Review

Pathogenic Microbes of the Oral Environment

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Improved health owes less to advances in medical science than to changes in the external environment, and to a favorable trend in the standard of living. We are healthier than our ancestors not because of what happens when we are ill but because we do not become ill; and we do not become ill not because of specific protective therapy but because we live in a healthier environment. In its preoccupation with the minutiae of diagnosis and the pathogenesis of disease, medicine is in danger of neglecting what has hitherto proved its most powerful resource—the manipulation of the external environment.

Grundy and Macintosh 1957

Disease background

Apart from a few situations, it is generally agreed that the microorganisms of today are as much an undisciplined force of nature as they were centuries ago. This is underlined by the fact that diseases most prevalent in modern civilized societies were also manifest in ancient man and still exist in modern primitive man. Because the nature of man has remained essentially the same for thousands of years, diseases have retained their universal characteristics. What has changed is the disease prevalence from one period of time to another, together with differences among social groups and geographical areas. Hence it can be said that differences in the environment as a whole and in the life-styles produce the diversity of disease.

Microbial diseases stem from two different origins. They can either be ‘exogenous’ or ‘endogenous’. A number of pathological processes are the outcome of exposure to a virulent pathogen. For example, the microbial agents responsible for diseases such as yellow fever, malaria, tuberculosis and syphilis can be traced. The pathological processes usually become manifest within a predictable period following exposure—thus they are truly infectious microbial diseases with an exogenous origin.

By definition, endogenous microbial disease refers to all pathological states caused by microorganisms which have been acquired at some prior time and which have existed in the body as part of its indigenous microbiota. Formerly harmless microbes such as coliforms and a number of Gram-negative bacilli, non-hemolytic streptococci, fungi, yeasts and viruses (many yet unidentified) display a low virulence, and yet serious pathological states can arise linked with changes in physiological circumstances. It appears that the majority of virulent organisms can become established in the body and remain quiescent without sign of overt disease until the general resistance of the host has been altered.

Man has evolved a close and constant association with a complex microflora and microfauna, and under natural conditions, his tissue development and function is influenced by a myriad of microorganisms such as those found in the digestive and respiratory tracts. Presumably the structures and physiological requirements of human organs have been at least partially determined by the microbiota present during evolutionary development, and that the body responds under certain circumstances to the microbiota present. Hence the microbiota is an environmental factor to which man has adapted.

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The indigenous microbiota, as found in the healthy state, consists of the flora and fauna which have been acquired during evolutionary development, and to which can be added a wide range of microorganisms that enter the body as a result of 'accidents of life'. Basically, the indigenous microbiota may be formed of several classes of microorganisms of contrasting origins.

The attainment of a symbiotic status between some microorganisms with their host has occurred over the long period of evolutionary change, and thus in some authorities have referred to them as the autochthonous biota. Others, which show a pathogenic capacity, can also establish themselves in tissue, but nevertheless display a state of biological equilibrium with the host under stable conditions. It is known that some of the most virulent pathogens can be harbored in tissues without producing pathological changes. This nonactive state for prolonged periods of time is shown by a considerable number of pathogens including viruses, fungi, bacteria, rickettsias, protozoa and some helminths. This 'persistence' has led to an extensive terminology such as "dormant," "latent," "carrier state" and the "masking and unmasking" of viruses.

Furthermore, there is the problem of microorganisms which are common in specific communities and are apparent in practically all individuals. These constitute the normal microbiota for those specific communities.

Thus it can be seen that the indigenous microbiota of each individual consists of the microorganisms derived during the evolutionary period (autochthonous microbiota), those microorganisms which are ubiquitous in the community (normal biota), and finally, the pathogens which have been accidentally induced but have the ability to 'persist'.

It must be emphasized that diseases are not symptoms or conditions nor are they instigators of symptoms or conditions, but are interactions between host and pathogen or between host and adverse environmental factors that result in such symptoms or abnormal conditions.

The oral cavity

The oral cavity presents an ideal environment for growth and maintenance of microorganisms. Basically it provides warmth, moisture and a continuous supply of nutrients from saliva, gingival (crevicular) fluid and from the passage of food. In addition, the oral cavity is able to exert control over the numbers of microorganisms present by limiting their rate of growth and providing means whereby excess microorganisms can be removed. Growth rates are limited, since the supply of nutrients is finite. In fact there is a shortage of nutrients most of the time, with occasional increases to excess. Saliva is the source of microbial nutrients in the oral cavity, but their concentrations tend to be low. The various microorganisms of the oral cavity give rise to complex patterns of food competition which limit nutrient availability. Growth rate is also reduced by the presence of antibacterial factors in the saliva, in addition to which, there is physical removal of microorganisms from the oral cavity by a combination of swallowing and the continuous exfoliation of epithelial squames from the various oral mucosal linings to which a number of organisms are attached. Thus factors involved in the promotion and limiting of microorganism growth in the oral cavity can be summarized as follows:

<table>
<thead>
<tr>
<th>Growth promotion</th>
<th>Growth limiting</th>
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<tbody>
<tr>
<td>Warmth</td>
<td>Salivary antibacterial factors</td>
</tr>
<tr>
<td>Moisture</td>
<td>Limited availability of nutrients</td>
</tr>
<tr>
<td>Nutrients (food, gingival fluid, saliva)</td>
<td>Epithelial cell exfoliation</td>
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<td></td>
<td>Saliva-swallowing</td>
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Origins of oral microbiota

The mammalian fetus in utero, and therefore the oral cavity, is normally free of microbiota unless there has been ingress via the maternal tissues. During birth, the fetus becomes exposed to the normal flora of the maternal genital tract, including organisms such as corynebacteria, lactobacilli, coliforms, micrococci,
facultatively anaerobic streptococci, anaerobic cocci, protozoa, yeasts and sometimes viruses. The oral cavity of the newborn child is considered to be sterile until at least the first breath is taken, followed by a rapid increase of detectable microorganisms within 8 h. The organisms appearing in the first few days of life to colonize the oral cavity are referred to as Pioneer Species. The earliest and most important of these is Streptococcus salivarius, which colonizes principally on the tongue and floats into saliva. Other species which can be identified include staphylococci; lactobacilli, streptococci, pneumococci, enterococci, coliforms, sarcinae, neisseriae, haemophilus and the yeast Candida albicans. Apart from Streptococcus salivarius, these do not manifest constantly or appear in high numbers.

Even though the oral cavity is exposed to a wide range of microorganisms in the early days of life, the oral habitat is selective, and not every microorganism which enters is able to establish an ecological niche for itself. Eventually, after a few weeks the composition of the oral flora settles down and becomes relatively constant. These organisms can now be regarded as an established Pioneer Community. By 3 months there is an identifiable resident microflora, but even at the end of a year, species of streptococci, veillonela, staphylococci and neisseriae are the only types found in all oral cavities; others such as Actinomyces, lactobacilli and fusobacteria are identified in half the oral cavities, while Bacteroides, corynebacteria, leptotrichiae and coliforms are even less in evidence.

The oral environment undergoes a major change at around 6 months due to eruption of the deciduous teeth. This provides opportunities for the establishment and growth of microorganisms adapted to living on hard enamel surfaces and at the dentogingival margin. Two species, Streptococcus sanguis and Streptococcus mutans, are specifically known to favor enamel surfaces. The increase in the number of species of microorganisms with the eruption of the deciduous and later the permanent teeth means that when eruption has been completed, the final microflora can be viewed as a Climax Community. Estimates of how many species may occur in the oral cavity have greatly increased over the past few years. It could be as many as 300 different species, and almost every year new additions are made to the list. The climax community—once established—may be maintained for many years with bacterial division and acquisition being continuously balanced by loss of bacteria (principally through swallowing).

With informed modern care, the longevity of the human dentition can now be extended over the complete life-span. If, however, teeth are lost through dental caries, periodontal disease or trauma, then the oral flora undergoes change and reverts to a type and composition reminiscent of that present before the emergence of the deciduous teeth. This new climax community based on the adult edentulous state has fewer species of microorganisms than when the oral cavity was dentate. The essential change is that those microorganisms originally associated with tooth surfaces and the dentogingival region no longer have a habitat. Further, it is interesting to note that the replacement of tooth loss by full dentures stimulates the re-emergence of some of the tooth surface-residing organisms such as Streptococcus mutans and Streptococcus sanguis together with lactobacilli and yeasts as part of the general flora.

Oral flora development

The oral cavity possesses several different habitats suitable for microbial growth such as the lips, palate, cheeks, tongue, gingivae, teeth and saliva. The exact microbial composition at each site depends on three important characteristics associated with oral microorganisms—"adhesion, growth and survival." Many microbes pass through the oral cavity, but only those that can lodge themselves or adhere and be retained have any opportunity of becoming established members of the oral flora. However, only those adhering microorganisms that can eventually grow, multiply and survive the body's attempts to dislodge them will maintain accepted status.

1. Adhesion of oral flora

Under normal masticatory actions and flow of saliva, most microorganisms will simply pass through the oral cavity together with those only partially attached to the various surfaces. The act of swallowing will
mean total removal. The flow of saliva in conjunction with mastication and swallowing means that shear forces will limit most populations to those habitats offering protection such as the gingival crevice, fissures and approximal areas between adjacent teeth. Only a few microorganisms have the ability to colonize exposed surfaces such as teeth, and these have evolved special mechanisms of adhesion such as the synthesis of insoluble extracellular polysaccharides from sucrose\cite{8-10}.

Adhesion of microorganisms to the oral tissues can be divided into three basic interactions, termed "cell-substratum adhesion," "homotypic cell-cell adhesion" and "heterotypic cell-cell adhesion." The first phase of adhesion covers the initial reaction between the microorganism and the substratum (hard tissue or large epithelial cells). This phase is also referred to as "deposition," since it involves the external surfaces of both the organism and the substrate, the deposition being influenced by the suspending medium. Deposition is influenced by the extracellular products (interactive polymers) formed by the organisms, those on substrates such as teeth and epithelium, and the influence and activity of saliva as a suspending fluid. Saliva is well known for its complex mixture of numerous polymers, although its composition varies in different mouths and with time.

Homotypic cell-cell adhesion is the linking of organisms of the same kind, i.e. the first organism interacts with the substratum (enamel or epithelium), and then the second organism attaches to the first. Heterotypic cell-cell adhesion is identical to homotypic adhesion except that the second organism or cell attachment is of a different type. Descriptions of adhesion activities in the oral cavity are based on the dental plaque with its complex initial form of organisms in saliva moving on to a pellicle-coated enamel surface\cite{11,12}.

**Adhesion Factors**

The exact mechanisms whereby different microorganisms attach themselves to each other and to hard and soft surfaces appear to be a complex problem involving a range of physicochemical positive and negative forces together with surface features including shape, chemical composition and charge, but with variations between microorganisms and their environment. Some microorganisms can achieve weak attachment but strengthen the link by means of polymer bridges, a layer of sticky external polysaccharide. Others exhibit numerous fine filaments which were known originally as "fimbriae" but now as "pili," which enable the organism to attach itself to similar or other species. A further group of cell surface molecules that specifically recognize receptors on tooth or epithelial surfaces, referred to as "adhesions," are demonstrated by lectins, which are carbohydrate-binding proteins that interact with carbohydrates in other organism.

Only some of the methods of attachment for some of the oral flora have been investigated, and these include the production of compatible receptor substances by the host and the organism\cite{15}, interactions between bacterial surface coatings of different species, the production of extracellular polymers by microorganisms and 'retention' rather than 'adhesion' by physical entrapment on various surfaces in the oral cavity.

**The role of host polymers**

Saliva acts as a suspending fluid through which adhesion of microorganisms occurs in the oral cavity. It is known to be composed of lipids, vitamins, ions, polysaccharides and immunoglobulins, together with a range of proteins, peptides and glycoproteins either dispersed or dissolved in water. Epithelial surfaces in general, but teeth in particular, form a very thin coating of proteinaceous material following cleansing. This film is formed from the saliva in a matter of seconds and builds up into a thin layer known as the "acquired pellicle." As far as can be ascertained, the pellicle material includes glycoproteins and antibodies. Many of the glycoproteins have aggregating factors so that the eventual adhesion of microorganisms involves interaction between the polymers on the surface of the organism and the polymers present in the acquired pellicle.

Since saliva contains aggregate-inducing substances and agglutinins, various organisms such as *Streptococcus sanguis*, *Streptococcus mutans*, *Streptococcus mitior* and species of *Actinomyces* have been
shown to become aggregated. The salivary substances responsible appear to be high-molecular-weight glyco-
coproteins. Although the formation of bacterial aggregates enables many of them to be swept away and
swallowed, giving these substances a protective role, it is known that aggregate-inducing substances do
adhere to hydroxyapatite. The existence of free dextran in saliva could be involved in cell-cell linkage.

The role of bacterial polymers

A number of oral microorganisms have the ability to produce extracellular polysaccharide from carbo-
hydrates, particularly from sucrose[5,13]. The production of these polysaccharides enables a number of oral
microorganisms including Streptococcus sanguis and Streptococcus mutans to adhere to hard surfaces such
as teeth.

Streptococcus mutans has been found to synthesize high-molecular-weight dextrans and other insoluble
glucans from sucrose. These polymers are involved in adhesion. Streptococcus mutans provides
enzymes which have been isolated and identified as "glucosyltransferase" and "fructosyltransferase." With
regard to sucrose, its basic chemical structure is formed from one D-glucose unit and one D-fructose unit.
Extracellular polymers are formed by the following reaction:

\[
sucrose \rightarrow glucan{(glucose)_{n+1}} + fructose.\]

The enzyme glucosyltransferase extends the glucan by adding an extra glucose molecule for each mole-
cule of sucrose cleared. The exact configuration of the glucose linkages affects both the solubility and
adhesive properties of the polysaccharide, although the nature of this relationship is not yet clear. Three
types of polymer from this reaction have been noted. First, a water-insoluble 'glucan' polymer which has
been termed "mutan," second, a water-soluble 'glucan' known as "dextran", and third, an unusual soluble
'fructan', which should not be confused with the fructan produced by other bacteria (Streptococcus salivar-
ius and Actinomyces viscosus).

Another extracellular polymer which can be derived from sucrose involves the fructose element,
which reacts with the fructosyltransferase enzyme produced by organisms such as S. salivarius. The for-
formation of fructan from sucrose occurs as follows:

\[
sucrose \rightarrow fructan{(fructose)_{n+1}} + glucose.\]

Fructosyltransferase elongates the fructan molecule by adding one fructose molecule for each molecule
of sucrose cleared. The most important bacterial fructans are 'levans'.

Lipoteicoic acid is another bacterial polymer, but sited in the wall of the organism. Many of the organ-
isms with this polymer are Gram-positive and are negatively charged due to the penetration of lipoteichoic
acid into their cell wall. These are then anionic polymers which consist of sugar phosphates which are usu-
ally glycerol and ribitol phosphate. Since the majority of surfaces in nature are negatively charged, it is pos-
tulated that microorganisms may be linked to their substrates by means of divalent cations. Applying this
principle to the oral cavity, it is presumed that the polymers are derived from saliva, and that by moving on
to the tooth surface they help to form the negatively-charged proteinaceous acquired pellicle, the final link-
age being achieved by calcium bridging involving positively charged calcium ions on the enamel surface.

Despite the large amount of work done in this field, it is still difficult to relate a particular bacterial
adhesive property to an individual polysaccharide. The potential importance of these polysaccharides in
adhesion is illustrated by the oral streptococci. Streptococcus mutans and Streptococcus sanguis, both of
which stick firmly to tooth surfaces, produce glucan polysaccharides such as 'mutan' and 'dextran'. On the
other hand, Streptococcus salivarius, which produces fructan (levan) as its main polysaccharide, is not
found on tooth surfaces but only on the tongue and in the saliva.
**Site - specific receptors (adhesions)**

Examination of *Streptococcus mitior* and *Streptococcus salivarius* by electron microscopy demonstrates that each has a characteristic fibrillar "fuzzy" surface coating referred to as the "fuzzy coat" or glycocalyx. This coat has been found to be trypsin-sensitive: when the enzyme is applied to organisms, much of the coat is lost, with impairment of bacterial adhesion to oral surfaces. The composition of this coat is unknown, except that it contains polysaccharides and lipoteichoic acid. There are molecules present on the bacterial surface that specifically recognize receptors on the tooth surface. One example are the lectins, which are protein adhesions specifically recognizing carbohydrate groups possibly present in the acquired pellicle. Lectins appear to be part of the glycocalyx.

**Fimbriae (pili)**

A number of microorganisms display hundreds of fimbriae or pili, which can be seen as very fine filamentous appendages. They stem from the cytoplasmic membrane and consist of the protein 'pilin'. Two types exist, the sex pilus which is considered to be a recognition device for mating between cell types, and the somatic pilus which plays a role in adhesion, since the localization of specific bacteria depends on these fibrillar coatings. Pili are a common feature of dental plaque bacteria and may have an important role in the differential adhesion of various species to the tooth surface.

**Physical retention**

Observations have revealed that a number of microorganisms are unable to colonize various oral surfaces and have poor adhesive properties. *Veillonella* is known to have limited adhesion properties, and yet is present in high numbers in the oral cavity. The only explanation is that this organism is retained and protected in some manner. Organisms such as lactobacilli are found in open carious lesions and around orthodontic bands. Others such as *Bacteroides* and spirochaetes are located in the gingival crevice. Obviously there is a whole range of "protected" areas or niches in the form of pits, fissures, carious lesions, approximal areas and gingival crevices. Populations of oral flora occupying such areas will only return low isolation numbers from more exposed surfaces such as teeth and the mucosa of the cheek and palate. If bacteria with poor adhesive properties are located in more exposed regions, they will be supported by factors such as extracellular polysaccharides and other means. A number of such organisms may well take refuge in dental plaque.

2. **Growth of oral flora**

(a) **Temperature**

Although most bacteria will grow within a fairly wide range of temperature, there is an optimum temperature with a narrow range at which the best growth is achieved. Because of the stability of this optimum range, bacteria can be separated into three basic divisions. First there are the psychrophils with a general range of 0-30°C and an optimum of around 29°C. This group therefore constitutes a problem with regard to the refrigeration of foodstuffs. Second, there are the mesophils, including many pathogens, which grow and multiply between 10 and 45°C. Pathogens in mammals are known to grow best close to 37°C. Third, there are the thermophils which operate successfully within the 25 to 75°C band with an optimum of 50 to 55°C.

The temperature in the oral cavity is known to remain constant, and in fact is only slightly lower than normal body temperature. Thus, mouth temperature is considered to lie within the range 35-36°C and is probably slightly lower at the front of the mouth than at the back. The level of temperature is important because of its effect on bacterial metabolism and enzymatic action, as well as on the habitat as a whole. Thus factors which may be influenced by temperature can include pH, ion activity, solubility of gases and the aggregation of macromolecules. The oral cavity appears to be a stable haven in the temperature sense, and so provides ideal conditions for the growth and multiplication of many microorganisms.
(b) **Acidity (pH)**

Most microorganisms in the oral cavity require a level of acidity or pH close to neutrality if growth is to be maintained\(^5\). The oral pH appears to be regulated mainly by the saliva, and the normal salivary pH lies between 6.5 and 7.0, thus meeting the optimum acidity needs for microbial growth as long as the environment is moistened by saliva. Acidity levels within dental plaque on tooth surfaces may differ considerably from the main mouth areas, and depend on the amount of acid produced by the plaque bacteria. Acid is an extracellular product of dietary sugar metabolism, and following the consumption of sugar, the pH of dental plaque falls rapidly to values as low as 5 or 4 due to the production of lactic acid. As expected, this pH value rises between intakes of sugar food. pH values are lower inside carious lesions of the teeth and in periodontal pockets. Under such conditions, bacteria can survive and grow, and these are referred to as aciduric (acid-tolerating). A number of oral streptococci and lactobacilli have an aciduric preference, and therefore seem to grow and multiply in areas such as carious lesions in dentine. Bacteria which produce large amounts of acid are known as acidogenic. Finally, saliva is thought to have a buffering or neutralizing effect on acid production in dental plaque. However, there is doubt whether saliva can penetrate into the deeper levels of plaque, which is a complex dense mass full of activity. The possibility remains that the upper surfaces of dental plaque may show various levels of acidity, i.e. the result of partial neutralization.

(c) **Oxidation-reduction potential (E\(_h\))**

Variations in the need for oxygen effectively separate bacterial organisms into a number of groups\(^5\). Those which are obligate anaerobes can grow only in the absence of oxygen or in a highly reducing medium. Facultative bacteria are versatile, since they can grow under conditions where oxygen is either absent (reduced) or present (oxidized). Obligate aerobes usually need oxygen, but some can survive with very small amounts. Microaerophilic bacteria must have low oxygen levels because their growth becomes suppressed if oxygen levels are high. Thus the oxidation-reduction potential (E\(_h\)) in a given area is critical for the growth of resident bacteria. The symbol E\(_h\) for oxidation-reduction potential is expressed in volts or millivolts, and therefore can be termed positive (+ volts), showing an oxidized state, or negative (- volts), showing a reduced form. It must be emphasized that although microorganisms are separable into various groups depending on their oxygen requirements, there is a wide spectrum of oxygen tolerance which makes the divisions somewhat arbitrary.

Although the oral cavity is rich in oxygen and supports a number of aerobic and facultatively anaerobic microorganisms, obligately anaerobic (oxygen-intolerant) bacteria can live in the oral cavity, since some have been isolated. Anaerobes need reducing conditions in which to achieve their normal metabolism, and so the level or degree of oxidation-reduction in a particular area will determine their survival. In the mouth, oxygen is readily available, as well as in the saliva and the more superficial layers of dental plaque. These conditions favor the growth and multiplication of aerobic species of bacteria and encourage the growth of facultative anaerobes. In specific parts of the mouth such as the gingival crevice (healthy) and periodontal pockets (infected), the environment becomes highly reducing and anaerobic. Estimates of E\(_h\) show that the typical healthy gingival crevice has a mean value of +73 mV whereas infected periodontal pockets show considerable reduction with a value of -48 mV. Many differences in E\(_h\) have been noted, but the range for periodontal pockets can be +12 mV to -57 mV, and that for the gingival crevice +10 mV to +113 mV; however in the gingival crevice values as extreme as -300 mV have been recorded.

In saliva, E\(_h\) varies but is always high, although the presence of spirochaetes, which require an E\(_h\) of -185 mV, indicates that extreme reducing areas exist. In the oral cavity and especially in dental plaque, there are gradients of oxygen concentration and E\(_h\), and in a complex deposit like dental plaque, bacteria can survive and exhibit a range of oxygen tolerance.
Oxidation-reduction potentials in the oral cavity
(redox potential, $E_h$)

<table>
<thead>
<tr>
<th>Saliva</th>
<th>Healthy gingival crevices</th>
<th>Deep periodontal pockets</th>
</tr>
</thead>
<tbody>
<tr>
<td>up to +550 mV (highly oxidizing)</td>
<td>+73 mV</td>
<td>-48 mV (highly reducing)</td>
</tr>
</tbody>
</table>

\(d\) Carbon dioxide

Growth initiation and continued growth are dependent on the presence of carbon dioxide, and a number of bacteria have been found to require a level of carbon dioxide greater than that in the atmosphere or that in culture medium simply for growth initiation. The concentration of atmospheric carbon dioxide is about 0.03%, and some microorganisms find this sufficient. If initial growth has commenced, bacterial cultures often provide additional carbon dioxide to maintain most of their growth needs. Many oral bacteria have been found to have an absolute requirement for increased levels of carbon dioxide to facilitate growth and metabolism, for example *Actinobacillus* and *Capnocytophaga*, both of which are linked to periodontal disease.

\(e\) Nutrients

In order for microorganisms to initiate growth and eventually cover maintenance, all nutrients must be available from the habitat occupied. The fact that some organisms are linked to a specific habitat can be construed as proof that the organisms have all their nutrient requirements. In the oral cavity the nutrients can be obtained from saliva and gingival (crevicular) fluid. Saliva provides many organic substances including amino acids, proteins, sugars, and glycoproteins, all essential for growth, and these can be broken down and metabolized by the bacteria. Furthermore, saliva contains dissolved carbon dioxide and vitamins, which are stimulatory for growth in many streptococci.

Conversely, a number of saliva components may inhibit or regulate bacterial growth in the oral cavity. Examples are the degradative enzyme lysozyme, which attacks the cell walls of Gram-positive bacteria, peroxidases, and salivary antibodies such as secretory IgA. The gingival (crevicular) fluid contains a number of important growth factors. It has a range of proteins such as the immunoglobulins IgG, IgA, IgM, and albumin. Of specific note are two important growth factors, haemin and $\alpha$ -2 globulin, which are of paramount importance for *Bacteroides* species and *Treponema denticola*, respectively. In addition, the gingival crevice harbors a large number of enzymes probably derived from both the host and the microorganisms which may play a role in their metabolism. Within dental plaque a number of microorganisms synthesize both intracellular and extracellular polysaccharides from sucrose. From the normal diet, an array of foodstuffs pass through the oral cavity periodically, and the fermentable carbohydrates, for example, sucrose, are used by microorganisms to produce acid and synthesize polymers. The intake of food is not regarded as an essential source unlike saliva and gingival crevice fluid. However, it is thought that certain microorganisms showing slow growth activity may show a switch to more rapid activity following the intake of dietary carbohydrate. There is a further interesting phenomenon arising from the fact that the majority of bacteria can be termed "primary feeders" because they only use the original sources available. These primary feeders have a number of end-products, and some bacteria utilize these, thus becoming "secondary feeders." Within the genus *Veillonella*, some members can use lactic acid produced through the metabolism of other bacterial species.

3. Survival of oral flora

Survival of microorganisms depends on their ability to withstand the defence systems of the body. Defences include neutrophils (polymorphonuclear leukocytes), complement and immunoglobulin antibodies\(^5\).
In the early phase of infection, polymorphonuclear leukocytes (PMNs) migrate towards the site of microbial invasion, stimulated by chemoattractants. On arrival, the PMNs attempt to destroy the microorganisms, and this action constitutes the first line of defence at a typical site such as the gingival margin. Various microorganisms respond in different ways, some inhibiting phagocyte "engulfment" while others prevent phagocyte "digestion." Resistance to phagocytosis by microorganisms can be related to the bacterial capsule, many of which are made up of polysaccharides in the form of a hydrophilic gel, the composition differing for each type of organism. Other types of capsule include peptide and glycoprotein. Another means of resisting phagocytosis which does not require a capsule depends on other surface components such as hyaluronic acid.

Microorganisms can be divided into two main groups based on their reaction to "digestion" after they have been engulfed by the phagocyte. Those which offer little or no resistance to phagocytosis but on ingestion establish a complex relationship where both the organism and phagocyte exist in balance are known as "intracellular pathogens." The other group are termed "extracellular pathogens" because as soon as they have been engulfed, they are destroyed. In this last group is Streptococcus pyogenes, which must either escape or resist phagocytosis, and in order to resist, it has evolved an antiphagocytic capsule or certain types of cell wall.

A number of oral bacteria such as Bacteroides produce factors that inhibit the migration and phagocytosis of PMNs. Phagocytosis is dependent on oxygen, and a number of Bacteroides are capable of counteracting this by producing enzymes such as catalase and superoxide dismutase which break down intracellular peroxide. Other effects on PMNs are known, and species of Streptococcus and Actinobacillus produce a leukocidin which specifically kills defence PMNs. The leukocidin produced by Staphylococcus is known to be an immunogenic and apparently disrupts the permeability of PMNs by altering the potassium pump. A number of Bacteroides resist complement-induced bacterial lysis by degrading the C-3 component of complement. The existence of immunoglobulin proteases is known and it is thought that Capnocytophaga, which is implicated in periodontal disease, has such proteases, although not necessarily specific, that can break down the IgA and IgG antibodies. Thus this particular bacterium produces changes in PMN morphology as well as impairing complement-induced PMN chemotaxis.

The reaction of tissues to the spread of bacteria in gingival regions is often manifested by a fibrin around the focus of infection. A number of oral Bacteroides are known to produce a non-specific fibrinolysin enzyme which breaks down the fibrin barrier in order to permit the survival of these organisms in the gingivae.

While these survival factors are important to all oral bacteria, they are especially significant for bacteria whose habitats are in the closest proximity to the soft tissues of the mouth. It is noteworthy that the organisms in which these factors seem most highly developed (Bacteroides, Capnocytophaga, and Actinobacillus) are those associated with the gingival crevice and periodontal pockets. They are not organisms normally associated with the tooth surface.

**Oral flora distribution**

The oral flora is composed of microorganisms that have been acquired, grown and have survived in the oral cavity. Conditions within the oral cavity are not uniform and there are various distinct habitats for characteristic communities of organisms. Each site or habitat is influenced by different acquisition, growth and survival factors, which results in different and characteristic floras at each site[5].

Regulation of the oral flora is a complex matter ranging from initial establishment of organisms on a usable substrate, i.e. an accessible carbohydrate, the intervention of diseases which may stimulate the pathogenic potential of a previously harmless organism, the level of oxygen available, alterations in saliva concentration and flow, oral hygiene, nutrition, tooth loss, use of antibiotics and physiological changes. These and many other factors all contribute to the state of the oral cavity relative to floral numbers, types and distribution[5,14]. These factors, although incomplete, can be summarized as follows:
Oral Flora Environmental Factors

Basically the oral cavity provides two specific types of tissue surface on which microorganisms can form colonies—hard, such as teeth, and soft epithelial surfaces. However, soft epithelial surfaces have inherent differences of their own, for example, the cheek has unkeratinized cells; and the palate has keratinized cells. The surface of the tongue with its papillae is a more specialized surface. Within all the various habitats and the range of possible environmental influences, it is not surprising that the floral communities present exhibit variation. The main features of floral distribution in the oral cavity are summarized as follows:

1) Lips

This area includes the transitional zone between the skin flora and the oral flora. The characteristic skin flora normally consists of staphylococci, micrococci and Gram-positive rods, and probably the most common species in the lip area is Staphylococcus aureus. The microorganisms of the oral cavity are mostly Gram-negative species. A number of streptococci, obviously members of the oral community have been isolated from the lips together with low numbers of Neisseria and Veillonella. In addition, there is a condition known as angular cheilitis (perleche) where there is damage to the lip mucosal surfaces at the corners of the mouth. Invariably the fungus Candida albicans colonizes the folds of the skin, but it is supported by other microorganisms such as Staphylococcus aureus, anaerobic cocci and Streptococcus pyogenes from the skin communities. This particular condition is an example of an opportunistic infection.

2) Cheeks

Microorganisms forming the cheek population have three predominant species represented by Streptococcus mitior (60% of all bacteria present), Streptococcus sanguis and Streptococcus salivaricus (both around 11%). The majority of other species are present in very low numbers, including Streptococcus mutans, Lactobacillus, Veillonella, Bacteroides, Streptococcus milleri, various enterococci and spirochaetes.

3) Palate

The palatal flora is not particularly well known, but is thought to be similar to that of the cheeks. A number of streptococci have been isolated, but the presence of other genera such as Lactobacillus and Hemophilus has been confirmed. From the data available, it appears that very few microorganisms colonize the palate under normal conditions. The use of dentures together with illness or defective oral hygiene can lead to an influx of Candida albicans, and a state of chronic atrophic candidiasis (denture stomatitis). Other species possibly involved include Candida tropicalis, Candida parapsilosis, Candida stellatoidea and Candida guilliermondii, together with species of Rhodotorula and Torulopsis. Recently, species of Klebsiella have been isolated from this type of lesion.

4) Tongue

The fact that, anatomically, the tongue dorsal surface consists of crypts and papillae means that it has not only a greater area for colonization in the oral cavity, but more a suitable surface. These points are supported by the finding that the tongue bears a greater bacterial density than elsewhere. The most outstanding microorganism on the tongue is probably Streptococcus salivarius, which can account for up to 50% of the bacteria present. This is followed by Streptococcus mitior and lesser numbers of Streptococcus milleri and
Streptococcus sanguis. Haemophili have been isolated from the tongue surface in addition to Lactobacillus, Veillonella, Neisseria, Bacteroides, Fusobacterium and spirochaetes. There remains one peculiar microorganism, originally known as Micrococcus mucilaginosus, and now reclassified into a new genus, Stomatococcus, species S. mucilaginosus. This has been isolated from the tongue, throat and nasopharynx and produces large amounts of polysaccharide “slime.” The pathogenic potential of this species is unproven as also is the role of the extracellular slime.

5) Saliva

Saliva in the oral cavity is a mixture of secretions of the major glands such as the parotid, submandibular and sublingual groups, to which can be added a number of smaller glands in the mucous membranes lining the mouth. The main organic constituents of saliva are proteins, especially mucin. Mucin is associated with the formation of glycoproteins which have a major role in the aggregation and adhesion of bacteria to various surfaces, particularly species such as Streptococcus sanguis and Streptococcus mitis. Although carbohydrates in the form of various sugars are present in the salivary secretions, they are not thought to have sufficient free forms to influence bacterial growth. As a result, the saliva tends to favor those bacteria which can use nitrogenous compounds. Urea appears in saliva, and nearly 20 amino acids have been detected, probably arising from the enzymatic breakdown of salivary proteins and polypeptides. In relation to this, urea emerges through the saliva following filtration from blood, although ammonia is hardly present. Hence ammonia may be derived from the urea, and provide a source of simple nitrogen suitable for microorganisms or it can be produced by deamination of the amino acids by bacterial enzymes. It is interesting to note that staphylococci produce urease and that many of the Gram-negative bacteria produce deaminases, but these enzymes are not present in glandular secretions.

In general, the saliva is not considered to have its own flora for a number of reasons including steady removal by swallowing. The organisms in saliva come from the various oral cavity surfaces as a result of the flow of saliva and crevicular fluid, mastication, and oral hygiene practices. Investigations have shown that the microorganism population in saliva is very similar to that of the tongue, i.e. all the species can be isolated in saliva, which leads to the current postulate that the salivary population is actually derived from the tongue bacteria.

6) Gingival crevice

The gingival crevice provides a fluid—the crevicular fluid—which differs considerably from saliva and harbors a community of microorganisms. The gingival crevice and its fluid support a flora which is both diverse and complex. The fluid is thought to exert a protective action by means of its "flushing," and this is perhaps more clearly demonstrated under conditions of periodontal disease whereby the flow is substantially increased. In constitution, the crevicular fluid is quite different from saliva and in many aspects is closely similar to serum. Apart from colonizing microorganisms, the fluid has a number of proteins such as albumin, immunoglobulins IgG, IgA and IgM, as well as complement. To this can be added normal tissue cells such as neutrophils, monocytes, lymphocytes of the T and B type and blast cells. The crevice and its fluid provide the essential growth factors required by colonizing bacteria, in addition to which are changes related to the depth of the crevice. As the depth increases there is an alteration of both $E_h$ and the mixture of organisms that can grow. Microorganisms are arbitrarily defined as aerobes and anaerobes depending on their ability to grow in the presence or absence of oxygen. Oxygen is the major factor curtailing the growth of anaerobes, and they require reducing conditions for their metabolism.

Microorganisms are not easily dislodged from the gingival crevice, whereas the gingival (crevicular) fluid provides an excellent nutrient growth medium. Some of the bacteria have specific nutritional needs, various Bacteroides species obtaining haemin from the fluid, while Treponema denticola obtains α-2-globulin. It is estimated that $10^8$ to $10^9$ bacteria may be present in each gingival crevice. The crevicular microorganisms include Streptococcus sanguis, Streptococcus mitior, Streptococcus salivarius, enterococci, a number of Gram-positive filaments, Veillonella and Neisseria species, Bacteroides, Fusobacterium and spirochaetes.
7) Teeth

The oral flora associated with teeth forms part of what is known as dental plaque. This plaque is a tenacious deposit on the tooth surface which consists of bacteria, their extracellular products and polymers of salivary origin\textsuperscript{16-19}. Dental plaque is usually described in terms of tooth morphology and sampling site. For example, there are smooth-surface, approximal, fissural and gingival plaques. In addition, samples taken from above the gingival margin are referred to as "supra-gingival plaque" or those beneath the gingival margin as "sub-gingival plaque."

In general, on tooth surfaces, the characteristic organisms are species of \textit{Actinomyces} together with a number of streptococci such as \textit{S. sanguis}, \textit{S. mitior}, \textit{S. mutans} and \textit{S. milleri}. There are a number of different environments or types of plaque from various tooth aspects, and probably the most important factor affecting the composition of these plaques in addition to their sites is diet and length of time since the surfaces were cleaned.

However, time can also mean the early establishment of plaque on a tooth surface and its later mature form. In "early" smooth surface occupation, \textit{Streptococcus sanguis} and \textit{Streptococcus mitior} dominate the flora along with \textit{Actinomyces naeslundii}. Members of the genera \textit{Arachnia}, \textit{Propionibacterium} and \textit{Rothia} are also present. After a time there is a phenomenon known as microbial succession, whereby \textit{Actinomyces} takes over the dominant role with the addition of another species, \textit{Actinomyces viscosus}. Examination of a "mature" smooth surface shows that the microorganisms within the plaque often include 3 species of \textit{Actinomyces}—\textit{A. israelii}, \textit{A. viscosus} and \textit{A. naeslundii}. Streptococci are also present: \textit{S. mutans}, \textit{S. sanguis}, \textit{S. salivarius} and \textit{S. milleri}. Both the \textit{Actinomyces} and \textit{Streptococcus} groups predominate the population, supported by representatives of other genera such as \textit{Neisseria}, \textit{Rothia}, \textit{Fusobacterium} and \textit{lactobacilli}. As already referred to under "Gingival crevice," a very different population occurs including anaerobes and spirochaetes, and so with these more obvious examples it can be seen that dental plaque has both qualitative and quantitative differences in its microbial colonies relative to tooth surfaces and to other communities elsewhere in the oral cavity.

\textit{Important members of the oral flora}

The number and variety of microorganisms which can appear in the oral cavity range from two to three hundred and include both indigenous and exogenous populations. With regard to regular oral inhabitants, at least 30 bacterial genera have been identified. Identification of each specific species in the mouth is not yet possible because of both the numbers and variety present, and also because some have exacting cultur-

<table>
<thead>
<tr>
<th>Table 1 Major microorganisms of the oral cavity</th>
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<tbody>
<tr>
<td><strong>1 GRAM-POSITIVE BACTERIA</strong></td>
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<tr>
<td>Facultatively Anaerobic Cocci</td>
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<tr>
<td>Genus Enterococcus</td>
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<td>Genus Stomatococcus</td>
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<td>Genus Streptococcus</td>
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<td>Obligately Anaerobic Cocci</td>
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<td>Genus Peptostreptococcus</td>
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<td>Regular and Nonsporulating Rods</td>
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<td>Genus Lactobacillus</td>
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<td>Irregular, Nonsporulating and</td>
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<tr>
<td>Facultatively Anaerobic Rods</td>
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<td>Genus Actinomyces</td>
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<td>Genus Arachnia</td>
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<td>Genus Bacterionema</td>
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<td>Genus Rothia</td>
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<td>Irregular, Nonsporulating and</td>
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<tr>
<td>Obligately Anaerobic Rods</td>
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<td>Genus Bifidobacterium</td>
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<td>Genus Eubacterium</td>
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<td>Genus Propionibacterium</td>
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<td><strong>2 GRAM-NEGATIVE BACTERIA</strong></td>
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<td>Facultatively Anaerobic Cocci</td>
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<td>Genus Neisseria</td>
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<td>Obligately Anaerobic Cocci</td>
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<td>Genus Veillonella</td>
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<td>Facultatively Anaerobic Rods</td>
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<td>Genus Actinobacillus</td>
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<td>Genus Eikenella</td>
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<td>Genus Haemophilus</td>
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<tr>
<td>Microaerophilic, Motile and</td>
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<tr>
<td>Helical/Vibrioid Rods</td>
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<tr>
<td>Genus Campylobacter</td>
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<tr>
<td>Obligately Anaerobic, straight,</td>
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<tr>
<td>Curved and Helical Rods</td>
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<tr>
<td>Genus Bacteroides</td>
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<td>Genus Fusobacterium</td>
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<td>Genus Leptotrichia</td>
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<td>Genus Wolinella</td>
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<td>Spirochaetes</td>
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<td>Genus Treponema</td>
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<td><strong>3 FUNGI</strong></td>
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<td>Genus Candida</td>
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<tr>
<td><strong>4 VIRUSES</strong></td>
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<tr>
<td>Herpesvirus group</td>
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al requirements which prohibit cultivation on artificial media.

The following account describes a number of the more important members of the oral flora placing emphasis on the indigenous population. For the sake of simplicity, these organisms will be described under the more general headings of Gram-positive bacteria, Gram-negative bacteria, fungi and viruses. A full list of the genera involved is included in Table 1.

The composition of the oral microflora depends directly on the environment, since the microorganisms have a number of nutritional and physiochemical requirements in addition to their ability to resist various physiological, mechanical and defence activities of the host. Since the mouth contains a number of potentially pathogenic organisms, shifts in the environmental balance may stimulate them to increase in number and inflict damage on the surrounding tissues. Microorganisms of exogenous populations may become opportunistic if the environmental conditions of the mouth alter sufficiently because of either local or systemic influences.

4. Gram-Positive Bacteria

Streptococci

These are characterized as Gram-positive cocci in the form of pairs or chains. In most instances they are nonsporulating and nonmotile. They exhibit a fermentative metabolism and are facultatively anaerobic with complex nutritional requirements. They are regarded as the most numerous single group in the oral cavity, and on cultivation comprise 25-50% of all bacteria\(^{20-23}\). In the widest sense, "oral streptococci" occur in the oral cavity and the upper respiratory tract of both man and animals, but a number of species have been located from other sites and from a number of clinical infections\(^{24-27}\).

Classically, streptococci have been separated into three major subgroups on the basis of their growth on blood agar:

- **Alpha (\(\alpha\))-hemolytic**: Producers of partial hemolysis of blood cells to give a green tinge to blood-containing media (giving rise to the "viridans" group of streptococci, after the Latin, *viridis*, for green).
- **Beta (\(\beta\))-hemolytic**: Producers of complete hemolysis of blood cells in media leaving a clear area around the bacterial colony.
- **Gamma (\(\gamma\))-hemolytic**: No hemolysis.

Classification problems have arisen in recent years, and although many of the oral streptococcal species fall within the \(\alpha\)-hemolytic division, many species do not show a constant or reliable behavior when cultured. It is true to say that oral streptococci are either \(\alpha\)-hemolytic, such as *Streptococcus salivarius*, or non-hemolytic, such as *Streptococcus (Enterococcus) faecalis*. The most common \(\beta\)-hemolytic streptococcus is *Streptococcus pyogenes*, which is known to cause tonsillitis and can be isolated from the throat during infection and from carriers, but is not usually considered a member of the normal flora. Recent studies have identified some well defined species, but there are many unresolved taxonomic and nomenclatural problems in spite of biochemical tests, cell-wall analysis and serological methods. Those species that have been characterized by testing have, in some instances, been further subdivided into serotypes, biotypes or bacteriocin types.

*Streptococcus mutans*

This species was first described by Clarke in 1924\(^{28}\) following its isolation from carious dentine. Its

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* Streptococcus faecalis has recently been reclassified and placed in the genus Enterococcus, *species* faecalis\(^{21}\). 

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primary habitat is the tooth surface of man, but it can be isolated from feces. The presence of colonies on the teeth is favored by high levels of dietary sucrose. Gram-positive cocci which occur in pairs, or as short or medium-length chains have been designated as "mutans" which refers to its ability to form very long chains when grown in a sucrose medium. Much has been written about Streptococcus mutans as a cause of dental caries, and it undoubtedly has this ability, as demonstrated by the production of carious lesions when it is introduced into the mouths of germ-free animals. The fact that laboratory monkeys specifically immunized with fractions isolated from Streptococcus mutans have less caries than non-immunized monkeys, is also evidence that the species has a role in the etiology of dental caries[30].

However, it has been extraordinarily difficult to demonstrate conclusively that Streptococcus mutans (or any other organism !) is responsible for dental caries in humans. Epidemiological studies have shown that high numbers of Streptococcus mutans are associated with increased dental caries, and this relationship is now used to predict dental caries from counts of Streptococcus mutans found in saliva. High bacterial counts indicate that caries is likely, and that sugar consumption needs to be reduced. This method has found considerable usage, and in Sweden, for example, over 30,000 counts are undertaken each year in the management of dental caries.

One complication is that the streptococci forming the Streptococcus mutans group are genetically heterogeneous and can be subdivided into several types. This has been made possible by studies of antigenic structures in the search for ways of immunizing humans against dental caries, and the employment of serological methods for rapid identification of fresh isolates. Basically, Streptococcus mutans can be separated into 8 serotypes designated by the letters a to h[5, 20, 30-35]. These divisions (Table 2) are supported by further investigations such as cell wall analysis, polyacrylamide gel electrophoresis of the proteins from whole cells, examination of the DNA base content (% G + C, the percentage of guanine & cytosine) and DNA hybridization studies. However, there are a number of physiological differences which occur in some of these serotypes involving, for example, fermentation and hydrolyzation, and this has led to speculation that some of the serotypes should be considered as either sub-species or species. Of the 8 serovars (a to h) S. mutans can be identified with c, e and f, while the remainder have been promoted to full species status: Streptococcus cricetus (a serovar), Streptococcus rattus (b serovar), Streptococcus sobrinus (d, g serovars), Streptococcus ferus (c serovar), Streptococcus macacae (h serovar), and Streptococcus downei (h serovar).

It is known that these different species or serovars (serotypes) vary in frequency in different parts of the world—the c serovar is common in Europe and North America, while the b serovar is common in North Africa, but the reasons for these variations are unknown. In addition, not all serovars are equally effective in producing dental caries in laboratory animals. The ability of Streptococcus mutans to cause dental caries when introduced into the mouths of germ-free (gnotobiotic) animals is described as "cariogenic." Streptococcus mutans is cariogenic because it is able to produce large amounts of acid (acidiogenic) by metabolizing sugars. S. mutans can synthesize soluble and insoluble extracellular polymers (soluble glucan, mutan, fructan) from sucrose, and it is these insoluble polymers which play an important role in enabling the bacterium to stick to tooth surfaces. Because of this, Streptococcus mutans is not isolated in the oral cavity before the emergence of the deciduous teeth.

Streptococcus sanguis
The primary habitat of this species appears to be dental plaque, where it may account for nearly half the total number of facultative streptococci, but is found in lower numbers elsewhere in the mouth. Colonization is only established in the mouths of infants after the emergence of the deciduous teeth, and cultural studies have shown that it is one of the first microorganisms to appear on clean tooth surfaces. Streptococcus sanguis can be isolated from blood and heart valves in cases of bacteremia and infective endocarditis, which at one time was known as Streptococcus s.b.e. (subacute bacterial endocarditis). Formed of spherical or oval cells, it occurs in medium or long chains, and most strains are alpha (α)-hemolytic but both beta (β)-hemolytic and gamma (γ)-hemolytic (nonhemolytic) forms are also found. This microorganism produces extracellular insoluble and soluble glucans from sucrose, which enable it to
stick to tooth surfaces. In addition it produces acids from sugars. Hence it can be seen that Streptococcus sanguis has properties in common with Streptococcus mutans, with which it shares tooth surfaces. Although S. sanguis is more commonly found on tooth surfaces than S. mutans, it is not generally believed to be as important an instigator of dental caries. However, experiments with Streptococcus sanguis have shown that it is able to cause dental caries when introduced into mouths of germ-free (gnotobiotic) animals.

Elsewhere S. sanguis may be detected in human feces and specific L-forms of the organism have some association with recurrent aphthous stomatitis.

"Streptococcus mitior"

At the present time there is still confusion regarding the correct nomenclature of streptococci referred to in the past as "S. mitior," "S. mitis," "S. viridans" and "S. sanguis type 2." Both the organisms of S. mitior and the originally termed S. mitis are very similar. In recent years S. mitis has been regarded by some as a rather ill-defined and heterogeneous group. Current thought tends to approve the retention of the name S. mitior for a group of α-hemolytic streptococci which can be defined with reasonable precision. Streptococcus mitis certainly differs from "S. mitior" but requires considerable clarification. "Streptococcus mitior" has been recognized by numerical taxonomic analysis of conventional biochemical and physiologic test data and by polyacrylamide gel electrophoresis for protein patterns of whole cells. Thus the clusters of strains corresponding to these tests have been accepted at present as "Streptococcus mitior," while "Streptococcus mitis" is simply retained for further investigation.

"Streptococcus mitior" consists of spherical or oval cocci which can be found singly, in pairs or in chains, although very long chains may occur in certain cultures. Most strains are α-hemolytic on blood agar medium, but some can produce clear zones of β-hemolysis. Very little, if any, extracellular polysaccharide is produced, which probably accounts for it being an organism that adheres to non-keratinized cells in the mouth (tongue, cheek and lips) and not to tooth surfaces. Generally "S. mitior" has been isolated from human saliva, dental plaque, sputum, feces and clinical infections, especially bacterial endocarditis.
Because of differences in the strains, the question of whether they induce dental caries remains unanswered. However, all strains are thought to produce acid from glucose, sucrose and maltose. In relation to infective endocarditis, it is interesting to note that "S. mitior" was isolated most frequently in recent studies, followed by S. sanguis and S. mutans.

"Streptococcus milleri"
This species has been promoted only relatively recently as a member of the oral flora, and based on physiological tests it appears to be fairly homogeneous but serologically heterogeneous. "Streptococcus milleri" is often included in a group of streptococci referred to variously as Streptococcus anginosus, Streptococcus MG, Streptococcus constellatus and Streptococcus intermedius. The group, as a whole, appears to share a number of similarities in biochemical properties. Separation is possible, for example, between S. constellatus and S. anginosus, which can be delineated from S. intermedius on the basis of lactose fermentation. Further analysis of the group members suggests that these various strains, currently known by several different names, have a great deal of similarity, although full genetic information is not available. It is postulated at present that most, if not all, of these streptococci bear a close enough resemblance to each other to enable them to be regarded as members of the same species. Probably the best defined species in the group is S. milleri, and a number of authorities are recommending that all these streptococci should be included under the species "Streptococcus milleri".

"Streptococcus salivarius"
This species appears as spherical or ovoid cells arranged in pairs or chains which may be either long or short. Hemolysis on blood agar is variable throughout the range of α, β, and γ. Acid is produced from glucose, sucrose and maltose among others, but it does not normally produce polymers from glucose. "Streptococcus milleri" appears to be a normal inhabitant of the mouth, and can be isolated from dental plaque, the gingival crevice and the throat. The species has been found to be cariogenic when introduced to gnotobiotic animals. In addition, "S. milleri" has been isolated from a number of infections in man, such as abscesses in various parts of the body including the mouth, brain, liver, appendix and bloodstream.

"Streptococcus rattus"
The species was originally described as a distinct serotype of Streptococcus mutans and designated as type b before being awarded species status. Although first discovered in the laboratory rat, it has subsequently been isolated from the human mouth. Streptococcus rattus is a Gram-positive coccus which may form pairs or chains. Fermentation occurs with mannitol, sorbitol, raffinose, sucrose, lactose, maltose and inulin, but not with glycerol or xylose. It produces an adhesive extracellular glucan from sucrose. The species is less commonly isolated from humans than the other Streptococcus mutans serovars (Table 2). Its biovar status allowing it to be separated from S. mutans has been supported by its ability to produce ammonia from arginine and its protein patterns evident on polyacrylamide gel electrophoresis.
Streptococcus cricetus

Named after the hamster, from which the original isolate was recovered, the cocci are Gram-positive and appear in pairs or chains. On blood agar some strains produce a zone of α-hemolysis, but the majority are nonhemolytic. The species grows best in an atmosphere of reduced oxygen and added carbon dioxide, and it has the property of fermenting mannitol sorbitol, raffinose, mannose, inulin, sucrose and lactose. Fermentation does not occur in the presence of arabinose, glycerol or xylose among others. *Streptococcus cricetus* is a newly promoted species originating from a *Streptococcus mutans* serovar type a. While it has distinct genetic differences from the other "serovars" it is difficult to separate from the "mutans" group by means of biovar (biotype). Unlike *Streptococcus rattus*, it cannot produce ammonia from arginine. *Streptococcus cricetus* has been isolated from the oral cavities of hamsters, wild rats and occasionally, man.

Streptococcus sobrinus

Although this species is given as a separate entity, some authorities prefer to regard these strains as a biovar or serovar within the *Streptococcus mutans* "group" because analysis shows a close relationship between the strains of serovar a (*S. cricetus*) and serovars d & g (*S. sobrinus*). Taxonomic studies and protein patterns also indicate a close relationship.

*Streptococcus sobrinus* displays Gram-positive cocci in pairs or chains, which may grow to a considerable length. Some strains exhibit α-hemolysis while others are nonhemolytic. Acid production stems from the fermentation of mannitol, inulin and lactose but varies with sorbitol and others. Ammonia is not produced from arginine. The main habitat of *S. sobrinus* is the surfaces of human teeth, and various strains have shown the ability to induce dental caries to the mouths of gnotobiotic animals.

Streptococcus macacae

This is one of the latest species authenticated, and has been derived from the *Streptococcus mutans* "group," where it was designated a serovar of the h type and a biovar of type VI. Little more is known about this species, but it has been isolated from the dental plaque of monkeys and it differs from the other oral strains in its reactions to sugars.

Streptococcus ferus

Prior to full species recognition, this microorganism was included in the *Streptococcus mutans* "group" with a serovar designation of c. It takes the form of Gram-positive cocci in the usual pairs or chains. From sucrose it can produce extracellular and intracellular glucans; mannitol and sorbitol are fermented whereas raffinose is not. Evidence supports this species as a distinct genetic group, not thought to be closely related to *S. mutans*. At present, *S. ferus* has been isolated only from the oral cavities of wild rats.

Streptococcus oralis

Although this species is quoted in the most recent microbiology papers, it is awaiting final confirmation of its status. Basically, it consists of Gram-positive cocci in short chains without capsules. In addition, they are nonmotile, nonsporing, facultatively anaerobic, fermentative and catalase-negative. When cultured on blood agar they produce α-hemolytic reactions. *S. oralis* has been isolated from the human mouth.

This new species shares some characteristics with "*S. milleri," S. sanguis and S. mitis" (the latter being a species also on probation with *S. oralis*). The properties of *S. oralis* detected so far do resemble *S. sanguis* in some respects, whereas some biochemical reactions resemble those of "*S. mitior." Obviously, this species needs considerable work in order to clarify its final status.

Streptococcus mitis

Another species on probation, *Streptococcus mitis*, is given full species status pending the availability of further genetic data with which to separate it from its close relatives, *Streptococcus sanguis* and "*Streptococcus mitior."" Testing of strains of *Streptococcus mitis* has revealed physiological, biochemical
and serological features which indicate that it could be placed within the species *Streptococcus sanguis*. On the other hand, DNA hybridization studies show links with "*Streptococcus mitior" but with sufficient differences to suggest that it may well represent a separate species-*Streptococcus mitis*.

The cocci of *S. mitis* appear spherical or ellipsoidal and form long chains in certain culture media. Acid is produced from the fermentation of glucose, maltose, sucrose and often lactose, but no acid is produced from inulin, mannitol, sorbitol, glycerol or xylose. When cultured aerobically on blood agar the species shows a marked α-reaction. In summary, the strains of *Streptococcus mitis* appear to form a heterogeneous group of α-hemolytic streptococci which are isolated from human saliva, the respiratory tract and feces.

**Peptostreptococcus**

The obligately anaerobic, Gram-positive, nonsporeforming cocci of this genus have recently undergone a revision of species. Apart from the removal of some species, the major change has been the transfer of four species of the genus *Peptococcus*, leaving it with one species only (*Peptococcus niger*). Thus the genus *Peptostreptococcus* now contains nine species, of which three have links with the oral cavity—*P. anaerobius*, *P. micros* and *P. prevotii*[^37^]. The species *P. anaerobius* has been isolated from a range of clinical specimens including abscesses of the jaw and ear. The species occur as part of the flora of the gingival crevice in the presence of gingivitis and periodontitis. So far, it has not been possible to establish the species constituting the normal flora of the healthy gingival crevice, but it is part of the normal flora of the vagina. It has been reported to constitute part of the flora of root canals and dental abscesses, but its role is not yet established. The second species involved in the oral cavity is *Peptostreptococcus micros*, which is often isolated from, among others, lesions of the head, jaw, neck and bite abscesses. *P. micros* is regarded as a major component of the flora of the gingival sulcus in periodontal disease, and in addition it is known to occur infrequently in the gingival crevice of the normal mouth. The appearance of *P. micros*, either alone or with other species, in infections which manifest above the diaphragm strongly suggests that the gingival sulcus in periodontitis could be its primary reservoir. *Peptostreptococcus prevotii* provides the final link with the oral cavity by occurring in the tonsil region, but nothing has been revealed about its role.

**Stomatococcus**

This is a newly described genus which has certain key characteristics which enable it to be separated from *Micrococcus* and *Staphylococcus* genera with which it shares a number of similarities. The new genus is represented by a single species known as *Stomatococcus mucilaginosus*[^39^]. This consists of facultatively anaerobic, nonmotile, nonspore-forming cocci which are Gram-positive. Its normal habitat appears to be the oral cavity and upper respiratory tract of man.

Investigations reveal that *Stomatococcus mucilaginosus* is an important component of the normal oral cavity flora, and has been isolated from the tongue, throat, nasopharynx, bronchial secretions and blood cultures. It produces considerable amounts of extracellular polysaccharide slime. Apart from its normal presence in the oral cavity, the pathogenic properties of *S. mucilaginosus* remain uncertain, although experimental subcutaneous injection has produced local abscesses in mice, while large doses can induce fatal disease.

**Enterococcus**

This constitutes a new genus designed to include four species originally classified under the streptococci, i.e. *Streptococcus faecalis*, *S. faecium*, "*S. avium"* and *S. gallinarum*. The first two of these—*S. faecalis* and *S. faecium*, occur in the intestinal tracts of humans and animals, while the remaining species—"*S. avium"* and *S. gallinarum*, appear in poultry. Of these, only *S. faecalis* has any relationship with the oral cavity.

**Enterococcus faecalis**

This species is the original *Streptococcus faecalis* relocated within a new genus[^21^]. The cells are ovoid
in form and elongated in the direction of the chain, occurring in pairs or short chains. They are usually non-motile. Lactic acid is produced from glucose fermentation, and under certain conditions there are increased amounts of formic and acetic acids in addition to ethanol. Sources of the species are the feces of humans and animals, certain insects and plants, and nonsterile foods. E. faecalis appears to act as a pathogenic agent in urinary tract infections and subacute endocarditis. However, it can be isolated from the oral cavity, irrespective of the fact that it is specially adapted to inhabiting the gastrointestinal tract through being tolerant to the levels of bile and salt that inhibit the growth of most streptococci. Enterococcus faecalis isolates, when induced into gnotobiotic animals, have been found to be cariogenic, although in man it is not usually considered to be an important cause of dental caries. Certain components of E. faecalis are very toxic to human cells grown in culture, and it is possible that this organism may contribute to the gingival inflammation observed when dental plaque accumulates.

Lactobacilli
    Current listings accept some 43 species within the genus Lactobacillus, of which 9 may be found in the oral cavity[39]. The cells vary from long, slender, often bent rods to short corniform coccobacilli, and chain formation can be observed. The length of the rods and the amount of curvature depends on age, the culture medium used and certain biochemical factors. Apart from one or two, most are nonmotile and nonsporulating. They give a Gram-positive response, but can become Gram-negative with age. There are complex nutritional needs which are generally characteristic for each species. Lactobacilli have two properties in that they are both "acidogenic" (acid-producing) and "aciduric" (survival and reproduction in acid conditions). Oral lactobacilli have been found to react differently in their management of glucose, and this has enabled them to be classified as "homofermentative" species which produce lactic acid, and "heterofermentative" species which produce much less lactic acid, but have a number of other products such as acetic acid, ethanol and a gas—carbon dioxide. Species from both groups are found in the mouth but it is the homofermentors which are probably most important in relation to caries[40].

In the oral cavity there are two species—Lactobacillus acidophilus and Lactobacillus salivarius, both of which are homofermentors and manage the breakdown of glucose via the Emden-Meyerhof glycolytic pathway with high lactic acid production. Two other species, represented by L. fermentum and L. brevis, are heterofermentors and degrade glucose by a 6-phosphogluconate pathway, producing lactic acid, ethanol or acetic acid. In addition, there are two further species, L. casei and L. plantarum, which use both pathways to degrade glucose and are therefore referred to as "facultative heterofermenters." The remaining species which have been isolated from the mouth include L. crispatus and L. gasseri (homofermentative) and L. buchneri (heterofermentative), but there is no pathogenic evidence linked to them.

Actinomyces
    These filamentous bacteria are obligately or facultatively anaerobic, capnophilic, Gram-positive, and nonacid-fast, and their filaments may disintegrate into coccobacilli[41]. All the identified species are regarded as members of normal oral flora in man and animals and none have been isolated from other environments.

The first Actinomyces species from the human mouth was located by Bergey in 1907, and since then a number of species have been characterized from both man and animals. The species A. viscosus, A. naeslundii and A. odontolyticus have limited pathogenic roles, whereas A. israelii and A. bovis are implicated in actinomycosis of man and lumpy jaw in cattle, respectively. A further species, A. meyeri, has also been linked to frank infection.

Actinomyces viscosus
    This organism has been isolated from the oral cavities of man, hamsters and rats, and its name is partially derived from its property of producing a mucinous extracellular slime upon laboratory culture. It is a proven facultative anaerobe, showing enhanced growth in the presence of CO₂. Animal investigations have
shown that A. viscosus induces periodontal disease with subgingival plaque in hamsters, and that isolates from them can produce the disease in other hamsters. The organism is also located in human dental calculus and is related to root surface caries. Basically, it is a common plaque constituent which is not thought to cause serious infections in man.

**Actinomyces naeslundii**

Basically, this is another common inhabitant of the oral cavity which has been recovered from man and a range of animals. The organism produces microcolonies demonstrating a thick mass of diphtheroid cells and tangled filaments at their centers, together with a periphery of radiating, curved and branched filaments. Although it is a facultative anaerobe, most A. naeslundii organisms can grow in air on solid media. Within the oral cavity there is a preference for tonsillar crypts, plaque and calculus as the most normal habitat. *Actinomyces naeslundii* and *Actinomyces viscosus* appear to be very similar in their human strains, especially from a biochemical viewpoint, and this has led to suggestions that the two species should be combined. In addition, both species have been shown experimentally to induce periodontal disease and dental caries in gnotobiotic animals. Current opinion is that A. naeslundii has a minor role in human infections.

**Actinomyces odontolyticus**

This species manifests in man and is located in carious teeth and specifically in deep-seated dentinal caries. Colonies of this organism grown on blood agar have produced a green area around them similar to hemolytic streptococci. Colonies tend to have a characteristic reddish-brown center. Little information exists at the present time, apart from the fact that it is a normal inhabitant of the oral cavity and its role in deep dentinal caries is unknown.

**Actinomyces israelii**

This organism is a normal member of the oral flora and can be isolated from tonsillar crypts and dental calculus when present. *Actinomyces israelii* is responsible, in conjunction with *Bacteroides* and *Arachnia propionica*, for the disease known as actinomycosis. The pathogenesis of actinomycosis is still not fully explored, but it does appear to be an endogenous (from within) infection and is not communicable. Since the organism is not especially invasive, it seems logical to accept that trauma may play a role as a method of entry. Hence it can be regarded as a true opportunistic infection, invading tissues and regions of the body as portals of entry occur.

Actinomycosis can be defined as a chronic suppurative and granulomatous bacterial infection characterized by contiguous spread, abscess formation and sinuses which discharge "sulfur granules". These "sulfur granules" are aggregates of actinomycyes filaments which lie in the suppurative material as tiny grains. The infection occurs widely and bears no relationship to occupation, climate, race or age, but is slightly more common in men. The disease is classified anatomically and thus there are several forms such as cervicofacial actinomycosis, which accounts for a high percentage of infections linked with dental caries, gingival and periodontal lesions, extractions, fractures and any other disturbance which breaks the integrity of the mucosal lining. The disease spreads by direct extension. Thoracic actinomycosis can arise due to aspiration of pharyngeal contents, dental plaque-calculus or from elsewhere in the body. Abdominal actinomycosis is another form and arises from a number of infected organs in addition to perforation of the gastrointestinal tract or appendicitis. The final major form is that known as disseminated actinomycosis, which may result from hematogenous spread from lesions elsewhere in the body, but especially from thoracic disease.

**Actinomyces bovis**

This organism is mentioned largely for completeness, and occurs in cattle where it induces "lumpy jaw," the cervicofacial actinomycosis of humans manifested in animals. It is not transmissible to man.
Arachnia

Within this genus there is a single species, *Arachnia propionica*, which has been isolated from actinomyces-like infections and from infections of the tear duct (lacrimal canaliculitis)\(^{[42]}\). The problem is that *Arachnia propionica* is inseparable morphologically from *Actinomyces israelii*. The method of separating these organisms is based on the fact that propionic acid is formed from glucose by *Arachnia* but not by *Actinomyces israelii*, and this is readily detected by means of gas-liquid chromatography. *Arachnia* has been located in dental plaques, carious dentine and necrotic pulp tissue.

Rothia

This genus is a Gram-positive organism with branching, filamentous morphology, but which may appear in cultures as coccoid, diphtheroid or bacillary forms, or in some instances as a mixture of all these. It is known to be aerobic, although some strains have responded to anaerobic conditions. The presence of CO\(_2\) is not stimulatory. *Rothia* forms white or cream pigmented colonies, often with a raised appearance. At present only one species is accepted, *Rothia dentocariosa*, and this is commonly isolated from the normal human oral flora, especially from saliva, dental plaque and calculus\(^{[43]}\). Reports also indicate findings in animals. Although the organism is not known to play a significant role in either the processes of dental caries or periodontal disease, and no natural infections have emerged from man or animals, experimentally it can produce abscesses in mice.

Propionibacteria

Within this genus many organisms were originally described as "anaerobic coryneforms." Inspection shows that they are anaerobic, gram-positive rods which produce a high peak of propionic acid when grown in glucose broth, in addition to being catalase-positive. These bacteria are regularly isolated from dental plaque and the oral cavity generally, and are regarded as members of the normal flora\(^{[44]}\), although primarily their main habitat is the skin. One such member of the skin flora, *Propionibacterium acnes*, has been isolated in the oral cavity in association with dental plaque, carious dentine and necrotic pulp tissue. Its presence has also been confirmed in oral samples by means of immunofluorescence. The association of propionibacteria with either dental caries or periodontal disease remains unproven.

Bacterionema

The genus was originally introduced to separate these branching filamentous organisms from *Leptotrichia*. There is one species within the genus, which is identified as *Bacterionema matruchotii*, a Gram-positive, nonacid-fast, nonmotile, often facultative organism with a clearly demarcated cellular morphology\(^{[45]}\). The cells are pleomorphic filaments about 2 \(\mu\)m wide and between 20-200 \(\mu\)m long, attached to a rectangular rod-shaped body 1.5-2.5 \(\mu\)m wide and 3-10 \(\mu\)m long. This particular morphology of rod and filament has led to the popular description of "whip-handle cells." However, it must be remembered that in microscopic preparations, filamentous and rod forms can appear as single entities. *B. matruchotii* has the property of being able to ferment carbohydrates to give acid, mostly acetate, propionate and a little lactic acid and CO\(_2\). Further investigations have been made of the species, and the available chemotaxonomic data suggest that *B. matruchotii* closely matches the properties of *Corynebacterium* and could be considered a member of that genus.

*Bacterionema matruchotii* is often isolated from the oral cavity of man, especially in dental plaque and calculus, and is frequently observed with large numbers of cocci attached to the whip-handle (corn-on-cob organisms)\(^{[5]}\). Although there is no current evidence relating *B. matruchotii* to either dental caries or periodontal disease, pathogenicity does occur since injection of live organisms into mice has resulted in abscesses or nodules. A more definite role that has emerged recently is possible involvement in the calcification of dental plaque to produce calculus, and the organism appears to act as an important focus for the calcification of dental plaque involving lipids in the bacterial membrane.
Eubacteria

Within this genus are a number of organisms which are often Gram-variable. By definition, any anaerobic, nonsporulating, pleomorphic, Gram-positive, filamentous or rod-shaped bacteria which do not fit into the approved classified forms are included in the genus Eubacterium[46]. There are a number of species related to the oral cavity, such as E. alactolyticum, E. saburreum and E. ingrens, all of which can be located in dental plaque[5]. E. ingrens displays a different colony morphology from the others, and when grown in fluid medium produces a mainly carbohydrate slime. In addition, E. saburreum contributes to the filamentous organisms found in mature supragingival deposits of plaque.

Some of these species have been identified from calculus, carious dentine and necrotic pulp tissue. Relative to periodontal disease, further species have been isolated as E. brachy, E. nodatum and E. timidum. Information on these species is still limited, but it is known that E. brachy consists of short Gram-positive rods linked into chains, whereas the cells of E. nodatum are said to have a close morphological resemblance to Actinomyces, from which it can be differentiated by its inability to ferment sugars and by the presence of butyric acid as a metabolic end-product. Eubacterium timidum has the outline of a small "diphtheroid," and when grown in peptone-yeast extract broth it may produce small quantities of formate, acetate, succinate or lactate, but does not ferment sugars.

Bifidobacteria

Difficulties in identification have meant that these obligately anaerobic, pleomorphic, Gram-positive rods have been placed within the Actinomycetaceae or the Lactobacillaceae as evidence emerged[45,47]. Fermentation products have helped, since in the Bifidobacteria there is a characteristic finding of 3 parts acetic to 2 parts lactic acid. Two species have been isolated from both dental plaque and carious dentine and are recorded as B. eriksonii and B. dentium. Numbers of these species appear low in the oral cavity.

5. Gram-Negative Bacteria

Neisseria

Neisseria are Gram-negative aerobic or facultatively anaerobic cocci. The two most important medical pathogens, N. gonococcus (gonococcus) and N. meningitidis (menigococcus) are not normal inhabitants of the oral flora[48]. Other Neisseria organisms have been located in various parts of the oral cavity such as the lips, tongue and cheek, together with involvement in plaque and saliva. However, there does not appear to be a particular affinity for any specific site or surface. In the oral cavity, two predominant species were originally identified as N. sicca and N. catarrhalis. The latter has been re-classified recently as Branhamella catarrhalis, i.e. has been moved into the genus Branhamella, due to marked differences in its basic biochemical, physiological and genetic data relative to the Neisseria genus. Current views accept that the Neisseria oral organisms may cause infections of the oral mucous membranes, but there remain doubts as to whether they play a significant role in either dental caries or periodontal disease[49].

Veillonella

This genus consists of cocci which are strictly anaerobic Gram-negative in type and comprise between 5% and 10% of organisms sampled and grown from saliva and tongue surfaces[50,51]. Cocci also account for up to 28% of organisms in dental plaque. Two species are known in the oral cavity – Veillonella parvula and Veillonella alcalescens[48]. The taxonomy of the genus is still subject to investigation, and some authorities indicate that V. parvula and V. alcalescens are possibly identical and that both should therefore be regarded as V. parvula. Recent research has isolated two further species from the oral cavity – V. dispar and V. atypica.

Interest has arisen in this genus, as it has been recognized that its species may have a beneficial role in protecting teeth against dental caries. Large numbers of these organisms have been found in dental plaque, and it is known that they cannot ferment carbohydrates because they do not possess glucokinase and fruc-
okinase. However, they do use certain intermediate metabolites such as pyruvate, lactate, malate, fumarate or oxaloacetate for growth, in addition to essential carbon dioxide. Lactate is taken up with the production of propionate, carbon dioxide, acetate and hydrogen. Hence, this metabolism of lactic acid must diminish the acidity existing within dental plaque.

Experimental mixtures of *Streptococcus mutans* and veillonellae have shown that pH values after addition of fermentable sugars do not fall as low as those incurred by *Streptococcus mutans* alone. Epidemiological studies have also shown that the presence of high levels of veillonellae can be linked with low levels of dental caries. Veillonellae apparently cause few infections and generally appear to be beneficial to the oral cavity of the host. It must be emphasized that this is an example of a dental plaque organism which is harmless, since it metabolizes potentially harmful substances. Other organisms may benefit the host through simpler mechanisms such as preventing the establishment of pathogenic species by their presence.

**Actinobacilli**

Within this genus there are only five known species, of which only one—*Actinobacilli actinomycetemcomitans*—is found as a member of the oral cavity flora [52]. Over the years, it has been frequently associated with cases of actinomycosis and the organism *Actinomyces israelii*. Basically, *Actinobacilli actinomycetemcomitans* is a small Gram-negative coccoid which has a very close resemblance to *Hemophilus aphrophilus*. At present, there are strong indications that *Actinobacilli actinomycetemcomitans* is one of the organisms involved in destructive periodontal disease, and has been noted in cases of juvenile periodontitis where rapid tissue destruction and bone loss has occurred in the periodontal pocket areas of adolescents. The organism is not often found in adult periodontitis and is rare in the healthy periodontium of adults or children.

Some strains of *Actinobacilli actinomycetemcomitans* have been found to produce an extracellular leukotoxin which is able to kill polymorphonuclear leukocytes (neutrophils) in periodontal pockets. The presence of such strains is likely to be especially pathogenic [5].

**Haemophili**

Most of the aerobic or facultatively anaerobic Gram-negative rods isolated from the oral cavity are haemophili [45, 53]. Recent studies have shown that they enter the oral cavity during infancy but that they have a low toxicity. In general, these are opportunistic pathogens which cause endogenous infections, but while not causing dental or periodontal disease in the direct sense, the oral haemophili may contribute to mandibular infections, otitis media and infective endocarditis. Haemophili are regularly represented in dental plaque, soft tissues surfaces and saliva, and the species most commonly isolated are *H. parainfluenzae*, *H. segnis* and *H. paraphrophilus*. One organism which is less common is *H. aphrophilus*. Of these species, *H. segnis* is a common member of the dental plaque flora and usually presents as smooth or granular, tall convex, grayish-white colonies, while the cells are pleomorphic, nonmotile and noncapsulated. The main appearance is that of irregular filaments.

**Eikenella**

There is one species of this Gram-negative facultatively anaerobic coccobacillus—*Eikenella corrodens* [45, 54]. The term "corrodens" arises from the property of this bacterium to 'corrode' or 'pit' the surface of agar plates during culture. The cells of this species are recorded as small, nonsporulating, nonencapsulated, nonmotile microaerophilic, Gram-negative organisms. *E. corrodens* can be isolated in the oral cavity and the upper respiratory tract as part of the normal commensal flora. In addition, it has been isolated from abscesses in various parts of the body as well as oral infections and abscesses, and from cases of infective endocarditis. Its presence has also been confirmed in periodontal pockets, where it is pathogenically suspect since it has been found to induce periodontal destruction when introduced into the mouths of gnotobiotic rats.
Bacteroides

These are Gram-negative anaerobic bacilli (GNABs) which, at present, consist of some 30 species according to various texts, and appear primarily in the intestinal tracts and mucous membranes of many mammals\(^{[51,55]}\). The important medical pathogen *Bacteroides fragilis* occurs in the large bowel and feces, and is not considered to be a normal member of the oral flora.

In relation to the oral cavity, the *Bacteroides* species are normally divided into pigmented or nonpigmented varieties, most interest being focused on the pigmented species. This has stemmed from the frequent isolation of pigmented *Bacteroides* from the oral cavity, especially the gingival sulcus or periodontal pocket. Many of those found have the ability to synthesize proteolytic and potentially tissue-destructive enzymes such as collagenase.

Originally the pigmented microorganisms were referred to as *Bacteroides melaninogenicus*, based on the mistaken belief that the black or brown pigment contained melanin (it is derived from bacteria), but as biochemical differences emerged between strains producing the pigmented colonies, *B. melaninogenicus* was divided into several subspecies designated as *B. melaninogenicus*, *B. intermedius*, *B. levii*, and *B. asaccharolyticus*\(^{[56]}\). To add to the confusion, the first three above have now been re-classified as species in their own right, while the asaccharolyticus subspecies has been revealed to be heterogeneous and has been subsequently recast into two entities with species ranking, viz. *B. gingivalis*\(^{[57]}\) and *B. asaccharolyticus*. It is thought that *B. gingivalis* is more commonly present in the oral cavity, and certainly both it and *B. intermedius* have been isolated from periodontal pockets in cases of destructive periodontitis. Obviously the classification and nomenclature of the *Bacteroides* species remains in a considerable state of flux, and a lot of work remains to be done in order to confirm their oral distribution and role in oral disease.

Fusobacteria

Fusobacteria are anaerobic, nonsporulating, Gram-negative rods with a characteristic fusiform (pointed end) shape.\(^{[5, 51, 58]}\) They often appear as paired rods, giving an elongated cigar-like appearance. Fusobacteria were first noted in ulcerative gingivitis in the 1880s, and later were linked to the presence of spirochaetes in what was known as Vincent's infection (AUG). In the oral cavity two species of fusobacteria have been isolated, *F. nucleatum* and *F. plauti*, the former producing small translucent colonies on agar—usually nonmotile, while the latter produces grey-white colonies with motility. *F. nucleatum* can be isolated from upper respiratory tract infections in addition to the oral cavity, where it is the most frequently isolated species; an increase in its numbers is associated with periodontitis conditions. Fusobacteria have been linked with fusospirochaetal infection. For example, the presence of the spirochaete, *Treponema vincentii* in the small ulcers of AUG enables the oral flora to be dominated by *Fusobacterium nucleatum*. Current postulates favor the idea that each bacterial species provides the other with essential nutrient factors, and that without these, neither species can multiply on its own.

Leptotrichia

*Leptotrichia*, following problems in its classification due to confusion and misnomers is, at present, confirmed as a genus with a single species, *Leptotrichia buccalis*, as the type species\(^{[5, 45, 59]}\). During the early classification problems it was considered to be Gram-negative, and its fine structure and lipopolysaccharides are known to be characteristic of Gram-negative organisms.

Characteristically, *Leptotrichia buccalis* is observed as an unbranched, nonmotile, nonsporulating straight or slightly curved rod, often with one or both ends shaped into a point. In young cultures it appears as short chains, while in older cultures it shows lengthy twisting chains. In cultures less than 6 h old, the organism shows a Gram-positive response, but by 24 h the position becomes reversed, with a Gram-negative response. Closer inspection will show that within the general Gram-negative reaction there are Gram-positive granules in the organism. Although regarded as anaerobic, 5% carbon dioxide is required for isolation and optimal growth. Fermentation of carbohydrate appears to be similar to that of the homofermentative lactobacilli. This thin thread-like or filamentous organism is commonly found in the oral cavity, but
it is not thought to be significantly pathogenic.

**Wolinella**

Wolinella is a new generic name applied to Gram-negative curved obligately anaerobic rods with a single polar flagellum. Originally, the organisms were named *Vibrio succinogenes*, now redesignated *Wolinella succinogenes*, to which has been added a new group stemming from periodontal pockets named *Wolinella recta*. These organisms do not appear to need or ferment carbohydrates, but are stimulated to grow in the presence of formate and fumarate-hydrogen and formate supplying the energy.

Studies have shown that the two species, *W. succinogenes* and *W. recta*, differ in a number of aspects such as cellular and ultrastructural morphology, susceptibility to dyes and antigenic structure. *W. succinogenes* has been found in human abdominal infections, blood cultures, dental abscesses and root canal samples. *W. recta* has been located in the human gingival crevice. Any pathogenic role relative to the oral cavity remains unknown at present.

**Capnocytophaga**

This is a relatively new genus which covers a number of important oral bacteria that were classified previously as *Bacteroides ochraceus*. Characteristically the organisms within the genus are Gram-negative, fusiform rods, canophilic (requiring CO2) and are recorded as being gliding or surface-translocating bacteria.

To date, three species have been identified from the oral cavity—*C. ochracea*, *C. sputigena* and *C. gingivalis*, and all of these can be identified on the basis of lactose and galactose fermentation and nitrate reduction. Although these organisms have been investigated intensively for a possible role in periodontitis and juvenile periodontitis, their association is still unclear.

**Selenomonas**

The genus *Selenomonas* is still regarded with uncertainty and includes three subspecies of *S. ruminantium* and one oral cavity species, *S. sputigena*. Basically they are crescent-shaped obligately anaerobic motile rods with a tuft of polar flagellae. The three subspecies are found in the gastrointestinal tract of both man and animals, while the oral species (*S. sputigena*) is located in the human periodontal pocket. The contribution of the oral species to periodontal disease remains unknown.

**Campylobacter**

Two species of this genus merit mention relative to the oral cavity namely, *Campylobacter sputorum* (originally known as *Vibrio sputorum*), and *Campylobacter concisus*. These are curved motile microaerophilic rods with a single flagellum at either or both ends. They apparently neither ferment nor oxidize carbohydrates, but can be species-separated on the basis of biochemical tests, growth inhibitors, temperature and resistance to antibiotics.

The most recent organism identified is *Campylobacter concisus*, which is known to grow in up to 5% oxygen, recognizable as curved cells with rounded ends.

Both species have been isolated from the human gingival crevice, and there is an increase in *Campylobacter sputorum* counts during gingival inflammation, although the significance of this rise has not yet been unequivocally linked to the condition.

**Spirochaetes**

Spirochaetal bacteria can occur in the oral cavity as medical pathogens which have been introduced by some means, or as normal inhabitants.

The pathogens include *Treponema pallidum*, the causative agent of syphilis, which is closely related to other spirochaetes causing yaws (*Treponema pertenue*), pinta (*Treponema carateum*), and bejel (unidentified treponeme), and is related to *Leptospira* (the cause of leptospirosis) and *Borrelia* (the cause of relaps-
ing fever). Of these, syphilis and bejel manifest in the mouth[64].

The isolation and classification of spirochaetes in the oral cavity started in the late 19th century by the observations of Koch, Miller, Plaut and Vincent in relation to periodontal and throat infections and in conjunction with fusiform bacilli. This particular fusospirochaetal combination appears as a common finding in mucosal and periodontal diseases[65].

Apart from differences in dimensions, the spirochaetes have a similar morphology, being helical cells with a number of rigid internal axial filaments (internal flagellum) inserted at the tapered ends of the cells. Motility is achieved in a rapid, jerky action. Spirochaete cell width ranges from 0.20-0.30 µm, while the length is between 5 -16 µm. The cells have a Gram-negative reaction but are too thin for good observation. Normally spirochaetes are studied by phase-contrast or dark-field microscopy, silver stain impregnation or fluorescent antibody methods.

Classification of oral spirochaetes rested originally on morphological features and activity, but has been superseded by metabolic activity tests, fermentation products and the number and arrangement of their axial filaments. Spirochaete cultivation is now undertaken with strict adherence to anaerobic conditions using medium supplemented with serum, blood or ascitic fluid to encourage growth.

The number of acceptable oral species appears to vary with claims ranging from as many as 40 to as few as 4. In recent years it has been accepted that oral spirochaetes are placed in the genus Treponema, with the current species of T. macrodentium, T. denticola, T. orale, T. vincentii, T. microdentium, T. scolioden
tium, T. mucosum and T. buccale. Of these, four have been given definite recognition with increased knowledge of their properties. For example, the growth of T. macrodentium does not rely on serum but does need isobutyric acid, polyamines and glucose. T. denticola has also been cultured without serum but has to be supplied with bovine serum albumin saturated with oleic and palmitic acids. The species T. orale can metabolize lactate.

This type of information suggests that the spirochaetes could be involved in various food-chains with other organisms. T. vincentii, often quoted in texts as Borrelia vincentii because formerly all large oral spirochaetes were placed in the genus Borrelia, is of particular interest because it is especially common in the acute painful lesions of acute ulcerative gingivitis (AUG). In this, the gingivae and other oral mucous membranes are edematous and ulcerous and have a necrotic membrane covering, the removal of which leaves a raw bleeding surface. The spirochaete is invariably found together with Fusobacterium nucleatum, and a smear from an infected region in a case of AUG will contain large numbers of spirochaetes and fusiforms—a fusospirochaetal complex. Surprisingly, neither organism as an entity appears to be able to induce the infection, which gives grounds for thinking that perhaps each organism provides essential nutritional factors needed by the other.

Experimentally, a specimen of the mixed flora of AUG can induce a similar lesion. The fusospirochaetae complex carries a high degree of general pathogenicity, which can lead to upper respiratory tract infections, infections resulting from transfer of fusospirochaetes from the mouth to subcutaneous tissue through bites, etc., and lung abscesses can also occur. In addition, the fusospirochaetal complex is involved in "tropical ulcers," a widespread cutaneous form found on the legs of malnourished children. Bacteriologic studies have implicated Bacillus fusiformis and Treponema vincentii. Finally, there is the enigma of noma (Cancrum oris; Gangrenous stomatitis), which may be caused or influenced by the fusospirochaetal complex. This is a rapidly spreading oral and facial gangrene that can appear in debilitated or nutritionally deficient persons. It is thought to originate from AUG which is apparently complicated by secondary invasion of other organisms such as staphylococci, streptococci and others.

The oral environment is receptive to spirochaetes in adults with normal dentition but in edentulous individuals they are very difficult to isolate, a situation which is repeated in infants before teeth erupt. About half of all older children harbor spirochaetes, whereas all adults carry a population. Without infections, the gingival crevice provides an ideal environment which is subsequently extended as gingival recession, pocket formation, gingivitis and periodontal disease intervene with a parallel rise in spirochaete number. It is still not clear whether spirochaetes are directly involved with the pathogenesis of gingivitis and
periodontal disease or if their presence is secondary to inflammation. Significantly, however, their presence indicates the state or degree of severity of the disease.

6. Fungi

Candida

This genus provides the only fungal species which are accepted as members of the oral commensal flora and give rise to pathogenic reactions when either the resistance of the host weakens or the oral flora becomes unbalanced due to some cause. A variety of pathological lesions can arise from Candida, and the term "candidiasis" (candidosis) is used to indicate both local and systemic processes sharing in common colonization or infection by species of the genus.[66]

Species are mostly biphasic, since the yeasts, which form the colonizing type, may develop a pseudomycelial configuration, especially during active tissue invasion. Hence Candida is a yeast-like fungus. The emergence of pseudomycelia results from the sequential budding of yeasts (blastospores) and the formation of branching chains of elongated organisms, demarcated by definite constrictions. The mycelial form is usually associated with pathological states, and thus the alteration from yeast to mycelial form is thought synonymous with alteration from commensalism to pathogenicity. Postulates for the cause of change are various including:

1. A fall in the available metabolites needed for yeast growth, which may induce Candida organisms to penetrate tissue, causing destruction in the search for substrates for its metabolism.
2. A fall in oxygen supply, which may induce mycelial formation.
3. A disturbance of the oral flora balance by agents such as antibiotics.

In the oral cavity a number of Candida species have been isolated, including C. albicans, C. tropicalis, C. stellatoidea, C. krusei, C. parapsilosis, C. guilliermondii and C. glabrata. The principal yeast form isolated in lesions involves C. albicans. Oral candidiasis occurs in the oral cavity in both acute and chronic forms:

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In acute pseudomembranous candidiasis (thrush) the infection manifests in all age groups but especially young children, the chronically ill or debilitated patients, as white plaques or pseudomembranes on the oral mucosa. Examination shows these plaques consist of dead mucosal cells with masses of fungal hyphae, while superficial removal may reveal normal, erythematous or bleeding mucous membrane.

In the other acute form, atrophic candidiasis, the cause is usually an imbalance of the normal bacterial flora due to the use of antibiotics. The lesions are red or erythematous with increased growth of fungi, mainly Candida albicans, while the mucosa becomes thin and atrophic. In chronic atrophic candidiasis there is a swollen, inflamed oral mucosa clearly demarcated by the limits of an upper denture and often linked with angular cheilitis. This common lesion is associated with a number of oral members of the genus Candida. Chronic hyperplastic candidiasis appears as firm, white persistent plaques on the lips, tongue and cheeks. They are not easily removed and consist of a thickened epithelial surface with hyphal penetration. A small percentage of these become malignant. Finally, chronic mucocutaneous candidiasis is usually observed in the young and elderly and is characterized by granulomatous plaques heavily infiltrated with Candida albi-
cans, which initially appears in the oral cavity but later spreads elsewhere.

Oral candidiasis, as described above, remains a localized process which can be extended to include eosophagitis which is often a sequel to the oropharyngeal form. The Candida genus, apart from being found in the oropharynx, also occurs in the gastrointestinal tract and vagina in a number of normal persons. Hence candidiasis can appear as intestinal, perianal, paronychia, vulvovaginitis and balanitis, all local forms. Systemic candidiasis involves, among other species, Candida albicans, C. tropicalis and C. glabrata, and these are linked to three "target organs"—the eyes, the kidneys and the skin. In addition, these candidal infections may be linked to vertebral osteomyelitis, arthritis, meningitis and cerebral, myocardial, hepatic, splenic and thyroid abscesses. Further, there is Candida albicans endocarditis due to prolonged intravenous therapy.

7. Viruses

In terms of the oral flora, only one virus—Herpes virus hominis (Herpes simplex)—is regarded as a member, while other viruses are regarded as transient. A number of viruses may be isolated from saliva during infections of various types, but are not necessarily producers of oral lesions\(^6\).

Naturally, a comprehensive classification of virus groups is not needed in the present context, but due to the role of viral infections which manifest in the oral cavity, a modified grouping will aid perspective.

In general terms a virus is judged by four characteristics which include the fact that the virus contains either DNA (deoxyribonucleic acid) or RNA (ribonucleic acid), it does not have enzymes to provide high-energy compounds needed for biosynthetic reactions, growth and division by binary fission do not occur, and its reproduction is achieved by the replication of its specific nucleic acid genetic material through metabolic support from the infected host cell. The criteria employed to classify viruses involve the kind of nucleic acid, the size of the virus, the viral shape, the presence of an envelope, chemical and physical nature of the virus and the site of assembly. A number of additional facts can be derived from properties such as low pH lability, antigenic differences and the kind of intracellular replication.

As a guide, the following classification is a simplification of the viral groups which may be present in the oral cavity producing recognizable lesions:

<table>
<thead>
<tr>
<th>RNA Group</th>
<th>DNA Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paramyxovirus</td>
<td>Herpesvirus</td>
</tr>
<tr>
<td>Rubeola (Measles)</td>
<td>Herpes simplex</td>
</tr>
<tr>
<td>Mumps virus</td>
<td>Varicella-Zoster virus (chickenpox &amp; zoster)</td>
</tr>
<tr>
<td>Picornavirus</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Epstein-Barr virus (infectious mononucleosis,</td>
</tr>
<tr>
<td>Coxsackievirus</td>
<td>Burkitt's lymphoma</td>
</tr>
<tr>
<td>Aphthovirus</td>
<td></td>
</tr>
<tr>
<td>Togavirus</td>
<td>Poxvirus</td>
</tr>
<tr>
<td>Rubella virus (Rubivirus)</td>
<td>(Molluscum contagiosum)</td>
</tr>
<tr>
<td></td>
<td>Papovavirus</td>
</tr>
<tr>
<td></td>
<td>Papillomavirus (warts)</td>
</tr>
</tbody>
</table>

Another virus of paramount importance in the practice of dentistry causes an inflammatory liver disease known as 'viral hepatitis'. This disease can be caused by one of three viruses: Hepatitis A, Hepatitis B and Hepatitis non-A and non-B (two or more agents). Hepatitis A is known as infectious hepatitis, catarrhal jaundice and epidemic hepatitis, while Hepatitis B is referred to as serum hepatitis or homologous serum jaundice. Hepatitis B is of specific interest since the virus is present in virtually all body fluids and excreta and can be transmitted by means of a percutaneous injection of infected blood, intimate personal contact, blood transfusions and other medical or nonmedical procedures which involve exposure to contaminated blood.
Hepatitis B can cause acute or chronic disease but perhaps the most important feature is the chronic persistent carrier state. Of the 'viral hepatitis' group, Hepatitis A virus (HAV) falls within the RNA group with characteristics of the picornaviruses. The Hepatitis B virus (HBV) is a DNA group virus. In the dental context, saliva as well as contaminated blood can transmit Hepatitis B virus, thus raising the risk of an occupationally acquired Hepatitis B infection.

Returning to the one accepted member of the oral community, Herpes simplex virus HSV) (Herpes virus hominis), it has been found that it exists in two serologically related types known as Herpes simplex virus 1 (HSV-1) and Herpes simplex virus 2 (HSV-2). The clinical effects include primary and recurrent infections of the skin, mucous membrane, eyes, and nervous system and sometimes a generalized infection.[68,69] The original target areas for these infections were once regarded as above the waist for HSV-1 and below the waist for HSV-2, being apparently distributed and acquired by direct contact with infected secretions such as saliva or genital lesion exudates. With changes in social habits, however, this division is no longer considered viable.

<table>
<thead>
<tr>
<th>Herpes type</th>
<th>Age group</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Embryonic</td>
<td>Disseminated disease of newborn</td>
</tr>
<tr>
<td>1</td>
<td>Child</td>
<td>Acute gingivostomatitis</td>
</tr>
<tr>
<td>1</td>
<td>Child</td>
<td>Disseminated skin lesions and eczema</td>
</tr>
<tr>
<td>1</td>
<td>Child</td>
<td>Recurrent mouth and facial lesions</td>
</tr>
<tr>
<td>1</td>
<td>Adult</td>
<td>Recurrent orofacial lesions</td>
</tr>
<tr>
<td>2,1</td>
<td>Adult</td>
<td>Primary genital infections</td>
</tr>
<tr>
<td>2,1</td>
<td>Adult</td>
<td>Recurrent genital infections</td>
</tr>
<tr>
<td>1</td>
<td>Adult</td>
<td>Encephalitis</td>
</tr>
<tr>
<td>2</td>
<td>Adult</td>
<td>Meningitis</td>
</tr>
</tbody>
</table>

Taking one of the most common types of herpes simplex infections—herpetic gingivostomatitis—the virus usually enters through a point of trauma in the mucous membrane or a non-keratinized cell. After incubation for about 5-8 days, the mouth becomes painful and the gingiva very inflamed. Eventually the oral cavity becomes involved, followed by the development of yellow vesicles which rupture and leave painful ulcers. Excessive salivation, fever and swollen glands will be noted. Remission and healing occur by about 15 days.

The herpes virus does not remain latent at the primary site in the mucosa or skin, but moves to the nerve ganglia for that area and remain latent there until reactivated. With HSV-1, this is usually the trigeminal nerve ganglion. Following this colonization of the nervous system, the patient will, at a more adult age, suffer recurrent herpetic stomatitis but without the fever and with a considerably reduced number of lesions.

The recurrent disease form is often stimulated by a wide range of factors such as trauma, pregnancy stress, and exposure to sunlight. The true mechanism is not known, but it appears that once reactivated, the virus moves out of the ganglia, and along the nerves to the oral mucosa and skin, where the epithelial cells are destroyed and the characteristic inflammatory reaction is induced. HSV-1 may be isolated from within the oral cavity when no disease symptoms are present and from dental plaque, but apart from typical herpes outbreaks, it remains primarily a colonizer of the nerve supply of the orofacial region.
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