus* tested had some cariogenic potential but even in the presence of a high sucrose diet* was unable to produce smooth surface lesions. It is possible that the *Lactobacillus* lacked the means to become attached to the tooth surface being able only to attach and establish itself by impaction in pits and fissures and enamel defects. The third reason is that these animal studies are not substantiated by clinical observations, they are inconsistent with the meticulous studies of Ikeda and his colleagues (1973), or other studies. (Goldsworthy and Spies, 1958; Snyder et al, 1962; Hill and Blayney, 1965; and Woods, 1971a)

3.4. GERM FREE STUDIES AND THE AETIOLOGY OF DENTAL CARIES

Using a germ-free technique Orland, Blayney, Harrison, Reyniers, Trexler, Wagner, Gordon and Luckey (1954) demonstrated beyond doubt that dental caries could not be established in the rat in the absence of bacteria. These experiments confirmed the informed speculations that had been made since Miller's studies nearly seventy years earlier.

The rats were from the Lobund colony of white rats at Notre Dame University. The caries susceptibility of these animals was such that almost 100 percent of them would develop dental caries when fed the usual cariogenic diet for 150 days beyond weaning.

In the first of two studies (Orland et al 1954), using 22 rats reared in a germ-free environment, demonstrated that these animals developed no evidence of dental caries when fed a known caries producing diet. The rats were exhaustively examined for

* Diet 585.

caries by macroscopic, histologic, and radiographic techniques. In a control group of 39 animals given the same diet as the experimental group, allowed to develop a normal oral flora, 38 developed caries.

From this study (Orland et al 1954) it was established that dental caries in the rat could not develop in the absence of bacteria.

In the second study (Orland, Blayney, Harrison, Reyniers, Trexler, Ervin, Gordon and Wagner 1955) employing a gnotobiotic* technique using an antibiotic suppression of the microflora, tested the effect of the introduction of a single strain of bacteria on the development of caries.

The micro-organisms tested were isolated from carious molar teeth of conventional rats and were selected after exhaustive assessment which included morphological, physiological and antigenic testing. Finally an *in-vitro* test, using a sterilized aqueous suspension of the test diet as a substrate for the proposed micro-organism, was employed to assess the ability of the test organism to produce caries in rat molars removed from a young germ-free animal. Under these conditions the test organism produced caries-like lesions. From this assessment a bacterium was selected to be introduced to the gnotobiotic animals. It was described as *Streptococcus faecalis* (Orland et al. 1955).

* "The Greek derived term "gnotobiotic" has reference to known life. Thus the term "gnotobiotic" refers to an organism which is grown by itself or in association with known kinds of organisms". (Orland et al. 1955)

In addition to confirming that bacteria were necessary for the development of dental caries in the rat the second project (Orland et al. 1955) established that it was possible for dental caries to develop in the presence of a single strain* of *Streptococcus*.

The results of these studies directed attention to the possible aetiological role of streptococci in dental caries.

* In addition to the single strain of *Enterococcus* introduced to the gnotobiotic animals, some animals also had present a pleomorphic bacterium which seems to have been a contaminant. A proteolytic bacillus was present in addition to the enterococci in one of the groups of five rats. Later, Orland (1959) reported that he had been able to establish caries in a group of gnotobiotic rats with the strain of *Enterococcus* alone.

3.5. STREPTOCOCCI AND DENTAL CARIES

3.5.1. Animal studies:

In their research Orland and his colleagues (Orland et al. 1954) directed attention to the role of streptococci in animal caries and demonstrated that some streptococci were capable of causing caries in rats. Fitzgerald, Jordan and Stanley (1960) produced extensive caries in germ-free rats inoculated with an unidentified streptococcus isolated from the oral cavity of rats fed a high sucrose cariogenic diet*. This streptococcus was not an enterococcus nor did it belong to any other group described in Bergey's Manual (Breed et al. 1957).**

In six experiments conducted with both hamsters and rats (Keyes 1960) no specific micro-organisms were mentioned. By suppressing the "cariogenic flora" with antibiotics and then re-inoculating groups of animals with specific material (faeces) from caries active animals, or by caging the suppressed animals with caries active animals, Keyes was able to show that experimental dental caries was transmissible and that caries could be transmitted from dam to offspring. It was concluded (Keyes 1960) that although Orland (Orland et al. 1954) had shown caries could not be developed in the complete absence of bacteria, the presence of a bacterial flora itself was not in sufficient to give rise to dental caries, the flora needed to have a particular quality or "cariogenic potential".

* Diet 585

** Bergey's Manual of Determinative Bacteriology, 7th ed. (1957) refers to *Streptococcus mutans* but does give a detailed description of *S. mutans* (Breed et al. 1957)

In addition Keyes (1960) concluded that "changes in the bacterial status of the animals can cause variations in caries activity and further that apparently the caries activity could be altered by diets and other factors during the prenatal, suckling or the experimental period".

Fitzgerald and Keyes (1960) published results of a series of experiments using albino hamsters which were initially "caries resistant" when placed on a test diet and caged apart from caries susceptible animals. Strains of streptococci and diphtheroids isolated from plaque scrapings of a carious hamster molar and lactobacilli from the oral cavity of hamsters were inoculated into the "caries resistant" animals. Caries occurred in the "caries inactive" hamsters when they were inoculated with single or pooled strains of streptococci from the caries active animals. However, it was not possible to demonstrate caries in those animals inoculated with either diphtheroids or lactobacilli. Further, no caries could be produced in animals inoculated with streptococci from "caries resistant" animals.

This experimental evidence once more directed attention to the role of streptococci in the aetiology of dental caries. Keyes (1960) went further, however, and listed some of the biochemical characteristics of the species of streptococci which could induce dental caries in hamsters.

The five streptococci species were identified as HS-1, HS-4, HS-6, HS-7, and HS-10. Of these HS-6 showed weak growth in 6.5 percent NaCl and might have been classified as an enterococcus in Bergey's Manual (Breed et al. 1957). All strains were able to ferment mannitol. There was no proteolytic activity in any of the species. The strains HS-1, HS-6, and HS-7 appeared to be highly cariogenic.

Fitzgerald and Keyes (1960) were successful in describing the transmissible agent earlier demonstrated by Keyes (1960) and, although approaching the problem differently from Orland (Orland et al. 1954, Orland et al. 1955), had arrived at a similar conclusion that the production of caries in experimental animals required a special type of cariogenic flora.

Fitzgerald and Keyes (1960) induced resistance to streptomycin* by serial passage of the test streptococci through increasing strengths of streptomycin until the organisms could grow in a concentration of 1,000 micrograms of streptomycin per millilitre. This procedure facilitated recovery and identification of the cariogenic streptococci which were designated HSR-1, HSR-4, HSR-6, HSR-7 and HSR-10.

Of the streptomycin-resistant streptococci strains HSR-1, HSR-6, and HSR-7 were able to induce appreciable caries activity and were consistently recovered from the oral cavity, carious plaques and dentine, as well as faeces of infected and carious animals. They were not recovered from the non-carious controls.

Employing the streptomycin-resistant cariogenic streptococcus HSR-6, Keyes and Fitzgerald (1960) were able to establish a streptomycin-resistant streptococcus from this group in hamsters who had a cariogenic flora. The introduced,

* Resistance to low concentrations of streptomycin is not uncommon in streptococci, occurring in about 90 to 95 percent of the streptococci classified as viridans or pyogenic and about half of the faecalis group (Woods 1975, 1988). Development of resistance to high concentrations of streptomycin is consistent with established resistance characteristics of some streptococci.

streptomycin-resistant streptococcus did not usually appear to persist in their oral cavities. It was shown however, that in those cases where streptomycin-resistant streptococcus HSR-6 became established it was transmitted horizontally to other animals in the same cage, and further it was transmitted vertically from dam to the next generation. This was evidence of the nature of the transmissibility of known cariogenic streptococci.

Using a substrain of the streptomycin-resistant streptococcus HSR-6* described by Fitzgerald and Keyes (1960), Krasse (1965a, 1965b) demonstrated that differing diets could affect the establishment of this streptococcal strain when implanted. Krasse tested variations of Diet 2000 and in particular Diet 1999, in which the 56 percent sucrose of Diet 2000 was replaced with glucose.

When hamsters inoculated with the streptomycin-resistant streptococcus HSR-6, were fed Diet 1999 very few of the streptomycin-resistant streptococcus HSR-6 were recovered. This was in contrast to a group given a diet containing 56 per cent sucrose (Diet 2000) in which the streptococci became implanted in large numbers and the animals in the group showed a high caries incidence.

These studies of Krasse (1965a, 1965b) were able to show the essential role of sucrose in the diet, for the establishment and recovery of an identifiable, cariogenic streptococcus.

3.5.2. Human streptococci and dental caries

It had been established that caries was transmissible from one group of hamsters to another and, by using identifiable or labelled, streptomycin-resistant bacteria, it was possible to trace known cariogenic bacteria in a second generation of animals.

* Now designated strain 3720.

There was, in the mid 1960's, no evidence that dental caries could be transmitted between different species and it was widely thought the disease could only be transmitted between animals of the same species, that is, the infectious agents were species specific. This point of view was supported at the time by experiments of Englander and Keyes (1964) who were unable to induce caries in hamsters by introducing human oral flora. Similarly, it had not been possible at that time to induce caries in hamsters using streptococci which had been able to induce rampant caries in rats (Fitzgerald and Keyes 1960).

The first successful induction of caries in hamsters using human streptococci was reported by Krasse (1966) who isolated strains of streptococci from the dental plaque of two very caries active persons. In addition a hamster streptococcus, strain 3720, which had been derived from strain HSR-6 (Fitzgerald and Keyes 1960), was tested and was found to be markedly cariogenic in the circumstances of Krasse's study.

Using mitis salivarius agar for isolation and to identify streptococci from human sources, organisms were selected on the basis of their similarity to the hamster strain 3720. Krasse (1970) described the appearance of strain 3720 on this agar as having easily recognisable colonies *"which have an internal structure reminiscent of frosted glass"*.

The strain of potentially cariogenic streptococci which was similar to strain 3720 was isolated from a patient, Ingbritt. (Krasse 1966) Streptomycin resistance was induced in this strain to facilitate identification and recovery and the streptomycin-resistant strain was designated strain 1600. The modified, human strain 1600 was introduced to groups of hamsters. This was achieved by introducing a dilution of the

culture into the mouth and buccal pouch and also to the drinking water for 48 hours. The procedure was described earlier (Krasse 1965b).

Compared with uninfected controls, animals infected with the modified (streptomycin-resistant) human streptococcus, strain 1600, developed rampant caries and the streptomycin-resistant, identifiable streptococci, strain 1600, were recovered.

Krasse (1966) had taken streptococci from a human patient who had dental caries, isolated them in pure culture and through a number of subcultures, and introduced them to a susceptible animal who developed lesions similar to the human dental caries lesion. The species of human streptococci which had been introduced was then re-isolated from the experimental animals. With this series of stages involving isolation of a designated and labelled streptococcus, its transmission to a laboratory animal which developed lesions characteristic of the disease and finally the recovery of the labelled streptococcus (strain 1600) the conditions set out in Koch's postulates (Burnett and Scherp 1968) had been fulfilled and it was reasonable to conclude that the *Streptococcus* strain 1600 was an infectious agent capable of causing dental caries. The importance of fulfilling the conditions of Koch's postulates had already been referred to by Keyes (1962).

While establishing the essential role of *Streptococcus* strain 1600 in dental caries, the fulfilment of Koch's postulates for a strain or even a species of microorganisms does not exclude a similar role for other microorganisms. Notwithstanding this statement it had been demonstrated in this series of studies by Krasse (1966) that *Streptococcus* strain 1600 had the necessary characteristics to initiate dental caries.

3.6. THE DESCRIPTION OF *STREPTOCOCCUS MUTANS*

Not only had it been demonstrated (Krasse 1966) that dental caries could be transmitted from human to animals*, the characteristic colony appearance of *S. mutans* when grown on mitis salivarius agar had also been described (Krasse 1966). The colonies were described as "highly convex with the edge undulate; the internal structure was finely granular, reminiscent of frosted glass...". The illustrations published by Krasse show very similar colonies of suspected cariogenic strains of streptococci described and photographed by Schamschula and Charlton (1971).

The formal description of *S. mutans* may have been hampered by the absence of a definite designation of the species. Bergey's Manual, (7th ed), (Breed et al. 1957) mentions the species only briefly, suggesting the need for further study and comparison with the species *Streptococcus salivarius*. The description of *S. mutans*, its characteristics and its relationship to other species, at the time of the investigations described, still needed to be established.

Originally Clarke (1924) reported a streptococcal species associated with dental caries and gave it the name *Streptococcus mutans*.

Clarke's original culture no longer exists. Edwardsson (1968), reviewing caries-inducing streptococci, mentioned that the National Collection of Type Cultures, London, possessed a "representative strain of *S. mutans* isolated by Dr W. Sims in 1965 from the deepest layer of carious dentine". The National

* Krasse also speculated that the cariogenic *Streptococcus* HS-6 of Fitzgerald and Keyes (1954) could have been an accidentally introduced human species. The speculation was based on the very similar biochemical characteristics of HS-6 and Krasse's strain, "Ingbritt".

Collection of Type Cultures (London), strain NCTC10449, was similar to strain Ingbritt. Edwardsson (1968) reported strain NCTC10449 was able to produce rampant caries in hamsters.

The biochemical characteristics of strain Ingbritt and strain 3720* were compared with with other streptococci (Edwardsson 1968).** Manitol was fermented by all strains of caries inducing streptococci isolated from human dental plaque and from caries-inducing strains IB and 3720 as well as from *Streptococcus faecalis* and *Streptococcus lactis*. Sorbitol could be fermented by seven of eight strains of caries-inducing streptococci isolated from human dental plaque; sorbitol was also fermented by strains Ingbritt and HS-6. Of the non-cariogenic streptococci tested sorbitol was not fermented by *S. sanguis*, *S. salivarius*, or *S. Lactis*, but was fermented by *S. faecalis*.

3.6.1. Cariogenic streptococci and sucrose

By substituting the sucrose in diet 2000 with glucose which was then designated diet 1999, it was shown that dietary sucrose was necessary to maintain the cariogenic streptococci with which hamsters had been inoculated (Krasse 1965a, 1965b). Hamsters inoculated with the streptomycin-resistant streptococcus HSR-6, and fed the glucose based Diet 1999 retained very few of the streptomycin-resistant streptococcus HSR-6 (Krasse 1965a, 1965b).

It was also reported (Krasse 1965a, 1965b) that the addition of sucrose to the diet of hamsters enhanced the cariogenic effect compared with glucose.

* Strain 3720 was derived from Fitzgerald and Keyes (1960) strain HS-6.

** Edwardsson (1968) also induced hamster caries with human streptococci which resembled *S. mutans*.

The unique relationship between cariogenic streptococci and sucrose was put forward by Gibbons and his colleagues (Gibbons, Berman, Knoettwar and Kapsimalis 1966) who investigated the biochemical characteristics of cariogenic streptococci. It was reported that these streptococci produced large quantities of extracellular, capsule-like, carbohydrate from sucrose. The non-cariogenic streptococci formed lesser amounts suggesting that this activity could be important in the caries process. By comparison cariogenic streptococci cultured in glucose and sugars other than sucrose, formed very little capsule-like material. The production of substantial amounts of capsule-like carbohydrate appeared to be unique to cariogenic streptococci.

A short communication (Wood and P.Critchley 1966) reported an analysis of the extracellular capsular material which was shown to consist of dextrans and levans; it was further concluded that synthesis of extracellular polysaccharides from sucrose is a major biochemical activity occurring in plaque.

Evidence was also presented that the extracellular polysaccharide produced by cariogenic streptococci was a dextran, capable of becoming tenaciously bound to both untreated and saliva-treated hydroxyapatite (Gibbons and Banghart 1968). The view was expressed that synthesis of extracellular dextran by cariogenic streptococci "appears to enable these organisms to form plaque which is necessary for the production of dental caries". This view is however factually incorrect since *S. salivarius*, considered to be non-cariogenic, also produces large amounts of extra cellular polysaccharide.

Examining the significance of the extracellular dextran it was demonstrated (Gibbons and Nygaard 1968) that only certain

types of dextrans* were capable of forming plaque. Significantly, the dextrans produced by *S. sanguis* or *S. salivarius* did not appear capable of forming plaque.

Additional evidence was produced showing that although dextrans and levans exist in both water soluble and insoluble forms. The insoluble form appears to be the more significant in plaque formation** (Gibbons and Nygaard 1968).

In studies on the effect of diet on early plaque formation in man (Carlsson and Egelberg 1965) the effect of glucose, sucrose and fructose was tested. Although plaque formed with all diets, a demonstrably greater amount of plaque developed when the subjects had a sucrose diet. The comment was made in these studies (Carlsson and Egelberg 1965) that the production of extracellular polysaccharide probably contributed to increased plaque. This was noted in a further investigation following up part of this report. In a later investigation analysis of the microflora present showed that in the ten dental students studied, *S. sanguis* was the most likely source of the plaque polysaccharide (Carlsson 1965).

* Although some of the cariogenic streptococci produced both dextrans and levans their ability to produce plaque was inhibited by the enzyme dextranase, indicating dextrans were of greater significance than levans in the production of plaque. Notwithstanding this observation Gibbons and Nygaard (1968) commented that because of the large amount of levan present in plaque it may enhance plaque development.

** The synthesis of insoluble dextran could be inhibited to a degree by the addition of various glycosyl acceptors, mostly other sugars. This appears to be the result of modification of the molecular size of the dextran synthesised by plaque producing bacteria.

Using a source of 89 strains of streptococci isolated from the plaque of the ten students (Carlsson 1965, Carlsson and Egelberg 1965), and 30 reference strains of streptococci including the known cariogenic streptococci, strains 3720, E49, and FA-1 (Fitzgerald and Keyes 1960) and strain "Ingbritt" (Krasse 1966), Carlsson (1968a), the biochemical characteristics of plaque streptococci and in particular the known cariogenic strains, were described.

The cariogenic strains* could all be classified in Carlsson's division II and he considered them to conform to the characteristics of *S. mutans*.

In addition to producing extracellular polysaccharide and forming zooglia when cultivated on mitis salivarius agar, the cariogenic group of streptococci could be distinguished by their ability to ferment mannitol; by their resistance to sulfonamides**, and to a lesser extent bacitracin. These characteristics provide the basis for recognition of species of *S. mutans*. Later Carlsson (1967) described a modification of mitis salivarius agar, by adding sulfadimidine 1 mg per mL, which could be used to identify species of *S. mutans* by their colony formation.

* The cariogenic strains were strains Ingbritt, 3720, E49 and FA-1.

** Sulfafurazole was the particular sulfonamide tested, although *S. mutans* is resistant to most sulfonamides.

Using Carlsson's modification of mitis salivarius agar* some of the biochemical characteristics of *S. mutans* isolated from 44 different patients were examined (Woods 1971a). It was found that mannitol could be fermented in 38 of 44 cases (86 per cent) although there was some variation in haemolysis on blood agar, alpha haemolysis occurring in 37 of the 44 cases (84 per cent), beta haemolysis in two cases (approximately 5 per cent) and gamma haemolysis (11 per cent) in five cases. Carlsson (1968a) reported beta haemolysis in three cases and gamma haemolysis in six cases.**

In further analyses of the biochemical characteristics of *S. mutans*, Krasse and Carlsson (1973) confirmed the distinguishing characteristics of the *Streptococcus*, that is ability to ferment mannitol and resistance to sulfonamides***.

In his review of the taxonomy of *S. mutans* Coykendall (1973) concluded that *S. mutans* is, in effect, a group of organisms with many common properties. He proposed, on the basis of serological differentiation, four subspecies, *mutans*, *rattus*, *cricetus* and *sobrinus*. These subgroups correspond to the serological groups "c", "b", "a", and "d", proposed by Bratthall (1970).

* Carlsson's modification of mitis salivarius agar (Carlsson 1967) was used in the studies to be described in this treatise and is the basis for the media used for the plaque screening tests for *S. mutans* employed in these studies test. (Woods 1971)

** Carlsson used human blood in his agar to test haemolysis, horse blood was employed in the author's study. (Woods 1971a)

*** Sulfafurazole was used in this case.

Cariogenic streptococci from animal and human sources have been isolated and studied and their characteristics including their biochemical characteristics, have been established. In particular the characteristics which can be used to distinguish them from other streptococci have been noted, especially their ability to produce extracellular dextran from sucrose* but not, other than minute amounts, from any other common sugar. This characteristic has been used to help identify *S. mutans* and gives rise to its characteristic colony formation on mitis salivarius agar (Krasse 1970).** The production of extracellular dextran by these streptococci may contribute to the potential for plaque development.

It has been suggested by Coykendall (1973) that the organism *S. mutans* was, in effect, a group of organisms. Coykendall (1973) suggested four species in the group corresponding to the four subspecies of *S. mutans* described by Bratthall (1970). This group of similar streptococci became referred as "mutans streptococci".

The resistance of mutans streptococci to sulfonamides has also been used to assist identification.

Cariogenic streptococci have been extensively studied and have been described; they conform to the description of *S. mutans*. A number of subgroups of mutans streptococci have been known to exist for some time, for instance *S. mutans* and *S. sobrinus* are commonly found in most populations. However a number of subgroups of mutans streptococci relevant to the caries initiation have been described (Bratthall 1970).

* Raffinose can also be utilised by these streptococci to produce extracellular polysaccharides.

** Mitis salivarius agar contains five percent sucrose.

3.7. *STREPTOCOCCUS MUTANS* AND DENTAL CARIES

In the series of experiments and studies reviewed the progressive steps which have led to the identification and establishment of *S. mutans* as the principal bacterial agent involved in the aetiology of dental caries have been described. The principal studies leading to the conclusion that bacteria are necessary for the establishment of dental caries; the recognition of the role of streptococci in the disease; and the description of *S. mutans* as the main micro-organism causing dental caries, have been reviewed.

The postulates enunciated by Koch (Burnett and Scherp 1968) designed to test the aetiological relationship between specific bacteria and the infectious diseases they cause, have been fulfilled with respect to *S. mutans* and dental caries. Even in this fulfillment it should be recognised that the conclusion that *S. mutans* is the cause of dental caries cannot rest entirely on this test. This is because Koch's postulates were formulated at a time when the concept that each infectious disease was caused by one type of bacterium was part of the bacteriologist's creed. In addition, the special nature of dental caries as an infectious disease has to be considered. The bacteria which initiate the lesion may not initially invade the body beyond dental enamel and dentine; their principal action could be on the tooth surface.

Koch's postulates, however, do not exclude the possibility that more than one species of bacteria could be responsible for the disease. In the light of the studies reviewed, *mutans* streptococci emerge as the principal infectious agent causing dental caries. The possibility remains however, that other species of bacteria or other micro-organisms might also cause dental caries. For instance, it has been possible to establish fissure caries in the molars of gnotobiotic rats with lactobacilli (Fitzgerald et al 1966). In addition there is root

surface and cementum caries to be considered which may in some cases, be initiated by other organisms. (Fitzgerald et al 1966, Jordan 1973)

Precedents exist for a single bacterial species present in a mixed bacterial flora to be the cause of an infectious disease. For instance pyogenic diseases may be caused by any one of a number of pyogenic bacteria, including streptococci and staphylococci. Notwithstanding the example that pyogenic diseases may be caused by any one of a variety of bacteria, the clinical signs and appearances of patients with these acute infections are similar, clinical differences are usually subtle and differentiation on the basis of clinical appearance is at best, difficult and at worst, unreliable.

Some types of dental caries, for instance, fissure, smooth surface or root caries, can be readily differentiated, although other subtle differences, yet to be recognized, may exist. Dental caries is a descriptive term referring to loss of hard tooth structure. It is the end result of a chain of events and is an irreversible, late stage in this series of events. Although other infectious agents may cause dental caries, on the evidence to hand at present, *S. mutans* is the principal bacterium responsible.

Because of the nature of dental caries which commences with dissolution of an external and exposed, hard tissue (dental enamel) which exists in an oral environment challenged by food, oral fluids and external surface bacteria, a strong case can be proposed that the most important aspect in the development of dental caries is the initiation of the lesion which occurs on the external tooth surface. The external surface is usually dental enamel although cementum and dentine are also surface tissues when exposed by gingival recession or tooth wear. Following the initiation of a carious lesion involving the surface of the tooth, the development and extension of the lesion could readily involve other bacteria which invade established lesions. These

invaders are likely to be aciduric which permits their establishment in the acid environment of a developing carious lesion. In this respect the aciduric qualities of *S. mutans* should not be overlooked, it is a quality which would assist the organism to continue involvement in the dental caries process beyond initiation, even if other opportunistic invading bacteria are present.

There is no shortage of clinical material linking *S. mutans* with dental caries and some of this material has already been mentioned. Many of these studies have been reviewed (Shklair 1973). Much of this material is of value because it substantiates classical studies in the microbiology of dental caries already referred to and adds to our clinical understanding of the nature of the disease.

To imply a causal relationship between *S. mutans* and dental caries, clinical studies must be able to demonstrate that *S. mutans* is present on the tooth surface prior to the development of a carious lesion and not concurrent with, or subsequent to, the development of such a lesion.

Early studies sought to examine the bacteria from established lesions. Not until this approach was superseded were advances made to identify the bacteria which initiated the lesion. It was presence of *S. mutans* on the sound tooth surface prior to the initiation of a lesion which support the aetiological role of *S. mutans* in the development of dental caries (Ikeda et al 1969). Judged by the same criterion the absence of similar data while not excluding a role, does not support any extensive aetiological role for lactobacilli, other than in special conditions, for instance the establishment of fissure caries.

When considering the aetiological role of *mutans* streptococci in the development of dental caries it should be appreciated that much of the work with human subjects was conducted in circumstances of relatively high caries incidence and on highly susceptible patients. With the reduction of caries which has occurred (Barnard and Clements 1976, Commonwealth Department of Health 1987, Armfield et al. 1999, 2000), circumstances could change.

As recognition of different types of dental caries emerges, for instance, fissure lesions, smooth surface lesions, and root surface lesions, it is possible other micro-organisms will be shown to have an aetiological role in these types of caries.

Summarising bacteria associated with root surface caries, (Bowden G and Edwardson S 1994) wrote:

"Despite findings of significantly increased levels of S. mutans associated with initial root surface lesions by some workers, others have not detected differences in the levels of this species between lesions and intact root surfaces. Similar results have been found for Lactobacillus which can be isolated from 28 per cent of samples from these lesions although they only reach a mean of 1 per cent of the total flora."

Because of the specific nature of immune reactions a different approach to establishing the identity of the micro-organism causing dental caries using immunological technique has provided a potentially more specific result. Immune studies in animals must be regarded as good evidence to confirm the aetiological role of *S. mutans* in dental caries. These studies can be conducted with one variable only, the immunization procedure.

Studies on immunization for dental caries (Lehner, Caldwell and Challacombe 1977) have provided evidence to support the aetiological role of *S. mutans*, Lehner et al. (1975, 1977) used monkeys maintained on a human diet containing 15 per cent

sucrose and containing indigenous *S. mutans*. The *S. mutans* could be recovered from about eight weeks of age. The monkeys experienced cervical and proximal caries in their deciduous teeth from about age two months. In these studies (Lehner et al. 1977), it was shown that immunization of rhesus monkeys with cells of a human strain of *S. mutans* produced significant serum antibodies. There was a slight increase in salivary antibodies which was not significant. The immunized monkeys developed no smooth surface caries during the study and experienced a marked reduction in fissure cavities in contrast to the animals who were not immunized and who developed both smooth surface and fissure caries.

Although the studies need replication the results illuminate the question of the bacterial cause of dental caries.

It may be noted that a topical vaccine against *Streptococcus mutans* developed by Guy's Hospital, London, is currently undergoing phase II clinical trials (Ma 1999).

Reviewing the current understanding of the dental caries process, Hanada (2000) recognized that the dental caries-associated oral streptococci are a group referred to as "mutans streptococci". Hanada (2000) reported *Streptococcus mutans* and *Streptococcus sobrinus* are the most caries-associated organisms in humans.

It was also observed by Hanada (2000) that vertical transmission from mother to child could be prevented by reducing the numbers of mutans streptococci in the mother's saliva.

In an extensive review of the microbial agents which cause dental caries, Balakrishnan, Simmonds and Tagg (2000) concluded that the mutans streptococci, a group of seven species of

streptococci, were the principal aetiological agents of dental caries. It was noted in this review that *S. mutans* and *S. sobrinus* were the most important members of the group in terms of human dental caries.

3.8. PREDICTION OF DENTAL CARIES

In a study of 148 pre-school children commencing at a mean age of 3.8 years, an association was demonstrated between the presence of salivary mutans streptococci and the development of dental caries in the following two years (Thibodeau and O'Sullivan 1995). The authors of this study concluded that salivary mutans streptococcus levels may be of use in predicting the caries risk in the deciduous dentition of some population.

Assessment of the value for predicting dental caries of a number of factors: mothers's education; social class, immigrant background, night meals or drinks, consumption of sugar containing beverages (≥ 2 /day), candy* (≥ 1 /week), toothbrushing (< 1 /day) and the presence of mutans streptococci (obtained from a tongue sample) (Grindejford et al. 1995). Clinical assessments and other information was gathered at age one year and related to the clinical assessment made at the age of 3.5 years. It was concluded that there was a high statistical probability ($P < 0.001$) of a direct relationship between the factors of immigrant background, mother's education, consumption of sugar-containing beverages and the presence of mutans streptococci. It was further concluded that prediction at age one year could provide information which could be used for preventive intervention (Thibodeau and O'Sullivan 1995).

* Candy may be referred to as confectionery in some countries.

In the second of two papers (Grindefjord et al. 1996) the initial study was reviewed recognizing the possibility of identifying children at the age of one year who are at risk of development of dental caries. A synergistic effect was identified between age and some of the factors considered, such as the presence of mutans streptococci, mother's education, immigrant background and candy* consumption.

With the evidence of a substantial reduction in dental caries in many countries becoming established it is increasingly important to be able to recognize caries susceptibility before irreversible damage to the dentition can occur.

The dental caries incidence (DMFT) in Australia for children of 12 years of age has fallen from 9.32** (country boys and girls 6.24**) in 1954-55, to 1.94 in 1970, 1.10 in 1993, 0.90 in 1996 and 0.86 in 1977. The percentage of children free of caries (deciduous plus permanent teeth) has risen from 1.0 per cent** in 1954-55, to 8.1 per cent in 1977, to 45.6 in 1993, and to 51.6 in 1977. (Barnard 1956, Commonwealth Department of Health 1987, AIHW Dental Statistics and Research Unit 1995, Armfield et al. 1999, 2000) Prediction of susceptibility to dental caries, particularly for infants and children could be an important aspect of future dental caries prevention.

A review of dental caries risk assessment (Reich et al. 1999) considered a number of factors: caries experience, fluoride use, extent of plaque present, diet, bacterial and salivary activity as well as social and behavioural factors. The authors (Reich et al. 1999) in assessing the potential of microbiological tests, wrote "Microbiological tests show close associations between odontopathogens and caries in subjects with high caries experience and conversely, low numbers of odontopathogens in low or non-caries subjects." Reference was made to the work of Bowden (1997).

* Candy may be referred to as confectionery in some countries or cultures.

** New South Wales schoolchildren

In a summary "Assessment of caries-risk" (Reich E et al 1999) it was stated "By combining past caries experience and values of mutans streptococci a more predictive model is obtained than with either individual test alone. Sensitivity was calculated as 71 per cent, specificity 81 per cent."

In a research abstract it was suggested that early identification of mutans streptococci-colonized children could be of value in identifying caries-risk children (Campus et al 1997).

Assessing caries risk tests which might be used in dental practice, Powell (1998) noted that the "any-risk" (dental caries either present or absent) model was better suited to predicting subjects who develop dental caries but also incorrectly identified many low-risk children as being high risk. She further observed risk models that include multiple variables offer better prediction because the disease (dental caries) is multifactorial. Generally, most caries risk models are better at selecting people who will not develop caries (that is, high specificity) than they are at selecting people who will (that is, high sensitivity). "Specificity" is the proportion of people without a disease who have negative results (Powell 1998).

Reviewing caries risk tests for children, Messer (2000) points out that "even highly sensitive and specific tests have a very low probability of detecting disease if the disease has a low prevalence, and a very low probability of detecting those few who will not develop caries given a high caries prevalence". Messer (2000) continues in her review, that "microbial tests for detecting mutans streptococci are very good at identifying caries-inactive children, but are less satisfactory for identifying caries active children, as some who have positive scores develop little caries." Messer points out that most

salivary diagnostic tests measure the cause of dental caries, or the actual risk factors, and have their "Sp" (specificity) and "Sn" (sensitivity) very close to 1.00 but that the subsequent development of caries is so complex that the presence of a single risk factor does not necessarily mean that caries will develop.

Again, Messer (2000) directs attention to the observation of Larmas (1992) regarding the correlation which exists between salivary concentration of mutans streptococci and their proportion in plaque, "When mutans streptococci represent more than 1 per cent of the total streptococci in plaque, the mean value per mL saliva is more than 1 million. Proportions of less than 0.3 per cent generally correspond to less than 300 000 per mL saliva. In conclusion Messer (2000) states "No one test is an adequate indicator of caries risk, and the "Sp (specificity)" or "Sn" (sensitivity) of tests are still not reliably diagnostic for one individual."

The role of mutans streptococci in predicting dental caries is still being defined, however studies are continuing to recognize the value of mutans streptococci in establishment of a caries-risk profile, sometimes associated with other bacteria, for instance lactobacilli (Llena-Puy MC et al. 2000).

If caries experience and the presence of mutans streptococci in dental plaque are two of the principal factors for assessment of caries risk, the relatively uniform low caries experience of a population may increase the relevance of the plaque mutans streptococci factor as a caries predictive factor by minimizing variation of one of the two factors, caries experience. This observation is supported in part by the observation of Messer (2000) who points out that "even highly sensitive and specific tests have a very low probability of detecting disease if the disease has a low prevalence ...".

Australian children have a low dental caries experience. In 1997 61.9 per cent of Australian children age 12 had caries free permanent dentitions (DMFT = 0) and at age 15 years 41.0 per cent had caries free permanent dentitions (DMFT = 0). (Armfield et al 1999, 2000) Australian dental caries experience may decline even further.

It is at present a reasonable expectation in low caries populations, that many children will complete school whilst remaining dental caries-free. In assessing the risk of developing dental caries, it is important to be able to identify those who are at risk of developing dental caries.

In low dental caries populations dental caries predictive techniques which utilise information on the presence of mutans streptococci in the oral environment, either in dental plaque, saliva or the surface of the tongue, are able to assist identification of caries susceptible individuals and to introduce effective preventive procedures prior to irreversible damage occurring through development of dental caries.*

* In the experience of the candidate, tests for mutans streptococci in dental plaque made regularly with six monthly examinations, can on occasions show an increase in the presence of mutans streptococci. Often this increase can be correlated with a change in the availability of dietary sucrose, often in an accessible form soft drinks or cordials, this in turn can often be related to a change in life-style, for instance changing from a primary school to a secondary school.

4. THE NATURAL HISTORY OF *STREPTOCOCCUS MUTANS*

Establishment of an aetiological relationship between mutans streptococci in dental plaque and the development of dental caries is a tangible advance in understanding the cause of the disease and formulation of rational programmes for its control. Following on from an understanding of this relationship it has become possible to consider a reasoned approach to the control of dental caries and to suggest a "medical model" to supplant the long practised "surgical model" for the control of this disease. A number of questions have to be answered. Where are the bacteria causing the disease found in nature? How are they transmitted from host to host? What is the relationship of the bacteria with their host? Are these bacteria parasites, low grade pathogens or both?* What are the circumstances that predispose a host to colonization by these bacteria?

4.1. THE GENUS *STREPTOCOCCUS*

The genus *Streptococcus* is classified in "Tribe I" of the family lactobacillaceae (Deible and Seeley 1974). *Streptococcus* is classified as Genus II. Various members of the family are found as parasites of the human and animal nasopharynx and throat. Members of the genus *Streptococcus* are parasites of the human and animal genito-urinary tract. Some members are also found associated with avian diseases and are bird parasites. Divisions I and II are parasites mainly found in the oral flora and throats of animals, they occur also on the skin and are associated with many suppurative and inflammatory lesions. Some species are present in the mammalian genito-urinary tract (Deible and Seeley 1974).

* Many infectious agents can exist as both parasites and pathogens; Burnet (1962) cites the example of cholera.

Division III of the family contains members that have been found in the gastro-intestinal tract and faeces of humans and animals as well as in the oral flora. This division includes the *faecalis* group of streptococci.

Members of Division IV have been found in nature and may be plant parasites; they have also been found as animal parasites present in the oral flora (Deible and Seeley 1974, Burnett, Scherp and Schuster 1976, p31).

Members of the genus are found in milk and milk products. Some members are used industrially in cheese manufacture. As contaminants streptococci, often from faeces, occur in water supplies and food, and are used as a measure of faecal contamination. In addition pathogenic streptococci are isolated from various exudates associated with disease in both human and animal.

4.2. DISTRIBUTION OF MUTANS STREPTOCOCCI

Mutans streptococci are widely distributed. Mutans streptococci have been isolated from communities in all parts of the world, from primitive communities, and from highly developed communities. There are reports of the isolation of *S. mutans* from Europe (Krasse, Jordan, Edwardsson, Svensson and Trelle 1968, Bratthall 1972a); Asia and Africa (Bratthall 1972a); Australia (Bratthall 1972a, 1972b, Carlsson, Grahnen, Jonsson and Wikner 1970,); New Guinea (Schamschula and Barmes 1970, Schamschula, Adkins, Barmes, Charlton and Davey 1978); North America (Bratthall 1972a, 1972b, Kozlowski, Shklair, Keene and Levine 1973, Littleton, Kahehashi and Fitzgerald 1970, Shklair, Keene and Cullen 1972); South America (Bratthall 1972a 1972b, Jordan, Englander and Lim 1969; Gibbons and Loesche 1967) and from the Pacific region (Woods, Barnard and Cutress 1977; Barnard, Woods and Cutress 1977).

Bratthall (1972a) studied samples of dental plaque sent from a number of countries. Species of *S. mutans* isolated were classified in five groups according to the serological criteria he described earlier. He found strains "c" and "d" in every area studied* and Group 'e'** from every area studied except one***.

Where *S. mutans* has been found in populations its prevalence has not always been reported. Small groups of subjects examined may not be representative of the whole population. There are, however, a number of studies where representative population groups have been examined; in most cases those examined were schoolchildren or adolescents, although there have been a number of studies which included adults and adolescents. These reports also include studies of some developing communities. The results of these studies have been summarized in Table 2.

The reported prevalence of *S. mutans* ranges from 59 percent in North America (Jordan, Englander and Lim (1969) and 60 percent in New Guinea (Schamschula and Barmes 1970), to over 90 percent in Sweden (Krasse, Jordan, Edwardson, Svensson and Trell 1968) and in North America (Shklair, Keene and Cullen 1972). The prevalence of *S. mutans* in populations may have changed as most of these data were reported over 25 years ago.

In Australia for instance, the reduction in dental caries being experienced is evidence of a change in the pattern of dental caries and associated factors which include the prevalence and distribution of *S. mutans*

* Europe (Gallivane, Goteborg, Lodz, Nijmegen, London, Lyon), Africa (Cairo), Asia (Osaka, Tokyo), Australia (Adelaide, Sydney), North America (San Francisco, Miami), South America (Rio de Janeiro)

** Bratthall could not rule out the possible concomitance of Group 'c' with his *S. mutans* Group e

*** *S. mutans* Group e was not shown to be present in samples for Osaka

The presence in various communities and From reports of the prevalence of *S. mutans*, it is clear that this micro-organism was widely distributed in the dental plaque of communities throughout the world in the years about 1970*. At that time it appears to have been present in more than half of the persons in the communities examined** including developing as well as developed communities (Table 2).

In his discussion on the world distribution of *S. mutans* Bratthall (1972a) presents a case to plot the distribution of *S. mutans* without reference to dental caries, however he continues, that before any conclusions could be reached from such studies the plaque concentrations of the bacteria found would also have to be taken into consideration.

4.2.1. *Streptococcus mutans* in dental plaque

There is no evidence of *S. mutans* living freely in nature. *S. mutans* appears to be an obligate parasite. Most studies of *S. mutans* have used dental plaque as a source. The search for this organism beyond dental plaque has not been very wide. It has been reported in faeces (Kilian, Theilade, and Schiott 1971, Shklair and Keene 1973) and also on the pencils of school children (Woods 1973).

* *S. mutans* was found to be present in 33.7 and 40.0 percent of New Guineans in two of four areas in Papua New Guinea. (Schamschula et al 1978) At the time of the report villagers in these areas had an average DMFT of 1.5 and zero respectively

** The proportion of children, adolescents and young adults with dental plaque seemingly free of *S. mutans* appears to be increasing accompanied by a comparable fall in those with plaque with *S. mutans* present. Preliminary, unpublished data from patients treated privately where plaque screening is part of their regular treatment suggests the increase in those free of *S. mutans* has been increasing.

As part of a more extensive study the faeces from four healthy adults was examined and dextran-producing streptococci not unlike *S. mutans* were demonstrated. (Gibbons and Banghart 1968) In addition (Kilian et al. 1971) *S. mutans* was isolated from 23 (21 percent) of 108 samples of faeces examined.

Examining the relationship between *S. mutans* in dental plaque and faeces, specimens from 94 young men, aged 17-21 years were tested. Of the 94 subjects tested, 59 (63 per cent) were caries-free and 35 had a history of dental caries. *Streptococcus mutans* was found in the faeces of 36 percent of the caries-free group and from 51 percent of the caries group*. From the data it was concluded that *S. mutans* is not a constant resident of the intestinal tract but is a transient (Shklair and Keene 1973).

Because *S. mutans* has been found in faeces it is likely to be found, even if as a transient microorganism, in sewage and other faecal contaminated areas, and on skin surfaces, particularly of fingers and close to the anus. It is not known how long *S. mutans* survives in these sites.

Samples of oral flora from a number of zoo animals were shown to contain *S. mutans* and the organism could be demonstrated in animals in contact with humans (Elvin-Lewis, Keudell, Klugman McGivney, Baldwin and Quinn 1975). *S. mutans*-like organisms were found in wild rats living in an urban dump and inferred that the distribution of *S. mutans* could be wider than had been expected (Dvarskas and Coykendall 1975). Coykendall, Specht and Samoi (1974) isolated *S. mutans* from the mouths of wild rats trapped in a sugar cane field. It was shown to be present in the

* *S. mutans* was isolated from plaque from 64 percent of the caries-free group and from plaque of 83 percent of the caries group.

oral flora of seven of the 14 rats examined, but not in their faeces. From this report it appears that *S. mutans* exists in the oral flora of mammals other than laboratory animals and man.

Streptococcus mutans is widely distributed in man. In most communities examined *S. mutans* is present in about half the population*. In addition it has been found in faeces and on skin surfaces; it has been demonstrated on pencil tops chewed by school children who have the micro-organism present in their plaque (Woods 1973).

Failure to demonstrate mutans streptococci cannot be equated with absence. Absence may arise from the insensitivity of the bacterial examination technique. *S. mutans* is also more likely to be found on some tooth surfaces than others; for instance it appears likely to be found in interdental areas which seem to be a preferential site for the organism.

* This statement is made on the basis of studies reported mostly performed in the years around 1970.

Table 2 Incidence of *Streptococcus mutans*: various countries

Author	Location	Subjects and age	Prevalence (Per cent)
Krasse <u>et al.</u> .1968	Sweden	49 children 7 yrs 46 children 13 yrs 50 dental students 21 yrs 37 dental students 24 yrs	82 93 74 59
Jordan <u>et al.</u> .1969	N. America	children	59-69
Schamschula <u>et al.</u> 1970	New Guinea	175 subjects from 18 primitive villages, including young adults and children.	60-70
Schamschula <u>et al.</u> 1978	Papua-New Guinea	146 subjects from four areas of Papua-New Guinea including children and young adults	33.7-98.9
Kozolowski <u>et al.</u> 1973	N. America	189 children (naval personnel dependants)	75
Shklair <u>et al.</u> 1972	N. America	93 naval recruits age 17-22 years	92
Rogers 1973	Central Australia	50 adolescent aboriginals	58
Woods <u>et al.</u> 1978	W Samoa (Apia, Poutassi)	25 children, 6.3 yrs 35 children, 13.6 yrs	80 83
Woods <u>et al.</u> 1979	Newtown (inner Sydney)	143 children 6-8 yrs (primary school)	55
Alaluusa <u>et al.</u> 1989	Finland (Helsinki)	149 children 5 yrs (<i>S. mutans</i> in saliva)	46
Woods 1976b	Boorowa (rural NSW)	primary school children age 5-9 yrs	41

4.2.2. Establishment of *Streptococcus mutans* in the
 human mouth

Although in general* babies are born without an established oral flora, in only a matter of hours after birth a simple oral flora can be demonstrated.

Examining the oral flora of 12-hour old babies established that strains of streptococci similar to *S. salivarius* were found in 11 of 51 infants (22 per cent) age 12 hours and in 20 of 25 (80 per cent) infants aged 25-54 hours (Zinner and Jablon 1969). In addition this study tested for the presence of strains AHT and BHT. Strains of streptococci similar to *S. mutans* were isolated from two of 12 children (16 per cent) examined at 1-2 months of age and from nine of 12 children (75 per cent) examined age 3-8 months (Zinner and Jablon 1969). No teeth had erupted in the children examined.

In nine of 10 children (90 per cent) age 9-14 months in whom some deciduous teeth had erupted, strains of *S. mutans* were present (Zinner and Jablon 1969). It was decided that in this case strains of streptococci similar to mutans streptococci had been isolated well prior to the eruption of deciduous teeth. It was concluded that mutans streptococci are part of the indigenous human oral flora (Zinner and Jablon 1969).

Berkowitz and Jordan (1975) on the other hand, were unable to demonstrate *S. mutans* in 91 predentate infants. They were able to show *S. mutans* in nine of 40 (23 per cent) children whose primary incisors had erupted and provided some evidence that similar strains were isolated from both mother and child, suggesting that there could be maternal transfer (vertical transmission) of *S. mutans*.

* "The newborn human infant draws its first breath into a mouth that is usually devoid of microbes. Within minutes to hours, the newborn mouth is colonized with viruses, bacteria yeast and protozoa that may remain in his or her mouth until death." (Slavkin 1997)

Other studies to isolate *S. mutans* from the mouths of infants were not successful. A study undertaken to isolate *S. mutans* from the mouths of infants (Carlsson et al. 1970) was unable to demonstrate *S. mutans* in the mouths of any infants examined, even after the eruption of some deciduous teeth and up to the age of 14 months. The failure to isolate *S. mutans* and the difference in their results and those of Zinner and Jablon (1969) was attributed to the different techniques used and to the fact that the streptococcus strain AHT, demonstrated by Zinner and Jablon (1969), had not, at the time of the study, been found in Sweden. A summary of the relevant studies is set out in Table 4.

Whether or not the finding of Zinner and Jablon (1969), that some *S. mutans*-like species were present before deciduous teeth erupted is not easy to test on the data presented. It is possible these organisms could have been present although there is insufficient data on the ages of the infants examined in the other studies cited (Berkowitz and Jordan 1975, Carlsson et al. 1970, and Catalanotto Shklair Keene and Levine 1974) to determine the issue. The structure of the studies cited, with the exception of Zinner and Jablon (1969), places greater emphasis on the division of subjects according to the presence or absence of teeth, than age.

Reviewing the studies cited (Zinner and Jablon 1969, Berkowitz et al 1975 and Catalanotto et al 1974) it is clear that *S. mutans* is not among the first micro-organisms to colonise the mouths of infants. The establishment of *S. mutans* is usually delayed for some time after birth, and is likely to be associated with eruption of the deciduous dentition which provides a hard surface suitable for colonization.

The views expressed by Zinner and Jablon (1969) and others have to be reconsidered in the light of a growing volume of evidence supporting the early establishment of *S. mutans* in the mouths of pre-dentate infants. In the study which involved 312 infants examined at The Mater Mothers' Hospital, South

Brisbane, Wan et al. (2003) isolated *S. mutans* from the mouths of 5 percent of infants soon after birth, from 49 percent aged 6 months, and from 79 percent aged 24 months. The study (Wan et al. 2003) employed a *S. mutans* - selective (TYCSB) agar with an increased potential for recovery of *S. mutans* compared with media used in earlier studies. Samples for analyses were taken using a sterile cotton tip from the dorsum of the tongue and all surfaces of teeth.

Wan et al. (2003) found the mean age for colonization of mouths of the infants by *S. mutans* was 15.7 ± 5.1 months. These authors have also questioned the concept of a "window of infectivity" with respect to establishment of *S. mutans*, finding instead a steady increase in the infection rate with age.

The study of dentate infants showed that pre-term infants had a 4.4 times greater chance of being colonized by *S. mutans* than full term infants (Wan et al. 2003). Pre-term infants were generally disadvantaged compared with full term infants in relation to establishment of *S. mutans*. In addition the authors found *S. mutans* colonization was greater in infants from families with a low total annual income; associated with conditions permitting repeated exposures; child rearing habits facilitating saliva transfer from adult to child for instance sharing food and utensils; and with habits permitting close (maternal) contact such as breast feeding and infants sleeping beside their mothers. The authors recognized mothers are the usual primary source of *S. mutans* colonizing their children.

The subtle differences between mutans streptococci isolated from oral mucosa and from tooth surfaces, if in fact differences exist, have yet to be delineated.

The full significance of understanding the initial colonization of the oral tissues of a new host by *S. mutans* during the immune tolerance period which occurs in early life,

is related also to assessing the likelihood of the child, a new, naive host, and development a lifelong affinity for the organism with susceptibility to colonization by the bacterium through life.

4.2.3. *Streptococcus Mutans* colonization of hard non-dental surfaces

With the exception of the study of Zinner and Jablon (1969) *S. mutans*, when isolated from the mouths of humans or animals, has been only isolated from the hard surfaces of teeth. The appearance of *S. mutans* in faeces (Elvin-Lewis et al 1975, Shklair et al 1972 and Duany, Mena, Jablon and Zinner 1971), although most likely to have been transient, could be a factor in its transmissibility. Certainly it is present, probably transiently, on the tops of pencils chewed by school children as a contaminant (Woods 1973).

Although unable to demonstrate *S. mutans* in the mouths of pre-dentate infants, the organism was isolated from two of 10 infants who had no erupted deciduous teeth but were wearing acrylic obturators for the treatment of cleft palate; the organism was isolated from the acrylic surface of the obturator in both cases. (Berkowitz and Jordan 1975)

The relationship between *S. mutans* and acrylic full dentures was examined with the assistance of seven patients who volunteered not to wear their full dentures for two days (Carlsson, Soderholm and Almfeldt 1969). Removal of the full dentures was found to reduce the proportion of *S. mutans* in saliva (which ranged from 0.1 to 6.0 percent of the cultivable flora) to an undetectable level in six cases (<0.01 percent) and to 0.02 percent in one case. When the dentures were re-inserted the former levels of *S. mutans* in saliva were re-established. In the 20 patients, including the seven patients referred to already (Carlsson et al. 1969), it was shown that *S. mutans* was established predominantly on the denture surface with only low concentrations in saliva.

The studies of Berkowitz and Jordan (1975), and Carlsson (1967) demonstrated that *S. mutans* exhibits a preference for colonising hard surfaces. Notwithstanding this observation it is possible the organism, or some strains, may be able to exist freely in predentate infants (Zinner and Jablon 1969) although this has yet to be established.

4.3. TRANSMISSION OF *STREPTOCOCCUS MUTANS*

Germ-free studies (Orland et al. 1954), demonstrated that bacteria were necessary to initiate dental caries and also by implication, showed that cariogenic bacteria were transmissible. The bacteria essential to initiate dental caries, like other pathogens, are also parasites and as such must be transmissible* . (Burnett et al. 1976, pp31, 144)

A more formal proof of the transmissible nature of cariogenic flora was the demonstration of transfer between members of the same species**, and further, that within species cariogenic flora could be transmitted vertically from dam to offspring (Keyes 1960). This confirmed the corollary arising from the work of (Orland et al. (1954) when the essential role of bacteria in the development of dental caries was established.

* For the preservation of species of parasites it is essential that they are transmissible in order to establish themselves in a new host either prior to or at the death of their host.

** In these experiments Keyes demonstrated transmission of cariogenic flora in the same species in both hamsters and Osborne-Mendel rats.

Vertical transmission of *S. mutans* in humans, from mothers to infants was suggested when following *S. mutans* suppression in mothers a lower prevalence of *S. mutans* was observed in the infants. (Kohler, Bratthall and Krasse 1983, Kohler, Andreen and Jonsson 1984)

Transmission of *S. mutans* in humans is not easily investigated. The widespread distribution of *S. mutans* in the populations examined (Table 2) suggests that the bacteria are widely transmitted and exchanged among hosts. The view has been expressed that *S. mutans* is transmitted interpersonally, possibly via saliva (Rogers 1977).

It is possible that *S. mutans* could be transmitted between humans by a combination of saliva or plaque, and indirect contact. The studies (Woods 1973) in which *S. mutans* was isolated from both the dental plaque and the pencil tops of school children aged seven to eight years, supported the possibility of transmission of *S. mutans* between schoolchildren by this route.* The presence of *S. mutans* demonstrated in faeces (Gibbons and Banghart 1968 and, Shklair and Keene 1973) combined with indirect contact, presents another possible route for transmission.

Transmission of *S. mutans* between members of a family was investigated as part of a study on establishment of *S. mutans* in the mouths of infants (Berkowitz et al. 1975). Isolation of the comparatively rare 'b' serotype** of this micro-organism from both a mother and an infant supported the possibility of transfer within the family of this micro-organism. In this study 'c' was the most common serotype* encountered.

* Transmission by pencil top contamination is supported by a brief report of the control of a small epidemic of diphtheria by preventing communal use of pencils (Burnet 1962).

** The 'b' and 'c' serotypes referred to were classified according to Bratthall (1972b).

Implantation of *S. mutans* labelled by inducing streptomycin resistance, (Jordan, Englander, Engler and Kulczyk 1972) failed to establish any uniform pattern of transmission. In one case, however, eight days following inoculation of a woman with the labelled strain of *S. mutans*, the labelled *S. mutans* was recovered from her husband, a clear demonstration of transmission.

There is strong evidence of intra-family transmission of *S. mutans* obtained by using a very specific means to identify specific *S. mutans* species* (Rogers 1977). Using very specific *S. mutans* labelling it was possible to show that generally an individual's mouth harbours only one type of *S. mutans* and that families tend to harbour the same species of *S. mutans* (Rogers 1977). Although Rogers (1977) demonstrated that families examined shared the same species of *S. mutans* there were exceptions. Notwithstanding this observation, the inconsistent results in some cases could be readily explained by transmission of the micro-organism from sources outside the family, for instance from school or the workplace.

The principle of vertical transmission of mutans streptococci from mothers to babies has been confirmed (Soderling et al. 2000). The xylitol was used by mothers in xylitol-sweetened gum used two or three times daily, commencing three months after the birth of their children. Mothers used xylitol-sweetened gum by mothers appeared to be associated with a reduction of mutans streptococci in their children.

* Rogers (1977) used a highly specific technique to identify *S. mutans* which employs bacteriocin typing as a basis for identification

Streptococcus mutans does not appear to exist freely in nature other than as a transient micro-organism (Rogers 1977, Coykendall et al. 1974) It exists as a parasite in certain animals, both laboratory and wild, and in man. Because of its parasitic nature *S. mutans* must, as such be transmissible (Burnett et al. 1976, p153).

The routes for transmission of *S. mutans* by indirect contact through saliva, plaque material, and faeces, have been explored and are possible. In addition there is evidence that *S. mutans* is transmitted vertically and within families.

4.3.1. Transmission of *Streptococcus mutans* within family groups

Vertical transmission of cariogenic bacteria is consistent with the observations of Keyes (1960) that it was possible to establish caries in both hamsters and rats by suppressing the flora of the dams and re-innoculating the dams with faeces from caries active animals. In this way Keyes (1960) demonstrated dental caries was transmitted from dams to offspring and also demonstrated vertical transmission of cariogenic flora.

Bacteriocin typing of *Streptococcus mutans* strains isolated from family groups established that similarly to other oral streptococci (*Streptococcus salivarius* and *Streptococcus pneumoniae*) *Streptococcus. mutans* was transmitted within the family (Rogers 1980). Regarding initial transmission of *S. mutans* Rogers (1980) comments "It is most unlikely that direct salivary transfer takes place but it may well occur indirectly via spoons, or toothpaste tubes and inadvertently-shared toothbrushes."

* The bacteriocin typing technique was described by Rogers (1975).

In 29 families examined using bacteriocin typing technique a common *S. mutans* type was found in ten families. The transmission of *S. mutans* in the remaining 19 families in the study was probably from the mother in five cases, in six cases probably the father and in the remaining five cases it could have been from either parent and in three cases it was clearly from neither parent (Rogers 1980).

Reviewing transmission of infectious oral diseases, Slavkin (1997) considered the establishment of oral microbial flora. He described a few "pioneer" species creating a habitat friendly to other (microbial) species which join the microbial ecosystem. When the first deciduous tooth commences eruption, another group of microbes including the cariogenic mutans streptococci, become established.

Continuing his review, Slavkin (1997) refers to the acquisition by children of *S. mutans* from their mothers in the first two years of life when bacteria are initially transferred from mother to child. Using genotyping or DNA 'fingerprinting', Slavkin referred to work from the University of Alabama which found *S. mutans* isolated from infants at the time of acquisition of the bacteria, to be homologous to those isolated from their mother's saliva. There was no instance where homology existed between the father's and the mother's genotype, or between the father's and the child's genotype, supporting the hypothesis that transmission and acquisition of *S. mutans* followed maternal lines very early in infancy and early childhood.

By monitoring salivary mutans streptococci in 65 mothers selected from 310 pregnant women whose salivary mutans streptococci level was greater than 105 colony forming units per millilitre, during the second and third trimesters of

pregnancy, the salivary levels of the mothers' mutans streptococci were reduced significantly. (Brambilla *et al.* 1998). Forty eight per cent of children whose mothers had used sodium fluoride and chlorhexidine mouthwashes daily became infected with mutans streptococci, compared with the control group where 83 per cent became permanently infected with mutans streptococci. (Brambilla *et al.* 1998) Interruption of vertical transmission of mutans streptococci from mothers to their infant children is being explored coupled with other procedures, to reduce dental caries susceptibility in children.

Using a different approach Soderling *et al.* (2000) interrupted vertical transmission of mutans streptococci from mothers with a high salivary level of mutans streptococci, to their infants when the mothers used a xylitol-sweetened chewing gum (65 per cent xylitol w/w) at least two or three times daily. At two years of age mutans streptococci were detectable in 9.7 per cent of a group of children whose mothers chewed xylitol-sweetened gum. This was compared with detection of mutans streptococci in 26.6 per cent whose mothers had chlorhexidine varnish applied, and 48.5 per cent whose mothers had a fluoride varnish applied. Consumption of xylitol appears to be selective for impairment of colonisation by mutans streptococci (Soderling *et al.*, 2000).

The majority of plaque bacteria ferment dietary sugars, however, xylitol appears to have specific mutans streptococci inhibiting effects. In addition, most plaque bacteria are not capable of fermenting xylitol. (Trahan 1995). Intracellular accumulation of an un-metabolisable metabolite of xylitol by mutans streptococci occurs and their growth is inhibited. In consequence a reduction occurs in the amount of plaque and the numbers of mutans streptococci in plaque and saliva (Trahan 1995).

Employing genotyping, a very sensitive technique, for identification of mutans streptococci, transmission of mutans streptococci from parents to their children was investigated by Emanuelsson and Bratthall (1998). Plaque samples from buccal and occlusal surfaces of 25 three year old children, their mothers and 18 fathers, were collected and analysed for mutans streptococci. In 11 of the families mutans isolates were found in parents and children.

When analysed, using a genotyping technique it was shown that of the 25 children, five harboured mutans streptococci genotypes different from their parents, six showed genotypes identical to those of their mothers, and none of the children had genotypes similar to their fathers. There were no genotype matches between any of the 11 pairs of parents. It was concluded mothers who have a high level of mutans streptococci in plaque or saliva, as the primary care providers of their children, were more often observed in the group with identical genotypes within mother-child pairs than in the non-matching group. The study indicated that fathers and children had not exchanged strains of mutans streptococci nor had there been any exchange between spouses. Children however appear to be able to obtain mutans streptococci from their mothers and also from outside the family (Emanuelsson and Bratthall 1998).

The possibility of the existence of a "window of infectivity" has been proposed (Straetemans, van Leven, de Soet, de Graff and ten Cate 1998.) These investigators studying 109 children who could be traced from an earlier study of 186, five year old children (Roeters, van der Hoeven, Burgersdijk and Schaeken 1995), were able to show a relationship between the presence of mutans streptococci before age five years and dental caries experience at age 11 years. Considering both deciduous and permanent dentitions, of children who had no mutans streptococci before the age of five years, 95 per cent were caries free at age 5 and 59 per cent were still caries free when

examined at age 11 years. Of the children from whom mutans streptococci were isolated by age 5 years, 49 per cent were caries free, and at age 11 years the percentage of caries free children in this group had fallen to 24 per cent.

The study by Straetemans *et al.* (1998) confirmed observations that children whose teeth are colonized early in life (by five years of age) by mutans streptococci show a higher caries experience in the deciduous dentition than children in whom the teeth are colonized later or not at all. It was also shown by Straetemans *et al.* (1998), who followed children's caries experience from age five to age 11 years, that the mean dental caries score was lower for those children who had no mutans streptococci before age five years (DMFS 0.44 ± 0.88) than for those who had mutans streptococci present before age five years (DMFS 1.20 ± 1.91)

Transmission of mutans streptococci vertically from mothers to their children is supported by a number of studies, it appears likely that little or no transmission of mutans streptococci to their children originates from their fathers.

Studies of Straetemans *et al.* (1998), Roeters *et al.* (1995) and Kohler and Andreen (1994) have taken the observation further and have related the establishment of mutans streptococci in children by age five years, to a higher caries susceptibility than where children have no mutans streptococci by age five.

The possibility of a "window in infectivity" between 19 and 31 months has been suggested by Straetemans *et al.* (1998) who have also suggested a second window of infectivity may exist between the ages of six and 12 years which could be related to the development of the permanent dentition.

Kohler and Andreen (1994) reported a trial involving mothers of children up to age three years who were provided with a preventive dentistry program. A test and control group of mothers was established. Salivary tests were used to determine the presence of mutans streptococci in the mouth.

The program was directed to reducing the presence of mutans streptococci in the mouths of the mothers and included dietary counselling, professional tooth cleaning and oral hygiene instruction, fluoride treatment and treatment of dental caries. Additionally, mothers with greater than 3×10^5 colony forming units of mutans streptococci in their saliva used a one per cent chlorhexidine digluconate mouthwash (Hibitane ICI) daily for five minutes for a period of two weeks.

The caries experience of the children at age seven years, whose mothers were in the test group who took part in the program to reduce mutans streptococci while the children were less than three years of age, showed significant reduction in dental caries when compared with the control group.

The 26 children in the test group at age seven years had a mean caries score (defs) of 5.2; those in the control group had a score (defs) of 8.6. There was also a greater percentage of caries free children in the test group (23 per cent) than in the control group (9 per cent). Additionally, children in the test group had lower mutans streptococci and lactobacilli counts than those in the control group.

These results support the concept of transmission of mutans streptococci and lactobacilli from mother to child and the establishment of the organisms in the child before the age of three years. The measures to reduce the presence of oral mutans streptococci and lactobacilli applied to mothers of children up to the age of three years appears to be related to the presence of these bacteria in the mouths of children four years later when the children were seven years of age. The children in the test group also experienced reduced dental caries and had more caries free children than were present in the control group.

The findings suggesting vertical transmission and establishment of oral microorganisms, in this case mutans streptococci and lactobacilli, are consistent with immune tolerance exhibited by young animals and described by Burnet (1962) and by Burnett Scherp and Schuster (1976). Microflora established in the very young is likely to be tolerated by the immune system and become a permanent part of the child's microflora.

Table 3 *Streptococcus mutans* in the mouths of infants

Author	Subjects		Deciduous teeth present	Cases where <u>S. mutans</u> was present [per cent]
	No.	age		
Zinner and Jablon (1969)	51	< 12 days	no	0
	18	13-24 days	no	0
	25	25-54 days	no	0
Zinner and Jablon (1969)	12	1-2 months	no	2 [17]
	12	3-8 months	no	9 [75]
	10	9-14 months	yes	9 [90]
Berkowitz <u>et al.</u> (1975)	91	(3 weeks to	no	0*
	40	14 months)	yes	9 [23]
<u>Catalanotto</u> <u>et al.</u> (1969)	14	from birth**	no	0
	10	from birth**	incisors	0
	9	from birth**	incisors***	2 [22]
Carlsson <u>et al.</u> (1970)	27	1-14 days	no	0
	27	3-14 days	yes	0

* *Streptococcus mutans* was isolated from 10 pre-dentate children wearing obturators.

** Infants, upper age not stated

*** also isolated from the first deciduous molar

4.4. THE ENDEMIC NATURE OF *STREPTOCOCCUS MUTANS* DISTRIBUTION

There is no evidence that *S. mutans* exists other than as a parasite associated with humans as well as some laboratory and wild animals. It appears that this bacterium colonizes the hard surfaces of teeth, dental prostheses or appliances and that this site is its 'niche' in the parasitic ecology where it is part of dental plaque flora. The presence of *S. mutans* in saliva and mucosal and tongue surfaces is likely also to be transitory and a reflection of its presence in its real home, dental plaque attached to the hard dental surfaces, including surfaces of appliances.

There is no community where *S. mutans* has been sought in dental plaque that it has not, in at least some members, been found.* In most communities *S. mutans* is present in more than 60 percent of the subjects examined. It has been found in populations all over the world*. On the basis of its wide distribution in populations throughout the world and the prevalence of the bacterium in these populations, the distribution of *S. mutans* appears to be endemic.

Endemic disease has been defined (Burnet 1962, p171)

"as an infectious disease present in a community in which the social circumstances do not offer any effective barrier to its spread".

The fact that it occurs in over 60 per cent of members of the populations examined, with the exception of of two primitive groups in remote areas of Papua-New Guinea mentioned already (Schamschula et al 1978), indicates it is widely and freely transmitted in almost all communities.

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- * With the exception of two primitive and isolated communities in Papua-New Guinea. (Schamschula et al 1978)
 - * Table 2, presents summaries of a number of community studies in various countries. The prevalence of *S. mutans* in these studies ranges from 55 to 93 percent.

The principle employed to control an infectious, endemic disease is to increase the resistance and immunity of every individual in the community to the infectious agent. Endemic disease is controlled by public health measures which anticipate exposure of all individuals in a community to the infectious agent and aim at increasing the ability of all individuals to resist contracting the infection.

The converse to endemic is epidemic, where the principle used to control an epidemic of an infectious disease is some form of isolation of the affected individual to prevent transmission of the disease and its contraction by new hosts.

Essentially management of endemic disease relies heavily on public health measures for its prevention, epidemic disease on the other hand relies on individual assessment and diagnosis, then appropriate personal treatment.

Public health measures used to prevent endemic disease include immunization of communities, for instance the immunization of children for diphtheria, tetanus, pertussis and poliomyelitis. Fluoridation of public water supplies is a very good example of a public health programme. Fluoridation reaches the entire community although it must be noted that the means by which it achieves its effect is not by preventing infection with mutans streptococci.

The endemic/epidemic classification is rarely absolute, some infectious agents which are endemic in one region may not be endemic in another region. Sometimes infectious agents have both endemic and epidemic characteristics. Cholera is for instance, endemic in some parts of the world where the "the social circumstances do not offer any effective barrier to its spread" (Burnet 1962 p171), in Australia when cholera occurs, it occurs as a small epidemic.

4.5. DISTRIBUTION OF *STREPTOCOCCUS MUTANS* - SUMMARY

Distribution of *Streptococcus mutans* in almost all populations is endemic, the known exceptions being several isolated populations in Papua New Guinea. (Schamschula et al. 1978). It has been possible to demonstrate *S. mutans* in the dental plaque of approximately 60 percent of almost every population examined. In addition it has been shown to occur in the dental plaque of both laboratory and some wild animals. Notwithstanding this observation it is likely that the classification of dental caries in those communities with a low prevalence of the disease may be shifting a little towards the epidemic area of the classification. It is entirely possible to have both endemic and epidemic characteristics of the same infections disease.

There are a number of methods by which *S. mutans* could be transmitted through the community, however, transmission by indirect contact associated with dental plaque is most likely the predominant form of transmission. Transmission by indirect contact via faeces is possible but in most communities which observe basic hygiene it is unlikely to be a principal form of transmission.

There is strong evidence that transmission of *S. mutans* occurs within family groups where its transmission is vertical, from mother to child, probably occurring when the child's deciduous dentition is first erupting.

There is also strong evidence to support the vertical transmission of mutans streptococci as well as lactobacilli, to children by the time they are three years of age, from their mothers. A period of infectivity has been suggested from 19 to 31 months (Straetemans et al. (1998).

Colonisation by *S. mutans* occurs on hard surfaces such as tooth surface or plastic prostheses. The organism occurs in saliva where its presence is most probably transient. For this reason its establishment as an oral parasite may be delayed until the eruption of the deciduous dentition.

Although *S. mutans* can be demonstrated outside the body it is most probable that in these locations it is transient. There is no evidence that *S. mutans* can exist, other than in transition, outside a host; it appears to be truly parasitic.

Because of the endemic nature of the distribution of *S. mutans* in communities it is reasonable to expect that most members will have experienced contact with the micro-organism shortly after school age. Contact with and exposure to *S. mutans* in early life is presumably governed by the presence of the organism in other family members, mothers in particular because of their close contact with their babies. Transmission of *S. mutans* is likely to occur between mothers and their newborn infants, because of their close contact, although colonization and establishment of the organism is likely to be delayed until the eruption of the deciduous dentition.

Whether or not *S. mutans* will become established in a new host after contact has been made will depend on the potential susceptibility of the host including the host's diet and dietary sugar availability, factors of host resistance, the size of the bacterial inoculum making the contact and the frequency of contacts.

Early established microflora may influence the establishment of mutans streptococci if for no other reason than because the established flora will have obtained access to a nutrient source. Microorganisms arriving subsequently will have to compete with those already established. Microflora established soon after birth has few if any microbial competitors and can more readily established.

In the newborn there is a specific immune defence against cariogenic mutans streptococci, mediated through salivary secreted IgA antibodies generated by the common mucosal immune system. (Russell *et al.* 1999) It was considered that the mechanisms of action could include interference with sucrose-independent and sucrose-dependent attachment of mutans streptococci to tooth surfaces (Russell *et al.* 1999).

Immune tolerance is exhibited by young animals and occurs in a foetus and for a brief period after birth (Burnet 1962, Burnett *et al.* 1976). Microflora established in the very young during the immune tolerance period are likely to be tolerated or accepted by the immune system and to become a permanent part of the child's, and later the adult's, microflora.

Initial establishment of mutans streptococci appears to be favoured by the availability of dietary sucrose. Less obviously, sucrose may also be available from some medication and certain vitamin supplements taken regularly.

4.6. A "MEDICAL MODEL" FOR THE PREVENTION AND CONTROL OF DENTAL CARIES

A description of the infective agents responsible for dental caries, their transmission, means to arrest, minimise or control transmission and means to assess an individual's dental caries susceptibility, has made possible the development of a 'medical model' for the treatment and control of dental caries. The 'medical model' for control of dental caries is a departure from the traditional 'surgical model'* in that the dental caries is considered as an infectious disease rather than solely as a lesion. The events leading to the development of a lesion can be controlled in a way to prevent development of the lesion.

The medical model, in general terms, aims to reduce or eliminate pathogens from patients diagnosed being at risk of developing dental caries (Fiset and Grembowski 1997). It is possible to introduce and apply the principles of control of infectious disease as a medical model, to control dental caries.

The principles of the medical model include prevention of the early establishment of mutans streptococci in the oral environment; monitoring caries susceptibility and the presence of mutans streptococci; reduction or elimination of oral mutans streptococci where they occur and support of procedures which bring about these objectives using appropriate techniques including such measures as fissure sealants which are directed to minimise caries development in susceptible areas also use of fluorides in their various forms ranging from water fluoridation to topical applications and the use of fluoride toothpaste. These measures will need to be supported by a program of health education. Protocols for these objectives have been developed and are already available.

* The 'surgical model' of treatment relies on removal of diseased tissue and where appropriate its replacement.

It has been established for some time that mutans streptococci can be transmitted within family groups, principally from mothers to their children (Rogers 1975, 1977, 1980). More recently, interest has been directed to establishing the age of a child when mutans streptococci originating from their mother, might first become established. A "window of infectivity" has been described (Straetemans et al 1998).

Kohler and Andreen (1994) observed preventive measures which reduced the salivary levels of mutans streptococci in mothers. The preventive measures included use of a chlorhexadine mouthwash and were described by Kohler, Bratthall and Krasse (1983). The maternal preventive programme was discontinued when the children reached three years of age. In this study it was shown that the reduction of a mother's mutans streptococci during the emergence of her child's primary teeth had a long term effect on the colonization of the child by mutans streptococci and the child's dental caries experience (Kohler and Andreen 1994). The transmission of *S. mutans* was from the mother, to child; the mother is regarded as the main source of the organism. This is likely to be related to the close contact between mother and child during the post natal period and is consistent with observations by Rogers (1980).

The possibility that other organisms which could impare colonisation by mutans streptococci may become established in advance of the 'window of infectivity' for mutans streptococci has been explored by Caufield, Dasanayake Li, Oan, Hsu, and Hardin (2000). These authors concluded that perhaps their most interesting finding is the relationship between levels and times of colonization of *S. sanguinis* and subsequent colonization by mutans streptococci. They found in their study that early colonization of infants by *S. sanguinis* delayed later colonization by mutans streptococci. The possibility of controlling colonization by mutans streptococci in this way has yet to be explored.

It is important to recognize that the studies of Straetemans et al. (1998) and Brambilla et al., (1998) demonstrated that a reduction in mutans streptococci in mothers could be related to reduced dental caries risk in the next generation. Of equal importance is the recognition of a post natal 'immune tolerance period' (Burnett et al. 1976) (p201). In essence, micro-organisms transmitted to the very young are frequently tolerated by the immune system and can ultimately become part of the natural flora of those individuals. The immune tolerance period extends from before birth to several years and very likely diminishes slowly until in humans by approximately the age of four or five years. Organisms becoming established during this period do not stimulate an immune response or may only stimulate a limited immune response. Organisms becoming established in this period appear not to be recognized as alien and in fact appear to enjoy a form of tolerance by their hosts throughout the lives of their hosts.

Controlling the initial colonization of infants by mutans streptococci largely originating from their mothers, offers a sound and realistic control method and is consistent with a 'medical model' for the control of dental caries.

5. FACTORS AFFECTING *STREPTOCOCCUS MUTANS* IN PLAQUE

5.1. SUCROSE AND *STREPTOCOCCUS MUTANS* IN PLAQUE

Using a triad of factors (Figure 1) Keyes (1962, 1969) illustrated the interrelated roles of diet, microflora, and host susceptibility.

For dental caries to occur an appropriate combination of diet (substrate), microflora, and a susceptible host, must occur. It was suggested by Keyes (1969) that sucrose in the presence of *S. mutans* was highly conducive to caries*. Other authors, (Krasse 1965a, 1965b, Gibbons and Banghart 1968, and Gibbons et al. 1966) demonstrated the relationship between sucrose and *S. mutans* in the aetiology of dental caries. Reviewing the role of sucrose in dental caries Newbrun (1969) concluded that a specific elimination of dietary sucrose was preferable to total carbohydrate prohibition for prevention of caries. Observations, based on studies made at Vipeholm (Gustafsson, Quensel, Larke, Lundqvist, Grahnen and Bonow. 1954) and Hopewood House, New South Wales (Goldsworthy 1958, Goldsworthy and Spies 1958) showed that the absence of dietary sucrose limited caries to a fraction of that which occurred in the general population at the time of the studies. These studies were made at a time of high community dental caries experience.

The Vipeholm diet was a low sugar diet and the groups studied had sugar added to this basic diet in various forms, in drinks, with meals, and as cakes or confectionery eaten between meals. (Gustafsson et al. 1954)

* In his assessment Keyes (1969) suggested that sucrose had a high cariogenic potential associated with *Lactobacillus acidophilus* and *Odontomyces viscosus* as well as *S. mutans*

FIG. 1: Keyes' Triad of Factors (Keyes 1962)

Refined carbohydrate was almost completely absent from the diets of children at Hopewood House, much of their diet was of a vegetable nature and eaten uncooked (Goldsworthy 1958).

The link between sucrose and *S. mutans* in dental plaque was to be established later to confirm the earlier empirical studies, for instance at Viperholm and Hopewood House, on diet and caries. It should be appreciated that most of the animal studies which have been reviewed, and which led to the demonstration of the aetiological role of *S. mutans* in dental caries, employed diets which contained high levels of sucrose*.

The experiments of Krasse (1965a) showed that *S. mutans* could be more readily established in the mouths of hamsters when they were fed a diet containing sucrose or lactose than when the diet contained glucose. Krasse (1965b) confirmed that a sucrose based diet led more readily to the establishment of cariogenic streptococci** than a glucose based diet.

Reviewing his series of studies on plaque microflora (Carlsson 1968b) concluded that the ability of *S. mutans* to produce extracellular polysaccharides could explain why the micro-organism could be found in large numbers on the tooth surface when the diet had a high sucrose content.

Referring again to Keyes' triad of factors (Keyes 1962, 1969), the three main factors he described interact with each other. For instance, the diet, especially the sucrose component, has a profound effect on the plaque microflora; the

* As an example Diet 580 contains 66 per cent sucrose, Diet 2000 contains 56 per cent sucrose

** Strain HSR-6, later identified as a strain of *S. mutans*

factors of diet (or substrate in the diagrammatic triad of factors - Fig. 1) and microflora are closely linked.

Other factors which can be applied to the host, such as the various forms of systemic or topical fluoride can have an effect on both the plaque microflora (Woods 1971b) and the consequential caries. These factors could be considered as substrate, or if they alter the nature of dental enamel, a host factor in terms of Keyes' triad.

Keyes' triad of factors which, when all factors are present indicate the likely development of dental caries, can be expanded to provide a model of the conditions leading to dental caries which could accommodate a greater number of factors. An expanded table listing additional factors in three groups, 'host', 'environment' and 'agent' can be prepared. A suggested, expansion of the factorial triad of factors is presented (Table 4).

The prevalence of *S. mutans* in plaque is considered to be endemic as it is widely distributed through the community. In a community where a microorganism is endemic, individuals in that community are regularly exposed to the microorganism. Whether the microorganism can establish itself in an individual will be influenced by a number of factors, including the individual's personal immunity and body surface resistance to the microorganism. The principle for control of endemic infectious disease is to increase the resistance of all individuals in a community to the infectious agent.

The quality and stability of the resident plaque microbiota can influence the ability of a pathogen, for instance *S. mutans*, to become established.

Changes in the composition of the dental enamel, for instance an increased fluoride content or a very regular enamel crystal composition, will make it more difficult to establish bacterial colonisation on the enamel surface (Woods 1971b). Likewise the absence of available sucrose in the diet will, because of the relationship between sucrose and *S. mutans*, increase the difficulty of establishing *S. mutans* in the dental plaque flora and will reduce the caries challenge from the microflora (Krasse, 1965a; 1956).

Each of the expanded factors in Table 4 has the potential affect the balance of the sum of the factors either to favour or resist the development of dental caries by altering the character of the three basic factors, host (tooth), the environment (substrate) or the agent (flora).

Development of this model helps understand the factors leading to the establishment of dental caries. It is also an attempt to demonstrate the multifactorial nature of dental caries. Notwithstanding that dental caries is a multifactorial disease the essential component is the bacterial flora; without a caries inducing flora development of dental caries will not occur. This has been clearly demonstrated (Orland et al. 1955)

The relationship between dietary sugar, mutans streptococci and dental caries has continued to be demonstrated in studies of both children and adults, including longitudinal multi-factor studies (Mattos-Graner 1998; Grindefjord, Dahllof G, Nilsson B, Modeer T 1996; Fure 1998) and more recently Nobre dos Santos, Melo dos Santos, Francisco and Cury (2002).

Table 4 Factors in a model for dental caries expanded and classified in the three groups proposed by Keyes (1962, 1969)

<u>Host</u> (tooth*)	<u>Environment</u> (substrate*)	<u>Agent</u> (flora*)
• Enamel and crystal structure. • Enamel minerals Ca,P,F. • Saliva quantity and quality • Immune response • Host behaviour • Host attitudes • Systemic disease • Diseases and medication affecting saliva. • Anatomical aspects • Deep, retentive fissures in posterior teeth • Broad teeth with increased interproximal contact • Hypoplastic enamel (may be related to hyperpyrexia disease during enamel formation)	• Plaque quantity • Plaque quality • Enzymes • Minerals • Bacterial substrate available sucrose in beverages • continuous infant reservoir feeding • Socioeconomic culture • Fluoride drinking water; toothpastes; supplements; diet; topical applications.	• <i>S.mutans</i> • Lactobacilli • Other cariogenic bacteria • Establishment of cariogenic flora during "window of infectivity" (See Section 5.3)

* Keyes original factors (Keyes 1962, 1969)

5.2. FLUORIDES AND *STREPTOCOCCUS MUTANS* IN DENTAL PLAQUE

Fluorides can be administered either systemically, or applied topically to erupted teeth. The ability of fluoride therapy to reduce dental caries has been established beyond doubt. Referring to Keyes' triad (Keyes 1969), in what way do fluorides interact with plaque microflora? What is the effect of fluorides on *S. mutans* in dental plaque?

Reviewing fluoride therapy, (Stookey 1966) it was considered that there were two possible mechanisms which could explain its anti-caries effect. First, the bacteriostatic effect on the oral flora based on the well known enzyme inhibition properties of fluoride released from enamel, and second, the incorporation of fluoride within the hydroxyapatite of enamel resulting in a lowered solubility of crystals of enamel apatite in weak organic acids.

The loss of fluoride ions from dental enamel after topical treatment with both acidulated phosphate fluoride and stannous fluoride solutions has been reported (Mellberg, Laakso and Nicholson 1966). They demonstrated using both *in-vitro* and *in-vivo* studies a high initial loss of fluoride from the treated surface. They were also able to show that *in-vitro*, a secondary fluoride loss occurred when the samples of treated enamel were rinsed in water. They were unable to demonstrate the secondary loss of fluoride *in-vivo*. These studies (Mellberg et al. 1966) demonstrated that fluoride taken up by dental enamel after topical fluoride application is not permanent. It was suggested that the initial rapid loss of fluoride was due to removal of un-reacted fluoride while the secondary loss from longer washing was due to slow dissolution of a sparingly soluble compound, possibly a calcium salt.

Immediately after topical fluoride treatment fluoride ions commence moving from the treated enamel which results in higher concentrations of fluoride ions adjacent to the tooth surfaces immediately following treatment. The escape of fluoride ions could be a simple equilibration. A high concentration of the fluoride solution adjacent to the enamel during the application is succeeded by a lower concentration after the application is completed. This could change the fluoride ion gradient and reverse the direction fluoride ion transport which was in the direction surface fluid to enamel while the topical fluoride application was taking place, but following the application to the reverse direction of enamel to enamel surface fluid and dental plaque. Where the dental plaque has been removed prior to topical fluoride application, re-establishment of the plaque, commences immediately with colonisation from the oral flora, and the plaque establishment occurs in the presence of fluoride ions released from the tooth enamel following exposure to fluoride.

The effects of the fluoride treatment of enamel on acid production of *Lactobacillus casei* were investigated using *in-vitro* techniques (Briner and Francis 1962). Both normal and decalcified teeth containing fluoride from topical treatment with either sodium fluoride or stannous fluoride, as well as teeth containing fluoride from natural sources*, were shown to liberate sufficient fluoride from the enamel to cause a decrease in acid production by *L. casei* in contact with the enamel surface.

Examining the effect of fluoride ions on acid production of a number of species of streptococci and lactobacilli in an *in-vitro* situation it was shown that fluoride ions greatly reduced the rate of acid production and the total amount of acid produced (Sims 1966).

* Teeth heavily mottled with dental fluorosis from ingestion of natural fluoride in the drinking water

The fluoride uptake of enamel and the levels of fluoride in saliva after topical fluoride application was investigated by Margalet and Gedalis (1969). It was demonstrated *in-vivo* that there was an elevated fluoride concentration in saliva which persisted for ten days following plaque removal and a four minute topical fluoride application*. Salivary concentrations of fluoride ranging from 0.46 ppm to the lower levels of 0.12 ppm, which was equivalent to the control level of fluoride in saliva, were recorded.

The results of the two studies by Margalet and Gedalis (1969) ARE set out in Table 5

* The fluoride application was made with an "Air Cushion Fluoridator" (The Ion Co. Los Angeles California)

Table 5 Fluoride in saliva following two types of topical fluoride applications (Margalet and Gedalis 1969).

Time of salivary fluoride determination	Fluoride in saliva following topical fluoride application (parts per million)	
	NaF + Na ₂ HPO ₄	NaF
Control*	0.12	0.12
Day 1**	0.46	0.39
Day 2	0.36	0.45
Day 3	0.34	1.41
Day 4	0.25	0.27
Day 5	0.16	0.19
Day 6	0.14	0.17
Day 7	0.13	0.15
Day 8	0.15	0.16
Day 9	0.13	0.15
Day 10	0.13	0.12

-
- * Prior to topical fluoride application
** Six hours after treatment

An *in-vitro* study demonstrated that enamel from extracted teeth readily took up fluoride when it was applied topically and that this fluoride was not permanent but could be removed by washing the treated enamel (Richardson 1967). In this study a greater retention of fluoride in the enamel was exhibited if washing was delayed.

The loss of fluoride from enamel following topical applications with acidulated fluorides, stannous fluoride, and sodium fluoride, tested separately, was demonstrated (Lovelock 1973). These studies were made on enamel from extracted teeth and used radioactive ^{18}F as a tracer. The fluoride was lost when the enamel was washed and, because of the linear rate of loss, it was suggested that the fluoride loss could have been as a result of diffusion (Lovelock 1973).

In an *in-vivo* study an appreciable fluoride uptake by tooth enamel after topical treatment with two per cent sodium fluoride, eight per cent stannous fluoride, five per cent sodium monofluoride phosphate and two per cent acidulated fluoro-phosphate gel was shown (Petersson 1976). In this study no uptake could be shown after rinsing with low concentrations (0.025 or 0.05 per cent) of sodium fluoride.

An *in-vitro* study demonstrated a marked enamel uptake of fluoride after exposure to fluoride solutions*, providing further evidence of the ability of fluoride ions to be taken up by tooth enamel (Brudevold, Savory, Gardner, Spenilli and Speirs. 1963).

These studies provide substantial evidence to support a temporary uptake of fluoride ions by dental enamel following a topical fluoride application (Lovelock 1973, Peterson 1976, Margalit and Gedalis 1969 and Brudevold et al. 1963)

* Acid phosphate solutions

It has been shown that acid production from *L. casei* could be reduced by release of fluoride ions from enamel (Briner and Francis 1962). It was also noted that fluoride ions could interfere with the Embden-Meyerhof pathways of glycolysis which could explain the reduced acid production by *L. casei* demonstrated (Briner and Francis 1962).

Streptococci rely on the Embden-Meyerhof pathway of glycolysis to provide their energy source (Burnett *et al.* 1976, Bisset and Davis 1960, and Wagg 1955). Streptococci are described as homofermentative, that is they depend on one metabolic pathway. The principal end product of their metabolism is lactic acid and in fact the amount of lactic acid produced is a measure of the energy they have used (Wagg 1955). Although he proposed other mechanisms could be possible, interruption of the Embden-Meyerhof metabolic pathways was favoured by Hamilton (1977). Hamilton (1997) suggested that fluoride ions could affect metabolism of streptococci by interrupting the transmission of glucose across the bacterial cell membrane.

Carlsson and Hamilton (1994) have described bacterial glycolytic pathways with specific reference to oral streptococci. While the principal function of the Embden-Meyerhof pathways of glycolysis remains the generation of energy and the precursors for synthesis of cellular material, many micro-organisms also possess the pentose phosphate shunt pathway which is directed to the production of cellular precursors, including the reducing power for biosynthetic reactions.

The oral streptococci *S. mutans* and *S. salivarius* do not possess the oxidative portion of the pentose phosphate pathway and generate their reducing power via a specific mechanism involving glyceraldehyde 3-P dehydrogenase. To obtain cellular precursors of the pentose phosphate pathway *S. mutans* and *S. salivarius* have the non-oxidative portion of the pathway.

Lactobacilli, for instance *L. acidophilus* or *L. salivarius* are regarded as 'homofermentive' as the principal end product of their glycolysis is lactic acid. Other lactobacilli for instance *L. fermentum* or *L. brevis* are 'heterofermentive' and degrade glucose by the pentose phosphate pathway to lactic and acetic acids and carbon dioxide. Bacteria equipped with both pathways are called 'facultative heterofermentive' and generate lactic, acetic and formic acids as well as ethanol.

The degradation of glucose in the glycolytic pathway provides the cell with an energy source in the form of adenosine triphosphate (ATP) or its equivalent. A system employing 'electron-transportation' which through a series of steps stores energy in the form of an electrochemical proton gradient across the cell membrane. This energy facilitates the synthesis of ATP.

Streptococci do not possess an electron-transport system in their cell membrane and extrude protons via the cell membrane with end products such as lactic acid.

Transport of electrons which extrude protons across the cell membrane which creates an electrical charge differential across the membrane. Bacteria such as streptococci which do not possess an electron-transport mechanism, may transport protons across the cell membrane using enzymes (H^+ /ATPase).

Protons may also be extruded with metabolic end products. These reactions contribute to conservation of metabolic energy in those bacteria without electron-transport mechanisms.

It has been established that the glycolytic Embden-Meyerhof pathway can be interrupted by fluoride ions which inhibit the enzyme enolase. In addition, the function of the cell membrane can be interfered with by fluoride ions in at least two ways. The permeability of the membrane can be increased and the enzyme H⁺/ATPase can be inhibited by fluoride ions (Carlsson and Hamilton 1994).

Regarding the likely levels of fluoride ions in dental plaque, Sims (1966)* states that the fluoride concentration would be unlikely to reach the necessary level to completely inhibit the growth of lactobacilli or streptococci, however, he admits that the growth of streptococci would certainly be retarded by the concentrations achieved.

Fluoride concentrations in dental plaque were found to be high but variable, ranging from 6.0 to 180 parts per million. The form of the fluoride reported was not known (Hardwick and Leach 1962). These values are higher than those referred to by Sims (1966).

The salivary concentrations of fluoride reported by Margalit and Gedalis (1969) were calculated in the first three or four days following topical fluoride application and were similar to the concentrations referred to by Sims (1966).

* Sims referred to reports of fluoride concentrations in plaque (from an area where the water supply contained 2 ppm fluoride) to be 47 ppm. (Dawes, Jenkins, Hardwick and Leach 1965).

There are two matters to consider. First, the retardation of streptococcal metabolism may not apply equally to all species of streptococci. Second, the effect of unequal retardation of activity including cell division, of some streptococci could influence the balance of various streptococcal species present in a plaque system containing many different streptococcal species.

Consistent with the principles of ecology and with the competitive exclusion principle enunciated by Hardin (1960) such ecologically disadvantaged species will diminish in number and finally, subject to a minor incident which could be a simple change in environment, the particular species could disappear altogether.

Applying this concept to the mixed flora of plaque it seems reasonable to speculate that a species of streptococcus could be so disadvantaged by the presence of fluoride ions, that its relative proportion in the total flora would be markedly reduced or it may be eliminated from this locality.

The effect is likely to be greater when the plaque is removed as new plaque would be developing and in these circumstances of rapid bacterial multiplication. Factors dependent on competitive cell multiplication would have a greater impact than they would in established plaque where bacterial populations are established and multiplication is minimal.