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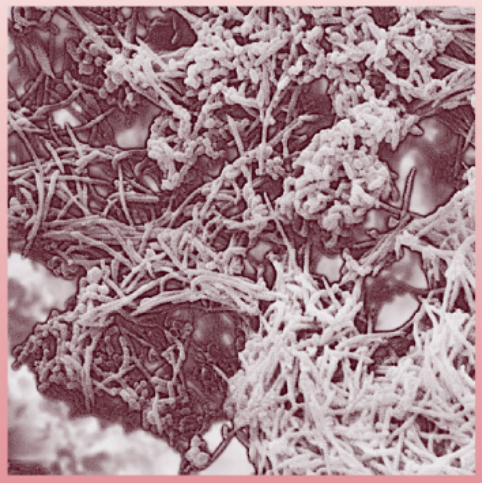

Food constituents and oral health

Current status and future prospects

Edited by Michael Wilson

CRC

WP

Food constituents and oral health

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Preface

The oral cavity is a complex system of tissues and organs whose collective functions are to select food that is acceptable for intake and to process that food into a form suitable for digestion by the rest of the gastrointestinal tract. Consequently, the maintenance of this multifunctional organ complex in a healthy state is very important for the wellbeing of the individual. Physical damage to any of the oral cavity's many components may have a detrimental effect on one or more of its functions but, in addition, the large variety of dietary constituents processed by the mouth throughout the lifetime of the individual may also have an impact on its ability to perform its many tasks. It has long been known that dietary sugars can have a detrimental effect on oral health as they are a major factor in the aetiology of dental caries. However, it is now apparent that other constituents of the human diet can also affect oral health – some exerting a beneficial effect while others are potentially detrimental. The purpose of this book is to provide a state-of-the-art overview of what is known about the role that the diet and its various constituents play in the oral health of humans and to summarise the ways in which such knowledge may be used, to not only provide new ways of improving oral health, but also to reduce the adverse effects of certain dietary constituents and practices.

The book consists of three main sections. In Part I, the aetiology, characteristic features and epidemiology of the main diseases of the oral cavity are described. This section will be of particular interest to readers with little knowledge of dentistry or oral medicine and provides essential background for understanding and appreciating subsequent chapters in the book.

In Part II the effects of important constituents of the diet on various aspects of oral health are described and discussed and the first chapter of

the section provides an orientating overview of the effect of diet on oral health. This part of the book will be of interest to readers with expertise in widely varying fields including dentists, doctors, nutritionists, nurses, food scientists, dieticians and non-specialist readers.

Part III focuses on how our knowledge of the effects of dietary constituents and dietary practices on oral health has been used to develop new approaches to maintaining oral health as well as to reduce the impact of potentially damaging constituents. It will, consequently, appeal to a wide variety of readers.

The contributors are all well-known experts in their respective fields and this book has drawn them together in a unique collaboration to provide an all-encompassing review of the current state of our knowledge of the effect that diet has on our oral health.

Michael Wilson

1

Diseases caused by oral bacteria

P. D. Marsh, HPA CEPR & Leeds Dental Institute, UK

Abstract: The chapter describes the characteristic and diverse collection of microorganisms that generally live in harmony with, and provide benefit to, the host by contributing to the normal development of the physiology and host defences of the oral cavity. The chapter then explains how this harmony can break down on occasions owing to changes in the local environment, which increases the competitiveness of previously minor components of the microbiota, thereby increasing the risk of dental caries and periodontal diseases. The chapter concludes by describing how many oral microbes are also opportunistic pathogens and can cause acute and chronic infection either within the mouth or at distant sites in the body.

Key words: abscesses, biofilms, dental caries, dental plaque, ecology, periodontal diseases, systemic infections.

1.1 The mouth as a microbial habitat

The mouth is an attractive habitat for microbial growth and a diverse range of Gram-positive and Gram-negative bacterial species can be isolated from the healthy oral cavity; in addition, yeasts, mycoplasmas and protozoa can be recovered on occasions (Marsh and Martin, 2009; Wilson, 2005). Oral surfaces are continuously bathed with saliva, which supplies nutrients, and keeps conditions warm (35–36°C) and moist at a neutral pH, which is suitable for the growth of many microorganisms. However, conditions in the mouth can also be hostile to many microorganisms, so that only a small proportion of the microbes that gain access to the mouth are able to colonize. The flow of saliva and the forces of mastication will remove by swallowing any microorganism not firmly attached to a surface; saliva also delivers an array of antimicrobial molecules. Thus, microorganisms that predominate on the skin (micrococci, coryneforms, staphylococci) do not generally become established in the mouth, and less than 30 out of the 700+

types of microorganism indigenous to the mouth are able to colonize the gastrointestinal tract successfully. This is despite the continual passage of oral microbes through the digestive tract in swallowed saliva, confirming the unique properties of the mouth as a microbial habitat.

The mouth is not a homogeneous environment for microbial colonization. Distinct habitats exist, such as mucosal surfaces (lips, cheek, palate and tongue) and teeth which, because of their different biological and physical properties, support the growth of characteristic microbial communities. As at other sites in the body, desquamation ensures that the bacterial load is relatively light on mucosal surfaces, although the papillated surface of the tongue provides a protective site permitting substantial numbers of microorganisms to establish themselves (see later). Teeth are unique sites in the human body for microbial colonization because they are non-shedding surfaces enabling extensive formation of complex biofilms (dental plaque), especially at protected and stagnant sites between teeth and around the gum margins. Dentures also provide a protected habitat, especially beneath the fitting surface, which results in colonization and growth by a range of bacteria and yeasts.

The most significant parameters affecting the oral microbiota are nutrient availability, pH and the integrity of the host defences. Saliva plays a major role in determining whether the resident oral microbiota has a beneficial or a damaging relationship with the host. The buffering action of saliva ensures that the pH of oral surfaces is maintained around neutrality, which is optimal for the growth of the majority of bacteria associated with oral health and maintains the integrity of the tooth surface. Saliva also delivers components of the innate and adaptive host responses, which play a key role in regulating the development of the resident microbiota and contributes to the exclusion of exogenous and often pathogenic microorganisms. The main immunoglobulin in saliva is secretory immunoglobulin A (sIgA), while innate factors include lysozyme, lactoferrin, sialoperoxidase and antimicrobial peptides (e.g. histatins) (Devine, 2004). Saliva also provides proteins and glycoproteins which (a) encourage the attachment of these bacteria to oral surfaces, while aggregating and clearing others (via swallowing), and (b) act as nutrients for the resident oral microorganisms. The metabolism of these endogenous substrates often requires the concerted and sequential catabolic activity of consortia of oral bacteria (i.e. the oral microbiota acts as a true microbial community) (Fig. 1.1). Superimposed upon these endogenous nutrients is the complex array of foodstuffs ingested periodically in the diet. Despite the complexity of the diet, fermentable carbohydrates are the only class of compound that markedly influence the ecology of the mouth. These carbohydrates can be broken down via glycolysis to acids while, additionally, sucrose can be converted by bacterial enzymes (glucosyltransferases, GTF, and fructosyltransferases, FTF) into two main classes of exopolymer (glucans and fructans) which can be used to consolidate attachment or act as extracellular nutrient storage

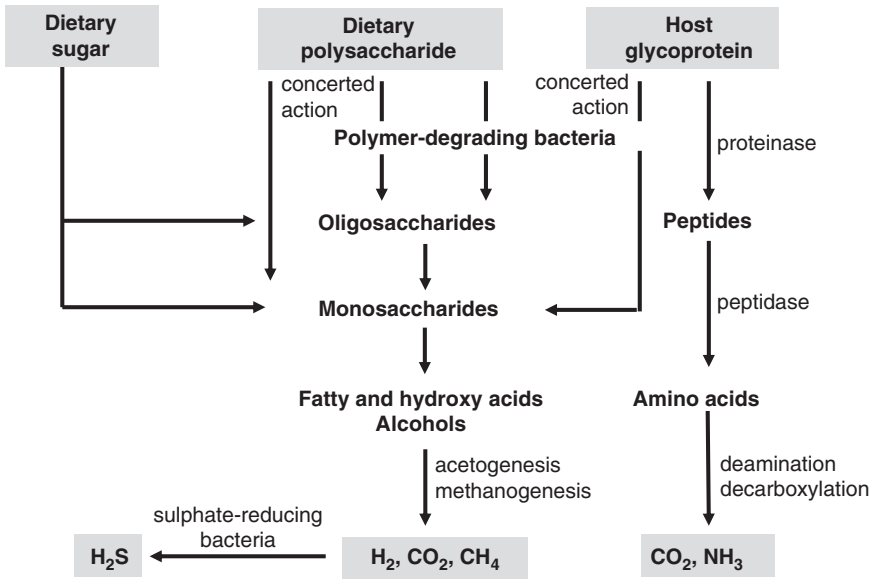

Fig. 1.1 Metabolic fate of endogenous and exogenous substrates following the concerted activity of oral microbial communities.

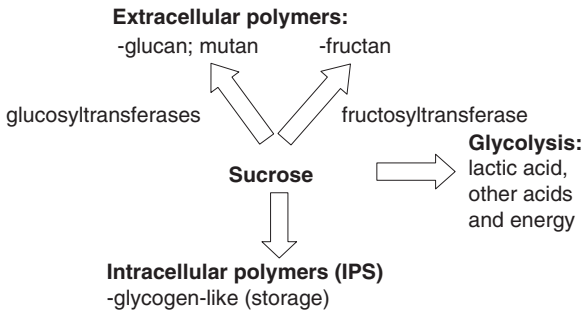

Fig. 1.2 Metabolic fate of sucrose.

compounds, respectively (Fig. 1.2). Dairy products (milk, cheese) may have some influence on the microbial ecology of the mouth owing to the buffering capacity of milk proteins or decarboxylation of amino acids after proteolysis. Milk proteins and casein derivatives can also adsorb on to the tooth surface and reduce the adhesion of bacteria (mutans streptococci) implicated in dental caries. Some non-fermentable sweeteners (aspartame, polyols, etc) can also reduce the growth and metabolism of oral bacteria.

Unlike the metabolism of dietary sugars, the utilization of saliva-derived substrates encourages stability (homeostasis) among the components of the

resident microbiota because organisms are mutually dependent on each other to breakdown these complex substrates, and only modest amounts of acid are made and at a rate that does not damage enamel. Given the pivotal role of saliva in oral health, any significant impairment of saliva flow will profoundly alter the composition of the oral microbiota and increase the risk of disease.

Gingival crevicular fluid (GCF) is a major influence on the microbiology of the gingival crevice (Uitto, 2003). GCF is a serum-like exudate that reaches the mouth by the flow of a serum-like fluid through the junctional epithelium of the gingivae. If plaque accumulates beyond levels compatible with health, the host mounts an inflammatory response and the flow of GCF is increased in order to deliver components of the systemic host response (IgG, complement, neutrophils). If this response does not resolve the microbial challenge, then the growth of many previously minor anaerobic bacteria is inadvertently favoured by the delivery of host molecules in GCF (e.g. transferrin, haemoglobin, haptoglobin, etc), which can be exploited as a novel nutrient source. Furthermore, the proteolytic activity of the subgingival bacteria drives a rise in local pH, which also favours the growth and metabolism of bacteria that are implicated in periodontal disease (Marsh, 2003).

1.2 The resident oral microbiota

Conventionally, the numbers and types of microorganism found in dental plaque have been determined by traditional culture techniques (e.g. colony counting on selective and non-selective agar plates, with identification based on simple biochemical or serological tests). The application of culture-independent, molecular approaches, such as 16S rRNA gene amplification, cloning and sequencing, has confirmed that only approximately 50% of the oral microbiota can be cultured at present. Many of these 'unculturable' organisms are found at subgingival sites, especially in disease, and represent novel groups of bacteria (often unnamed at present, such as the TM7 group) the properties of which we know little about. These sensitive molecular techniques are not without bias themselves, but they have expanded our concept of the diversity of the microbiota of the mouth. Over 700 distinct types (taxa) have now been distinguished, although they are never all found in a single mouth (Aas *et al.*, 2005). A small sample of plaque biofilm from a healthy site would typically contain around 30–50 species of bacteria.

The most numerous group of microorganisms in the mouth are bacteria and some of the more common genera are listed in Table 1.1, many of which contain multiple species (Marsh and Martin, 2009). Some oral bacteria are fastidious in their nutritional requirements while others have particular atmospheric requirements (e.g. some are capnophilic and require carbon dioxide, while others are facultatively or obligately anaerobic). Even more

Table 1.1 Bacterial genera commonly found in the oral cavity

Gram positive	Gram negative
Cocci	
Gemella	*Neisseria*
Granulicatella	*Veillonella*
Peptostreptococcus	
Streptococcus	
Rods	
Actinomyces	*Aggregatibacter*
Bifidobacterium	*Campylobacter*
Corynebacterium	*Capnocytophaga*
Eubacterium	*Eikenella*
Lactobacillus	*Fusobacterium*
Propionibacterium	*Haemophilus*
Rothia	*Leptotrichia*
	Porphyromonas
	Prevotella
	Selenomonas
	Simonsiella
	Tannerella
	Treponema
	Wolinella

Other bacterial genera are found on occasion, especially in periodontal diseases. *Mycoplasma* are also isolated from the mouth (see Table 1.2). There are also unculturable bacteria that have yet to placed in a genus; some belong to the phylum, TM7.

genera are found occasionally, especially in plaque samples from periodontal pockets, and include sulphate-reducing bacteria, methanogens, *Bulleida*, *Cantonella*, *Cryptobacterium*, *Dialister*, *Filifactor*, *Mogibacterium*, *Olsenella*, *Pseudoramibacter*, *Shuttleworthia*, *Slackia* and *Solobacterium* spp.

Yeasts are also isolated regularly, especially from the tongue. Most belong to the genus, *Candida*, and *C. albicans* is the commonest species (Marsh and Martin, 2009; Williams and Lewis, 2000); other yeasts are listed in Table 1.2. Carriage rates for yeasts range from 2–71% in asymptomatic adults, but this approaches 100% in medically compromised patients or those on broad spectrum antibacterial agents. Oral mycoplasmas are found in 6–32% of individuals and common species are listed in Table 1.2. Human herpes viruses (*Herpesviridae*) are detected frequently in orofacial tissues; examples are listed in Table 1.2. Hepatitis and human immunodeficiency virus (HIV) can be detected in the saliva of infected patients, where their presence poses a significant cross-infection risk. Bacteriophages have been observed in samples of saliva and dental plaque, but few have been isolated. Some 'phage with activity against non-oral bacteria (e.g. *Proteus mirabilis*)

Table 1.2 Common mycoplasmas, yeasts and viruses isolated from the mouth

Mycoplasma	Yeast	Virus[a]
M. salivarium	*Candida albicans*	Herpes simplex type 1
M. pneumoniae	*C. glabrata*	Herpes simplex type 2
M. hominis	*C. tropicalis*	Cytomegalovirus
M. buccale	*C. krusei*	Papilloma virus
M. orale	*C. parapsilosis*	Coxsackievirus
	C. guilliermondii	
	Rhodotorula spp.	
	Saccharomyces spp.	

[a] Other viruses (e.g. Hepatitis, HIV and paramyxoviruses) can be detected in saliva from subjects with disease; some of these viruses can pose a cross-infection risk.

has been detected and this might contribute to the ability of the resident oral microbiota to exclude exogenous species (colonization resistance – see later). Protozoa such as *Entamoeba gingivalis* and *Trichomonas tenax* are isolated on occasion and may survive, in part, by grazing on the bacteria that form biofilms over the oral surfaces (Marsh and Martin, 2009).

1.3 Development of the oral microbiota

Microbial colonization starts from birth and distinct species of bacteria (mainly streptococci) are recovered from the mouth of infants only a few hours old. At this stage only mucosal surfaces are available for colonization. The complexity of the developing microbiota increases over time, particularly following tooth eruption. The most diverse collections of microorganisms are found as biofilms on teeth (dental plaque) (Aas *et al.*, 2005) (Fig. 1.3). These biofilms develop following a specific pattern (microbial succession) (Kolenbrander *et al.*, 2006). Molecules (adhesins) on early colonizers such as *Streptococcus sanguinis* and *S. mitis* bind to specific receptors on saliva-coated tooth surfaces. More fastidious bacteria bind to these early colonizers and their collective metabolism modifies the local environment (e.g. by making it more anaerobic) making conditions more suitable for growth of later colonizers. Some bacteria produce extracellular polymers (glucans and fructans) that form the plaque matrix and support the structure of the biofilm. *Fusobacterium nucleatum* may be a critical organism in oral biofilm maturation by acting as a bridge between early and late colonizers. These biofilms are spatially and functionally organized and the conditions within the biofilm induce novel patterns of bacterial gene expression. An important property of biofilms is their reduced sensitivity to antimicrobial agents which can affect treatment outcomes (Socransky and Haffajee, 2002; Marsh, 2005).

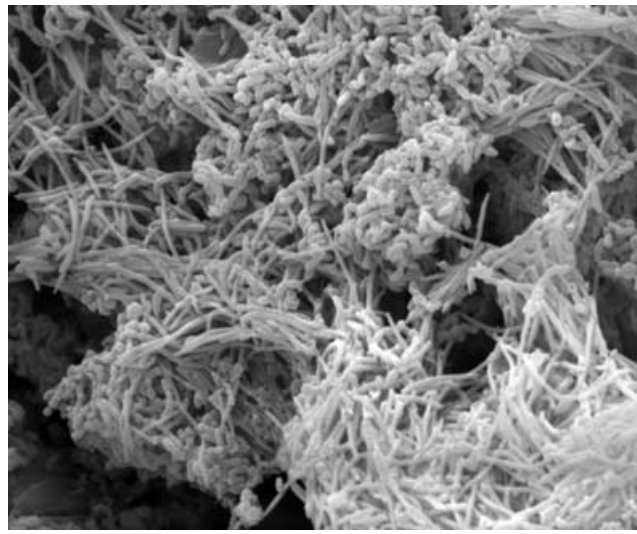

Fig. 1.3 An electron micrograph of human dental plaque, showing the diverse range of morphological types of microorganism found in these biofilms (printed with kind permission from Nicky Mordan and Michael Wilson at the UCL Eastman Dental Institute, London).

The microbial composition of the oral microbiota remains relatively stable over time, but in older age the carriage of non-oral bacteria such as staphylococci and enterobacteria is more common; yeasts are also more prevalent in the elderly. The presence of these microorganisms can be due to both direct (e.g. waning of the activity of the host defences) and indirect (e.g. the increased wearing of dentures or the increased likelihood of medication in the elderly) effects of ageing (Percival, 2009).

1.4 Distribution of the oral microbiota

The microbial communities found on distinct surfaces vary in composition owing to differences in the biological and physical properties of each site (Aas *et al*., 2005; Wilson, 2005; Marsh and Martin, 2009). Mucosal surfaces generally have a lighter microbial load. The predominant bacteria from the cheek (buccal mucosa) are facultative anaerobes such as streptococci (mainly *S. mitis* and *S. oralis*), *Granulicatella* spp., *Gemella* spp. and *Haemophilus parainfluenzae*; obligate anaerobes are not present in high numbers, although *Veillonella* and *Prevotella* spp. can be detected. Similar species are found on the palate. However, recent studies, in which fluorescent *in situ* hybridization (FISH) was combined with confocal microscopy, have shown that some of the species implicated in periodontal diseases

(*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Tannerella forsythia*) can gain refuge inside buccal epithelial cells in healthy people, where they persist as intracellular polymicrobial communities, implying that oral mucosal cells could serve as a reservoir for periodontal pathogens (Rudney *et al*., 2005).

The dorsum of the tongue has a large surface area owing to its highly papillated surface and supports a higher bacterial density and a more diverse microbiota than other oral mucosal surfaces. Streptococci (*S. mitis*, *S. oralis*, *S. salivarius*) are again the most numerous bacteria. Anaerobic streptococci have also been isolated, while *Rothia mucilagenosa* is found almost exclusively on the tongue. *Veillonella* spp., Gram-positive rods (e.g. *Actinomyces naeslundii* and *A. odontolyticus*), and haemophili are also commonly isolated. Anaerobes (e.g. *Prevotella intermedia* and *P. melaninogenica*) can be recovered from the tongue and this site is also regarded as a potential reservoir for some of the Gram-negative anaerobic bacteria implicated in periodontal diseases. Other organisms, including lactobacilli, yeasts, fusobacteria, spirochaetes and other motile bacteria, have been found in low numbers (<1% of the total microbiota) on the tongue. Oral malodour is associated with the microbiota from this site. A higher bacterial load on the tongue, especially of Gram-negative anaerobes (including *Porphyromonas*, *Prevotella* and *Fusobacterium* spp.), is found in subjects with high odour. An even more diverse microbiota was found when culture independent molecular methods were applied to the analysis of tongue samples (Kazor *et al*., 2003). The chemical basis of odour is not fully understood, but includes the production of volatile sulphur compounds by the resident microbiota.

Teeth have distinct surfaces, each of which is optimal for colonization and growth by different populations of microorganisms owing to the physical and biological properties of the site. Bacteria such as mutans streptococci and *S. sanguinis* only appear in the mouth once teeth have erupted, because of their requirement for a hard surface for colonization. The areas between adjacent teeth (approximal) and in the gingival crevice afford protection from natural removal forces (mastication, saliva flow and oral hygiene) and both sites are anaerobic. Streptococci and Gram-positive rods such as *Actinomyces* spp., predominate at approximal sites; the most numerous Gram-negative species are *Veillonella*, *Neisseria, Haemophilus, Prevotella* and *Fusobacterium* spp. The gingival crevice region is bathed in the nutritionally rich gingival crevicular fluid (GCF), particularly during inflammation, and supports a more diverse community including even higher proportions of obligately anaerobic and proteolytic bacteria belonging to genera such as *Prevotella*, *Porphyromonas*, *Fusobacterium* and *Treponema*. In contrast, although fissures also offer protection, the environment is influenced by saliva and has a higher Eh, resulting in a streptococci-dominated microbiota with few Gram-negative anaerobes.

1.5 Benefits of the resident oral microbiota

The resident human microbiota contributes directly and indirectly to the normal development of the physiology of the host and functions as part of the innate host defences by acting as a barrier to permanent colonization by exogenous organisms, some of which are potentially pathogenic. The mechanisms responsible for this 'colonization resistance' by resident oral organisms include (a) saturation of microbial attachment sites, (b) more effective competition for essential nutrients, (c) creation of conditions unfavourable to the growth of invading microbes and (d) the production of inhibitory factors, such as bacteriocins, bacteriophage, hydrogen peroxide and acidic end products of metabolism.

If this microbial barrier is broken down, for example, as a result of long-term, broad-spectrum antibiotic therapy, humans may become susceptible to overgrowth by exogenously acquired and potentially pathogenic microorganisms which can cause a range of infections in the mouth.

1.6 The oral microbiota and disease

1.6.1 Dental plaque and disease

Dental plaque is implicated in two of the most common diseases of industrialized countries: caries and periodontal diseases. In the absence of effective oral hygiene, plaque can accumulate to levels that are no longer compatible with health and microbial homeostasis breaks down, thereby predisposing sites to disease (Marsh, 2003; Jenkinson and Lamont, 2005). There is a shift in the balance of the microbiota away from those species that are found at healthy sites (Fig. 1.4). Breakdown of homeostasis can be due to a variety of factors but is generally a response to a substantial change in a key ecological determinant, such as an increase in the frequency of dietary sugar intake, a decrease in saliva flow or a perturbation of the host defences (Fig. 1.4). Comparisons have been made of the composition of the plaque microbiota from healthy and diseased sites in order to identify those species directly implicated in disease. Interpretation of the data from such studies is challenging because plaque-mediated diseases occur at sites with a pre-existing diverse resident microbiota and the traits associated with pathogenicity are not restricted to a single species.

Dental caries

Caries is a result of excess acid production from the microbial fermentation of sugars present in the diet. A key determinant for caries is the frequency of exposure to sugar-containing foods, drinks and snacks. Sucrose is a key component in a cariogenic diet because plaque bacteria can convert the disaccharide to acid via glycolysis, extracellular polysaccharides (fructans and a range of soluble and insoluble glucans) and intracellular storage

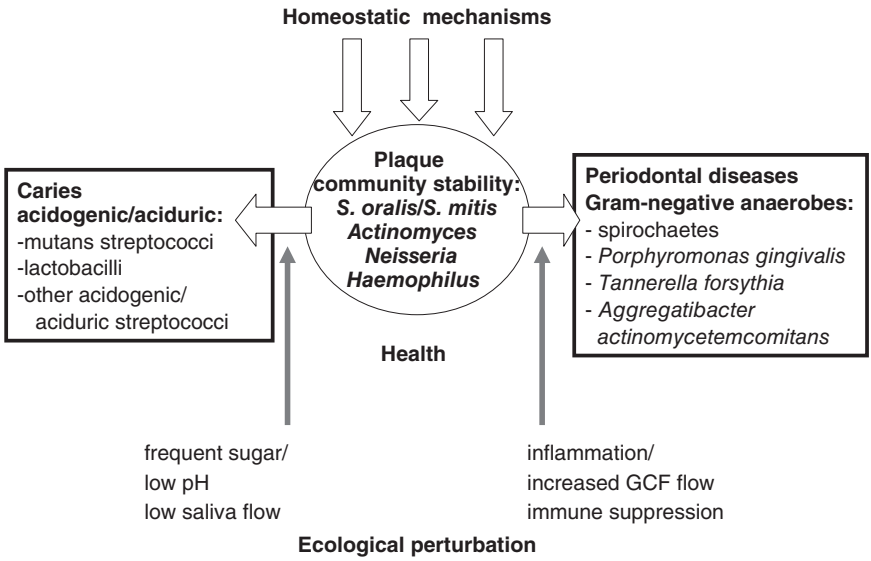

Fig. 1.4 Ecological shifts in the dental plaque microbiota that lead to disease.

polysaccharides (Fig. 1.2) which can subsequently be broken down to acid later when dietary carbohydrates are no longer available, thereby extending the exposure of enamel to low pH. Extensive studies over several decades of a range of age groups on different diets have shown an increase in the proportions of acid-producing (acidogenic) and acid-tolerating (aciduric) bacteria in plaque biofilms at sites with caries. The most common bacteria with these properties are mutans streptococci (*Streptococcus mutans*, *S. sobrinus*) and lactobacilli, but the relationship between these organisms and caries is neither absolute nor diagnostic (Marsh and Nyvad, 2008). These bacteria are not detected at all carious sites, implicating other bacteria with similar properties in disease, while they can also be found at sites without evidence of demineralization.

The microbiota become more diverse as the caries lesion progresses into the dentine, with proteolytic bacteria being isolated alongside the acidogenic species. This reflects the change in environment and the availability of new substrates (e.g. collagen), thereby changing the competitiveness of bacteria within the community.

Periodontal diseases

Periodontal diseases are a collection of conditions in which the supporting tissues of the teeth are attacked. More teeth are lost through periodontal disease than dental caries. Plaque accumulates at the gingival margin beyond levels that are compatible with health and there is an increase in numbers of obligately anaerobic, proteolytic and often Gram-negative bacteria

(Socransky and Haffajee, 2005). Gingivitis is a mild reversible condition, but if unresolved, can develop into periodontitis in which gradually over time there is periodontal pocket formation, bleeding, loss of attachment of tooth to the periodontium and even bone loss. The microbiota are highly diverse and involve consortia of bacteria that collaborate to cause disease (pathogenic synergism). One approach to managing the complexity of the microbiota has been to cluster particular bacterial combinations or complexes to specific stages of the disease (Socransky and Haffajee, 2005). The 'red complex' consisting of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* was commonly found in deep periodontal pockets, although molecular techniques have subsequently detected a large number of other fastidious or unculturable groups of bacteria, whose role in disease has yet to be determined.

The host mounts an inflammatory response to this microbial insult and the flow of GCF is increased to deliver antibodies, complement and phagocytic cells. If the microbial challenge is not controlled, the subgingival microbial consortium can subvert the host response by producing cytotoxins, inflammatory mediators and proteases that degrade host regulatory proteins (Curtis *et al*., 2005). This leads to an inappropriate and damaging inflammatory response resulting in by-stander damage to host tissues, while the aggressively proteolytic bacteria become increasingly competitive by catabolizing the molecules present in GCF.

Aggressive periodontitis is a rare condition (affecting only around 0.1% of the susceptible age group) which usually occurs in adolescents. The microbiota of plaque from patients is relatively sparse considering the severity and rapidity of the tissue destruction and bone loss, but patients usually have neutrophils with various functional abnormalities in which there is reduced chemotaxis and phagocytosis, but increased superoxide radical production. High numbers of *Aggregatibacter actinomycetemcomitans* are recovered from localized aggressive periodontitis; cells produce a range of relevant virulence factors, including a powerful leukotoxin (a protein toxic for polymorphs), lipopolysaccharide which can stimulate bone resorption and a cell surface-associated material which also induces resorption of bone (Henderson *et al*., 2003); cells can also invade host mucosal cells (Rudney *et al*., 2005). In addition, *A. actinomycetemcomitans* produces enzymes with the ability to degrade collagen, as well as other, less well-defined factors, that modulate the activity of the host defences. A specific clone (JP2) of *A. actinomycetemcomitans* is responsible for rapid bone loss in adolescents living in, or originating from, north and west Africa (Haubek *et al*., 2008).

Recently, an ecological hypothesis was proposed to explain the relationship between plaque composition and caries and periodontal disease (Marsh, 2003). Briefly, a major change in local environmental conditions can alter the competitiveness of plaque bacteria, leading to the enrichment of organisms most suited to the altered environment. In caries, an increased

frequency of sugar intake, or a reduction in saliva flow, results in plaque biofilms spending more time at low pH. This selects for acidogenic and aciduric species (most commonly mutans streptococci, but not exclusively so) at the expense of health-associated bacteria that prefer pH values around neutrality. Increases in the acidogenic populations leads to even greater production of acid and further raises the risk of demineralization.

In periodontal disease, the inflammatory response to plaque accumulation results in an increased flow of GCF which, in addition to introducing components of the host defences, also delivers host molecules (haemoglobin, transferrin) that can be catabolized by anaerobic and proteolytic bacteria. This type of metabolism makes the site more anaerobic and the local pH increases, and this drives the selection of complex consortia with an inflammatory phenotype. A key principle of this hypothesis is that disease should be treated not only by removing plaque, improving oral hygiene or targeting the putative pathogens directly, but also by interfering with the environmental pressures that select for the pathogenic microorganisms. In caries, this could be by reducing the low pH challenge by recommending snack foods containing non-fermentable sweeteners, using fluoride or antimicrobial-containing products to reduce acid production, or boosting saliva flow with sugar-free gums to encourage remineralization. Alternatively, the use of oxidizing or redox agents to make sites less anaerobic, or the application of anti-inflammatory compounds to reduce GCF flow would change the local environment and thereby restrict the growth of periodontal pathogens (Marsh, 2003).

1.6.2 Acute and chronic infections

There is a dynamic relationship between the host and the resident oral microbiota. Many of these microorganisms are opportunistic pathogens and are capable of causing infection if environmental conditions change in the mouth and provide them with a competitive advantage, or if they enter the blood stream and are disseminated to vulnerable sites in the body. For example, oral streptococci (especially *Streptococcus sanguinis*, *S. oralis* and *S. gordonii*) are commonly isolated from cases of infective endocarditis. Oral bacteria enter the blood stream following dental treatment and the streptococci in particular are able to attach and grow on clots that develop on damaged heart valves to form vegetations that further impair blood flow and valve function. The vegetations can also break away and occlude the blood supply to vital organs.

Oral candida are opportunistic pathogens and can cause infections (a) when oral bacteria are suppressed following treatment with broad-spectrum antibiotics (acute erythematous candidosis), (b) following the wearing of ill-fitting dentures (chronic erythematous candidosis), and (c) in medically compromised patients (pseudomembranous candidosis; ‘oral thrush’) including those infected with HIV (Marsh and Martin, 2009;

Williams and Lewis, 2000). Chronic hyperplastic candidosis is a rare form of oral candidosis with its highest prevalence seen in middle-aged men who are tobacco smokers. The condition is generally asymptomatic but, if left untreated, a minority (5–10%) may develop oral cancer. Unlike other forms of oral candidosis, chronic hyperplastic candidosis is characterized by the invasion of the oral epithelium by hyphal forms of *Candida*.

Dento-alveolar abscesses can result from death of the tooth pulp, usually from the progression of dental caries. The microbial aetiology is polymicrobial and a range of facultatively and obligately anaerobic bacteria can be isolated from aspirated pus from these abscesses, including various streptococci (especially *Streptococcus anginosus*), *Actinomyces* spp., *Haemophilus* spp., anaerobic cocci, *Prevotella* spp. and *Porphyromonas* spp. The organisms isolated from these abscesses may also display pathogenic synergism, in which the component species are only weakly virulent in pure culture, but function as a more pathogenic unit when present as a consortium. Molecular techniques have disclosed an even more diverse microbiota and novel unculturable taxa have been detected. Similar groups of culturable and unculturable bacteria are isolated from endodontic (root canal) infections. The placement of implants into the maxilla or mandible is now common. The junction between the implant and the oral tissue can become infected (peri-implantitis); the microbiota resembles that seen in advanced periodontitis, with many Gram-negative and obligately anaerobic bacteria such as *Prevotella* and *Porphyromonas* spp. being isolated.

A number of chronic infections can develop in the mouth, including actinomycosis, which typically presents as a granulomatous lesion from which *Actinomyces* species, such as *A. israelii*, can be isolated, although disseminated infections can occur in medically or immunocompromised patients. Salivary glands can also become infected, usually as a consequence of impairment of saliva flow, and a range of microorganisms are detected. Medically and immunocompromised patients can also become colonized with non-oral bacteria, including *Pseudomonas*, *Acinetobacter, Klebsiella* spp. and staphylococci. Carriage of these bacteria can be associated with septicaemia, aspirational pneumonia and other life-threatening conditions.

Oral viral infections are common, and include cold sores (Herpes simplex type 1), ulceration (Herpes simplex type 2), chickenpox (Varicella zoster virus) and hand, foot and mouth disease (Coxsackie virus) (Marsh and Martin, 2009).

1.6.3 Systemic infections

Evidence linking oral and general health is accumulating, particularly with respect to cardiovascular and respiratory diseases, diabetes mellitus and a risk of pre-term labour and low birth weight infants. The theory behind this association is that (a) many oral bacteria can act as opportunistic pathogens if they gain access to sites not normally accessible to them, or if host

defences are compromised, and (b) subgingival biofilms in periodontal disease contain numerous Gram-negative species which have inflammatory cell surface components such as lipopolysaccharide, and shed toxic metabolites which induce prostaglandins and pro-inflammatory cytokines. The vascular nature of the periodontium means that these inflammatory mediators can affect distant sites in the body.

Oral microorganisms can enter the blood stream during transient bacteraemias and play a role in systemic disease (e.g. infective endocarditis). Molecular techniques have also detected DNA from oral Gram-negative bacteria in atheromatous plaques and recent research suggests that periodontal bacteria may be an added risk factor for cardiovascular disease (Demmer and Desvarieux, 2006). Periodontal diseases can represent a small risk factor for pre-term or low birth weight babies, either as a direct consequence of pre-term labour or owing to premature rupture of membranes (Vergnes and Sixou, 2007), although as yet there is no strong evidence that treatment of periodontitis can improve birth outcomes. Elevated levels of prostaglandins have been found in the GCF of mothers with preterm, low birth weight babies, although the association with periodontal disease has not been confirmed in all population groups that have been investigated. Microorganisms associated with periodontal diseases may also cause aspiration pneumonia in susceptible patients since anaerobic bacteria from periodontal pockets have been isolated from infected lungs and dental plaque can act as a reservoir for respiratory pathogens (Raghavendran *et al*., 2007).

There is a need for more well-controlled investigations in a range of populations in this important area in which the statistical analyses are adequately adjusted for other life-style confounding risk factors (smoking, alcohol consumption, maternal education, etc). Longitudinal, prospective studies are also needed to determine whether periodontal disease is causal for these medical conditions. The outcome of intervention studies, in which the impact of periodontal treatment on the subsequent development of systemic disease is monitored, will be crucial in confirming the impact of oral disease on general health.

1.7 Conclusions

The mouth supports a diverse and characteristic collection of microorganisms, and many species that predominate in neighbouring habitats (skin, gastrointestinal tract) are non-competitive and generally fail to colonize successfully. Generally, these oral microbes live in harmony with the host and contribute to the normal development of the physiology and defence systems of the mouth. Although the composition of the oral microbiota remains generally stable over time, the relationship with the host is dynamic and a change in environment can perturb this natural balance and predis-

pose the mouth to disease. If dental plaque reaches levels that are incompatible with health then, under certain conditions, dental caries or periodontal disease can occur. Many oral bacteria are opportunistic pathogens and can cause infections either in the mouth or at distant sites. Recent studies have re-affirmed an earlier concept that oral health is inextricably linked to general health and *vice versa*. It is essential, therefore, that our oral health is actively managed so as to maintain microbial homeostasis throughout life to ensure that we reap the benefits of our resident oral microbiota and avoid suffering from their misbehaviour.

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2

Viral and fungal infections of the oral cavity

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Abstract: A wide range of viral infections can give rise to oral manifestations and/or may reside within the mouth. A smaller range of fungal infections, notably *Candida* species, may affect the mouth, although deep mycotic infections such as histoplasmosis, aspergillosis, mucormycoses and paracoccidioidomycosis may occasionally give rise to oral manifestations. The present chapter will consider the common and/or clinically significant viral and fungal infections of relevance to the mouth.

Key words: oral, viral, fungal, infection.

2.1 Introduction to viral infections

A wide range of viral infections can affect the oral cavity. Probably most common amongst these worldwide are infection with herpes simplex 1 (HSV-1), varicella zoster virus (VZV; herpes zoster), Epstein–Barr virus (EBV), human herpes virus 8 (HHV-8) and human immunodeficiency viruses (HIVs). Other viral infections likely to have an impact upon the mouth include the coxsackie group, mumps virus, measles and perhaps hepatitis C virus.

2.2 Herpes virus infection

Herpes viruses are a family of DNA viruses found commonly in humans and animals. They are widely disseminated in nature and most animal species carry at least one herpes virus, and frequently several, diverse herpes

viruses. The members of the herpesviridae have been classified into three subfamilies (alpha, beta and gamma *herpesvirinae*), based principally upon host range and tropism as well as *in vitro* growth rate and characteristics of viral latency.

There are eight human herpes viruses. The alpha herpes viruses comprise herpes simplex 1 (HSV-1), herpes simplex 2 (HSV-2) and varicella zoster virus (VZV). These viruses have a relatively short reproductive cycle, rapid spreading culture and are able to establish latent infection primarily, but not exclusively, in sensory ganglia. Cytomegalovirus (CMV) is the only beta herpes virus to infect man. This group of viruses replicate in a variety of cell types *in vivo* and give rise to cell enlargement (cytomegaly) with an appearance of characteristic nuclear eosinophilic inclusion bodies. Latent infection commonly affects lymphoreticular cells or secretory glands (e.g. the salivary glands) and renal tissue. The gamma herpes viruses affecting man comprise Epstein–Barr virus (EBV) and human herpes virus 8 (HHV-8). The gamma herpes viruses are characterised by tropism for lymphoid tissue, their ability to induce cell proliferation *in vivo* resulting in transient or chronic lymphoproliferative disorders and *in vitro*, where they may cause immortalisation of the infected cells.

2.2.1 Alpha herpes viruses

Herpes simplex 1 (HSV-1)

Infection by HSV is widespread and worldwide. HSV-1 and HSV-2 can affect the mouth or genitals, although in general HSV-1 is considered to give rise to infection 'above the waist', while HSV-2 is 'below the waist'. Nevertheless, there is considerable variation in the site of infection by these viruses, indeed HSV-1 accounts for the majority of new primary genital infections (Samra *et al.*, 2003; Roberts, 2005). The major route of transmission of HSV-1 is presumably salivary. Sexual, transplacental and bloodborne transmission can occur, but these are not as epidemiologically significant as salivary transmission (Arduino and Porter, 2008). The prevalence of HSV-1 infection increases gradually from childhood, reaching up to 90% in the later adult years (Gil *et al.*, 1998; Wutlzer *et al.*, 2000; Lafferty, 2002). The seroprevalence of HSV-1 infection is greater in lower than in higher socioeconomic groups (Corey and Spear, 1986).

Primary infection by HSV-1 usually arises in children and young adults. Most primary HSV infection in children is either asymptomatic or so mild that children or parents do not notice the illness; indeed perhaps only 10–12% of children who are infected have signs or symptoms severe enough to be recalled by the child or parent (Chauvin and Ajar, 2002). Following an incubation period of 2–20 days, non-specific symptoms such as malaise and myalgia can arise, these being followed within 1–3 days by mucocutaneous vesicular eruptions (Huber, 2003).

Primary infection, termed primary herpetic gingivostomatitis, typically affects the tongue, lips, gingivae, buccal mucosa and the hard and soft palates. 1–2 mm blisters develop and rapidly break down to give rise to irregular coalescent superficial ulceration. These ulcers heal within 10–14 days without scarring. Additionally, the gingivae may become profusely swollen and purple, particularly about the margin, although occasionally the attached gingivae, particularly anteriorally, can also be involved. The oral mucosal and gingival features are usually accompanied by pyrexia, lethargy, fractiousness and hypersalivation. Sometimes it can cause irritability, headache and bilateral cervical lymphadenopathy. Cutaneous rash is not uncommon and there can be dysphagia (Arduino and Porter, 2008).

There has been a trend in recent years for the mean age of primary clinical disease to have increased to the teenage years which perhaps reflects previous poor hygiene within schools and nurseries such that the first time that salivary transmission is likely to take place is during teenage dating (Main, 1989; Katz *et al.*, 1991; Chauvin and Ajar, 2002). Most initial episodes of orofacial HSV infection are caused by HSV-1; nevertheless, infective primary gingivostomatitis of some older patients is probably due to HSV-2 infection, this being transmitted sexually. Oral infection with HSV-2 can also be a complication of long-term iatrogenic or human immunodeficiency virus (HIV)-related immunosuppression (Liang *et al.*, 1993; Gilmour *et al.*, 2001).

Following local replication within mucosal surfaces, HSV-1 enters sensory nerve endings and is transported by retrograde intra-axonal transport to neuronal cell bodies. With orofacial infection this will inevitably involve the trigeminal ganglion. Here a more restrictive replication cycle occurs, most often culminating in the lifelong latent infection of neurons (Quinn *et al.*, 2000). Detailed discussion of the latency of HSV-1 can be found elsewhere (Arduino and Porter, 2008).

Reactivation of the virus in the sensory ganglion causes cutaneous and mucocutaneous recurrent herpetic infection. This reactivation can be spontaneous or be triggered by a number of factors (e.g. UV light, psychological stress, concurrent infection, pregnancy). Such recurrent infection occurs at variable time intervals ranging from months to years. Clinically detectable secondary infection arises in 20–40% of HSV-1 seropositive individuals. The lesions (herpes labialis – sometimes termed 'cold sores') typically occur at mucocutaneous junctions of the face (usually the lips) and give rise to initial symptoms of paraesthesia and itching that last for approximately 6 hours. About 25% of these painful lesions do not progress beyond this phase. The majority of lesions do, however, give rise to areas of erythema that become pustular and eventually ulcers. The cold sore heals within 1–10 days of onset of initial symptoms (Arduino and Porter, 2008).

A small number of individuals seem to develop recurrent HSV-1 infection within the mouth, this typically comprising a small number of mildly painful superficial ulcers. A common precipitant of this presentation is

palatal local anaesthetic injection (Raborn and Grace, 2003), although reduced ferritin has been suggested as a predisposing factor for recurrent intra-oral herpes, perhaps reflecting an iron-related deficiency-induced cell-mediated immunodeficiency (Lamey and Biagioni, 1995).

Immunodeficiency can give rise to recurrent HSV-1 infection comprising slowly healing and extremely painful areas of ulceration. A dendritic type of ulceration of the dorsum of tongue has been reported to be a possible feature of recurrent HSV-1 infection in immunocompromised persons (Epstein *et al.*, 1990; Cohen *et al.*, 1995).

HSV-1 can give rise to a variety of non-oral disorders (e.g. cutaneous, ocular, genital and neonatal infections (Arduino and Porter, 2008)). In addition, HSV-1 has been proposed to be associated with, or of aetiological relevance to, a number of orofacial disorders including erythema multiforme minor. Behcet's disease, Bell's palsy (Furuta *et al.*, 1998) and Ménière's disease (Vrabec, 2003). Trigeminal sensory neuropathy possibly caused by HSV has occasionally been observed (Yura *et al.*, 2000).

Details of the diagnosis of HSV-1 infection can be found elsewhere (Arduino and Porter, 2008). A review of the current treatment measures of HSV-1 can likewise be found elsewhere (Arduino and Porter, 2008). Of note, however, antiviral therapy for primary infection in otherwise well individuals is rarely warranted and probably unlikely to be of clinical benefit. At present there remains no effective vaccine for HSV-1 infection, although there continue to be a number of suggested prototype vaccines (Arduino and Porter, 2008).

Varicella zoster virus

Varicella zoster virus (VZV) has a worldwide distribution. In a temperate climate, the primary infection, chicken pox, gives rise to epidemics in late winter and early spring (Arvin, 1996). Children in these regions usually acquire chicken pox between the age of 5 and 10 years and indeed almost all children eventually become infected. In tropical climates there is a higher median age of infection and lower VZV seroprevalence compared with temperate climates (Garnett *et al.*, 1993). It has been suggested that the higher temperatures and humidity of tropical regions may inactivate the virus and interfere with its transmission, hence the differing epidemiology compared with temperate climates (Garnett *et al.*, 1993).

Varicella zoster virus is transmitted via the droplet route and has an incubation time of approximately 14–16 days (Arvin, 1996). Following incubation, non-specific prodromal symptoms of fever, malaise, headache, anorexia and abdominal pain can arise and approximately 2 days later the characteristic chicken pox rash develops. This rash develops in crops and tends to be more diffuse and dense in hollows and protected areas of the body, although there is eventual spread which affects the scalp, trunk and extremities. The rash commences as areas of pruritic macules which then develop into superficial vesicles that rupture to give rise to areas of

ulceration of pustules. The lesions eventually dry, crust and exfoliate within 1–3 weeks of the onset of infection and can leave slightly depressed areas of skin. There is variable pyrexia, this being absent in mild disease, but can rise to 40°C with severe illness. Pruritis associated with chicken pox can be notably distressing.

Oral lesions in chicken pox occur frequently and typically affect the vermilion border of the lip and the palate. The labial mucosa, buccal mucosa and tongue can also be affected (Kolakatronis *et al.*, 2001). Oral mucosal lesions commence as small vesicles or ulcers of approximately 3–4 mm in diameter which eventually give rise to large shallow ulcers with an erythematous halo. The presence of this oral mucosal disease correlates with the severity of chicken pox.

As with HSV-1 infection, most instances of primary VZV infection are benign and self-limiting. Nevertheless, complications can arise including bacterial infection of the skin, central nervous system (CNS) involvement (of which encephalitis is by far the most serious complication) and lower motor neurone palsy of the 7th cranial nerve (van der Flier *et al.*, 1999). Erythema multiforme may rarely arise in association with chicken pox (Prais *et al.*, 2001).

Chicken pox is generally more severe in immunocompromised children, particularly those with HIV infection, leukaemia, and patients with severe asthma treated with corticosteroids or those receiving iatrogenic immunosuppression secondary to allograft receipt (Cohen *et al.*, 1999).

Shingles

Following the primary infection, VZV establishes latency within the trigeminal and dorsal ganglia. Reactivation of the virus results in a more localised and painful vesicular and dermatomal rash known as herpes zoster shingles. Most secondary infection affects the dermatomes of T3-L2 giving rise to chest and abdominal lesions, but in approximately 13% of patients, branches of the trigeminal region may be involved (Millar and Troulis, 1994).

Patients with cell-mediated immunosuppression (e.g. HIV disease and iatrogenic immunosuppression) are at particular risk of VZV reactivation. Other risk factors include psychological stress, old age and exposure to immunotoxic materials (Thomas and Hall, 2004). A detailed discussion of the clinical manifestations of shingles can be found elsewhere (Fitzpatrick *et al.*, 1993).

In the head and neck region, the trigeminal nerve is the most frequently involved cranial nerve, the ophthalmic division accounting for almost 50% of trigeminal nerve involvement (Tidwell *et al.*, 1999). The maxillary and mandibular branches (which thus give rise to oral manifestations) are affected in 15–20% of instances of trigeminal shingles (Greenberg, 1996). Shingles in the mouth may be preceded by a prodromal pain on one side of the face or pain localised on a single tooth (McKenzie and Gobetti,

1990). In some instances this odontalgia may be the only prodromal symptom (Law and Lilly, 1995). Maxillary or mandibular division involvement gives rise to cutaneous rash on the face before involvement of the oral mucosa (Tidwell *et al.*, 1999). Intra-orally, vesicles develop at sites following distribution of the involved nerve. These vesicles then break down to give rise to areas of superficial ulceration that may coalesce to produce large irregular ulcers. Healing takes approximately 7–10 days. There have been rare instances of shingles in persons with malignant disease giving rise to spontaneous maxillary or mandibular alveolar bone destruction (Arikawa *et al.*, 2004). Post-herpetic neuralgia (pain and/or paraesthesia persisting for three or more months after cessation of the mucocutaneous features) can arise in up to one-third of patients, particularly when aged over 80 years (Choo *et al.*, 1995). Varicella zoster virus infection of the geniculate ganglion may give rise to Ramsay–Hunt syndrome. A wide range of complications, typically associated with dorsal root ganglion involvement, can arise especially in immunocompromised groups; this may include CNS and visceral involvement.

The treatment of chicken pox in healthy children is typically symptomatic, although aciclovir may be considered for treatment of children of 12 or more years, those with chronic cutaneous or pulmonary disease or individuals who have been receiving corticosteroids (Arvin, 2002). Valaciclovir, famciclovir and penciclovir are not presently suggested for the treatment of chicken pox (Arvin, 2002). Recently sorivudin has been suggested to be an effective means of management of treating chicken pox in healthy adults. Chicken pox of immunocompromised children and adults can be effectively treated with intravenous aciclovir. Vidaribine has also proved to be effective in the treatment of chicken pox in immunocompromised individuals (Arvin, 2002). The antivirals of benefit for the treatment of shingles include aciclovir, valaciclovir, famciclovir and brivudin. These agents may be provided to patients with and without immunocompetence.

A live attenuated varicella vaccine is now available in several countries. This vaccine is an effective prophylactic therapy for susceptible immunocompetent individuals and, indeed, the incidence of chicken pox reduced significantly in the USA following the introduction of varicella vaccine in 1995. Viral reactivation following vaccination appears to be uncommon and, indeed, varicella vaccine may provide protection against viral reactivation in elderly persons (Oxman *et al.*, 2005).

2.2.2 Beta herpes viruses

Cytomegalovirus

Cytomegalovirus (CMV) belongs to the beta herpes viriniae; a detailed discussion of virology and pathology of this can be found elsewhere (Mocarski, 1996; Griffiths, 2000). Cytomegalovirus infection arises world-

wide, with about 60% of individuals resident in developed countries being infected by adulthood. In homosexual males, poor socioeconomic groups and residents of developing countries, the seroprevalence rates are higher, being 90%+ (Guinan *et al.*, 1984; Collier *et al.*, 1987; Munro *et al.*, 2005). Infection is generally asymptomatic, although it leads to persistence. Its impact upon morbidity and mortality has become more prominent in recent times owing to the increased use of iatrogenic immunosuppressive regimes and, of course, HIV disease. Cytomegalovirus is also a leading cause of birth defects (Griffiths, 2000; Pass, 2001).

Cytomegalovirus is transmitted horizontally and vertically and is present in many body fluids. Transmission may occur following primary infection, or reactivation, the virus being shed into various body fluids including saliva, semen, urine and cervicovaginal secretions. Salivary transmission is likely to be significant within families (Beyari *et al.*, 2005), particularly among children; indeed infectious toddlers may transmit the virus to adult nursery staff or to mothers and oral fluids are a more likely source of CMV than urine (Beyari *et al.*, 2005). Of concern, cytomegalovirus is a significant post-allograft infection; indeed the frequency of CMV infection following allogeneic bone marrow transplant may be as high as 70%. Salivary shedding of CMV post-haematopoietic stem cell transplant can be as high as 45% (Correia-Silvre *et al.*, 2007).

Most perinatally infected infants do not develop acute symptoms and post-natal infection in immunocompetent children or adults is usually symptomatic, although it may account for approximately 8% of all cases of mononucleosis-like illness (Nesmith and Pass, 1995). Infection following renal allograft receipt can cause chronic rejection and renal arterial stenosis, while coronary artery stenosis may arise in cardiac allograft recipients and bile duct anomalies in liver transplant recipients (Steininger, 2007a). CMV-related pneumonitis is a potential complication of bone marrow transplantation and associations between CMV and onset of chronic graft-versus-host disease have been proposed (Steininger, 2007a). Retinitis is one of the potential manifestations of CMV infection in HIV disease, particularly in persons who have not received antiretroviral therapy (Hoover *et al.*, 1993; Steininger, 2007a).

Cytomegalovirus is the leading cause of congenital viral infection, having an incidence of 0.5–3% of live births worldwide (Hassan and Connell, 2007). Primary CMV infection in pregnancy carries the greatest risk of intrauterine transmission, the resultant foetal damage including hepatosplenamegaly, jaundice, CNS abnormalities and growth retardation (Hassan and Connell, 2007). The majority of infected newborns are asymptomatic at birth, but 30% of them will develop severe hearing deficits (Stagno *et al.*, 1986). Hypoplasia of the deciduous dentition is a potential oral feature of congenital CMV infection. Improvements in immunosuppressive therapy and the availability of anti-CMV therapies such as ganciclovir and valganciclovir have reduced the development of CMV disease significantly in

immunocompromised groups, while in HIV disease the availability of antiretroviral therapy (ART) (at least in the developed world) in parallel with the availability of ganciclovir has reduced the morbidity and mortality associated with CMV infection. A range of novel therapies for CMV infection are in development (Steininger, 2007b).

Cytomegaloviral infection can be a common antecedent viral infection of patients with Guillain–Barré syndrome. This infection may represent primary infection or re-infection with a different CMV serotype. It is unclear, however, that CMV infection truly does trigger Guillain–Barré syndrome (reviewed by Steininger, 2007a).

Human herpes virus 6

Human herpes virus 6 is a beta herpes virus. This virus exists as two closely related variants, HHV-6a and HHV-6b. The former has not been aetiologically linked to any disorder; however, the latter is a causative agent of an exanthema subitum disorder characterised by high fever, mild skin rash and occasionally seizures or encephalitis (De Bolle *et al.*, 2005). HHV-6 is not known to give rise to any oral manifestations; however, it is suggested that the salivary glands are a potential site of HHV-6 persistence and saliva is the principle vehicle for transmission of the virus. It is probable that HHV-6 is transmitted via this route early in childhood either from a mother to a child or between children.

While not giving rise to significant disease in immunocompetent individuals (although suggested to be a potential trigger of chronic fatigue syndrome (Komaroff, 2006)), HHV-6 activation of re-infection in immunocompromised groups such as bone marrow and stem cell transplant recipients can give rise to encephalitis or encephalopathy, pneumonitis and delayed engraftment (De Bolle *et al.*, 2005). Similarly, although much less common than the aforementioned patient group, HHV-6 may cause encephalitis and pneumonitis in small numbers of patients with HIV disease (Knox and Carrigan, 1995; Knox *et al.*, 1995). There remains no specific therapy for HHV-6, although agents such as ganciclovir and valganciclovir may be of some benefit, while aciclovir, valaciclovir, cidofovir and foscarnet are less effective (De Clerq and Naesens, 2006).

Human herpes virus 7

Human herpes virus 7 (HHV-7) is a beta herpes virus which, like HHV-6, is acquired in early childhood. Transmission occurs via saliva, as the salivary glands are a site of persistent viral replication (Black and Pellett, 1999; Caselli and de Luca, 2007). HHV-7 has been associated with some cases of exanthema subitum; in addition, it has been suggested that primary HHV-7 infection may cause CNS features such as hemiplegia and febrile convulsions. Associations between HHV-7 and pityriasis rosea have been suggested. Unlike HHV-6, HHV-7 does not seem to be associated with significant complications in allograft recipients, although it may exacerbate

disease associated with cytomegalovirus (Osman *et al.*, 1996). Similarly in HIV disease, HHV-7 does not appear to have any significant clinical impact. Potential synergism between HHV-6 and HHV-7 may occasionally occur, predisposing to seizures in children (Hall *et al.*, 2006).

2.2.3 Gamma herpes viruses

Epstein–Barr virus

Epstein–Barr virus (EBV) belongs to the gamma-1 herpes viruses. This virus has strong associations with the mouth by virtue of the oral epithelium being a site of persistence, replication and infection. A detailed discussion of the spectrum of EBV-associated disease, epidemiology and pathogenesis can be found elsewhere (Kutok and Wang, 2006). The present discussion will focus upon the oral consequences of EBV.

Epstein–Barr virus affects 95% of humans in the first few decades of life. In developing countries there can be early exposure to EBV and the associated primary infections are usually unremarkable. In developed countries, infection is often delayed such that EBV infection first presents in adolescence or early adulthood, giving rise to a self-limiting disorder termed infectious mononucleosis (IM) (Kutok and Wang, 2006). Following primary infection, EBV persists asymptomatically, being particularly persistent within circulating B cells and perhaps oral epithelial cells. Transmission of EBV is typically via saliva.

Infectious mononucleosis

Infectious mononucleosis represents primary infection of EBV. Following an incubation time of several weeks, patients developed pharyngitis, pyrexia, cervical lymphadenopathy and possibly hepatosplenomegaly. There may be petechiae of the palate and some patients have a small number of superficial oral mucosal ulcers (Scully *et al.*, 2004). At the time of this acute infection, cell-free virus is present in throat washings and saliva indicating a high degree of active viral replication (Gerber *et al.*, 1972). This replication may occur in B lymphocytes, for example within tonsil lymphoid tissue, and possibly within oral epithelial cells of the mouth and pharynx (Sixbey *et al.*, 1983; Karajannis *et al.*, 1997).

Following this primary infection, EBV persists as a latent infection in peripheral blood lymphocytes (typically B cells) and as a lytic infection of the oral mucosa (Kutok and Wang, 2006). Reactivation of the virus giving rise to oral and other systemic disorders may be likely with a variety of immunodeficient states, particularly those involving iatrogenic immunosuppression, HIV disease and a number of primary immune deficiencies (e.g. X-linked lymphoproliferative disorders (XLP) (Sayos *et al.*, 1998). There is no effective specific therapy for infectious mononucleosis, treatment being directed towards a control of symptoms.

Oral hairy leukoplakia

Oral hairy leukoplakia is a common oral manifestation of HIV disease that can also arise in a variety of other immunosuppressive states, particularly iatrogenic immunosuppression. Oral hairy leukoplakia gives rise to adherent white patches which occur bilaterally on the lateral borders of the tongue and the floor of the mouth. Occasionally there may be extensive involvement of the dorsum of the tongue and there have been reports of involvement of the gingiva, buccal mucosa and pharyngeal mucosa. These lesions are asymptomatic and have no malignant potential (Triantos *et al.*, 1997; Scully *et al.*, 1998). Histopathologically there is extensive acanthosis and some koilocyte presentation, this reflecting the expression of lytic viral genes (Triantos *et al.*, 1997). The extent of oral hairy leukoplakia varies with the degree of systemic immunosuppression; indeed the frequency and severity of oral hairy leukoplakia in HIV disease have reduced following the advent of ART (Frezzini *et al.*, 2005). Aciclovir and other antiherpetic regimes (e.g. valaciclovir and ganciclovir) can cause reduction or indeed resolution of oral hairy leukoplakia but, as these lesions are asymptomatic and have no malignant potential, therapy is not advised (Frezzini *et al.*, 2005).

Non-Hodgkin's lymphoma

Epstein–Barr virus is strongly associated with the endemic form of Burkitt's lymphoma (Kutok and Wang, 2006). Similarly, EBV is associated with 40% of classic Hodgkin's lymphoma (Kutok and Wang, 2006). Of greater relevance to the oral cavity, approximately 50% of B cell type non-Hodgkin's lymphoma types of HIV disease are associated with EBV, suggesting perhaps that EBV drives the development of these tumours, particularly when cell-mediated immunity is failing (Frezzini *et al.*, 2005).

The nasal type of extranodal natural killer cell/T cell lymphoma (ENKTCL) is strongly associated with Epstein–Barr virus (reviewed by Al-Hakeem *et al.*, 2007). Although rarely arising within the mouth, ENKTCL can extend from the nose and paranasal sinus to the hard and soft palate. The uvula, posterior wall of the pharynx, posterior third of the tongue and maxillary gingivae can also be affected. Palatal ulceration mainly commences in the anterior hard palate and slowly extends to the soft palate ultimately causing destruction of the uvula. Spread of palatal tumours to the buccinator space can arise and trismus can occur following spread of tumour to the muscles of mastication. Gingival involvement (typically of the maxilla) of ENKTCL gives rise to painful necrotic ulceration with later bony destruction, tooth mobility and exfoliation of teeth. There may be notable oral malodour as a consequence of intra-oral necrosis.

Although arising in all geographic regions of the world, ENKTCL is much more common in persons resident in south east Asia (Aozasa *et al.*, 1992; Seki *et al.*, 2001), meso-America and southern America (Arber *et al.*,

1993). This geographic distribution perhaps reflects the prevalence of different EBV strains or variants with a high malignant transformation efficiency and/or perhaps ethnicity, or geography-associated susceptibility to endemic EBV infection (Al-Hakeem *et al.*, 2007).

EBV has a central role for a number of lymphoid tumours within and outwith the mouth, particularly in immunosuppressed hosts. In addition, however, EBV is associated with epithelial tumours such as the non-keratinising type of nasopharyngeal carcinoma (Wei and Sham, 2005) and lymphoepithelioma-like tumours (Imai *et al.*, 1994). Nevertheless, it does not appear to be associated with oral squamous cell carcinoma.

2.2.4 Human herpes virus 8

Human herpes virus 8 (HHV-8) is a gamma herpes virus and the cause of all types of Kaposi's sarcoma (Leao *et al.*, 2006). Additionally, HHV-8 is the causative agent of pleural effusion lymphoma (PEL) and multicentric Castleman's disease (Leao *et al*., 2006). Oral Kaposi's sarcoma arises in patients with disease secondary to HIV infection and iatrogenic immunosuppression and may also be a feature of patients with endemic Kaposi's sarcoma. Lesions manifest as blue, red, purple macules, papules, nodules or destructive ulcers typically at the junction of the hard and soft palate or on the gingivae (particularly upper gingivae) (Frezzini *et al.*, 2005). Confusingly, occasionally Kaposi's sarcoma may be non-pigmented and hence mimic oral squamous cell carcinoma.

The primary mode of transmission of HHV-8 remains unclear; certainly there is strong evidence for both sexual and non-sexual transmission. There have been many reports suggesting that the mouth and oropharynx are dominant sites of HHV-8 shedding and that saliva is an important vehicle of HHV-8 transmission (Beyari *et al.*, 2005).

The virus is present in whole saliva, but is much less common in parotid fluid. HHV-8 DNA has previously been found in the buccal epithelial cells of healthy immunocompetent individuals and buccal and palatal exfoliates of patients with non-oral Kaposi's sarcoma (Beyari *et al.*, 2005). The virus has the ability to infect primary human keratinocytes (Duus *et al.*, 2004). A model of reactivation and shedding similar to Epstein–Barr virus has been suggested in which HHV-8 is shed into the mouth from reactivation in latently infected B cells infecting the epithelial cells that line the lymph nodes (Ivarsson *et al.*, 1999). Alternative models suggest that HHV-8 persists and replicates in oral epithelial cells and indeed is a possible principal source of HHV-8 in saliva (Viera *et al.*, 1997; Duus *et al.*, 2004). Saliva may be a significant vehicle of mother-to-child transmission of HHV-8 (Dedicoat *et al.*, 2004) and use of saliva in healing medical practices, religious initiation or ritual practices may also be a potential route of transmission of this virus (Wojcicki, 2003; Wojcicki *et al.*, 2007).

2.3 Human papilloma virus

Human papilloma viruses (HPV) comprise a group of DNA viruses of approximately 200 different types. These viruses are classified according to the tissue tropism into dermatotropic and mucosotropic. Detailed reviews of HPV can be found elsewhere (Zur Hausen, 2002). HPV infection can give rise to a wide range of usually benign skin and squamous mucosal lesions. These include plantar warts, flat warts, anal and genital condyloma acuminata and oral papillomas. Of significance, however, HPV type 16, 18 and 45 are the principal viruses associated with cervical malignancy (Zur Hausen, 2002). Human papilloma virus is typically transmitted by direct contact with an infected lesion, for example squamous papillomas may arise as a result of auto-inoculation following biting of a wart of the skin. Similarly, condyloma may arise as a consequence of oral sex. Non-sexual transmission via oral fluids or food utensils may account for acquisition of multifocal epithelial hyperplasia (as detailed below).

Human papilloma virus can give rise to, or be associated with, a number of oral lesions. Probably the most common are squamous cell papillomas (SCP). These manifest as small finger-like projections resulting in a lesion with a rough or cauliflower-like surface. HPV 6 and HPV 11 are the most commonly isolated genotypes in these lesions (Praetorius, 1997). Condyloma acuminata, considered to be sexually transmitted as a consequence of sexual acquisition (e.g. via oral sex and possibly maternal transmission) gives rise to similar lesions to those of SCP. HPV types 2, 6 and 11 seem to be the more commonly isolated genotypes of condyloma acuminata (Zeuss *et al.*, 1991; Praetorius, 1997; Syrjanen, 2003). Verucca vulgaris, often indistinguishable from SCP and condyloma acuminata, may arise on the oral mucosa. HPV types 2 and 57 are the most commonly associated genotypes, although HPV 6 and 11 have sometimes been detected (Praetorius, 1997; Syrjanen, 2003).

Multifocal epithelial hyperplasia (MEH), sometimes termed Heck's disease, gives rise to multiple soft, flat or rounded elevated nodules and is probably the most technically significant HPV disorder of the mouth. These lesions are typically asymptomatic, persist for several years and regress spontaneously. The lesions of MEH occur exclusively on the oral mucosa, being commonly located on the mucosa of the lower lip and the buccal mucosa. Lesions can arise at other sites, but involvement of the floor of the mouth, soft palate and oropharynx is unusual (Harris and van Wyk, 1993; Carlos and Sedano, 1994; Terzhalmy *et al.*, 2001).

Worldwide, MEH is rare, although it can be observed among certain ethnic and racial groups. It is particularly found among Inuit Indians resident in North, Central and South America, Eskimos from Greenland and North Canada and descendants of Khoi-San in South Africa (Harris and van Wyk, 1993; Herbert and Lopez, 1997; Jaramillo and Rodriguez, 1991; Michael *et al.*, 1999; Tan *et al.*, 1995). Multifocal epithelial hyperplasia is

more common in children in adolescence than in adults in most examined groups (Carlos and Sedano, 1994), although it has been reported that in Eskimos MEH is more common in adults than in children. The precise reason why MEH is more common in the first two decades of life than in other age groups is unclear, although a probably unlikely suggestion is that the impaired immune development may underlie the development of MEH and the later development of adaptive immunity eliminates the virus and causes regression of disease.

While many HPV genotypes have been detected in MEH lesions, types 13 and 32 are the most commonly associated types, representing 75–100% of detected HPV (Syrjanen and Syrjanen, 2000). The clustering of MEH, particularly in deprived groups and ethnically related cohorts, suggests that both social and genetic factors may underlie transmission and acquisition of the causative HPV genotypes (Harris and van Wyk, 1993; Carlos and Sedano, 1994; Borborema-Santos *et al.*, 2006).

Associations between HPV and oral squamous cell carcinoma (OSCC) (and other head and neck carcinomas) have been proposed (Syrjanen, 2003; Syrjanen, 2005). Typically, most head and neck squamous cell carcinomas (HNSCCs) reflect tobacco and/or alcohol usage. Nevertheless, there are a group of individuals who develop HNSCC in the absence of exposure to these risk factors. It has been observed that HPV-positive HNSCCs were statistically associated with individuals with a history of multiple sexual partners, practising oral sex and with a previous history of genital warts, all perhaps suggested sexual transmission (Schwartz *et al.*, 1998; Herrero *et al.*, 2003). Approximately 20% of all OSCCs contained HPV, this being typically HPV-6 or perhaps HPV-18 (Syrjanen, 2005). The relationship between HPV-positive HNSCC and prognosis/survival rate is not consistent and it has been suggested that patients with HPV-infected tumours have a better outcome compared with those without such infection (Smith *et al.*, 2004). Other studies have, however, observed no such differences (Clayman *et al.*, 1994).

Oral squamous cell carcinoma is sometimes preceded by a potentially malignant disease such as oral lichen planus and, more commonly, oral epithelial dysplasia. If HPV was to have an oncogenic role in OSCC, it might be expected that the virus is present in these lesions. Interestingly, HPV has been detected in 15–87% of examined oral lichen planus samples, 10–89% of proliferative verrucous leukoplakia and up to 85% of lesions with clinical and/or histopathological features of likely oral epithelial dysplasia (reviewed by Ha and Califano, 2004). Nevertheless, although the most commonly isolated genotype of these lesions is HPV 16, it is evident that the vast majority of squamous cell carcinomas are not HPV driven, but instead reflect social factors such as high tobacco and/or alcohol usage. Perhaps the true oncogenic potential of HPV 16 (and 18) in the development of OSCC and other HNSCCs will become apparent in about 40 years time when the clinical benefits of HPV vaccination may become evident.

There remains no specific therapy for HPV infection of the mouth. Typical therapies include both surgical or laser excision and cryotherapy (Scully *et al.*, 2004). Interferons, either administered systemically or topically, have proven effective in small numbers of patients with MEH (Kose *et al.*, 2001; Steinhoff *et al.*, 2001). The precise benefits of imiquimod for the treatment of HPV infection of the mouth are unknown.

2.4 Hepatitis viruses

Although oral lichen planus has very rarely been associated with hepatitis B (HBV) infection, similarly there have been case reports of vaccination against HBV giving rise to lichen planus of the mouth. In recent years there has been considerable interest of the impact of hepatitis C virus (HCV) upon mouth and salivary glands. HCV infection is a major health care problem worldwide; worldwide prevalence of HCV may be 2%, the majority of infected individuals being outside the Americas and Europe. In the developed world, HCV is the leading indication for liver transplantation. Detailed discussions of the epidemiology, virology (Simmonds *et al.*, 2005) and oral aspects of HCV can be found elsewhere.

The oral manifestations of HCV disease principally centre upon HCV-associated sialadenitis (Porter, 2008). Xerostomia and/or salivary gland enlargement affect up to 80% of examined individuals with HCV infection (Carrozzo and Gandolfo, 2003). While lacrimal gland dysfunction can also occur, HCV does not cause Sjogren's syndrome (SS). The histopathology of HCV sialadenitis does not include the same pattern or lymphocyte infiltration as SS; however, a transgenic mouse model carrying HCV envelope genes E1 and E2 did develop sialadenitis with lymphocytic infiltrates that resembled those of SS (Haddad *et al.*, 1992). The precise pathogenic mechanism of HCV sialadenitis remains unknown.

Oral lichen planus (OLP) has been observed in a minority of patients with HCV infection. The exact, if any, aetiological link between HCV-associated OLP and this virus infection remains controversial. Anti-epithelial antibodies have been detected in patients with HCV-related OLP (Lodi *et al.*, 1997); however, these may not be of aetiological relevance as interferon alpha therapy can induce such antibodies (Fleischmann *et al.*, 1996). Both positive and negative strands of HCV have been detected in 82–93% and 21–36% of examined HCV-associated OLP lesions (Carrozzo *et al.*, 2002), but HCV-RNA has also been detected in the normal oral epithelium of HCV-infected individuals (Arrieta *et al.*, 2000). Some geographic variation in the prevalence of HCV-related OLP has been observed, perhaps suggesting an immunogenetic basis to this association. A recent study demonstrated there was an increased frequency of HLA DR 6 in Italian individuals with HCV-related OLP when compared with Italians with HIV, but not HCV or OLP, and in UK individuals with OLP only (Carrozzo *et al.*, 2005).

It is known that non-Hodgkin's lymphoma (NHL) can be an uncommon, non-hepatic feature of HCV infection. Perhaps unsurprisingly, NHL within parotid glands has been observed in a small number of patients with HCV disease (Ascoli *et al.*, 1998; Luppi *et al.*, 1996). Associations between OSCC and HCV infection have been suggested (e.g. Nagao *et al.*, 1995); however, HCV infection is not common in oral epithelial dysplasia (Jaber *et al.*, 2003).

2.5 Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV) infection affects people in all countries in the world, but the great majority of infected individuals reside in the developing world. HIV disease is the most common cause of early death in Africa and possibly the fourth most common cause of such deaths globally (UNAIDS, 2007). This disease has a significant impact upon the mouth by virtue of the wide range of opportunistic infections that may and can arise, and the oral consequences of ART. The present discussion will centre upon common oral manifestations of HIV disease and the carriage of HIV within the mouth.

The oral manifestations of HIV disease worldwide depend upon the availability of ART. As a consequence, HIV disease can be considered to be two world disorders; in the developed world therapies are easily available and manifestations may be infrequent or not severe, but in the developing world as ART has a limited availability, clinical manifestations are frequent and significant. As a consequence of HIV-associated immunosuppression, particularly of cell-mediated immunity, the potential oral manifestations of HIV disease centre upon an increased liability to fungal, viral and mycobacterial infection. In addition, there is an increased risk of a variety of bacterial infections, perhaps including periodontal disease. Finally, patients with untreated, poorly responsive HIV disease have an increased liability to Kaposi's sarcoma and non-Hodgkin's lymphoma (Frezzini *et al.*, 2005).

2.5.1 Fungal infection in HIV disease

Superficial mycotic infection, particularly oral candidosis, is the most prevalent oral disorder of HIV infection. Pseudomembranous candidosis is the most common presentation, followed by erythematous candidosis, angular cheilitis and hyperplastic candidosis (Frezzini *et al.*, 2005) (see below for additional details of clinical presentations). The frequency of oral candidosis is associated with the CD4+T-lymphocyte count and/or HIV load, although other risk factors may include age under 35 years, injecting drug use and smoking more than 20 cigarettes per day (Campo *et al.*, 2002; Kerdpon *et al.*, 2004). *Candida albicans* is the predominant yeast colonising

in the oral cavity in HIV-infected individuals (Schoofs *et al.*, 1998), although species such as *C. glabrata, C. kruseii and C. tropicalis* may be isolated (Coleman *et al.*, 1998). There has been considerable interest in the isolation of *C. dubliniensis* from persons with HIV disease, although this infection is not restricted to HIV infection (Frezzini *et al.*, 2005). Multiple strains and genetically different isolates of *C. albicans* may be harboured in the mouth of HIV-infected individuals, and these isolates may produce significantly higher levels of secreted aspartyl proteinase (SEP) than those of non-HIV-infected individuals. The widespread use of fluconazole as treatment and prophylaxis of fungal infection of HIV disease has led to the emergence of strains resistant to fluconazole and other azoles, although there may be continued sensitivity to non-azole antifungals. This antifungal susceptibility seems to vary with the country of residence (perhaps reflecting clinical practice) and candidal strain.

Systemic mycotic infections are rare in the mouth, although when described they have generally been in individuals with HIV disease or other immunosuppressed states (see later discussion). There have been small numbers of reports of oral lesions alone or secondary to disseminated histoplasmosis in persons resident in Africa or Brazil (Hodgson and Rachanis, 2002). HIV-related orofacial aspergillosis manifesting as sinusitis has been observed and chronic ulceration of the oral mucosa or lips secondary to blastomycosis of HIV disease has been documented (Scully *et al.*, 1998). Likewise, mucormycosis giving rise to thrush-like disease or bony destruction has been observed in small numbers of HIV-infected individuals. Mucosal and gingival ulceration secondary to *Cryptococcus neoformans* has been reported in patients both in the developing and developed world (Hodgson and Rachanis, 2002). Finally, Paracoccidioidomycosis giving rise to 'moriform' stomatitis and alveolar bone destruction and tooth loss has been observed in HIV-infected residents of South America, particularly in parts of Brazil (Almeida *et al.*, 2003).

2.5.2 Viral infections in HIV disease

Infections by herpes simplex virus 1 and VZV have been suggested to be features of HIV disease, although these appear to be unusual events (Frezzini *et al.*, 2005). Instances of atypical presentation (e.g. persistent ulceration secondary to HSV-1) have been reported (Santos *et al.*, 2001) and trigeminal herpes zoster infection may occur in 17% of patients with reactivation of mucocutaneous HSV infection secondary to ART-associated immune reconstitution.

As discussed previously, EBV gives rise to oral hairy leukoplakia; indeed this lesion was first observed in patients with HIV infection. The prevalence of oral Hodgkin's lymphoma (OHL) in HIV-infected adults varies from 0.42 to 38% and in most developed and developing countries OHL appears

to be more common in males than in females (Frezzini *et al.*, 2005). There is no strong association between number of insertive oral sex male partners in the instance of OHL, and exposure to multiple strains of EBV may not increase the risk of developing OHL. The development of OHL is associated with a low CD4+T-cell count and a high viral load, but data are conflicting. As discussed previously, therapy for OHL in HIV disease is not warranted.

EBV is associated with NHL of HIV disease; indeed 50% of all HIV-related lymphomas appear to be associated with EBV. Oral plasmablastic lymphoma is possibly associated with dual viral infection by HHV-8 and EBV. EBV may account also for some of the small numbers of individuals who have OHL secondary to HIV infection.

2.5.3 Kaposi's sarcoma

(HHV-8)-Kaposi's sarcoma remains the most common oral malignancy of HIV disease. The prevalence of oral Kaposi's sarcoma (KS) varies from 0 to 12% in Africa and from 0 to 38% in the USA and Europe. In the developed world, the incidence of HIV-related oral KS is declining, partly as a consequence of the availability of ART, although in countries such as Africa, oral KS remains a frequent complication of HIV disease (Frezzini *et al.*, 2005).

2.5.4 Human papilloma virus infection in HIV disease

Estimates of oral HPV prevalence in non-HIV-infected individuals are highly variable, although it is estimated that perhaps just under 20% of examined patients may have HPV in exfoliated oral cells (Schwartz *et al.*, 1998). HIV-infected individuals may be more likely to carry HPV in the mouth than immunocompetent individuals, be infected by more than one HPV type and to carry a high risk genotype (e.g. HPV-16). Oral HPV infection (either carried and/or clinical presentation) seems to be associated with male gender, HSV-2 seropositivity and oro-genital contacts. In one study, oral sex with more than one partner during a preceding 12-month period was associated with an estimated 13-fold increase in the odds of having an oral HPV infection. Oral HPV infection may frequently present clinically in patients who have recently received ART. This enhanced HPV infection of the mouth may represent some functional ineffectiveness of newly generated CD4+T cells. Of interest and perhaps concern, HPV-related lesions in HIV disease may be associated with moderate or severe oral epithelial dysplasia, and similar to OSCC, dysplastic oral warts in HIV disease may have an overexpression of Ki67. It remains to be seen if ART-associated HPV infection will truly drive the emergence of OSCC in HIV disease (Frezzini *et al.*, 2005).

2.5.5 HIV-related salivary gland disease

HIV-related salivary gland disease (HIV-SGD) is characterised by salivary gland swelling, typically in one or more parotid glands, with or without xerostomia (Porter, 2008). Salivary gland swelling arises in approximately 3–10% of reported adults infected with HIV, this possibly being higher in children. The swelling arises as a consequence of a variety of aetiologies including reactive-inflammatory conditions, infections (e.g. acute suppurative sialadenitis) and neoplasms (KS or NHL) (Frezzini *et al.*, 2005). In some instances, the enlargement particularly represents a manifestation of diffuse infiltrative CD8+ lymphocytosis syndrome (DILS) or lymphoepithelial cyst.

Xerostomia or salivary gland hypofunction occurs in 2–30% of HIV-infected patients. This reduced salivary function may arise as a consequence of HIV infection, as an adverse side effect of anti-HIV therapy or associated with significant major salivary gland disease, particularly DILS. A detailed discussion of HIV-SGD can be found elsewhere (Frezzini *et al.*, 2005; Porter, 2008).

2.5.6 Gingival and periodontal disease in HIV disease

The majority of gingival and periodontal disease in HIV infection reflects typical plaque-related gingivitis and periodontitis. Nevertheless, small groups of individuals may have more aggressive disease manifesting as acute necrotising ulcerative gingivitis (ANUG) and periodonitis (NUP) and necrotising stomatitis. Gingival inflammation giving rise to a band of erythema both of the free and attached gingivae has been reported in 0–11.9% of adults with HIV infection, although a much higher prevalence rate has been observed in India (Frezzini *et al.*, 2005; Porter, 2008). Necrotising ulcerative gingivitis and periodontitis have been reported in up to 10% of HIV-infected people, although a very high rate of 27.7% was observed in Cambodia (Bendick *et al.*, 2002).

A detailed discussion of the immunological and microbiological aspects of gingival and periodontal disease in HIV infection can be found elsewhere (Frezzini *et al.*, 2005). Of note, although the microbiology of HIV-related periodontal disease is generally similar to that expected in otherwise healthy individuals, associations between periodontitis and HIV-seropositive patients in co-infection of EBV and human herpes viruses 6, 7 and 8 have been proposed. Mono- or co-infection with herpes viruses appears to be positively associated with elevated levels of periodontopathic bacteria such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythensis, Prevotella nigrescens* and *Treponema denticola*. It is possible that the immunosuppressive influence of HIV disease could facilitate the selective overgrowth of specific pathogenic bacteria and force host cells to release tissue-destructive cytokines.

2.5.7 Dental disease in HIV infection

HIV-infected children may be more liable to dental caries than healthy subjects; however, this remains controversial; it may be that HIV-infected children display a different decay pattern to that of healthy children, as a consequence of HIV-associated xerostomia. Other possible reasons for this change in caries pattern include high carbohydrate and sugar intake in order to provide sufficient calorific intake and/or the ingestion of sucrose-based medications. Poor socioeconomic status, low use of fluoride and/or ART-related xerostomia may also contribute to an increased risk of dental caries in children with HIV disease (Frezzini *et al.*, 2005).

2.5.8 Oral aspects of HIV therapy

Antiretroviral therapy without doubt reduces the frequency and/or severity of many of the oral consequences of HIV disease. It has been suggested that ART may increase the instance of HIV salivary gland disease and, at least in the short-term, increase the risk of HPV infection of the mouth. A wide range of adverse oral side effects can arise with ART; in particular, nucleoside analogue reverse transcriptase inhibitors (NRTIs) can give rise to neutropenic oral ulcers, erythema multiforme, toxic epidermal necrolysis, lichenoid reactions and mucocutaneous pigmentation. Protease inhibitors (PIs) can give rise to dysgeusia and oral perioral paraesthesia (Frezzini *et al.*, 2005; Scully *et al.*, 2004).

2.6 Fungal infections

2.6.1 Superficial infection

Infection with candida species, particularly *Candida albicans*, is common in the mouth. *C. albicans* is by far the most common oral candidal species present in up to 60% of healthy individuals.

A large number of non-albicans candida species can also be found in the mouth including *Candida glabrata, C. tropicalis, C. parapsilosis, C. kruseii, C. dubliniensis, C. lusitaniae* and *C. guilliermondii.* Some of these non-albicans candida species have the potential to give rise to clinical disease, particularly in immunocompromised persons, but *C. albicans* remains the most common fungal pathogen of the mouth. The present discussion will focus upon the clinical manifestations of candidal infection of the mouth. A detailed review of the contemporary management of candidal infections can be found elsewhere (Zonios and Bennett, 2008).

Candida species can give rise to a wide spectrum of disorders in humans, particularly in immunocompromised individuals. These vary from superficial mucosal disease to rare life-threatening disseminated invasive infection. Factors that predispose to clinical manifestation of candidal infection include immunosuppression, in particular in HIV disease and iatrogenic

immunosuppression (Frezzini *et al.*, 2005), the presence of dental prostheses (Coelho *et al.*, 2004), xerostomia (Porter *et al.*, 2004), tobacco smoking (Rindum *et al.*, 2004) and the short-term use of broad spectrum antibiotics. It has been suggested that nutritional deficiency such as iron, folic acid, other vitamins and diets rich in carbohydrates may predispose to oral candidosis (Samaranayake, 1986), but there really is little supportive evidence of this.

Because of variable clinical manifestations of oral candidosis and its multi-factor aetiology, the classification oral candidal infection is difficult. A number of classifications have been proposed (Ellepola and Samanarayake, 2000), but the present discussion will focus on the likely oral consequences of candidal infection.

2.6.2 Pseudomembranous candidosis (thrush)

Pseudomembranous candidosis gives rise to soft, friable, white plaques resembling milk curd. These typically arise on the hard and soft palates and posterior buccal mucosa, being easily scraped off to leave underlying areas of erythematous and sometimes bleeding oral mucosa. Note that thrush is usually painless. This disorder commonly arises in HIV disease, undiagnosed leukaemia, long-standing xerostomia, recurrent use of topical and systemic broad spectrum antibiotics, corticosteroids, other types of iatrogenic immunosuppression and perhaps diabetes mellitus (Scully *et al.*, 1994).

2.6.3 Atrophic (erythematous) candidosis

This usually manifests as a painless erythematous area of the buccal mucosa and dorsum of the tongue. The palate may be simultaneously affected giving rise to a contact or 'kissing' lesion (Reichart *et al.*, 2000). Erythematous candidosis may arise *de novo* or as a consequence of shedding of the pseudomembrane of thrush. It is suggested that erythematous candidosis is common in HIV disease, although it may also arise with iatrogenic immunosuppression and recurring use of broad spectrum antibiotics (Scully *et al.*, 1994; Frezzini *et al.*, 2005).

2.6.4 Hyperplastic candidosis (candidal leukoplakia)

Hyperplastic candidosis manifests as adherent white patches, typically at the commissures of the mouth (Sitheeque and Samaranayake, 2003). They may arise at other sites, particularly if the patient has chronic mucocutaneous candidosis. Hyperplastic candidosis also manifests as homogenous or speckled lesions. This disorder typically arises as a consequence of tobacco smoking (Epstein and Polsky, 1998), although it may also be a feature of HIV disease and other immunosuppressive states. It has been suggested

that chronic hyperplastic candidosis may be potentially malignant as there have been occasional reports of malignant transformation of such lesions and candida isolated from such disease may generate a question mark. Note that this suggestive malignant potential of *C. albicans* is not limited to chronic hyperplastic candidosis; indeed associations between severity of oral epithelial dysplasia at other sites and the presence of *C. albicans* have been proposed (McCullough *et al.*, 2002).

2.6.5 Denture-related (associated) stomatitis

Denture-related stomatitis (DRS) manifests as a painless erythematous area of a denture-wearing area of mucosa (Muzyka and Glick, 1995). It typically arises beneath maxillary dentures (Farah *et al.*, 2000), although can occasionally arise in people with orthodontic appliances. It has been suggested that a combination of *C. albicans* and other microorganisms may be responsible for DRS, in particular bacteria such as *Streptococcus, Veillonella, Lactobacillus, Prevotella* and *Actinomyces* species as well as non-albicans candida species (Koopmans *et al.*, 1988). Denture-related stomatitis typically arises in individuals with poor denture hygiene, the candida species giving rise to a biofilm from which proteolytic agents are then released (Lamfon *et al.*, 2003). Patients with DRS sometimes have angular cheilitis (see below); this probably because the denture has become worn and hence lip support lessening.

2.6.6 Angular cheilitis

Angular cheilitis manifests as erythematous cracks/fissures at one or both angles of the mouth (Ellepola and Samaranayake, 2000). In some instances these abnormalities may extend to the facial folds. Angular cheilitis probably arises as a consequence of accumulation of saliva containing candida, occasionally streptococci and *Staphylococcus aureus* at the corners of the mouth. Angular cheilitis typically arises due to a decreased vertical dimension of the denture occlusion caused by resorption of the underlying bone, or wear of the occlusal surfaces of dentures. Less common contributing factors may include deficiency of iron and/or vitamins B6, B12 and folic acid (Blanck *et al.*, 2002). Poorly controlled diabetes mellitus, HIV disease and iatrogenic immunosuppression may be additional contributory factors (Ranganathan *et al.*, 2004).

2.6.7 Median rhomboid glossitis

Median rhomboid glossitis manifests as a painless, usually symmetrical, area of erythema of the dorsum of the tongue anterior to the circumvalate papilla. The erythema has a rhomboidal or elliptical shape. Occasionally the mucosa can have a hyperplastic exophytic appearance. Median rhomboid

glossitis typically arises in patients with xerostomia, long-term tobacco smoking and it may be feature of HIV disease, long-term topical corticosteroid therapy (e.g. asthmatic inhalers) and iatrogenic immunosuppression (Scully *et al.*, 1994).

2.6.8 Chronic mucocutaneous candidosis

Chronic mucocutaneous candidosis comprises a group of rare disorders characterised by recurrent and/or persistent candidal infection of the mucocutaneous surfaces. This group may be subclassified as familial, diffuse, candidosis endrocrinopathy syndrome and candidosis thymoma syndrome. Affected patients usually have recurrent episodes of any of the aforementioned candidal infections of the mouth. Of note, there may also be extensive adherent white patches that histopathologically resemble chronic hyperplastic candidosis. A detailed discussion of chronic mucocutaneous candidosis can be found elsewhere (Scully *et al.*, 1994).

2.7 Paracoccidioidomycosis

Paracoccidioidomycosis (Pmycosis, formerly termed Lutz's disease) usually arises in Brazil, with São Paulo, Paraná, Rio Grande do Sul, Goiás and Rio de Janeiro being the most prevalent areas, but it is also seen in other countries, particularly in Colombia and Venezuela and also in Chile, Guayana French Guayana and Surinam (Almeida *et al.*, 2003). Paracoccidioidomycosis is caused by *Paracoccidioides brasiliensis*, a fungus found in the soil of certain areas of Latin America, from Mexico in the north to Argentina in the south. In soil, the fungus *P. brasiliensis* grows as a mycelium. Humans seems to be susceptible to infection with conidia (formed from the mycelium when nutrition and water are poor) and is the only host susceptible to the disease, although nine-banded armadillos can harbour the fungus. Armadillos can be an important reservoir and transmission is perhaps via armadillo faeces in the soil. Inhalation of the conidium form of the fungus and transformation into a yeast in the tissues, with a primary infection of the lungs and dissemination via lymphatic and blood vessels, are the most usual mechanisms of infection. There is no evidence of human-to-human transmission of *P. brasiliensis*. Immunosuppressive disease, particularly iatrogenic immunosuppression, and immunodeficiency such as HIV infection can predispose to parracoccidiodomycosis, possible via reactivation of disease, but the majority of persons with Pmycosis are not notably immunosuppressed (Almeida *et al.*, 2003).

Pmycosis gives rise to chronic disease, usually of the lungs, oropharynx and lymph nodes (including the cervical chain). The gastrointestinal tract is also often involved. Haematogenous dissemination of Pmycosis to the abdominal lymph nodes, spleen, liver, adrenal glands, bones, skin, or brain

can result in life-threatening complications. There may be eventual spread to the central nervous system, bones or other tissues. Latent infections can flourish after many years, and relapse is common. Rarely, the patient can present an asymptomatic pulmonary lesion called a paracoccidioidoma.

Oral involvement is common, arising in up to 48% of patients. Usually, the oral lesions are multiple, involving the lip, gingivae, buccal mucosa, palate, tongue and floor of the mouth. The oral lesions typically show an erythematous finely granular hyperplasia, speckled with pinpoint haemorrhages, and a mulberry-like surface called 'moriforme' stomatitis. Ulceration is common. Involvement of the lips causes a pronounced increase in thickness and consistency. The juvenile form can cause alveolar bone destruction and tooth loss. Oral paracoccidioidomycosis may rarely cause perforation of the hard palate.

The gingivae, particularly of the upper jaw, are a common site of involvement of oral paracoccidioidomycosis. Lesions manifest as chronic areas of ulceration, having a mulberry-like appearance with pin-point haemorrhages. There may be destruction of the underlying bone. The ulceration can be of variable size and can potentially be mistaken for oral squamous cell carcinoma.

Antifungal therapy is required, although relapse is possible. Initial treatment lasts from 2 to 6 months and includes sulphonamides, amphotericin B, or imidazoles. Systemic azoles are considered to be the most effective agents, although there remains some uncertainty as to the most effective therapeutic regime (Menezes *et al.*, 2006). Following initial treatment, maintenance with sulphadimethoxine or sulphadoxine for about two years is required (Almeida *et al.*, 2003).

2.8 Aspergillosis

Aspergillosis typically affects patients with prolonged or profound neutropenia and, less frequently, invasive aspergillosis may occur in patients with diabetes mellitus. Infection can affect any part of the respiratory tract including the paranasal sinuses, larynx and lungs. Primary lesions can also be localised in the eyes, ears and oral cavity.

Invasive aspergillosis is a major cause of morbidity and death in patients with a haematological malignancy of bone marrow following bone marrow transplantation. Invasive aspergillosis usually associated with *Aspergillus fumigatus* can affect the maxillary antrum causing antral pain, facial swelling and destruction of bony walls leading to oral invasion or occasionally intracranial extension. Oral aspergillosis may reflect extension of maxillary disease or arise as a consequence of primary or secondary infection of the oral mucosa. Such infection typically gives rise to yellow or black necrotic ulcers of the palate (Scully and de Almeida, 1992; Scully *et al.*, 1998). Therapy now focuses upon agents such as voraconazole, liposomal amphotericin B, caspofungin and posaconazole (Maschmeyer *et al.*, 2007).

2.9 Mucormycosis

Mucormycosis is a rare fungal infection caused by organisms of the order *Mucorales*, the largest order of Zygomycete fungi. Mucormycosis primarily arises in patients with poorly controlled diabetes mellitus and haematological malignancy (Auluck, 2007). Patients with extensive burn injuries, chronic renal failure, prolonged corticosteroid use and deferoxamine treatment have also been reported to have mucormycosis. Approximately 50% of patients with rhino-cerebral mucormycosis have diabetes mellitus. Mucormycosis gives rise to rhino-cerebral pulmonary disease; it has also been known to infect cardiac tissue and the gastrointestinal and genitourinary tracts.

Oral mucormycosis initially arises in the nose or palate and manifests as a bloody ulceration of the nose or pseudomembrane with ulceration of the palate. The palatal ulceration comprises an extension of nasal and maxillary sinus disease, is characteristically unilateral and causes loss of the underlying bone. Ulcers caused by mucormycosis have also been observed on the gingivae, lip and alveolar ridge (Scully and de Almeida, 1992). Mucormycosis is limited to the parotid gland as described in a very small number of patients (Chandu *et al.*, 2005).

Treatment of mucormycosis requires early and aggressive surgical removal of necrotic tissue and restoration of immune function and/or diabetic control and appropriate antifungal therapy. Of note, voraconazole and caspofungin may not be particularly effective in the management of this disorder (Karanth *et al.*, 2005), although posaconazole may be of some benefit (Rogers, 2008).

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3

Non-infectious diseases of the oral mucosa

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Abstract: The oral mucosa represents the lining of the oral cavity and is commonly affected by disorders that may be specific to the mouth or may be associated with systemic conditions. The most common conditions of the oral mucosa are infections, white patches, fibrous overgrowths, oral ulceration, blistering conditions and sore mouth or stomatitis. Squamous cell carcinoma, although not common, is an important cause of oral lesions. The minor salivary glands, especially in the lips, soft palate and cheeks may also give rise to mucosal swellings. The purpose of this chapter is to present an overview of non-infectious disorders which affect the oral mucosa.

Key words: disorders of the minor salivary glands, non-infectious diseases of the mouth, oral cancer, oral mucosa.

3.1 Introduction

The oral mucosa represents the lining of the oral cavity and is composed of a stratified squamous epithelium supported in most areas by loose fibrous connective tissue. The mucosa is divided into three types. Most of the mouth, including the cheeks, the lips, floor of the mouth and underside of the tongue is covered by a simple *lining* mucosa covered by non-keratinised or parakeratinised epithelium. Elsewhere the mucosal surface must withstand the forces of mastication and the palate and gingival margins are covered by *masticatory* mucosa. This is orthokeratinised and is tightly bound down to the underlying connective tissues, in places forming a mucoperiosteum. The dorsum of the tongue is covered by *specialised* mucosa which has a papillary surface to aid mastication and swallowing and also contains many taste buds.

Diseases of the oral mucosa are common and may be specific to the mouth or may be associated with systemic conditions. The most common

conditions of the oral mucosa are infections, white patches, fibrous overgrowths, oral ulceration, vesiculobullous or blistering conditions and sore mouth or stomatitis. Carcinoma may also affect the oral mucosa and, although not common, is an important cause of oral lesions. The minor salivary glands, which are found just below the mucosa, especially in the lips, soft palate and cheeks may also give rise to mucosal swellings. The purpose of this chapter is to present an overview of non-infectious disorders which may affect the oral mucosa. Readers who desire more detailed information may wish to consult specialist textbooks (for example, Jordan and Lewis, 2004; Regezi *et al.*, 2008; Scully, 2008).

3.2 White patches of the oral mucosa

The healthy oral mucosa is pale pink, but pathological changes may result in white patches usually caused by an increase in the thickness of the keratin layer or by keratinisation in those regions of the mouth which are normally non-keratinised. There are many causes of white patches but the most common are listed in Table 3.1.

3.2.1 Frictional keratosis

Increased keratinisation may arise in response to persistent chronic trauma from such causes as cheek biting, the rubbing of a sharp tooth or from an appliance such as a denture or an orthodontic bracket. Traumatic lesions are common on the buccal mucosa, often as a result of cheek biting or on

Table 3.1 Common causes and key features of white patches of the oral mucosa

Frictional keratosis	Caused by persistent trauma; site of the lesion corresponds to cause, e.g. a sharp tooth, denture, cheek biting. The lesion disappears when the cause is removed.
Lichen planus	No obvious cause but thought to be immune-mediated. Affects middle aged and more common in females. Typically see bilateral reticular lesions in the cheeks and lateral tongue. May also be skin lesions.
Lichenoid reactions	Similar appearance to lichen planus but may be single lesions and associated with drugs. May see solitary lesions adjacent to large amalgam restorations.
Leukoplakia	White lesions, not associated with trauma and with no identifiable cause. Lesions may be homogeneous with smooth, even colouration, or non-homogeneous which may have a raised nodular or red and white speckled surface. Leukoplakia has an increased risk of malignant change and non-homogeneous lesions have the highest risk.

the tongue from other causes. A diagnosis of frictional keratosis can only be made if the source of the trauma is identified and the position of the white patch should correspond to the trauma. The cause should, if possible, be removed, and the lesion will then resolve. This effects a cure and also confirms the diagnosis.

3.2.2 Lichen planus

Lichen planus is a relatively common disorder which affects middle-aged women more than men. It is characterised by bilateral lesions most often affecting the cheeks and lateral borders of the tongue. Typically the lesions are reticular or lace-like white striations but plaque-like, erosive and ulcerated forms may be seen. Erosive lichen planus typically affects the gingivae to produce fiery red lesions called desquamative gingivitis. The common reticular form is usually symptomless but erosive lesions may cause considerable soreness and discomfort. Lichen planus may affect the skin as well as the oral mucosa and may present white striae or violet papules on the wrists or shins.

Histologically, lichen planus may show epithelial atrophy, but with areas of hyperkeratosis corresponding to the white striations seen clinically. The upper connective tissues contain a band of lymphocytes beneath the epithelium which is associated with destruction of the epithelial basal layers. This suggests that the lesions and the epithelial damage may be a result of a cell-mediated immune reaction, but the cause of this remains unknown.

3.2.3 Lichenoid reactions

Clinically, the term lichenoid is used to describe lesions which resemble lichen planus but in which a cause may be identified, usually drugs, or dental amalgam. Lichenoid drug reactions are most frequently associated with antihypertensive drugs taken for high blood pressure or oral hypoglycaemic drugs taken for diabetes. The lesions become apparent after commencement of drug therapy and resolve when the drug is withdrawn. Such lesions may be bilateral and can be difficult to distinguish from lichen planus unless a careful medical and drug history is taken.

Lichenoid reactions to an amalgam restoration are also common, but in these cases the lesions are unilateral and are found on the mucosa adjacent to, and in contact with, the restoration. Often it is the buccal mucosa and lateral borders of the tongue that are affected. Removal of the amalgam usually results in resolution of the lesion.

3.2.4 Leukoplakia

Leukoplakia is defined as a white patch which has no obvious cause and cannot be diagnosed as any identifiable disease. Thus it is a diagnosis of

exclusion which can only be made after other types of white patch such as frictional keratosis or lichen planus have been eliminated (Axell *et al*., 1996). Leukoplakia is important because it is a potentially malignant lesion. A small proportion have a higher risk of turning malignant (developing into squamous cell carcinoma) than does the normal mucosa. The clinical appearance of leukoplakia is very variable and they have been grouped into homogenous and non-homogenous types.

Homogenous leukoplakias are uniformly white and flat, although they may have an undulating or corrugated surface. This type of leukoplakia is regarded as low risk and has a negligible risk of turning malignant. Non-homogenous leukoplakias show variations in colour and texture. They may be nodular or verruciform and have a speckled red and white colour. These lesions are regarded as high risk and up to 25% may progress to squamous cell carcinoma. Lesions on the lateral border of the tongue, floor of the mouth and the retromolar area are more likely to progress to cancer. Patients who use tobacco or drink excess alcohol are also at a higher risk. Chewing tobacco or betel quid also results in a higher risk and in these patients the lesions may be seen at the site where the quid is held in the mouth, usually in the cheek.

Histological changes in leukoplakia

All white patches in which a clinical cause cannot be identified must be biopsied. At the present time, histological examination and particularly identification of the features of epithelial dysplasia are the most reliable guide to the potential for these lesions to become malignant. The risk of malignant transformation is related to how abnormal the individual epithelial cells appear (cellular atypia) and the extent and arrangement of the atypical cells throughout the thickness of the epithelium (architectural changes). These changes together are referred to as dysplasia which is graded as mild, moderate or severe (Bouquot *et al*., 2006). Lesions showing severe epithelial dysplasia have the highest risk of malignant transformation, of about 25%, and are usually completely removed. Lesions showing mild or moderate dysplasia may be kept under review.

3.3 Oral cancer

Oral cancer, or oral squamous cell carcinoma, arises from the surface epithelium of the oral mucosa and is the most common malignant neoplasm which affects the mouth. The most commonly affected sites, comprising about 80% of all lesions, are the lateral border of the tongue, floor of mouth and the retromolar region. Intra-oral cancer is associated with tobacco use and alcohol consumption and it is thought that the carcinogens from tobacco dissolve in saliva and pool in these lower regions of the mouth. In south east Asia, the use of betel quid, which contains the Areca nut and often also tobacco, is associated with a high incidence of oral cancer. Lesions occur on

the cheeks adjacent to where the quid is placed. Studies have shown that immigrant populations from south east Asia in the UK continue this habit and have a higher risk of developing oral cancer (Warnakulasuriya, 2002). Other risk factors for oral cancer include excessive exposure to sunlight which is associated with lesions on the lower lip and there is an increased risk in individuals whose diet is low in fresh fruit and vegetables.

3.3.1 Clinical features of oral cancer

The majority of cases of oral cancer occur in patients over 40 years of age, with a peak incidence in the seventh decade. Lesions are associated with alcohol and tobacco use and are more common in men than in women with a ratio of almost 2:1. In the western world the incidence of oral cancer is increasing especially in younger age groups, under 60 years. It is thought this is due to increased alcohol consumption by younger people rather than an increase in smoking (Hindle *et al*., 2000). Studies have shown that, in patients under 45 years, a significant proportion have no known risk factors (Llewellyn *et al*., 2003).

Most lesions of oral cancer, possibly over 60%, present late when lesions are large and ulcerated. Thus a typical oral cancer may appear as a non-healing fungating ulcer, 1–2 cm in diameter, with a raised margin and a firm or indurated base. Smaller lesions appear as white patches, similar to non-homogeneous leukoplakia, or as a red patch or erythroplakia. Up to 20% of patients may present with metastases in the lymph nodes of the neck. Nodes which contain tumour are usually painless, but they feel very hard and may be fixed to surrounding tissues.

Oral cancer has a poor prognosis and about 50% of patients die of the disease within five years. Early recognition of oral cancer is important and early diagnosis, particularly before the cancer has spread to the lymph nodes in the neck, may save lives. Thus all red and white patches, especially non-homogeneous lesions should be treated with suspicion.

3.3.2 Pathology of oral cancer

The epithelium proliferates excessively and malignant cells grow down into the underlying connective tissue and show considerable cytological atypia and many mitotic figures. The tumour invades and spreads into underlying tissues including muscle, salivary glands and the bone of the jaws. Malignant cells will also invade lymphatic vessels and metastasise to the cervical lymph nodes in the neck (Speight *et al*., 1996).

3.3.3 Treatment

Oral cancer is diagnosed by biopsy and histopathological examination. Management is by a multidisciplinary team including surgeons, oncologists, radiologists and specialist nurses. Treatment is usually by a combination of

surgery and radiotherapy, but the exact modality to be used depends on the size and extent of the disease at diagnosis.

3.4 Swellings of the oral mucosa

Swellings are common in the oral cavity and may occur anywhere on the oral mucosa. Most are benign lesions which form as fibrous overgrowths as a result of injury or chronic trauma. Benign neoplasms or developmental lesions may occasionally arise and, less frequently, a swelling may be due to a malignant neoplasm. Swellings may arise from the mucosa, the overlying epithelium or the underlying connective tissues or they may be related to the teeth, bone or salivary glands.

3.4.1 Swellings on the gingivae

A swelling on the gum is called an epulis and the three most common types are fibrous hyperplasia (fibrous epulis), pyogenic granuloma or a peripheral giant cell granuloma. An overview of gingival swellings and their key features is given in Table 3.2.

Fibrous epulis

These swellings are caused by an overgrowth of fibrous connective tissue in response to chronic irritation from dental plaque. The swelling is localised and usually presents interdentally between two teeth. The cause of the local accumulation of plaque may be due to accumulation of subgingival calculus, a restoration, misalignment of the teeth or the presence of an orthodontic appliance or denture. Often these lesions are pedunculated or nodular and are referred to clinically as a fibro-epithelial polyp. Fibrous hyperplasias are covered by oral epithelium and appear pale and the same colour as the surrounding mucosa. They are treated by removing the cause of the irritation and by excision.

Table 3.2 Common lesions of the gingivae

Fibrous hyperplasia (fibro-epithelial polyp)	Smooth often pedunculated. Normal colour related to chronic irritation from a local accumulation of plaque.
Pyogenic granuloma	Red, vascular lesion which bleeds easily, similar aetiology to above. May be associated with pregnancy or at puberty.
Giant cell granuloma	Red/blue vascular swelling in anterior mouth. Contains accumulations of giant cells.
Dental abscess	Red/yellow, soft/ fluctuant, associated with non-vital tooth.

Pyogenic granuloma
Pyogenic granulomas are also formed in response to chronic irritation from a local accumulation of plaque or calculus and are the result of overgrowth of immature granulation tissue. They grow rapidly and are ulcerated and not covered by epithelium. The lesions are very vascular and appear red and may bleed easily. Pyogenic granulomas are particularly common in pregnancy and during puberty when there appears to be an exaggerated response to inflammation caused by hormonal changes. They are sometimes referred as a pregnancy epulis. Treatment is to remove the cause after which some lesions will regress, but often it is necessary to excise them.

Peripheral giant cell granuloma
Giant cell granulomas occur on the gingivae, usually in the anterior parts of the mouth. They are red/blue in colour and clinically may be difficult to distinguish from a pyogenic granuloma. Lesions may grow to a large size and extend over the labial aspect of the teeth. The cause is unknown and treatment is excision. Histologically the lesions are composed of inflamed vascular fibrous tissue containing focal accumulations of multinucleated giant cells. The lesions may be indistinguishable from central giant cell lesions and it is important to exclude a central lesion by radiology.

3.4.2 Swellings at other oral sites

Fibrous overgrowths
Fibrous overgrowths and pyogenic granulomas may arise at any intra-oral site and are usually associated with trauma from biting or rubbing of an appliance such as a denture or orthodontic brackets. A common cause is persistent trauma from an over-extended or ill-fitting denture. This may result in large growths of hyperplastic tissue under the denture or at the denture margins. The histological features are similar to the lesions on the gingivae. Treatment is to remove the cause and surgically to remove the excess tissue.

Squamous papilloma
Squamous papillomas are formed as a result of proliferation of the epithelium caused by infection by human papillomavirus (HPV). The epithelium shows heavy keratinisation and is thrown into folds or fronds resulting in lesions with a white cauliflower-like appearance clinically. Common sites are the lips and palate but any site may be affected. Treatment is simple removal and lesions rarely recur.

Lipoma
These are benign tumours of adipose tissue and may be found in the cheek or tongue. They may appear yellow but are often of normal colour. They

are difficult to distinguish from fibrous hyperplasia but they are not related to chronic trauma.

Neural tumours
Benign neural tumours may occasionally arise on the oral mucosa. A common site is the tongue, but any site in the mouth may be affected. Lesions are usually solitary and most commonly are benign Schwannomas or solitary neurofibromas. Clinically they may be indistinguishable from fibrous overgrowths or fibro-epithelial polyps.

Mucous cysts
Mucocoeles are an important cause of mucosal swelling and are most common on the lower lip where they should be considered in the differential diagnosis. They are discussed below under salivary gland disease.

3.5 Oral ulceration

An ulcer is defined as a break or discontinuity in the epithelial covering of the oral mucosa. This results in an acute inflammatory response and the surrounding mucosa becomes red and inflamed. The surface of the ulcer is covered by a white/grey fibrinous slough. There are many causes of oral ulceration but the important lesions are summarised in Table 3.3.

3.5.1 Traumatic ulceration

Traumatic ulcers are most often caused by biting or irritation from a sharp tooth or are associated with the margin of an ill-fitting or over-extended denture. Occasional ulcers may be caused by chemical irritation or by a

Table 3.3 Common and important causes of oral ulceration

Traumatic ulcers	The site of the ulcer is related to the cause, usually an over-extended denture, sharp tooth or cheek biting. Typically of short duration and surrounded by a red inflamed area.
Aphthous ulceration	Common recurrent ulcers, often in young adults. Painful, small lesions, often on the lips or floor of mouth. Heal in about 10–14 days.
Squamous cell carcinoma	Non-healing ulcer with no obvious cause. Large lesions have raised margins and are firm or indurated. Common sites are lateral tongue, floor of mouth and retromolar area.
Vesiculobullous lesions	Blistering lesions which rapidly break down to give large ulcers or erosions. Usually in older individuals. May also have skin lesions.

burn from hot food. Diagnosis of a traumatic ulcer is usually not difficult and the position, shape and size of the ulceration will correspond to the suspected cause. The ulcer usually heals in 10–14 days if the cause is removed.

3.5.2 Aphthous ulcers (recurrent aphthous stomatitis)

Aphthous ulcers are common and have been described in detail in a recent review (Jurge *et al*., 2006). The cause of aphthous ulceration is not known, but in almost 50% of patients there may be a family history. Other associated factors include stress, trauma and menstruation. It is important to note that in some patients there is a relationship to iron deficiency which in itself may be associated with an underlying gastrointestinal disorder or a poor diet. Clinically, aphthous ulcers most often affect young adults and are characterised by a history of recurrence, often starting in childhood and increasing in frequency with age. The most common type of aphthous ulcers are called minor aphthae but major aphthae and herpetiform ulcers may also occur.

Minor aphthae are the most common type and are typically small, up to 5 mm across and covered by a grey or yellow slough. The surrounding mucosa may be reddened. The ulcers usually occur in crops of 2–3 at a time but may be solitary. The non-keratinised mucosa of the lateral tongue, floor of mouth or cheeks is most often affected. Lesions are painful but usually heal in about ten days. Periods of recurrence vary from occasional to almost constant repeated episodes. Herpetiform ulcers are similar, but may be a little smaller and usually arise in larger numbers with up to ten or more lesions at a time. Major aphthae are less common and differ in that they may be solitary and are usually over 10 mm in diameter. The palate and other keratinised areas are most often affected and the lesion may persist for up to 4 weeks or longer, after which they heal and may leave a scar.

3.5.3 Vesiculobullous lesions

There is a large group of lesions which are associated with blisters or vesicles on the oral mucosa which rapidly break down to cause ulcers or erosions. Many of these are caused by viruses and have been covered in Chapter 2. The remaining vesiculobullous lesions are caused by a group of diseases which are autoimmune in nature and are described as mucocutaneous because they may affect both the skin and mucous membranes. Vesiculobullous lesions which may affect the oral mucosa are summarised in Table 3.4.

The most commonly encountered are pemphigus vulgaris and mucous membrane pemphigoid. Both affect the middle aged to elderly and women more often than men. The typical clinical presentation is of widespread ulcers or erosions affecting the tongue, gingivae, lips and palate. There is

Table 3.4 Important vesiculobullous disorders which may affect the oral mucosa

	Aetiology and pathology	Clinical features
Pemphigus vulgaris	Autoimmune disease associated with antibodies against intercellular desmosomes (desmoglein). Blisters are intraepithelial due to breakdown (acantholysis) of the epithelium.	Affects skin and mucosa, but over 50% affect oral mucosa first. Large vesicles or blisters rapidly break down into painful erosions. Lesions commonly seen on tongue, lips and gingivae.
Mucous membrane pemphigoid	Autoimmune disease associated with antibodies against basement membrane proteins. Blisters are subepithelial due to separation of epithelium from the connective tissue.	Primarily affects oral mucosa and conjunctiva. Other mucosal sites may be involved but rarely the skin. Presents with blisters and ulcers. Gingivae and palate are common sites.
Bullous pemphigoid	Similar to mucous membrane pemphigoid.	Primarily affects the skin, but oral lesions may arise. Oral lesions are indistinguishable from mucous membrane pemphigoid.
Dermatitis herpetiformis	IgA autoantibodies accumulate subepithelially and cause papules and blisters. Often associated with gluten enteropathy (coeliac disease).	Rare, but affects younger adults. Primarily skin lesions with itchy papules and small blisters. Oral lesions may be small blisters but usually multiple ulcers.
Epidermolysis bullosa	A group of disorders, mostly hereditary, associated with genetic defects in intercellular or basement membrane proteins. The acquired type (epidermolysis aquisita) shows autoantibodies to basement membrane proteins.	See fragility of the skin with blister formation in response to mild trauma. Hereditary types affect infants and may cause severe cutaneous blistering followed by scars. Some types are rapidly fatal. Oral lesions may cause severe blistering and scarring followed by loss of function.

often severe pain and bleeding. Although other mucosal or cutaneous sites may be involved, the oral cavity is often the first region to be affected. It is therefore important to recognise the importance of ulcers and erosions in adults, since early diagnosis and treatment may prevent or limit the onset of widespread disease. Management is overall by the use of immunosuppressive drugs and steroids.

3.5.4 Other types of ulcer

Squamous cell carcinoma is a relatively rare but important cause of oral ulceration and has been discussed previously. Ulceration following breakdown of vesicles is a feature of viral lesions and these have been discussed in Chapter 2.

3.6 Disorders of the salivary glands

Saliva is produced by the major salivary glands – the parotid, submandibular and sublingual glands – and by many minor salivary glands which are found throughout the oral mucosa. The most common and important disorders of the salivary glands are discussed here.

3.6.1 Mucous cysts

Mucous cysts or mucocoeles are cystic swellings affecting the minor salivary glands, most commonly in the lower lip. They are most often seen in children and young people and are associated with trauma. The lesions present as sessile pale pink/blue swellings which may be fluctuant. There is often a history of rupture with exudation of a clear fluid, followed by recurrence. There are two types of mucocoele, mucous extravasation cysts and mucous retention cysts.

Mucous extravasation cysts are the most common type and are caused by trauma which ruptures the minor salivary gland duct resulting in spillage of saliva into the connective tissues. A cystic space forms which is lined by inflamed granulation tissue. Mucous extravasation cysts are seen mostly in children and are most common on the lower lip or in the floor of the mouth.

Mucous retention cysts are more rare and may be more frequently encountered in adults. They are also caused by trauma, but in this case the duct becomes blocked by fibrosis or scar tissue. The blocked duct becomes dilated to form a cyst lined by ductal epithelium. Mucous retention cysts are found more frequently on the palate and ventral surface of the tongue. In elderly patients they may be associated with trauma from a denture.

3.6.2 Salivary stones and sialadenitis

Stones or calculi may form by precipitation of calcium salts from the saliva which may become stagnated in a salivary duct. Often the calculus forms around a nidus of cellular debris within a duct. Over 80% of all calculi arise in the duct of the submandibular gland, which passes just below the mucosa of the floor of the mouth. The stone may partially block the duct, leading to accumulation and back pressure of saliva in the gland. The typical clinical presentation, therefore, is of repeated and sometimes painful swelling of

the submandibular gland. If the stone is in the superficial part of the duct, it may be visible in the floor of the mouth as a hard yellow-coloured swelling or nodule.

3.6.3 Sjogren's syndrome

Sjogren's syndrome is an autoimmune disease which may affect the salivary glands, lacrimal glands and many other organs in the body. Over 80% of patients are middle aged and female. There are two types of Sjogren's syndrome. *Primary Sjogren's syndrome* is a systemic autoimmune disease of unknown cause which is associated with dry eyes and a dry mouth. *Secondary Sjogren's syndrome* is characterised by the presence of dry eyes and a dry mouth in association with another identifiable autoimmune disease, usually rheumatoid arthritis. The cause of Sjogren's syndrome is not known, but patients have circulating auto-antibodies in their blood and their salivary glands are destroyed by heavy infiltrates of lymphocytes. This leads to a lack of saliva and a dry mouth (xerostomia) which is one of the most distressing clinical aspects of Sjogren's syndrome.

The primary clinical feature of Sjogren's syndrome is dry mouth or xerostomia. Patients typically have trouble in eating, speaking and swallowing and cannot taste their food. Loss of the protective effects of saliva results in an increased incidence of cervical and smooth surface caries, periodontal disease and opportunistic infections by *Candida albicans*. On examination, the oral mucosa may be red and atrophic and dry or sticky to touch. Up to 50% of patients may also have swelling of the parotid gland and swelling of the cervical lymph nodes is sometimes also seen.

Sjogren's syndrome is important because it may present with distressing clinical symptoms and be very difficult to manage, but also because a minority of patients (about 5%) may develop lymphoma within the salivary glands.

3.6.4 Salivary gland tumours

Salivary gland neoplasms may arise in any of the major or minor salivary glands. About 75% overall arise in the parotid gland and 85% of all tumours are benign. The most common type is the *pleomorphic adenoma*.

Most benign tumours present as slow growing swellings with well-defined margins and few symptoms. The most common site and presentation is as a swelling in the lower pole of the parotid gland, just in front of the ear. Intra-orally, benign neoplasms present as sessile swellings covered by normal mucosa. The most common sites are the palate, upper lip and cheeks. The lesions are usually painless but occasionally they may be ulcerated if they have been subjected to trauma. Histologically, most benign tumours are pleomorphic adenomas, but in the lips, basal cell adenomas are frequently encountered.

Malignant neoplasms usually show rapid growth and present as indistinct swellings that may be ulcerated. They infiltrate adjacent tissues and may be fixed or may cause symptoms including pain or loss of sensation due to nerve damage. M*ucoepidermoid carcinomas* and *adenoid cystic carcinomas* are the most common malignant tumours and the parotid gland is the most common site overall. In the oral mucosa, the palate is the most common site, where about 50% of salivary tumours are malignant. At other oral sites, including the tongue and retromolar region, malignant lesions are more common than benign lesions.

3.7 Oral lesions associated with systemic disease

Patients suffering from some general systemic disorders may show oral signs and symptoms. Systemic disorders which may have oral manifestations are outlined in Table 3.5.

The most common oral findings are mucosal atrophy and ulceration. These signs are typified in patients with anaemias who may show marked aphthous-type ulceration associated with smooth reddened mucosa. Similar lesions are seen in patients with gastrointestinal disorders, either as a direct

Table 3.5 Common oral manifestations of systemic disorders

Haematological disorders	Iron deficiency anaemia	Oral mucosal atrophy Aphthous ulceration Angular cheilitis *Candida* infections
	Leukaemias	Gingival haemorrhage Lymphomatous masses Signs of anaemia
	Other anaemias (e.g. pernicious anaemia)	Mucosal atrophy, smooth tongue Signs as for iron deficiency
Gastrointestinal disorders	Crohn's disease	'Oral Crohn's disease': swelling of the lips, oral ulcers and mucosal 'tags' in the buccal sulci
	Coeliac disease	Apthous type ulcers Oral signs of dermatitis herpetiformis
	Ulcerative colitis	Oral ulcers Signs of anaemia
Endocrine disorders	Diabetes	Susceptibility to infections including periodontal disease, *Candida* infections. Dry mouth
	Multiple endocrine neoplasia syndromes	Multiple small mucosal neuromas, present as sessile normal coloured swellings.

manifestation of the disorder or owing to superimposed anaemia. In Crohn's disease the mouth may be affected by lesions which are similar to those seen in the intestines, including linear ulcers and mucosal tags. Histologically these show the typical histiocytic granulomas of this disease.

Opportunistic infections are also a common manifestation of disorders where the host immune response is compromised. In particular, acute pseudomembranous candidosis (oral thrush) is a common manifestation of anaemias, dietary deficiencies and gastrointestinal disorders. Patients who are immunocompromised as a result of chemotherapy for malignant disease, after transplant surgery or suffering from HIV disease may also show oral *Candida* infections.

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4

Dental erosion

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Abstract: Erosive tooth wear is becoming increasingly important when considering the long-term health of the dentition. Dental erosion is a multifactorial condition; the interplay of chemical, biological and behavioural factors is crucial and helps explain why some individuals exhibit more erosion than others. It is important that diagnosis of the tooth wear process in children and adults is made early. Clinical detection of erosive tooth wear is important once dissolution has started. The clinical appearance is the most important sign for dental professionals to diagnose erosion. Adequate preventive measures can only be initiated when the different risk factors are known and interactions between them are present. This chapter shows the importance of early diagnosis of dental erosion as well as of the risk factors. Food constituents may play a role in increasing, but also in protecting, this condition.

Key words: diagnosis, erosion, prevention, risk factors.

4.1 Introduction

Erosive tooth wear is becoming an increasingly important factor when considering the long-term health of the dentition. There is some evidence that the presence of this condition is growing steadily. Dental erosion is a multifactorial condition; the interplay of chemical, biological and behavioural factors is crucial and helps explain why some individuals exhibit more erosion than others. The erosive potential of erosive agents like acidic drinks or foodstuffs depends on chemical factors, for example pH, titratable acidity, mineral content, clearance on tooth surface and on its calcium-chelation properties. Biological factors such as saliva, acquired pellicle, tooth structure and positioning in relation to soft tissues and tongue are related to the pathogenesis of dental erosion. Furthermore, behavioural factors like eating and drinking habits, regular exercise with dehydration and decrease of salivary flow, excessive oral hygiene and an unhealthy lifestyle, for example chronic alcoholism are predisposing factors for dental erosion.

It is important that diagnosis of the tooth wear process in children and adults is made early. Clinical detection of erosive tooth wear is important once dissolution has started. The clinical appearance is the most important sign for dental professionals to diagnose erosion. This is of particular importance in the early stages of erosive tooth wear. Adequate preventive measures can only be initiated when the different risk factors are known and interactions between them are present. Consequently an individually tailored preventive programme may be suggested.

4.2 Diagnosis

Diagnosis of early forms of erosion is difficult, as it is accompanied by few signs and fewer, if any, symptoms. There is no device available in routine dental practice for the specific detection of dental erosion and its progression. Therefore, clinical appearance is the most important feature for dental professionals to diagnose this condition. This is of particular importance in the early stage of dental erosion (Lussi, 2006). The teeth should be dried thoroughly and well illuminated to note minor surface changes. The appearance of smooth silky-glazed, sometimes dull, enamel with the absence of perikymata and intact enamel along the gingival margin are typical signs. It has been hypothesized that the preserved enamel band along the oral and facial gingival margin could be due to some plaque remnants, which could act as a diffusion barrier for acids. This phenomenon could also be due to an acid neutralizing effect of the sulcular fluid (Lussi *et al*., 2004). The initial features of erosion on occlusal and incisal surfaces are the same as described above. Further progression of occlusal erosion leads to a rounding of the cusps and restorations rising above the level of the adjacent tooth surfaces. In severe cases, the whole occlusal morphology disappears.

It is sometimes challenging to distinguish between the influences of erosion, attrition or abrasion during a clinical examination. Attrition-affected areas are often flat, have glossy areas with distinct margins and corresponding features at the antagonistic teeth. Facial erosion should be distinguished from wedge-shaped defects which are located at, or apical to, the enamel–cementum junction. The coronal part of wedge-shaped defects ideally has a sharp margin and cuts at a right angle into the enamel surface, whereas the apical part bottoms out to the root surface. Thereby the depth of the defect exceeds its width. Figures 4.1, 4.2 and 4.3 show typical patterns of dental erosion process.

The basic erosive wear examination (BEWE) provides a simple scoring system that can be used with the diagnostic criteria of all current indices (Bartlett *et al*., 2008). The most severely affected surface in a sextant is recorded with a four-level score (Table 4.1) and the cumulative score is classified in complexity levels guiding the management of the condition (Table 4.2). The maximum score per subject is 18. The management includes

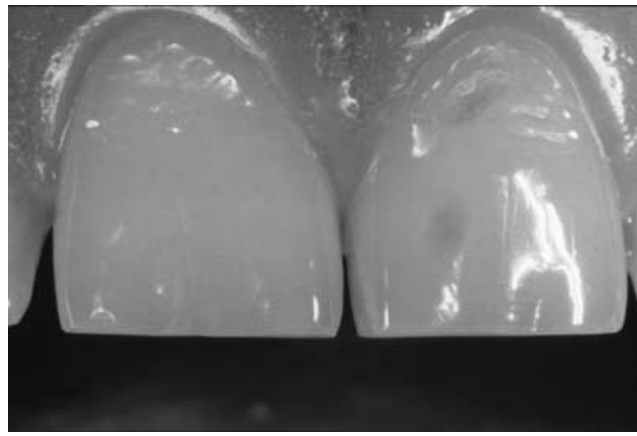

Fig. 4.1 Facial erosion: enamel with a smooth tooth surface and the absence of perikymata is clearly visible as well as undulating borders of the lesion (left). Tooth 22 (right) shows involvement of dentine. Both teeth have intact enamel along the gingival margin.

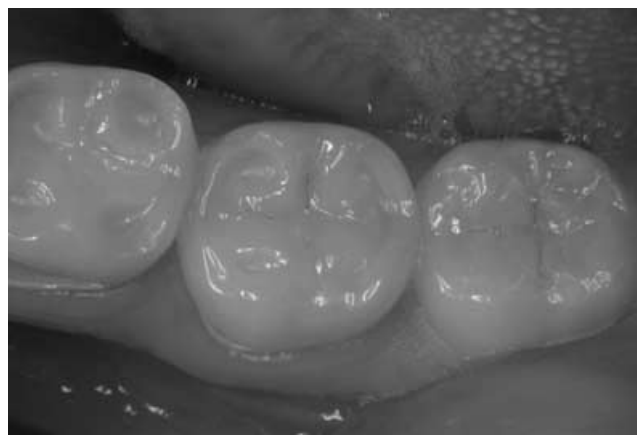

Fig. 4.2 Occlusal erosion: cupping and grooving of the occlusal surfaces. Note: the wisdom tooth which erupted after erosion had taken place is not affected.

identification and elimination of the main aetiological factor(s), prevention and monitoring, as well as symptomatic and operative intervention where appropriate. The BEWE further aims to increase the awareness of tooth erosion amongst clinicians and general dental practitioners and to provide a guide to its management.

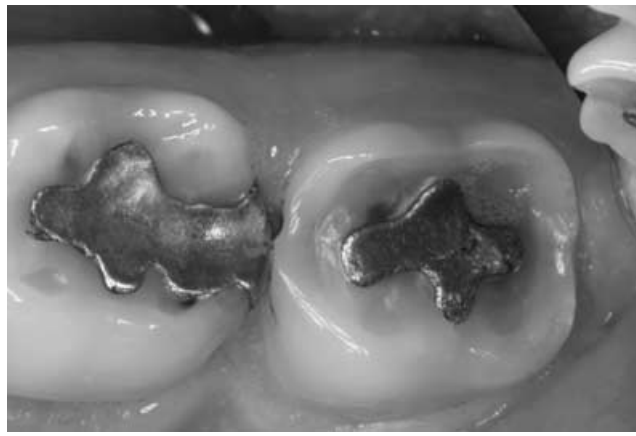

Fig. 4.3 Occlusal erosion: cupping and grooving of the occlusal surfaces with amalgam fillings rising above the level of the adjacent tooth surface are signs of these defects.

Table 4.1 Criteria for grading erosive wear

Score	
0	No erosive tooth wear
1	Initial loss of surface texture
2*	Distinct defect, hard tissue loss < 50% of the surface area
3*	Hard tissue loss ≥ 50% of the surface area

* In scores 2 and 3 dentine is often involved.

4.3 Risk factors

When an acidic solution comes in contact with enamel, it has to diffuse first through the acquired pellicle and only thereafter can it interact with enamel. On the surface of the enamel, the hydrogen ion component of the acid will start to dissolve the enamel crystal. Thereafter, fresh, unionized acid will eventually diffuse into the interprismatic areas of enamel and dissolve further mineral in the region underneath the surface (Eisenburger *et al.*, 2001a; Lussi and Hellwig, 2001). This will lead to an outflow of ions (dissolution) and subsequently to a local pH rise in the tooth substance immediately below, and in the liquid surface layer adjacent to, the enamel surface (Lussi and Hellwig, 2001). The events in dentine are, in principle, the same but are even more complex. The acquired pellicle is an organic film, free of bacteria, covering oral hard and soft tissues. It is composed of mucins, glycoproteins and proteins, including several enzymes (Hannig *et al.*, 2005). The acquired

Table 4.2 Complexity levels as a guide to clinical management

Complexity level	Cumulative score of all sextants	Management
0	Less than or equal to 2	• Routine maintenance and observation. • Repeat at 3-year intervals.
1	Between 3 and 8	• Oral hygiene and dietary assessment, and advice, routine maintenance and observation. • Repeat at 2-year intervals.
2	Between 9 and 13	• Oral hygiene and dietary assessment, and advice, identify the main aetiological factor(s) for tissue loss and develop strategies to eliminate respective impacts. • Consider fluoridation measures or other strategies to increase the resistance of tooth surfaces. • Ideally, avoid the placement of restorations and monitor erosive wear with study casts, photographs, or silicone impressions. • Repeat at 6–12-month intervals.
3	14 and over	• Oral hygiene and dietary assessment, and advice, identify the main aetiological factor(s) for tissue loss and develop strategies to eliminate respective impacts. • Consider fluoridation measures or other strategies to increase the resistance of tooth surfaces. • Ideally, avoid restorations and monitor tooth wear with study casts, photographs, or silicone impressions. • Especially in cases of severe progression consider special care that may involve restorations. • Repeat at 6-month intervals.

pellicle may protect against erosion by acting as a diffusion barrier or a perm-selective membrane preventing direct contact between the acids and the tooth surface, reducing the dissolution rate of dental hard tissue.

There are many factors involved in the erosive tooth wear process. Figure 4.4 shows the different predisposing factors and aetiologies of the erosive condition. Biological, behavioural and chemical factors interact with the tooth surface, which over time may either wear it away, or indeed protect it. The interplay of all these factors is crucial and helps to explain why some individuals exhibit more erosion than others, even if they are exposed to exactly the same acid challenge in their diets. Other factors listed in the outer circle of Fig. 4.4 will further influence the whole process of erosion development or defence (Lussi, 2006). It is useful to consult a check list in order to unveil risk factors (Table 4.3).

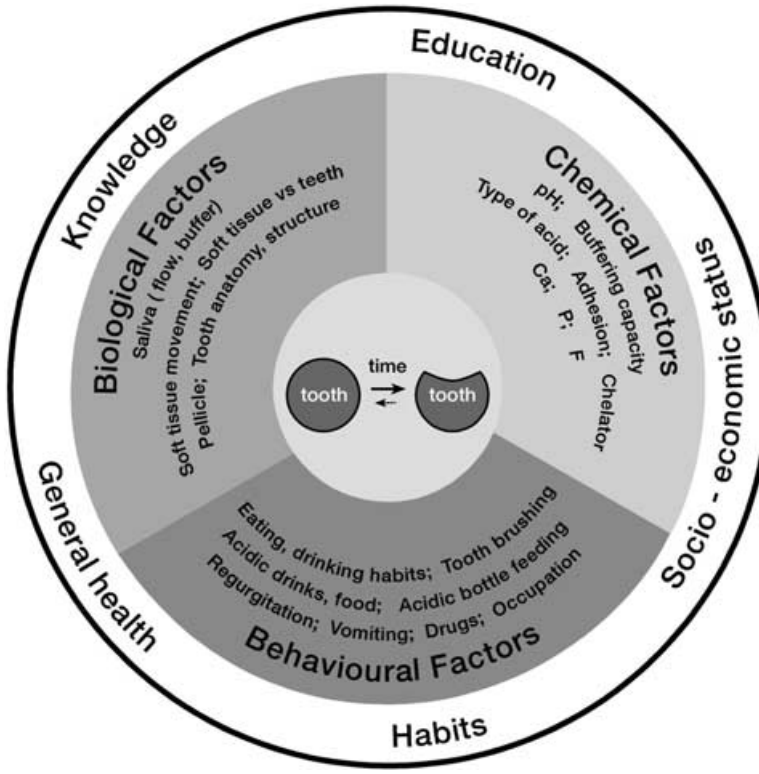

Fig. 4.4 Interactions of the different factors in the development of erosive tooth wear.

Table 4.3 Check list in order to ascertain the aetiological factors for erosions (in part from Lussi *et al*., 2004; Lussi and Hellwig, 2006)

- Take case history (medical and dental).
- Diagnose the severity and the site-specific distribution.
- Record the dietary intake over four days and estimate the erosive potential.
  Question the patient for specific factors which they may not be aware of:
  - Diet: herbal teas, acidic candies, alcohol, sports drinks, effervescent vitamin C tablets, etc.
  - Gastric symptoms: vomiting, acid taste in the mouth and gastric pain (especially when awakening), stomach ache, any sign of anorexia nervosa.
  - Drugs: alcohol, tranquillizer, anti-emetics, antihistamines, lemonade tablets. (Change of acidic or saliva-reducing drugs is possible in consultation with patient's physician.)
- Determine the flow rate and buffering capacity of saliva.
- Reveal the oral hygiene habits, abrasivity of toothpaste and technique.
- Question patient about occupational exposure to acidic environments.
- Question patient about X-ray therapy of the head and neck area.
- Assess further progression with silicone impressions, study models and/or photographs.

4.4 Biological factors

Biological factors such as saliva, acquired pellicle, tooth structure and positioning in relation to soft tissues and the tongue are related to dental erosion development.

A very important biological parameter is saliva. Several salivary protective mechanisms come into play during an erosive challenge: dilution and clearance of an erosive agent from the mouth, neutralization and buffering of acids, and slowing down the rate of enamel dissolution through the common ion effect by salivary calcium and phosphate (Zero and Lussi, 2000). Practical experience demonstrates the importance of saliva in patients suffering from salivary flow impairment. Studies have shown that erosion may be associated with low salivary flow or/and low buffering capacity (Jarvinen *et al*., 1991; Lussi and Schaffner, 2000; Rytomaa *et al*., 1998). The dry mouth condition is usually related to ageing (Dodds *et al*., 2005; Navazesh *et al*., 1992; Percival *et al*., 1994) even though some other studies have not found this correlation (Ben-Aryeh *et al*., 1986; Heintze *et al*., 1983). It is well established that patients taking medication can also present decreased saliva output (Wynn and Meiller, 2001), as well as those who have received radiation therapy for neck and head cancer (Dreizen *et al*., 1977).

Tests of the stimulated and unstimulated flow rate, as well as of the buffer capacity, of saliva may provide some information about the susceptibility of an individual to dental erosion. However, it has to be kept in mind that these parameters are two of a multifactorial condition. Sialometric evaluations should be carried at a fixed time-point or in a limited time interval in the morning, avoiding intra-individual variations that are due to the circadian cycle. Studies have shown that sour foodstuff has a strong influence on the anticipatory salivary flow (Christensen and Navazesh, 1984; Lee and Linden, 1992), which can be significantly increased when compared to the normal unstimulated flow rate (Engelen *et al*., 2003).

Hypersalivation also occurs in advance of vomiting as a response from the 'vomiting centre' of the brain (Lee and Feldman, 1998), as frequently seen in individuals suffering from anorexia and bulimia nervosa, rumination or chronic alcoholism. It is suggested that this could minimise the erosion caused by acids of gastric origin. On the other hand, patients with symptoms of gastro-esophageal reflux disease (GERD) should not expect the salivary output to increase before the gastric juice regurgitation, because this is an involuntary response not coordinated by the autonomic nervous system (Hara *et al*., 2006). Therefore, there may be insufficient time for saliva to act before erosion occurs.

The influence of saliva on the remineralisation/rehardening of erosive damaged dental hard tissue is a controversial issue. There is evidence that acid-softened enamel can reharden after exposure to saliva or remineralisation solution and that dietary products and fluoride can enhance the rehardening process (Amaechi and Higham, 2001; Feagin *et al*., 1969; Gedalia

et al., 1991a). Other investigations could not find a significant rehardening effect of saliva *in situ* (Collys *et al*., 1993; Garberoglio and Cozzani, 1979; Gedalia *et al*., 1991b; Lippert *et al*., 2004). Some limited increase in the abrasion resistance of softened enamel was found after intra-oral exposure to saliva (Attin *et al*., 2001; Jaeggi and Lussi, 1999). It seems that *in vitro* some rehardening could be expected when a supersaturated solution or saliva with no protein added is used (Eisenburger, 2001a, 2001b), whereas *in situ* this is only the case to a very small extent.

Millward and co-workers (1997) monitored the pH at the surface of teeth of healthy volunteers after drinking 1% citric acid. They observed that the pH recovered to above pH 5.5 within 2 minutes from a site adjacent to the palatal surface of the upper central incisor and within 4–5 minutes from another palatal surface of the upper first molar. Other observations have revealed a longer clearance time on the upper incisors for patients with active erosions and normal saliva values compared to patients with no erosion (Lussi, unpublished). These differences could be due to the anatomy of the teeth and soft tissues which may influence the retention/clearance pattern of erosive agents. Also, soft tissue movements of the tongue and buccal mucosa and the swallowing pattern can influence the clearance rate. The importance of the tongue in modifying the tooth wear process has long been the subject of speculation. Holst and Lange (1939) considered mechanical abrasion caused by the tongue to be a contributing factor in erosion caused by vomiting. Observations from animal studies also provide support in that beverages produced erosion mainly on the lingual surfaces of rat molar teeth in areas where the tongue contacts the teeth (Stephan, 1966).

4.5 Chemical factors

Several *in vitro* and *in situ* studies have shown that the erosive potential of an acidic drink or foodstuff is not exclusively dependent on its pH value, but is also strongly influenced by its mineral content, its titratable acidity ('the buffering capacity') and by the calcium-chelation properties. The pH value, calcium, phosphate and fluoride content of a drink or foodstuff determine the degree of saturation with respect to tooth mineral, which is the driving force for dissolution. Solutions oversaturated with respect to dental hard tissue will not dissolve it. A low degree of undersaturation with respect to enamel or dentine leads to initial surface demineralisation which is followed by a local rise in pH and increased mineral content in the liquid surface layer adjacent to the tooth surface. This layer will then become saturated with respect to enamel (or dentine) and will not demineralise further.

Acids such as citric acid exist in water as a mixture of hydrogen ions, acid anions (e.g. citrate) and undissociated acid molecules, with the amounts of each determined by the acid dissociation constant and the pH of the solu-

tion. The hydrogen ion directly attacks the crystal surface. Over and above the effect of the hydrogen ion, the citrate anion may complex with calcium, also removing it from the crystal surface. Each acid anion has a different strength of calcium complexation dependent on the structure of the molecule and how easily it can attract the calcium ion (Featherstone and Lussi, 2006). Consequently, acids such as citric acid have a double action and may be very damaging to the tooth surface. Up to 32% of the calcium in saliva can be complexed by citrate at concentrations common in fruit juices, thus reducing the supersaturation of saliva and increasing the driving force for dissolution with respect to tooth minerals (Meurman and ten Cate, 1996).

The dissolution with water of drinks containing organic acids with high buffering capacity will hardly reduce the pH but will reduce the titratable acidity. This is of some importance, as the greater the buffering capacity of the drink, the longer it will take for saliva to neutralize the acid. But dilution will also reduce concentrations of calcium and phosphorus (if present), which have a protective effect.

The calcium and phosphate content of a foodstuff or beverage are important factors for the erosive potential as they influence the concentration gradient within the local environment of the tooth surface. The addition of calcium (and phosphate) salts to erosive drinks has shown promising results. Addition of calcium to a low pH blackcurrant juice drink has been shown to reduce the erosive effect of the drink (West *et al*., 2003). When calcium was added to a sports drink, a reduction in the erosive potential was found (Hooper *et al*., 2004). Today, several Ca-enriched orange juices and sports drinks are on the market which do barely soften the enamel surface. Yoghurt is another example of a food with a low pH (~4.0), yet it has hardly any erosive effect owing to its high calcium and phosphate content, which makes it supersaturated with respect to apatite. It has to be kept in mind that the addition of minerals to sports drinks does not completely prevent enamel dissolution. However, the progression can be retarded, which has some implications for the patient and the clinician.

Theoretically, fluoride has some protective effect in a drink with a pH higher than that indicated by the saturation curve of fluorapatite at given Ca and PO_4 concentrations. Lussi *et al*. (1993, 1995) and Mahoney *et al*. (2003) found an inverse correlation of the erosive potential of different beverages with their fluoride content. It is unlikely that fluoride alone at the concentration present in beverages has any great beneficial effect on erosion, because the challenge is high. However, it is possible that, under conditions in which the other erosive factors are not excessive, fluoride in solution may exert some protective effect. It appears that topical fluoride application can positively affect the tooth wear process when it is incorporated into, and deposited on, the enamel during treatment.

The adhesiveness and displacement of the liquid are other factors to be considered in the erosive process. There appear to be differences in the ability of beverages to adhere to enamel based on their thermodynamic

properties, for example the thermodynamic work of adhesion (Ireland *et al*., 1995).

In summary, the two very often cited parameters, the pH and the titratable acidity, do not readily explain the erosive potential of food and drink. The mineral content is also an important parameter, as is the ability of any of the components to complex calcium and to remove it from the mineral surface.

4.6 Behavioural factors

During and after an erosive challenge, behavioural factors play a role in modifying the extent of tooth wear. The manner that dietary acids are introduced into the mouth will affect which teeth are contacted by the erosive challenge and, possibly, the clearance pattern. As lifestyles have changed through the decades, the total amount and frequency of consumption of acidic foods and drinks have also changed. Soft drink consumption in the USA increased by 300% in 20 years (Calvadini *et al*., 2000), and serving sizes increased from 185 g in the 1950s, to 340 g in the 1960s, to 570 g in the late 1990s. Around 1995, between 56% and 85% of children at school in the USA consumed at least one soft drink daily, with the highest amounts ingested by adolescent males. Of this group, 20% consumed four or more servings daily (Gleason and Suitor, 2001). Studies in children and adults have shown that this number of servings per day is associated with the presence and the progression of erosion when other risk factors such as swishing drinks are present (O'Sullivan and Curzon, 2000; Lussi and Schaffner, 2000). High erosion was associated with a method of drinking whereby the drink was kept in the mouth for a longer period (Johansson *et al*., 2002). Multiple regression analysis showed that the consumption of erosive drinks and foodstuffs was associated with the development of facial and occlusal erosions (Lussi *et al*., 1991).

Considerable risk of erosion was found when citrus fruits were eaten more than twice a day and soft drinks were drunk daily (Jarvinen *et al*., 1991). On the other hand, other studies were not able to find an association between dental erosion and behavioural factors (Jaeggi *et al*., 1999; Ganss *et al*., 1999) or they found only a weak association (Nunn *et al*., 2003). One can only speculate about the reasons. A possible explanation is the mode of questioning the persons (orally versus a written questionnaire) the statistics employed (multivariate versus univariate) and the population group under study (selected versus randomly).

Excessive consumption of acidic candies combined with a low salivary buffering capacity may aggravate erosive lesions (Distler *et al*., 1993; Lussi *et al*., 1997). The high intake of herbal teas, widely perceived as a healthy drink, may have an erosive potential exceeding that of orange juice (Phelan and Rees, 2003).

A healthier lifestyle, paradoxically, can lead to dental health problems, in the form of dental erosion as it often involves regular exercise and what is considered to be healthy diets with more fruit and vegetables. A lacto-vegetarian diet, which includes the consumption of acidic foods, has been associated with a higher prevalence of dental erosion (Ganss *et al*., 1999). The benefits of exercise are well proven; however, exercise increases the loss of body fluids and may lead to dehydration and decreased salivary flow. A few case reports and studies have reported an association between sports activities and erosive tooth wear. The cause could be direct acid exposure or strenuous exercise which may increase gastroesophageal reflux. Risk groups are swimmers exercising in water with low pH and athletes frequently consuming erosive sports drinks. Sports drinks are often erosive (Sorvari *et al*., 1996; Hooper *et al*., 2004, 2005; Venables *et al*., 2005) and when consumed during strenuous activity, when the person is in a state of some dehydration, the possible destructive effects may be enhanced further. Health-conscious individuals also tend to have better than average oral hygiene. While good oral hygiene is of proven value in the prevention of periodontal disease and dental caries, frequent tooth brushing with abrasive oral hygiene products may enhance erosive tooth wear.

At the other end of the spectrum, an unhealthy lifestyle may also be associated with dental erosion (Zero and Lussi, 2006). Wine has properties such as low pH, low content of phosphorus and calcium, which confers on it an erosive potential. Alcoholics may be at particular risk for dental erosion and tooth wear. Robb and Smith (1990) reported significantly more tooth wear in 37 alcoholic patients than in age- and sex-matched controls. Tooth wear was most pronounced in males and those with frequent alcohol consumption. Although a direct impact of the alcohol cannot be ruled out, one has to keep in mind that alcoholics often have regurgitation.

Professional wine tasting is very common all over the world. In some countries (e.g. Sweden, Finland) wine tasters are employed by the state to support their state-owned wine shops. Full-time Swedish wine tasters test on average 20–50 different wines, nearly 5 days a week. Wiktorsson *et al*. (1997) investigated the prevalence and severity of tooth erosion in 19 qualified wine tasters in relation to the number of years of wine tasting, salivary flow rate and buffer capacity. Salivary flow rate and buffer capacity of unstimulated and stimulated saliva were measured. Data on occupational background and dental and medical histories were collected. Fourteen subjects had tooth erosion mainly on the labio-cervical surfaces of maxillary incisors and canines. The severity of the erosion tended to increase with years of occupational exposure. Caries activity in all subjects was low. It was concluded that full-time wine tasting is an occupation associated with an increased risk of tooth erosion.

Although no detrimental effects were described on a population level, one has to keep in mind that factors like sports drinks consumption and occupation can be, for some patients, a cofactor in the development or in

the increase of dental erosion when other factors are present. It is unlikely that one or two isolated factors (e.g. sports drink, dehydration) will be responsible for a multifactorial condition like erosion.

4.7 Summary

This chapter shows the importance of early diagnosis of dental erosion as well as of the risk factors. Food constituents may play a role in increasing but also in protecting against this condition.

4.8 Acknowledgement

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5

Nutrition and its effect on oral health and disease

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Abstract: This chapter provides an overview of the multifaceted impact that nutrition and diet have on oral health and the role of diet in oral disease prevention. Recent research into diet and caries confirms the importance of limiting free sugars in the diet. Whole fruits, vegetables and milk are not important in caries development and furthermore, milk, cheese and plant-based foods may have cariostatic effects. Current evidence indicates that soft drinks are the most important dietary factor contributing to enamel erosion. Global dietary guidelines promote a diet that is low in fat and free sugars and high in fruits, vegetables and fibre: this type of diet will protect against oral diseases.

Key words: cariostatic factors, dietary recommendations, diet and oral cancer, erosion, nutrition, nutrition and AIDS, soft drinks, sugars.

5.1 Introduction

Diet and nutrition have an impact upon oral health in many ways and provide a key link between oral and systemic well-being. Nutrition plays a key role in the aetiology of dental caries, tooth erosion and oral mucosal diseases and is an important factor in the development of the teeth and maintenance of the periodontal tissues. Dental diseases ultimately result in tooth loss which may result in difficulty in eating, thereby reducing the ability to consume a healthier diet and having an impact upon eating-related quality of life. The aim of this chapter is to provide an overall insight into the multifaceted impact that nutrition and diet have on oral health and the potential role of diet in oral disease prevention.

5.2 Enamel developmental defects

Diet and nutritional status have an impact upon the development of the dental tissues in the pre-eruptive stage. Malnutrition is a contributing factor to enamel developmental defects; those enamel developmental defects affected by nutrition may be broadly classified into opacities and enamel hypoplasia (Rugg-Gunn and Nunn, 1999).

5.2.1 Enamel hypoplasia

Enamel hypoplasia is characterised by the interruption of the smooth enamel surface by pits, fissures or larger areas of missing enamel that become stained after eruption and render the teeth more susceptible to dental caries. Enamel hypoplasia is associated with protein energy malnutrition (PEM); the prevalence increasing with the severity of malnutrition (Enwonwu, 1973). Diarrhoeal disease is common in malnourished communities and results in hypocalcaemia which has been postulated as the mechanism by which malnutrition leads to enamel hypoplasia (Nikiforuk and Fraser, 1981). Evidence from animal studies suggests that deficiencies of vitamin A and vitamin D are a cause of enamel hypoplasia (Harris and Navia, 1980; Rugg-Gunn and Nunn, 1999). The role of vitamin D in the prevention of enamel hypoplasia was confirmed by an intervention study of pregnant women in which vitamin D supplementation during pregnancy subsequently raised the plasma calcium concentration of the infant and subsequently reduced the occurrence of hypoplasia in the primary dentition (Cockburn *et al*., 1980). The impact of vitamin supplementation on oral health is considered further in Chapter 15.

5.2.2 Diet, nutrition and fluorosis

Excess ingestion of fluoride during the development of the teeth may cause enamel fluorosis, an enamel developmental defect that manifests as small white diffuse opacities and, in severe cases, pitting. The susceptibility of the aesthetically important upper central permanent incisors is greatest during the first four years of life (Rao, 1984; Evans and Stamm, 1991) when fluoride intake should not exceed the suggested optimum range of 0.05–0.07 mg F/kg body weight/day (Burt, 1992). Malnutrition has been shown to exacerbate fluorosis of the permanent dentition (Rugg-Gunn *et al*., 1997; Yoder *et al*., 1998). However, in a study of 6-year-old Brazilian children, no relationship between the presence of malnutrition and the severity of fluorosis in the primary dentition was found (Sampaio *et al*., 1999).

5.3 The role of diet in dental caries

Diet has a profound effect on caries development: the amount and frequency of consumption of sugars is the most important dietary factor in the aetiology of dental caries. Evidence for the relationship between sugars intake and dental caries comes from a wide range of studies including human epidemiological studies, animal studies, human experimental studies and laboratory investigations. Human epidemiological studies include observational studies and intervention studies and provide the strongest evidence. When assessing the overall cariogenicity of a foodstuff, the evidence from all types of investigation should be considered and the findings of animal studies and experimental studies need to be interpreted alongside the epidemiological data. A more detailed account of the impact of food sugars on dental caries is provided in Chapter 9.

5.3.1 Epidemiological studies of the relationship between carbohydrates and dental caries

Using data from the World Health Organisation (WHO) Global Oral Health Databank on the mean number of decayed missing and filled teeth (DMFT) of 12-year-olds and Food and Agricultural Organisation sugar supply data, early studies showed sugar supply explained over 50% of the variance in caries levels (Sreebny, 1982). A more recent analysis showed this relationship was not as strong; however, sugar supply still accounted for 28% of variance in caries levels (Woodward and Walker, 1994). Overall, such global comparisons consistently show that countries with sugar intake below 18 kg per person per year have a low level of caries experience (Sreebny, 1982; Woodward and Walker, 1994).

Patterns of change in the intake of sugars by populations over time often mirror changes in dental caries levels. The earliest examples of this come from studies carried out around the Second World War which showed that patterns of dental caries fell during the war years when there was a reduction in sugars availability owing to food shortages, but subsequently increased when sugar consumption increased after the War (Takeuchi, 1961). Although a reduction in sugar intake was not the only dietary change resulting from food rationing, it is noteworthy that the intake of starchy staple foods increased in many countries, suggesting an insignificant role of such foods in the aetiology of caries. A more recent study has shown that the dramatic reduction in sugars intake due to the UN Sanctions in Iraq in 1990 resulted in the mean number of decayed, missing and filled teeth falling by up to 72% of the pre-war values (Jamel *et al*., 2004).

Another example of the impact of patterns of sugar consumption on the levels of dental caries is provided by data from Japan demonstrating a significant strong correlation between sugar supply and average DMFT at age

12 between 1957 and 1987 (Miyazaki and Morimoto, 1996). Data from England also show that the rise and fall of levels of dental caries was mirrored by changes in sugar consumption with a correlation between the two variables being high (Downer, 1998). There are many epidemiological studies that have shown a marked increase in dental caries in populations following transition from their traditional low sugars diet to a Westernised diet, which is typically high in sugars.

Numerous observational studies have demonstrated a relationship between the amount of sugars in the diet and the development of dental caries. Those conducted since the introduction of fluoride are summarised here.

Stecksen-Blinks and Gustafsson (Stecksen-Blinks *et al*., 2003) measured dietary intake of sugars and caries increment over one year in Swedish children aged 8–13 years and found those who developed two or less decayed surfaces had a significantly lower sugars intake compared with children who developed three or more carious surfaces. In another longitudinal study of caries levels in a cohort of almost 700 Swedish children between the ages of 1 and 3.5 years, a significant relationship was found between the consumption of confectionery and sugar-containing beverages and caries increment (Grindefjord *et al*., 1996). Furthermore, in a cohort study of Finnish children from birth to 10 years, significantly higher caries, approximately double the level, were found in the top 5% (DMFT 3.9) of sucrose consumers compared with the bottom 5% (DMFT 1.9) (Ruottinen *et al*., 2004).

Intervention studies which measure the impact of reducing sugars intake on the development of dental caries are rare, probably owing to the difficulties inherent in achieving compliance to dietary regimens over the period of time required to measure changes in caries development. Two often quoted early studies are the Vipeholm Study (Gustafsson *et al*., 1954) and the Turku Sugars Studies (Scheinin and Makinen, 1975), which both showed that caries development was related to sugar consumption. A more recent intervention study by Rodrigues and Sheiham (2000) studied the impact of introducing guidelines on sugars consumption into nursery schools in Brazil: children attending nurseries where guidelines on sugar intake were adhered to had significantly lower intake of free sugars and significantly fewer dental caries than those attending nurseries that had not adopted the guidelines. Those children attending nurseries that had not adopted the guidelines had almost five times the risk of developing decay. In children in whom dietary sugars provided in excess of 10% of their energy intake, the risk of developing high levels of caries was increased by three-fold.

5.3.2 The importance of frequency versus amount of sugar consumed

In the past there has been debate about whether the amount of sugars consumed or the frequency with which they are consumed is more impor-

tant in terms of caries development. Information from both animal experiments (Hefti and Schmid, 1979; Konig *et al*., 1968) and epidemiological studies (Rugg-Gunn *et al*., 1984; Jamel *et al*., 1996) indicate that both variables are related to caries development. Furthermore, evidence shows that a strong correlation exists between amount and frequency of sugars consumption and so efforts to control one of these factors should control the other (Rugg-Gunn *et al*., 1984; Jamel *et al*., 1996). It is important to set recommendations for a safe threshold for sugars intake in terms of amount as this provides a standard which drives health promotion and against which the intake of populations may be judged and the effectiveness of health promotion monitored. However, at the level of the individual, guidance on limiting the frequency of consumption of sugars may be easier for the patient to conceptualise. However, the overall important goal with respect to health is to decrease the amount of sugars-rich, energy-dense foods and sugared soft drinks that are consumed as these items are associated with increased risk of obesity and obesity-related disorders such as diabetes and cancer (World Health Organisation, 2003; World Cancer Research Fund, 2007).

5.3.3 Classification of sugars for health purposes

Lactose, has been shown to be potentially less cariogenic compared with other mono- and disaccharides (Jenkins and Ferguson, 1966; Rugg-Gunn and Nunn, 1999) and most recent epidemiological studies show that milk, despite containing ~5% lactose, is negatively or neutrally associated with caries (Petti *et al*., 1997; Mattos-Graner *et al*., 1998; Levy *et al*., 2003). There is little evidence of differing cariogenicity between other sugars. However, sucrose is the sole substrate for extracellular glucan synthesis, and recent clinical evidence suggests that glucans enhance *Streptococcus mutans* in plaque by increasing plaque porosity which results in increased acid accumulation at the enamel surface (Zero, 2004).

A global dietary recommendation is that intake of fruits and vegetables should be at least 400 g per day (World Health Organization, 2003). As fruit is a natural source of sugars (fructose, glucose and sucrose), the cariogenic potential of fruit is an important consideration. Animal studies, in general, have shown that fruit causes caries but significantly less so than sucrose, and in these studies frequency of consumption was often high (e.g. 17 intake per day) (Rugg-Gunn, 1993). Some plaque pH studies have also shown that consumption of fruit lowers plaque pH but significantly less so than sucrose and not to below the critical pH of 5.5 (Pollard, 1995). Furthermore, epidemiological studies have generally shown a negative association between consumption of fruit and levels of dental caries and, therefore, fresh fruit is not thought to increase the risk of dental caries (Moynihan and Petersen, 2004). When juiced, however, the concentration of sugars and the amount per standard portion, increases substantially and drinking juice does not

provide the mechanical stimulus to salivary flow that whole fruit consumption does. In addition, there may be protective factors in the plant cell walls of whole fresh fruit that are removed in the juicing process (Moynihan, 2006).

In view of the evidence relating to types of sugar, milk and fruit, the WHO (World Health Organisation, 2003) classified sugars that were potentially harmful to health as 'free sugars', which include all sugars added by manufacturer, cook or consumer, plus those sugars found in fresh fruit juices, honey and syrups. Based on the best available evidence, the WHO recommends that the intake of free sugars should not exceed 10% of energy intake, which equates to approximately 60 g per day for an adult or 33 g per day for a young child.

5.3.4 Overview of foods and factors that protect against dental caries

There are a number of foods that contain cariostatic factors and may, therefore, have a preventive effect on dental caries development. These include milk, cheese, peanuts, some berry fruits, tea and high fibre foods.

Milk and cheese

Milk contains calcium, phosphate, casein and other protein components which protect against decay. Early animal experiments provide good evidence of the anticariogenic properties of milk and show it causes very low caries, even in rats from whom the salivary glands had been removed to make them caries prone (Sperling *et al*., 1955; Bowen *et al*., 1991). Recent *in vitro* experiments have demonstrated that whey protein extracts from milk prevent demineralisation of enamel (Grenby *et al*., 2001).

The evidence for a cariostatic effect of cheese comes from animal experiments, human experiments and epidemiological intervention studies. Mastication of hard-type cheeses provides a good gustatory stimulus to salivary flow and increases its buffering capacity. Consumption of cheese on its own, or as part of a cooked meal, increases plaque calcium concentration, a factor that is thought to be related to caries risk. This may be explained by the formation of casein phosphopeptide amorphous calcium phosphate nanocomplexes which play an important role in the remineralisation process (Reynolds *et al*., 2003) and is described in detail in Chapter 10. In an oral health intervention study of children, consumption of 5 g hard cheese per day after breakfast was shown to be effective in preventing dental caries (Gedalia *et al*., 1994). This small amount of cheese would not make a substantial contribution to intake of saturated fat. The impact of dairy foods on oral health is considered further in Chapter 8.

Plant-based foods

Despite consistent evidence from early animal studies of a protective effect of organic and inorganic phosphates derived from plant foods, clinical trials

proved inconclusive. Current interest is focusing on the protective properties of polyphenolic compounds which are widely found in plant-based foods. Rich sources include berry fruits, for example cranberries which have been shown to have antimicrobial effects and may reduce the adhesion of *S. mutans* (Weisse, Lev-Dor, *et al*., 2002; Koo, Guzman *et al*., 2006). Apples are also rich in polyphenolic compounds; however, early clinical trials into the effect of apple consumption on caries development proved inconclusive. Polyphenolic compounds in green and oolong teas suppress the growth of *S. mutans* and reduce the ability of these bacteria to synthesise extracellular glucan. Black tea is also a rich source of fluoride and this is an additional way in which it may exert an anticaries effect. In Chapter 19, the beneficial effect of tea on oral health is considered in more depth. Many plant foods are fibrous in nature and their mastication provides a mechanical stimulus to salivary flow which increases the capacity to neutralise plaque acids and also promotes oral clearance of food debris.

5.4 Diet and dental erosion

Dental erosion is the irreversible loss of dental hard tissue by acids in a process that does not involve bacteria. The acids may be of intrinsic (i.e. from the gastrointestinal track) or extrinsic (i.e. diet and or environment) origin. Dietary acids include acetic, ascorbic, carbonic, citric, malic, oxalic, phosphoric and tartaric acid which are found in soft drinks, fruit and fruit juices, some vegetables (e.g. rhubarb), wine, vitamin C tablets, and items containing vinegar. The most significant contribution in westernised diets comes from acidic soft drinks, including both still and carbonated, sugared and sugar-free. In recent years, substantial evidence has emerged indicating an association between dental erosion and the consumption of such acidic drinks. Levels of erosion are higher in those children who report more frequent consumption of soft drinks. A three-fold increase in risk of dental erosion of the primary dentition has been reported in young children who consume more than one soft drink per day (Harding *et al*., 2003).

The pH of the drink, the type and amount of acid present and the amounts of calcium, phosphate and fluoride present in the soft drink may influence the potential of the drink to cause erosion (Larsen and Nyvad, 1999). In addition, drinking habits may also have an impact on the degree of erosion caused by a drink; for example, holding the drink in the mouth and/or swishing the drink around the mouth before swallowing, and sipping the drink over a long time result in a prolonged exposure to the acids which may increase the likelihood of the drink causing erosion (Johansson *et al*., 2002). On the other hand, consuming chilled drinks may lower the erosive potential compared with consuming drinks at room temperature (Amaechi *et al*., 1999). The factors that have an impact upon the erosive potential of acidic drinks are considered further in Chapter 4 which

provides an in-depth account of the role of diet in the aetiology of tooth surface loss.

A few studies have shown that high levels of consumption of some types of fresh fruit, primarily citrus fruits, apples and berries can cause dental erosion (Kunzel *et al.*, 2000). There are also reports of individual cases of erosion demonstrated in patients with unusual patterns of fruit consumption. For example, severe erosion was demonstrated in a woman who consumed the juice of 22 oranges per day and a kilo of stewed rhubarb per week (Levine, 1973). However, apart from reports of the impact of high or unusual patterns of fruit consumption, there is little evidence that, as part of a normal mixed diet, fresh fruit consumption causes erosion. In the UK, children have low levels of intake of fresh fruit, yet approximately 30% of 12-year-olds have erosion, indicating that it is unlikely that fruit consumption is responsible for the current levels of erosion. The WHO report concluded that, despite soft drinks being a probable cause of dental erosion, there was insufficient evidence of fresh fruit consumption being a significant risk factor.

It is possible that some dietary components may be protective against dental erosion, but this is an area where there has been little research. One study has shown that consumption of a hard-type cheese reverses some of the enamel damage caused by acidic drink consumption (Gedalia *et al.*, 1991). However, further research on dietary factors that protect against dental erosion is needed.

5.5 The impact of nutrition on oral mucosal conditions

Nutritional status may have a profound effect on the aetiology and severity of several oral mucosal diseases including oral cancer, glossitis, stomatitis and the oral symptoms of HIV AIDS.

5.5.1 Nutrition and oral cancer

Despite use of tobacco and alcohol (see Chapter 17) being the main aetiological factors in the development of oral cancers, diet is recognised as the third most important modifiable risk factor. Population studies show increased risk of oral cancer is associated with a low, compared with a high, consumption of fruits and vegetables, with reductions in risk ranging from between 40 and 80%. The protective effect of high fruit and vegetable consumption is greatest for fruits, in particular citrus fruits, and raw and yellow/orange vegetables. The reduction in risk of oral cancer conveyed by fruits and vegetables is independent of tobacco use and alcohol consumption and is greater in smokers compared with non-smokers. In 2007, the World Cancer Research Fund (WCRF) published a report that summarised all the evidence to date for an association between diet and cancer and

ranked the strength of the evidence with respect to different dietary components as convincing, probable, or non-conclusive. The report stated it was probable that non-starchy fruits and vegetables decreased the risk of oral and pharyngeal cancers (World Cancer Research Fund, 2007).

Population studies have also shown a negative association between intake of dietary fibre and risk of oral cancers and there is some evidence of a protective effect of whole grain foods, although some have failed to find such associations. The WCRF report concluded that there was no conclusive evidence for a protective effect of dietary fibre or wholegrain foods (World Cancer Research Fund, 2007).

Research into the effect of deficiencies of specific vitamins on the risk of oral cancer have generally shown inconsistent results with respect to vitamins A, B group, C and E. A higher incidence of oral leukoplakia has been associated with a low carotenoid status and low intake has also been negatively associated with the risk of oral cancer. The WCRF report concluded it was probable that a high intake of foods containing carotenoids protects against oral and pharyngeal cancers (World Cancer Research, Fund 2007). With respect to mineral status, most attention has focused on the impact of iron and selenium status but, again, the results are conflicting and there is insufficient evidence to draw firm conclusions.

Evidence of different food types that may increase the risk of oral cancer has focused on the positive association between oral cancer and the consumption of scalding hot foods, charcoal grilled meats and processed meat products; however, no firm conclusions can be drawn based on the best available evidence to date. The relationship between diet and oral cancer risk is considered again in Chapter 14.

5.5.2 The oral symptoms of micronutrient deficiencies

Micronutrient deficiencies cause atrophy of the oral mucosa and increased susceptibility to ulceration, inflammation and infection of the buccal mucosa, loss of papillae on the lingual mucosa and atrophy and fissures and inflammation to the labial mucosa of the lips. The oral mucosal conditions caused by nutrient deficiencies are summarised in Table 5.1 and a more detailed account of the role of vitamins and trace elements in oral health is provided in Chapters 15 and 16, resepectively. Many oral mucosal conditions are not specific to deficiency of any individual micronutrient and further investigation is required for a nutritional diagnosis.

5.5.3 The interrelationship between nutritional status and the oral symptoms of HIV/AIDS

A sore dry mouth and swallowing difficulties can have a serious impact upon the nutritional intake of anyone. However, ironically, these oral impacts tend to occur in those at most need of good nutritional intake and

Table 5.1 Oral mucosal conditions and associated nutrient deficiencies

Oral condition	Associated micronutrient deficiency
Angular cheilitis	Riboflavin (vitamin B2) Nicotinic acid (vitamin B3) Folic acid Cobalamin (vitamin B12) Ascorbic acid (vitamin C) Iron
Burning mouth syndrome	Pyridoxine (vitamin B6)
Candidiasis	Folic acid Cobalamin Iron
Glossitis	Riboflavin Nicotinic acid Pyridoxine Folic acid Cobalamin Iron Protein energy malnutrition
Lip fissures	Pyridoxine
Oral sensitivity	Thiamine Pyridoxine
Recurrent aphthae	Riboflavin Folic acid Cobalamin Ascorbic acid
Stomatitis	Nicotinic acid Folic acid Cobalamin

there is no better example than those with HIV/AIDS. The oral symptoms of AIDS have an impact upon eating ability, lead to reduced oral intake (Patel and Glick, 2005) and include candidiasis, stomatitis, ulcerations (e.g. associated with herpes simplex virus), xerostomia, and in some instances Karposi's sarcoma (depending on size and location). All of these symptoms may cause difficulties in masticating and swallowing food. It has been recognised that nutritional care is an essential component of the management of HIV/AIDS, which enhances the quality of life and minimises disease symptoms (Thuita and Mirie, 1999). Despite this awareness there has, in fact, been little documented research in this field, and nutrition and oral care have been identified as the least addressed needs of HIV positive women (Segurado *et al.*, 2003). There is a need for more research on the impact of oral health in those with HIV/AIDS on nutrition intake. Furthermore, the

impact of minimising the oral symptoms of HIV/AIDS on nutritional well being requires further study. Good nutrition is likely to contribute to improved systemic well-being, response to treatment and quality of life of HIV patients. Therefore, if poor oral health is a barrier to achieving this, it requires urgent attention.

5.6 The impact of nutrition on periodontal health and disease

Many nutrients play a key role in the development and maintenance of healthy periodontal tissues and early evidence from experiments in animals showed that severe deficiencies of vitamins A, B group, C and E have marked effects on the periodontium. However, these early observations were not supported by evidence from epidemiological studies which often showed no, or only weak, associations between nutrient intake or status and periodontal status. Today, nutritional methodologies have improved, as has our understanding of the periodontal disease process, and the application of this to modern-day research has resulted in renewed interest in the field of nutrition and periodontal disease.

Analysis of dietary and oral health data from the third US National Health and Nutrition Examination Survey (NHANES III) has shown significant associations between several nutrients and the risk of adult periodontitis. Intake of calcium in females of below 499 mg per day was associated with more than a 50% increase in the risk of periodontitis (Nishida *et al*., 2000a). In the same survey, a low intake of folic acid was shown to be a risk factor for adult periodontitis (Yu *et al*., 2007). However, despite a key role in epithelial formation, no association was found between serum vitamin A and periodontal disease risk (Chapple *et al*., 2007).

The role of vitamin C in protecting the tissues of the periodontium is well established as this nutrient has a role both as an antioxidant and is important for optimum immune function. Most recent studies show a weak, but significant, association between the status of this vitamin and indices of periodontal health (Nishida *et al*., 2000b; Amarasena *et al*., 2005).

Current interest is focusing on the potential protective role of antioxidant nutrients (vitamins A, C, E, β-carotene, selenium and dietary flavonoids) on the periodontal tissues. Antioxidant nutrients play an important role in the host defence mechanism by scavenging excess reactive oxygen species, thereby preventing damage to the tissues of the periodontium. Concentrations of antioxidants and total antioxidant capacity in both gingival crevicular fluid and in serum are compromised in patients with periodontitis (Brook, Butterworth *et al*., 2004). However, whether this is a cause or effect of periodontal disease requires clarification. A more detailed account of the role of antioxidants in periodontal disease is given in Chapter 11.

Increased severity of periodontal disease, notably aggressive periodontal disease, has been associated with PEM. Protein energy malnutrition compromises immune status, which may increase the susceptibility of the tissues of the periodontium to plaque-induced inflammation (Enwonwu, 1994). At the other end of the scale of nutritional status, it has been postulated that obesity is a risk factor for periodontal disease (Al-Zahrani *et al.*, 2003; Genco *et al.*, 2005). This theory is based on the knowledge that adipose tissue secretes a number of inflammatory mediators (e.g. IL6, leptin), which are known to cause a low-grade systemic inflammation. This may contribute to periodontal inflammation (Ritchie and Kinane, 2003), possibly through causing insulin resistance (Genco *et al.*, 2005). However, the evidence to support a link between obesity and periodontal disease is largely based on cross-sectional studies and confirmation of causality is required through prospective trials to determine whether lifestyle intervention to address overweight and obesity benefits periodontal health.

5.7 Diet and general health

A joint World Health Organisation and Food and Agricultural Organisation consultation on 'Diet Nutrition and the Prevention of Chronic Disease' met in Geneva in 2002, in response to the growing epidemic of chronic diseases affecting both developed and developing countries, which are strongly related to diet (World Health Organisation, 2003). The task of the consultation was to review the evidence for diet as a risk factor for diseases and make recommendations for governments, international agencies and relevant partners in the public and private sector.

Almost all countries, developed and developing, are experiencing an obesity epidemic and this is of concern because it is associated with cardiovascular disease (CVD), hypertension, diabetes and some cancers. For example, one study in the USA showed that 53% of deaths in women with obesity were directly related to their obesity. On reviewing available evidence on the association between diet and obesity, the WHO Expert Committee concluded that there was convincing evidence that a diet high in fibre prevents obesity. It also concluded that there was convincing evidence that diets high in energy-dense foods, that is, foods that are high in fat or free sugars or both, and low in fruit and vegetables and wholegrain cereals increase the risk of obesity. It was also stated that it was probable that a high intake of sugared soft drinks and fruit juices increase the risk of obesity. These findings highlight that there are common dietary risk factors for oral health diseases such as dental caries and enamel erosion and obesity.

However, diet is second only to tobacco as a preventable cause of cancer. Cancer rates are also increasing in developing countries in parallel with economic development.

On reviewing available evidence on the association between diet and cancer, the WHO Expert Committee concluded that it was probable that a diet low in fruit and vegetables increased the risk of cancer of the colon and of oral cancer. The WCRF recommend a population average consumption of 600 g per day of non-starchy vegetables. The evidence reviewed by WHO also suggests a possible link between a diet low in fibre and increased risk of cancer. In addition, obesity and high meat consumption were associated with cancer; the latter with cancer of the colon. This is of relevance to oral health as it indicates that the type of diet often reported in patients with tooth loss (typically low in fruits, vegetables and fibre) could be placing them at increased risk of some cancers. Furthermore, the WCRF report on diet and the prevention of cancer recommends limiting energy-dense foods and avoiding sugared drinks (to prevent obesity) as strategies for cancer prevention.

Table 5.2 Global dietary recommendations for general and oral health

Dietary factor	WHO recommendation*	WCRF recommendation**	Rationale
Total fat	15–30% energy intake	Limit energy dense foods	Obesity, CVD, diabetes
Saturated fat	0–10% energy intake		Diabetes, CVD
Free sugars	0–10% energy intake	Limit energy dense foods. Avoid sugared drinks	Dental caries Obesity Cancer (obesity)
Starchy foods	Total carbohydrate 55–75% energy intake	Limit refined starchy foods	Cancer (via obesity)
Fibre	From foods	>25 g per day	Obesity Diabetes CVD Cancer
Fruits and vegetables	>400 g per day	Population average 600 g per day non-starchy fruits and vegetables. Individual goal >400 g per day	CVD Cancer

* World Health Organisation (2003). *Diet, Nutrition and the Prevention of Chronic Disease*. Technical Report Series 916. World Health Organisation/Food and Agricultural Organisation, Geneva.

** World Cancer Research Fund (2007). *Food, Nutrition and Physical Activity and the Prevention of Cancer: a Global Perspective*, World Cancer Research Fund, American Institute of Cancer Research.

One-third of global deaths are due to CVD and an epidemic is emerging in developing countries owing to the adoption of a more westernised diet that is high in energy-dense foods. On reviewing available evidence on the association between diet and CVD, the WHO Expert Committee concluded that there was convincing evidence that myristic and palmitic acids, saturated fats mostly found in meat and dairy foods, increase low density lipoprotein cholesterol and if they are replaced with linoleic acid (which is found in vegetable oils) this would reduce the risk of CVD. There is very strong evidence that a high intake of fruit and vegetables protects against CVD and that low intakes of fibre, wholegrains and nuts, dietary practices often seen in the edentulous, increased the risk of CVD.

The dietary goals of the WHO Expert Consultation and of the WCRF Report are summarised in Table 5.2. It appears that many of the global dietary recommendations for the prevention of systemic chronic diseases would also benefit oral health. Therefore, consumption of a diet that is low in saturated fat, free of sugars and high in fruits, vegetables and fibre will safeguard oral health as well as general health and well-being, confirming that dietary goals for oral health should be consistent with those for overall health.

5.8 Conclusion

From this overview of the literature, the broad scope of the interaction among diet, nutritional status and oral health and disease can be seen. What is also apparent is that many of the dietary risk factors for oral disease are in common with other diet-related conditions. Nutrition health promotion in the dental health field need no longer focus on the mouth alone, but should adopt a more holistic approach which considers systemic health and well-being.

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6

Relating breath malodour to food constituents and oral health

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Abstract: Halitosis is classified according to its origin of generation within the body. The most common cause of halitosis is oral malodour which is of microbial aetiology and caused mainly by biofilms on the tongue and periodontal environment. Some breath odour is due to infections of the pharynx, bronchioles or lungs. A rare type is bloodborne halitosis where compounds from the gut, liver or kidney are circulated to the lungs and volatilized on breath. The mechanisms of odour generation, perception of odour, its objective measurement and how food, drink and nutrient deficiencies may affect its generation is reviewed in this chapter and methods for odour management outlined.

Key words: bloodborne halitosis, endogenous–exogenous substrates, malnourishment, odour-generating microbes, oral malodour, periodontal disease, vitamin deficiency, volatile organic compounds (VOCs), volatile sulphur compounds (VSC), xerostomia.

6.1 Breath malodour and its measurement

Bad breath (also known as *foetor oris*, *foetor ex oris*, halitosis and oral malodour) is used to describe the general condition of having odiferous breath. It seems likely that all human beings will suffer to a certain extent with odiferous breath at some period of time during their lives and for many this will affect their quality of life. Personal ambient odour may influence the perception of an individuals worth in society (Hirsch, 2008). Halitosis frequently causes embarrassment, may affect interpersonal social communication (Bosy, 1997) and may rank only behind dental caries and periodontal disease as the cause of a patient's visits to the dentist. It has also become an important market for the pharmacological and cosmetic industries, with

millions of pounds, euros and dollars spent annually on medications and over-the-counter products. The perception of halitosis is different in culturally diverse populations (Rayman and Almas, 2008). The prevalence of halitosis is unknown, particularly since it is not an all or nothing phenomenon and the degree to which it is severe enough to be considered socially unacceptable (also known as pathologic halitosis) will vary depending on how it is judged. Moreover, it is only recently that researchers have adopted common terminology (see Table 6.1) and classification to cover the different types and aspects of halitosis (Porter and Scully, 2006; Sanz *et al*. 2001). Available epidemiological data are difficult to evaluate, but suggest that halitosis is common and can affect people of all ages. The prevalence of persistent oral malodour in a recent Brazilian study (Nadanovsky *et al*., 2007) was 15% and nearly three times higher in men than in women. More-

Table 6.1 Terminology and classification of halitosis

Term	Definition	Notes
Halitosis	Any disagreeable odour of expired air, regardless of origin	
Bad breath	Lay term for halitosis	
Genuine halitosis	Where breath malodour can be verified objectively	Inter-subjective agreement in double blind assessments by judges, and/or objective measures from instruments
Intra-oral halitosis (oral malodour)	Intra-oral site of generation by microbes in the mouth	Physiologic halitosis (transient, including morning breath) Pathologic halitosis (persistent and strong)
Extra-oral halitosis	Extra-oral generation by microbes (in respiratory tract other than mouth) or bloodborne halitosis (via lungs)	Pathogenic halitosis (persistent and strong).
Pseudo-halitosis	The patient initially believes that they have halitosis until presented with objective evidence that they do not. Patient then agrees that they do not have the condition.	Error of self-diagnosis
Halitophobia	The patient persists in believing they have halitosis despite firm evidence to the contrary.	Psychological condition (delusional; monosymptomatic hypochondriasis)

over, the risk was more than three times higher in people over 20 years of age compared with those aged 20 years or under (controlled for gender). The large majority of studies report figures of about 30% (Liu *et al*., 2006; Sanz *et al*., 2001; Ueno *et al*., 2007), but some studies estimate that more than 50% of the population have halitosis (Tessier and Kulkarni, 1991).

Halitosis can be classified according to its site of origin (Myazaki *et al*., 1999; Yaegaki and Coil, 2000). If the site of generation is the oral cavity itself (and thus of microbial aetiology), it can be defined as 'oral malodour'. If the halitosis is from the respiratory tract, it can be detected equally well on breath from the mouth or from the nose and has an extra-oral source. This may include upper respiratory tract infections or be present from the lower respiratory tract (infected lung or bronchus). All these 'causes' are thus of microbial aetiology and between them account for over 90% of all halitosis cases. Alternatively, volatile organic compounds (VOCs) are being produced by the mammalian system (van den Veld *et al*., 2007a) and VOCs in the bloodstream are carried to the lungs where they enter the breath by volatilization. Potential sources of (non-microbial) halitosis include certain systemic diseases, metabolic disorders, medications and certain foods. Systemic diseases and metabolic disorders, together with the effect of certain medications and foods, may be grouped into the category of 'bloodborne halitosis'.

Levels of malodour (from zero to highly noticeable) equate with concentrations of volatile compounds (VCs); more specifically VOCs which are odiferous in nature. To be perceived as noticeable and unpleasant, the VOCs in question must be well above their threshold concentration for smell detection by the perceiver. The relative 'smell' of a pure compound can be determined by accurate dilution of the VOC in question to the point where a panel of judges can just detect it. The degree of dilution necessary to reach this point is a measure of the olfactory power (p_{ol} value) of the compound in question. The unit of measurement (p_{ol} by volume) is defined as the negative log of the threshold concentration expressed in volumic or molar fractions. For example, 1 ppm = p_{ol} 6, whilst 1 ppb = p_{ol} 9. Some approximate averaged standardized thresholds have been published (Devos *et al*., 1990). A list of p_{ol} values of some compounds of possible importance in breath odour are shown in Table 6.2. The full list of odiferous VOCs has not been established since there are thousands of potentially odiferous compounds in nature. The many VOCs that are found in breath (Van den Velde *et al*., 2007b) include a sub-class described as volatile sulphur compounds (VSCs). These are thought to be important contributors to oral malodour. VSCs and other VOCs can be detected by analytic instruments (sensors; gas chromatography, GC; gas chromatography–mass spectrometry, GC–MS direct mass spectrometry, D–MS) which allow for objective measurement (gas phase concentrations of VOC) which in turn may be related to their degree of odour as described by human perceivers (trained breath judges). An example of breath analysis by GC–MS is shown in Fig. 6.1.

Table 6.2 Averaged olfactory threshold for some putative malodour compounds in bad breath (Devos *et al*., 1990; El-Maaytah, 1996)

Compound	Synonym	p_{ol} value
Sulphides:		
Hydrogen sulphide	Hydrogen sulphide	7.75
Methyl mercaptan	Methyl sulphide	8.98
Methylthiomethane	Dimethyl sulphide	8.60
Methyldithiomethane	Dimethyl disulphide	7.91
Indoles:		
Indole	Indole	10.50
3-methyl indole	Skatole	9.25
Acids:		
Acetic	Acetic	6.84
Propanoic	Propionic (C3)	7.45
Butanoic	Butyric (C4)	8.41
2-methylpropanoic	Isobutyric (C4)	7.71
Pentanoic	Valeric (C5)	8.32
3-methylbutanoic	Isovaleric (C5)	8.61
Hexanoic	Caproic (C6)	7.90
4-methylpentanoic	Isocaproic (C6)	7.81
Heptanoic	Oenanthic (C7)	7.56
Octanoic	Caprylic (C8)	8.40
Amines:		
Ammonia		5.24
Methylamine		7.73
Trimethylamine		8.60
1,4 diaminobutane	Putrescine	7.45
1,5 diaminopentane	Cadaverine	8.20
Pyrrolidine		7.50
Butylamine		6.28

6.1.1 Perception of odour and breath assessment

Different VOCs have different smells. The spectrum of smells depends on the types of smell receptors present in the nose (see composite Fig. 6.2). The sense of smell in some animals (dogs, bears and rats) is generally regarded as superior to that of humans. Smells are sensed by olfactory sensory neurons in the olfactory epithelium. Humans have about 16 cm^2 of olfactory epithelium, whereas some dogs have 150 cm^2. A dog's olfactory epithelium is also considerably more densely innervated, with a hundred times more receptors per square centimeter giving (in total) about 2 billion olfactory receptors in dogs against around 40 million in humans. Whether animals have a wider range of receptors (to give a wider spectrum of smell), is uncertain. Moreover, animals may have better ways of focusing gas molecules onto the sensitive areas of the sensory epithelium (via the shape of the nasal passages, combined with the ability to sniff). The olfactory epithelium surfaces are covered in fluid (mucous) and gas molecules must

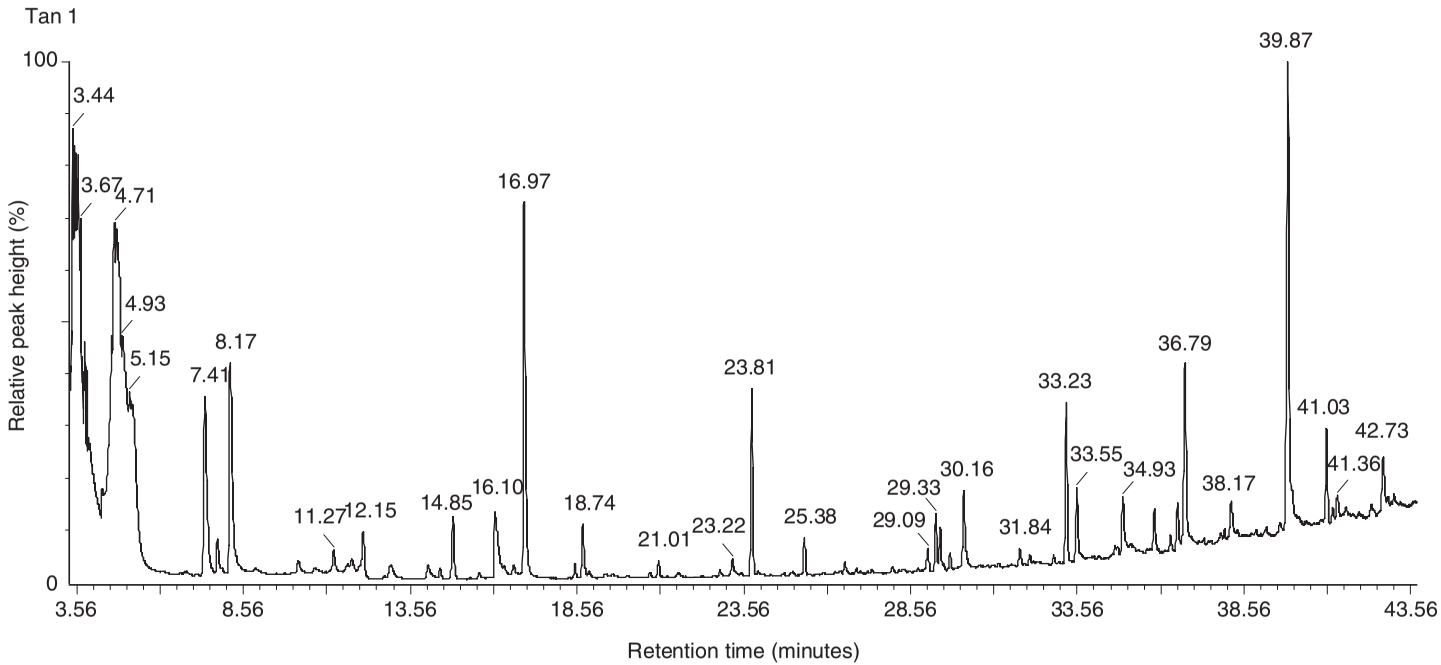

Fig. 6.1 Gas chromatography–mass spectroscopy (GC–MS) trace of a breath sample from a healthy person. The breath sample was pre-concentrated onto a 5-bed absorbent tube and was then thermally desorbed and analysed by GC–MS. Typically well over 50 different VOCs can be identified.

first dissolve in this and then diffuse across a thin film layer in order to reach the receptor surfaces. The mucous layer may be capable of selective adsorption, thus concentrating or focussing the VOCs taken from a (relatively) large volume of gas stream. A further sensitivity may arise in animals owing to neuronal development of brain areas associated with smell.

6.1.2 Odorant receptors

Sensory neurons in the olfactory epithelium contain receptor proteins at the end of small projections called cilia, which are bathed in mucus. Buck and Axel (1991) discovered both the family of transmembrane proteins that were believed to be the odour receptors and some of the genes that encode them. It is now known that there are about 350 odorant receptor genes and about 560 odorant receptor pseudogenes in humans (Buck and Axel, 1991). This number of genes and pseudogenes, specific to the olfactory system, comprises 2–3% of the 50 000 or so genes of the human genome.

The axons of receptor cells carrying the same type of receptor converge into the same glomerulus (see composite Fig. 6.2), where they contact the next level of nerve cells, the mitral cells. Each mitral cell is activated only by one glomerulus and the specificity in the information flow is thereby maintained. The mitral cells send the information to several parts of the brain (via long nerve processes) where the information from several types of odourant receptors is combined into a pattern characteristic for each odour.

In reality, each neurone with its receptors does not react to one odourous substance but to several related molecules with varying degrees depending on their odour-receptor binding affinity; thus, each receptor can recognize several odorant molecules. Conversely, a single odorant molecule can be recognized by multiple receptors, some with higher affinity than others. Thus, different combinations of receptors might be able to identify a multitude of odorants similar to the way different combinations of letters form words. By using around 350 distinct odorant receptors, the human nose can distinguish thousands of odorants.

However, certain substances named ‘irritants’ (ammonia, pepper, onions), when sniffed or inhaled are known to cause burning, stinging, tickling, cooling, warming and pungency (Wise *et al*., 2005). It is believed that these sensations are conveyed through the trigeminal nerve (whose receptors are located on free nerve endings within the nasal epithelium) and that the activation of the trigeminal nerve triggers protective reflexes such as apnea and sneezing (Finger *et al*., 2003).

The existence of the vomeronasal system, an accessory organ situated in the base of the nasal septum, has been the subject of great debate (Meredith, 2001). It is believed that this uni- or bilateral membranous structure exists in 50–97% of individuals (Zosel *et al*., 2004). It is also believed that the vomeronasal organ (VNO) is connected to the brain via the vomeronasal

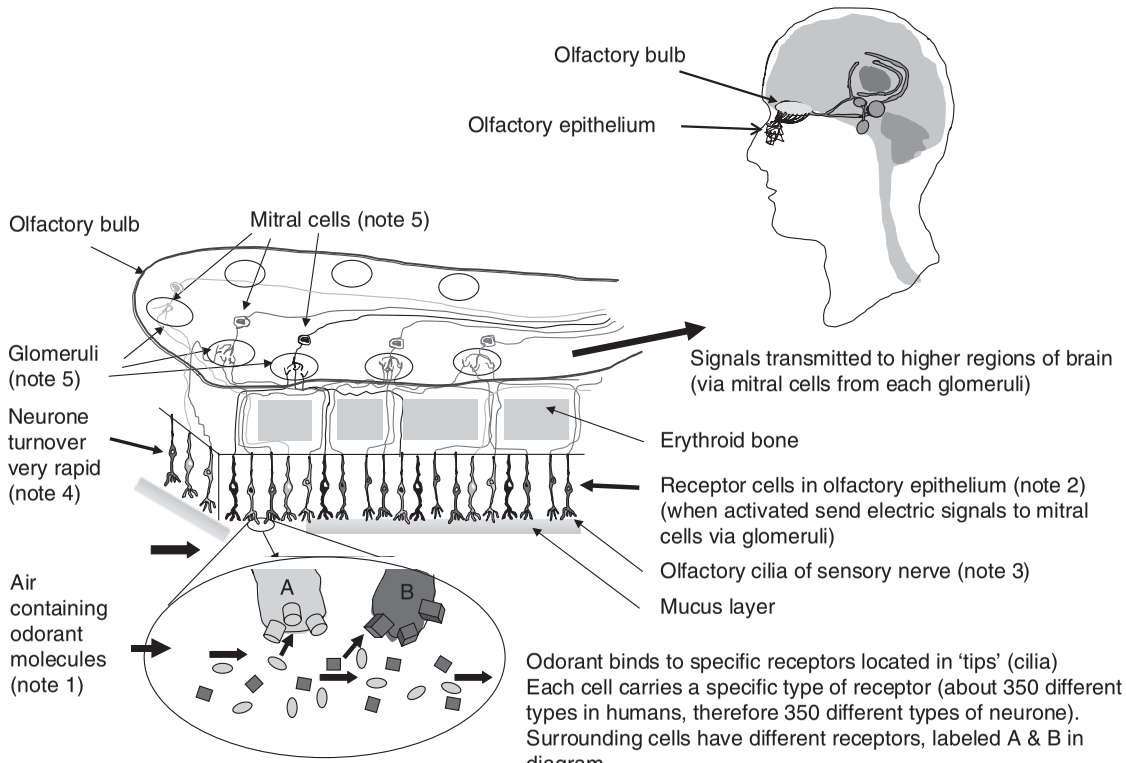
Olfactory bulb
Olfactory epithelium
Olfactory bulb
Mitral cells (note 5)
Glomeruli (note 5)
Neurone turnover very rapid (note 4)
Air containing odorant molecules (note 1)
A
B
Signals transmitted to higher regions of brain (via mitral cells from each glomeruli)
Erythroid bone
Receptor cells in olfactory epithelium (note 2) (when activated send electric signals to mitral cells via glomeruli)
Olfactory cilia of sensory nerve (note 3)
Mucus layer
Odorant binds to specific receptors located in 'tips' (cilia)
Each cell carries a specific type of receptor (about 350 different types in humans, therefore 350 different types of neurone).
Surrounding cells have different receptors, labeled A & B in diagram

Fig. 6.2 The sense of smell.

1. Odorant properties: Molecular weight <300; some water solubility, a sufficiently high vapor pressure, low polarity, some ability to dissolve in fat (lipophilicity), and surface activity.
2. Regio olfactoria (olfactory epithelium): Located in the roof of the two nasal cavities of the human nose, just below and between the eyes. These are small areas of about 2.5 cm^2 per nostril with about 50 million primary sensory receptor cells. Each olfactory receptor neuron has 8–20 cilia that are whip-like extensions 30–200 μm in length. These project down into a layer of mucous which is about 60 μm thick. This mucous layer is produced by the Bowman's glands, which reside in the olfactory epithelium. It is a lipid-rich secretion that bathes the surface of the receptors at the epithelium surface.
3. Olfactory cilia: These are the sites where molecular reception with the odorant occurs and sensory transduction (i.e. transmission) occurs. The receptors are termed G-protein-linked receptors since they trigger the biochemical synthesis of neurotransmitters, including cAMP and inositol triphosphate, which open cation channels that ultimately lead to action potentials and signalling.
4. Neurone turnover: In contrast to all other neurons, the olfactory receptor neurons naturally replace at a (relatively) great speed of regeneration with a turnover of approximately 40 days. Of some significance is the finding that the new neurons do not replace the old ones in exactly the same position on the epithelial surface relative to other types of receptor, but they do remain fixed to the same glomeruli to converge with all the sensory neurons carrying the same type of receptor.
5. Glomeruli and mitral cells: While the olfactory receptor neurons extend through the epithelium to contact odorants in the atmosphere, on the opposite side within the epithelium, the neuronal cells form axons that are bundled in groups of 10–100 penetrating the ethmoidal cribriform plate of bone, reaching the olfactory bulb of the brain where they converge to terminate with post-synaptic cells forming synaptic structures called glomeruli. The glomeruli are connected in groups that converge into mitral cells. The total convergence is estimated to be about 1000:1. Physiologically, this convergence increases the sensitivity of the olfactory signal sent to the brain. From the mitral cells the message is sent directly to the higher levels of the central nervous system in the corticomedial amygdala portion of the brain (via the olfactory nerve tract) where the signalling process is decoded and an olfactory interpretation and response occurs.

One receptor must be able to interact with several discrete odorants. Conversely, an odour molecule must be capable of interacting with multiple receptors. By inference, an individual odour will activate multiple glomeruli in the olfactory bulb.

terminalis nerve and it is generally accepted that the signals detected by this system are not detected by the olfactory system and do not function in the same manner. The external chemical signals detected by the vomeronasal organ (namely pheromones or vomeropherins) are believed to mediate human autonomic, psychological and hormonal responses.

6.1.3 Organoleptic and hedonic judgement of odours

There is an assumption when judging the strength of particular target odours, the linkage between ligand binding by odour receptors and biological response (e.g. nerve firing) is direct or proportional. When considering *in vivo* tests on odorants, there is another assumption; that the sensory information about the strength of the odorant is somehow maintained when transformed into nerve firing rate. Each action potential (spike or impulse) has the same amplitude, but the firing rate changes frequency from just a few 'spikes' up to 1000s of spikes per second (per axon). Therefore, the higher the concentration of odorant, the greater the degree of stimulus and the greater the frequency of action potentials from the same family of sensory nerve cells/receptors. There is a final assumption that the whole network of repeating chains of electrical and chemical events (synapses, junctions and combination of hierarchies and parallel processes) still maintain objective information on the strength of odorant. Somehow, the message from the sensory nerves to the brain (via complex neuronal assemblages and gestalts) allows us to report a score that relates to strength of odorant.

Our sense of smell is important to our quality of life. The normal smell acuity in humans allows an individual to both recognize and distinguish hundreds of different smells when exposed to different types of compound and to describe the strength of the smells (faint or strong). Humans can be trained to assess and report the strength of a target smell using a method called organoleptic assessment (see below). However, by associative learning in early life, humans have come to associate some smells with pleasant things (edible fruit, roast dinner, fine wine) and some with unpleasant things (dead matter, rotting corpses and excrement). Whereas dogs and other animals may 'like' the smells of the latter (if wagging of the tail can be considered to convey an opinion), the human learns to regard some smells as pleasant and some as unpleasant depending on societal and family conditioning. This gives rise to a second (psychological level) method of smell judgement; whether a particular smell at a particular time is pleasant (or nice) or unpleasant (nasty). This type of smell judgement is termed hedonic assessment and should be distinguished from the organoleptic method, which simply assesses the strength of the target smell(s) without subjective opinion on how pleasant or unpleasant these may be.

In the organoleptic method, the judge is ideally giving a true report on a 'learnt' scale which correlates with the saturation of their smell receptors specific for the target VOCs in question. Smell acuity can be easily

compromised in humans owing to virus infections masking odours and gradual loss of smell acuity (loss of receptor within receptor-bearing cells, or loss of receptor bearing cells as cell lineages [clone ablation] or inhibition of turnover of receptors during middle and old age). The Smell Identification Test (SIT, Sensonics Inc., Haddon Heights, NJ, USA), the equivalent of the eye chart in vision, was designed to test the smell function of individuals using smell scratch cards with 40 different odorants. It was introduced in the workplace for employees working in hazardous environments and in the medical field as an accurate clinical assessment of smell loss (anosmia) which affects individuals with a great number of medical conditions as well as being an early diagnostic test for neurodegenerative conditions. Moreover, it has been used for the selection of the 'best' sensory panelists in the cosmetic, food and oral care industry.

Using smell scratch card data for populations, the SIT can quantify the degree of dysfunction caused by diseases, accidents and overexposure to chemicals. The scores are compared against sex- and age-related norms and the results are analysed using a chart relating scores to varying patient populations, including patients with multiple sclerosis, Korsakoff syndrome and those feigning anosmia. Those in the latter group tend to score much lower on the test than expected by chance. However, with full smell acuity, humans can be easily taught how to use their nose in an objective manner, which can be assessed using double-blind tests with pure odorants in order to verify this approach. In effect, the judge is treating their nose as if it was a sensor.

There are a number of different organoleptic scales that have been described in the literature (Allison and Katz, 1919; Rosenberg *et al*., 1991; Greenman *et al*., 2004, 2005). One that is commonly used is the 0 to 5 scale (Greenman *et al*., 2004) where the integers have descriptive explanations (see Table 6.3). Because there is fairly close association between quantitative (instrumental) methods and organoleptic methods for pure compounds and for a wide range of real breath samples (across the scale from zero to 5), it can be shown through codeterminations (Rosenberg *et al*., 1991; El Maaytah, 1996; Saad, 2006) that the strength scale that humans perceive is

Table 6.3 The 0 to 5 organoleptic scale of Rosenberg (Rosenberg *et al*., 1991) as modified by Greenman *et al*. (2004)

Score	Descriptive
0	No odour
1	Barely noticeable odour
2	Slight odour
3	Moderate odour
4	Strong odour
5	Extremely strong odour close to saturation

not linear but exponential in nature (El-Maaytah, 1996; Greenman *et al*., 2004, 2005). In this respect, it is similar to other sense organs (eyes for assessment of light levels using the lux scale; ears for hearing notes whose amplitudes are being judged in decibels) and obeys Stevens psychophysical power law (Stevens, 1957), namely:

$$S = kI^n \quad \text{or} \quad \log S = \log k + n \log I$$

where S = magnitude of agonist (odour concentration), I = magnitude of reported intensity and k and n are constants. Thus for oral malodour, a smell can be judged to be 'twice as strong' as a known control when in reality the gas concentrations are in the order of 5–7 times the concentration of the original control (El-Maaytah, 1996; Greenman *et al*., 2004, 2005). The exponential character of the organoleptic scale means that, when it is plotted against instrumental measurements of gas concentration, the latter should be plotted on a log scale to allow linear regression or correlation (and degrees of scatter) to be determined. Then it can be shown that a straight line relationship exists between log concentrations of odorant (as measured by a halimeter) and organoleptic scores (see Fig. 6.3).

6.1.4 Instrumental analysis

Objective instrumental methods of measuring VCs/VSCs include gas chromatography with sensitive detectors (flame photometric sulphur device (PSD), sulphur chemiluminescence detectors (SCD) and mass spectroscopy detectors). These instruments are often expensive and require technical maintenance and support. They are rarely portable, which means that gas

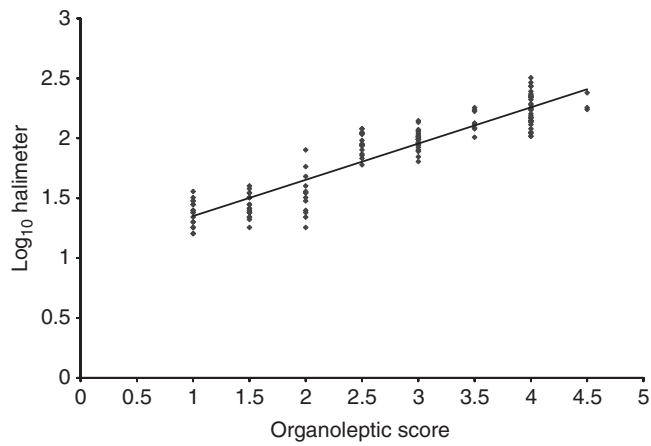

Fig. 6.3 The exponential nature of the organoleptic scale. The straight line relationship ($R^2 = 0.8566$) was observed by plotting organoleptic scores against log-transformed halimeter readings for $n = 120$ codeterminations (Saad, 2006).

sampling is required in a tube, teflon bag or storage loop, which can then be transported to the machine equipment. Alternatively, the gas sample may be adsorbed on to an inert material or cryogenically 'trapped' and later thermally desorbed into the GC inlet. Despite these problems, many workers have used GC to great advantage to identify and measure different molecular species. The methods which use PSD and SCD detectors are specific for sulphide gases. Gas chromatography in combination with a PSD has been used successfully to measure breath VSCs (Tonzetich, 1971; Tonzetich, 1977; Yaegaki and Sanada, 1992). The advantage of GC with an MS detector (GC–MS) is that it can potentially detect a whole range of VOCs and VSCs. However, water, carbon dioxide and air can often mask detection and, consequently, it has not been used routinely to measure bad breath.

In recent years a number of selective sensors have become available to detect one or more of the VSCs found in breath. One such sensor is a gold film electrode (Jerome 631-X analyser, Arizona Instruments Corporation, Phoenix, Arizona, USA), which specifically detects hydrogen sulphide and is extremely sensitive down to 1ppb. Another instrument widely used for measuring VSCs on breath is the halimeter (Rosenberg *et al*., 1991) which uses a sulphide sensitive voltammetric sensor. An instrument using a zinc oxide sensor has also been described (Shimura *et al*., 1996). Unfortunately, these instruments are sensitive to moisture and require pumps, traps or filters in order to draw mouth gas across the sensor and exclude moisture. Other interferents include alcohol, flavour oils or chlorine compounds that are commonly found in breath-freshening mouthwashes. Both these instruments detect VSCs but cannot discriminate between the levels of hydrogen sulphide and methyl mercaptan. The sensors are less sensitive to methyl mercaptan and yet this compound has a much higher odour power rating (p_{ol} value) than hydrogen sulphide.

Traditional laboratory GC or GC–MS are cumbersome, need inert column carrier gas (gas cylinders of nitrogen or helium) and require technicians or specialists with adequate training and are thus clinically impractical. However, a newly developed portable gas chromatograph (OralChroma™) has now been described (Tangerman and Winkel, 2007; van den Veld *et al*., 2007a), which utilizes an iridium-based semiconductor gas sensor, does not require the use of a special carrier gas (using air instead) and is highly sensitive to sulphides yet relatively low cost compared to a standard gas chromatograph.

6.1.5 Microbial putrefaction

Fundamental early work in the USA established oral malodour to be the result of bacterial putrefaction by oral microbes (Miller, 1890). Many later workers agree with this description (Sulser *et al*., 1939; Berg and Fosdick, 1946; Tonzetich, 1971; McNamara *et al*., 1972). Putrefaction is the decomposition of organic matter, particularly animal proteins by anaerobic micro-

organisms, described as putrefying bacteria. When animals die in their natural environment, putrefaction quickly ensues. Putrefaction is the destruction of the soft tissues of the body by the action of microorganisms (bacteria, fungi and protozoa) and results in the biotransformation of tissue into gases, liquids and simple molecules. Typically, the process starts via gut organisms seeping into the rest of the body with distention of the tissues (bloat) caused by gas production (carbon dioxide, hydrogen, methane, ammonia and hydrogen sulphide).

The destruction of soft tissues is mainly due to anaerobic fermentation. In addition to the gases, volatile fatty acids are produced such as propionate and butyrate. Shortly after purging of the gases, active decay sets in. Muscle, composed of protein, is hydrolysed into small peptides and amino acids by proteolytic activity and the amino acids are further broken down depending on the type. Yet more fatty acids are produced as well as indole, methylindole (skatole), putrescine, cadaverine and many other types of VOCs. Therefore, putrefaction usually results in volatile organic compounds (VOCs), which have a putrid odour. Material that is subject to putrefaction is called putrescible. It should be noted that any protein-rich materials left in the open environment would decay. This includes foods such as meat, milk or eggs as well as animals or fish. Indeed, almost all organic compounds are eventually broken down by some types of microorganisms somewhere. However, it is only the proteinaceous materials that putrefy. In general, low pH favours growth of yeasts and moulds, whilst bacteria are more dominant at neutral or alkaline pH. However, the same process of decomposition occurs wherever there is a rich diversity of microorganisms and organic matter including sewage breakdown and composting as well as the breakdown of trapped food in the mouth.

It is clear from previous work (McNamara *et al*., 1972; Tonzetich, 1971; Tonzetich and McBride, 1981; Kleinberg and Westbay, 1990; Kleinberg and Codipilly, 1995; Codipilly and Kleinberg, 2008) that anaerobes, and particularly the Gram-negative anaerobes, tend to produce a higher degree of malodourous VC/VSCs in culture than other groups. By incubating species of oral microbes in liquid culture containing elevated levels of amino acid substrates, Kleinberg and Codipilly (1995) were able to demonstrate odour production by a range of species against a range of substrates. In general, Gram-positive species were not particularly effective at producing odour whilst Gram-negative species, particularly the anaerobic species *Porphyromonas gingivalis* and *Prevotella intermedia*, were effective odour producers. The most effective substrates to induce odour were cystine/cysteine, methionine, ornithine, lysine and tryptophan. *P. gingivalis* produced increased odour with all these substrates, which implies that it may have the potential to produce hydrogen sulphide, methyl mercaptan, putrescine, cadaverine and indole from respective substrates. In addition to having a high smell threshold, H_2S contributes significantly to lowered redox potential (E_h) (Kleinberg and Codipilly, 2008).

Research over the last decade has shown the relationship between halitosis and sources of VOC generation within the human individual. In most cases of bad breath (>80%), it appears that the main generation site for odour is the oral cavity itself and is from a microbial source (oral malodour). Furthermore, the tongue surface (rather than teeth or other mucosal surfaces) appears to be the most important intra-oral site (Yaegaki and Sanada, 1992; Rosenberg and Leib, 1995, De Boever and Loesche, 1996). This is not surprising since a high density of microbes in the form of biofilms populates the tongue dorsum surface. Viable numbers may reach as high as 10^9 cm^{-2} of the dorsum surface (macrosurface area) (Hartley *et al.*, 1996a, 1996b; Saad, 2006). Typically the viable count (colony forming units, cfu) is only 10% of the total count, suggesting that the cell density of the tongue biofilm may be as high as 10^{10} cells cm^{-2}. The dorsum anterior to posterior surface is approximately 30 cm^2 giving a total microbiota of 10^{11}–10^{12} cells. Subjects who clean their teeth just prior to breath measurements (organoleptic or instrumental) are able to reduce bad breath by only a small fraction (20%), whilst removing the biofilm from the tongue (by careful brushing) may remove up to 80% of the odour (Tonzetich, 1977; Bosy *et al.*, 1994).

As previously stated, up to 80% of all cases of bad breath or halitosis are regarded to be oral malodour. Most of the rest (~15%) may still be mainly due to a microbial aetiology where the microbes are infecting the pharynx (nose, throat, tonsils) or the bronchioles and lungs. It has been estimated that up to 5% of all cases of bad breath halitosis may be due to VOCs produced by the host mammalian systems, which (via bloodstream circulation and alveolar exchange in the lungs) become exhaled on the breath. Such VOCs can be detected equally well from samples taken from mouth gas or gas from the nasal passages (during normal breathing). The various metabolic or physiological conditions that cause endogenous body odours (especially breath odour) are reviewed in Section 6.3. Therefore, in total, over 95% of all cases of halitosis have a microbial aetiology.

6.2 The central paradigm for intra-oral generation by microbes

The simple model of oral malodour explains the presence of VOCs on the breath to be due to the presence of microbes with particular enzymes or enzyme pathways (cellular activity) converting substrates into volatile odiferous products. The types of volatile products include hydrogen sulphide, methylmercaptan, organic acids, amines and indole. The types of substrates are described in Fig. 6.4 and can be classed as immediate substrates (e.g. cysteine, methionine, tryptophan) and secondary substrates (glutathione, proteins and peptides containing cys, met and trypt residues). The rate of volatile product formation can be limited by the amount of enzyme activity

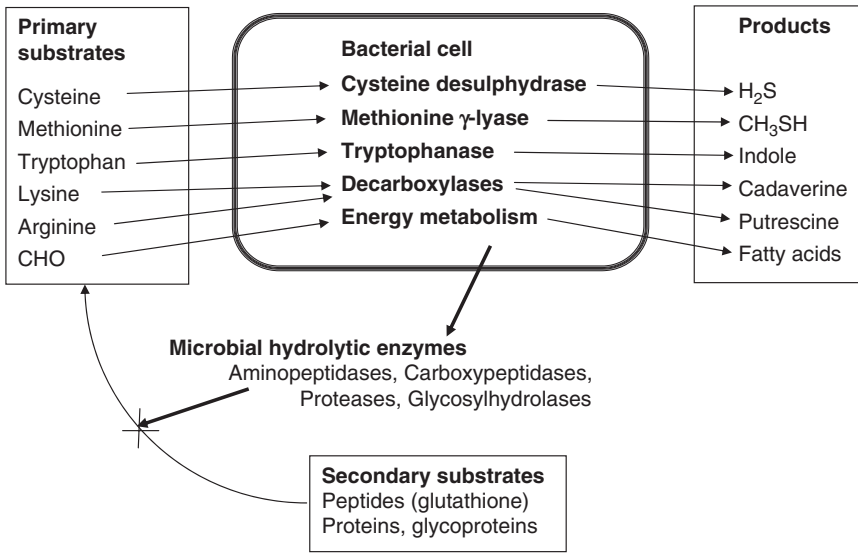

Fig. 6.4 Primary and secondary substrates for production of odiferous VOCs by microbes.

(which corresponds to the number of cells containing the transforming enzymes). There are other points to consider such as the concentrations of substrates available and the types of microbes present as an ecological mix and their physiological state. All these factors may influence malodour generation rates.

Whatever the ecological mix of species that may be present on the tongue biofilm, if the total amount is close to a zero quantity of microbial cells (i.e. enzymes), then there will be close to zero conversion rate of substrates to volatile products and zero malodour (Hartley *et al*., 1999). A mouth with greater biomass per square centimetre of surfaces will have greater conversion rates, and twice the biomass (twice the amount of enzyme) might be expected to increase the generation rate two-fold.

Measuring the amount of biofilm on the tongue dorsum surface and relating this to the surface area (defined as aerial density) has been attempted by a number of workers (Hartley *et al*., 1996a, 1996b; Saad, 2006). The most important feature of these studies was an attempt to relate whatever biomass measure was taken (microscopic count, viable count, total protein, dry weight, etc.) to the surface area from which the sample was taken. By sequential sampling of the same sample area, it can be shown that a single impressed toothbrush sample removes about 10% of what is present (Hartley *et al*., 1996a). After 10 sequential samples, the number of cfu recovered is less than 1 – log the starting value. Most importantly, Hartley and co-workers were able to show that a single sample, despite only removing a small fraction of resident microbes (tongue biofilm) was nevertheless proportionate to the whole. The fact that samples from a known area (if

taken in a similar manner) are proportional to what is there means that assessments relating biofilm to malodour are valid. Other methods of relating tongue biofilm and malodour rely on a measure known as the tongue coating index (Gross *et al*., 1975; Bosy *et al*., 1994; Miyazaki *et al*., 1995; Chen, 1987; Winkel *et al*., 2003; Gomez *et al*., 2001) using a visual assessment of tongue coating. Typically, this is assessed using a 0–5 (or 0–3 or 0–12) index. However, it is well known that different microbial species have large differences in their opacity to light, some being translucent and others being heavily pigmented. It is also known that exogenous staining (tea, coffee, smoking, food pigments) can markedly affect the visibility of the coating. It is unknown whether the tongue coating index is an exponential or a linear scale and it is not known how the tongue index relates to the biofilm aerial density (Saad and Greenman, 2008).

6.2.1 Specific and non-specific theories regarding causative species

Causation of VSC generation depends on the concentration of VC/VSC-generating enzyme activity, which in turn depends upon (a) the proportion of species that possess generating activity and (b) the physiological expression of enzyme activity per microbial cell (the physiological state of the microbe in relation to the prevailing physicochemical conditions). Regarding species specificity, there are two dichotomous theories (in common with dental caries and periodontal disease; see Marsh, 2003).

The specific theory of microbial aetiology states that one (or a small number, say two or three) of distinct microbial species (causative species) within the total ecological mix, are solely responsible for the malodour condition. The species in question will be negatively associated with low or no malodour but positively associated with high malodour. If none of the species are present, the individual will not have oral malodour. This theory is in opposition to the non-specific theory which states that there is no single species that is causative in itself since many microbial species can substitute for one another with respect to generation of VC/VSCs. In support of the latter theory, it has been observed that many species of microbes isolated from the tongue biofilm possess an indole-producing capability, many can produce sulphides, acids or amines and many are proteolytic or assacharolytic (Greenman, 1999). However, it has been shown that most of the above characteristics are strongly associated with anaerobes and anaerobic metabolism. However, the numbers of potential species (anaerobes) would still be so high that it could not be used to support a specific theory of aetiology.

6.2.2 Different origins of nutrients and substrates (endogenous and exogenous)

Endogenous substrates are supplied from saliva, desquamating oral epithelial cells and by diffusion from the blood across the oral epithelial coating

(including gingival crevicular fluid). The substrates include immediate (primary) substrates (free amino acids, cysteine, cystine, methionine, tryptophan, lysine, arginine, glucose) and indirect (secondary) substrates (polymers including proteins, peptides, glutathione), which release primary substrates by hydrolytic breakdown from microbial enzymes, particularly proteases, aminopeptidase and carboxypeptidase activities (see Fig. 6.4). The thicker the tongue biofilm, the more salivary proteins can be adsorbed or entrapped within its matrix. Salivary mucin is attacked by breakdown of the glycoside (sugar) moiety (initial steps involving glucosidases, N-acetylglucosaminidases, galactosidases) followed by scission from the protein moieties via fucosidase. This exposes the protein core to subsequent attack by proteolysis.

Exogenous substrates include immediate and secondary substrates supplied in food and drink. The degree to which exogenous dietary compounds can act as malodour substrates for bacteria is unknown. Cysteine rinses have a large effect on H_2S production (Kleinberg and Codipilly, 1999; Kleinberg and Codipilly, 2008) and so it is likely that any of the primary substrates (free amino acids) in food or drink would enhance oral malodour, at least over the short term (up to 10 or 15 minutes following exposure). Secondary substrates from food may also be available to microbes in the mouth depending on their physical characteristics and clearance rates. The physical characteristics (physical size of particles, ionic charge, solubility) may determine their attachment or adsorption into biofilm surfaces. Many food substrates may become entrapped (e.g. between teeth, under dentures).

6.3 Diet, oral health and malodour

6.3.1 Physical nature of food

The urge to swallow food is believed to be triggered by a threshold level in both food particle size and lubrication of the food bolus. Thus, both oral physiology (salivary flow rate, bite force and masticatory performance) and product characteristics may influence the swallowing threshold. It has been shown that salivary flow rates are significantly negatively correlated with the number of chewing cycles for dry foods (Engelen *et al.*, 2005). Hence, subjects with more saliva may need fewer chewing cycles for these dry products. Maximum bite force and masticatory performance also has an influence on the swallowing threshold for hard products such as carrots and peanuts.

Hard and dry products require more chewing cycles and a longer time in the mouth until swallowing in order for sufficient breakdown to take place and for enough saliva to be added to form a coherent bolus that is safe for swallowing. Lipids such as butter enhance lubrication and bolus formation of dry products, thus reducing the number of chewing cycles until

swallowing. Some foods (like caramel and jelly-beans) have high immediate retention (or stickiness) but this is followed by rapid oral clearance since the sticky components quickly dissolve. Conversely, foods like white bread, pretzels, chips and cereal may not feel 'sticky' but exhibit a slow rate of oral clearance when eaten alone.

There are profound and complex interactions between nutrition and oral health. Diet and eating patterns have a local effect on the teeth, saliva and soft tissues from birth to old age and the systemic action of diet can have consequences on the oral cavity. A good and balanced nutrient intake is essential for the development, growth, maintenance of tissues and prevention of cell damage. Diet also affects the efficiency of the immune system, the resistance to infectious and chronic diseases. Clinical manifestations of nutrient deficiencies can have a significant impact on the function of the oral cavity. This includes taste, salivation, mastication and swallowing food. An alteration of the structure and function of the oral cavity may compromise the nutrient-deficiency state. Intra-oral manifestations of malnutrition may include the alteration of the integrity and appearance of the teeth, the soft tissue and tongue. Vitamin deficiencies can cause the gingiva to become abnormally red, spongy and to bleed. A deficiency in folate, niacin, iron, B6 and B12 can be responsible for a glossitis with the tongue appearing red raw and fissured. Iron and folate deficiency can cause the tongue to become pale, atrophic and smooth, with atrophy of the filiform papilla. A deficiency in riboflavin may give the tongue a magenta colour.

Compromised host defence responses associated with malnutrition may make the periodontium more susceptible to infectious organisms that are a normal component of the oral microbiota leading to gingivitis, periodontal disease and periodontal malodour. The acute phase protein response to tissue injury is impaired to varying degrees in malnourished individuals. During periods of malnutrition, the magnitude of the inflammatory response is limited, resulting in an impaired host response. This could result in a greater amount of periodontal destruction, leading to a compromised dentition (Touger-Decker, 1998).

6.3.2 Damage to mucous membranes

The oral cavity is lined by mucous membrane consisting of three layers. The top layer is stratified squamous epithelium covering the second layer (lamina propria), a layer of loose connective tissue containing blood vessels, sensory nerve endings, lymphatic vessels, smooth muscle fibres and areas of lymphatic tissue. A third layer (the submucosa) also contains loose connective tissue containing larger blood vessels and lymphatics, exocrine glands and a network of nerve fibres. The epithelial stem cells replicate in the basement membrane which lies between the lamina propria and submucosa. These cells are continuously being renewed with a life-span of approximately 7–10 days. The outer epithelial layer is completely replaced every 7 to 14 days. This high cell turnover makes the epithelium very prone to

damage by cytotoxic agents such as radiation and chemotherapy. Following 12 days of radiotherapy, the epithelium begins to slough off with no renewal, leaving a denuded mucous membrane with large areas of bleeding and painful ulceration. These areas are very vulnerable to infection by both bacteria and fungi with consequential effects on oral odour.

The interactions between nutrition and oral health are even more marked in the elderly population and it has been suggested that oral malodour increases with age (Miyazaki *et al*., 1995). However, age alone is not always responsible for increased malodour. Other factors such as the decline of the sense of smell and taste might influence oral health and function of individuals (Ship, 1999). The oral health of older people is often compromised owing to the higher incidence of edentulism, the decreased number of natural teeth and the presence of dentures which they rely upon for oral function (Shah *et al*., 2004). Although the salivary flow and composition is not affected by age in the case of healthy, unmedicated individuals (Walls and Steele, 2004), nevertheless, this does not appear to be the case for those who suffer from a medical condition and are under medication. A great number of drugs are known to influence salivary secretion either through a direct effect on the salivary mechanism or indirectly through tissue hydration (Walls and Steele, 2004). Often, several drugs are administered with a synergistic action giving rise to xerostomia. Induced xerostomia can cause problems in chewing, swallowing, speech, taste, tolerance of dentures as well as dental caries and alterations of the oral mucosa. The soft tissue of the oral cavity will change. These problems will in turn affect the individual's diet with a shift towards a softer and more carbohydrate diet.

Another consequence of denture problems in the elderly population is that small particles of food are entrapped between the teeth or remain in the fissures of the tongue where they serve as secondary substrates for the bacteria present on these surfaces. In edentulous individuals, the tongue is often used to help in the chewing process which, when hard food is consumed, makes the tongue even more sore and inflamed owing to traumatic lesions. Inflammatory processes in the oral cavity such as stomatitis, glossitis, gingivitis and cryptic tonsils can lead to the development of fissures and ulcerations of the oral mucosa that further trap food particles, bacteria, desquamated cells and tissues giving rise to high bacterial activity at these sites and hence oral malodour. Salty, sour, pickles, certain dried fruits, hard sweets, mechanical irritation or injury from burns after ingestion of very hot food, rough edges of teeth, dental appliances, hot cigarette smoke and other trauma may cause a glossitis and or a glossodynia resulting in painful mastication and swallowing.

Trimethylaminuria, a condition characterized by increased levels of trimethylamine in the body is mainly due to the failure to remove the amine via the usual oxidation route to the non-odourous metabolite, trimethylamine N-oxide (Mitchell, 2005). The primary genetic form results from the failure of an isoform of the hepatic flavinmonooxygenase enzyme (FMO3)

to oxidize trimethylamine, provided via enterobacterial action on dietary precursors, into the non-odourous trimethylamine N-oxide. The acquired and irreversible form in adult life has been related to a history of hepatitis. In premature infants fed with choline-rich food supplements, the transient form of trimethylaminuria was attributed to the immaturity of the N-oxidase enzyme. It seems that trimethylaminuria is also associated with menstruation and appears just before and during menstruation and then disappears afterwards.

Trimethylamine is also derived from dietary precursors such as choline, carnitine and trimethylamine N-oxide through enterobacterial metabolism. Treatment with high doses of choline, as is the case in Huntington's disease and Alzeimer's disease, can induce a fishy odour in patients' breath, which is attributed to the overloading of their N-oxidation capacity (Mitchell, 2005).

6.3.3 Exogenous input of constituents that directly contribute to breath odour owing to their inherent aroma or fragrance

Almost any food or drink with strong flavour or aroma is likely to remain long enough in the oral cavity to contribute to the smells on the breath. Large particles of food are rapidly cleared in the mouth, but small particles may be retained between teeth and around stagnant sites. Very small particles can be adsorbed into the matrix of the tongue biofilm (e.g. cream droplets are retained on the tongue for many hours). Mint flavour, fruit esters, cheese, beer, garlic, onions, aromatic or spicy constituents of food may continue to be detected on the breath for up to several hours following ingestion. Some food constituents (e.g. garlic) can also be adsorbed from the stomach or intestine and circulated via the bloodstream. These can then be detected on lung breath.

6.3.4 Exogenous input of constituents that directly contribute to breath odour owing to them being primary or secondary substrates for microbes to transform into volatile compounds or volatile sulphur compounds

Amino acids and other monomeric substrates may be contained in food at different concentrations depending on the food. Exogenous input of constituents (mainly proteinaceous) that indirectly contribute to breath odour owing to their being secondary substrates for microbes to transform into VC/VSCs (via microbial proteolytic activity) requires that the components are retained in the mouth. Any protein trapped within a matrix will be utilized by surrounding microbes in a similar way to that proposed for salivary mucin. As previously stated, cysteine rinses have a big effect on VSC production within a few minutes of their use (Kleinberg and Codipilly, 1999). Amino acid rinses (cysteine, methionine, tryptophan and arginine) at 1%

(w/v) were all shown to increase breath malodour for up to 15 minutes following their use (El-Maaytah, 1996).

6.3.5 Other effects of food components or diets

If a low carbohydrate, low fat and high protein diet is used and depending how strictly the diet is followed, the fat stored in the body starts to be burnt giving rise to ketonic bodies which are then released through the breath. It is also believed that the transient passage in the mouth during mastication of food rich in protein does not affect oral malodour (apart from the inherent odour of the food itself) since there is insufficient time or enzyme activity to degrade the protein into peptides and amino acids which can be used as primary substrates by malodour species. This would not be the case if the proteins were adsorbed or trapped (retained) within the oral biofilms. However, it is also believed that a diet high in protein might affect oral malodour by increasing the levels of peptides and amino acids in the serum which gain entry to the mouth via gingival crevice fluid (GCF) or (for some compounds) via saliva. The presence of small peptides and amino acids in saliva will be made available to odour-producing bacteria for use as a primary substrate, which will be then transformed into malodorous compounds.

6.3.6 Volatile sulphur compounds from the colon

Colonic bacteria liberate large quantities of H_2S and CH_3SH from both endogenous S-sources (mucin, taurocholate and cysteine) and exogenous sulphate in the diet (the latter via a sub-group of microbes called sulphate-reducing bacteria). Since a variety of high molecular weight thiols are detoxified by the liver via methylation reactions catalysed by S-methyltransferase, it was thought that H_2S and CH_3SH were detoxified by the same process. However, the rate of H_2S methylation by rat colonic mucosal cells is much too low to account for its removal; it was thought, therefore, that other mechanisms of removal must be occurring. It is now known that colonic mucosal cells detoxify H_2S and CH_3SH, not by methylation, but rather by demethylation of CH_3SH to H_2S and oxidation of H_2S primarily to thiosulphate (Levitt *et al.*, 1999). This is confirmed by radiolabelling experiments where labelled CH_3SH can be detected as H_2S and H_2S in turn detected as thiosulphate (by rat mucosa).

6.3.7 Endogenous H_2S production

H_2S has been proposed as the third gaseous mediator in addition to carbon monoxide and nitric oxide. H_2S is principally synthesized by cystathionase, which is found in the liver, kidney, enterocytes and vascular smooth muscle cells. In contrast, it is synthesized by cystathionine beta synthase in the

brain and partially by 3-mercaptopyruvate sulphurtransferase in cardiac tissue (Kamoun, 2004). With regard to catabolism, H_2S is broken down rapidly by thiosulphate reductase (found in mitochondria). The sulphite generated is then oxidized to sulphate by sulphite oxidase. The amount of thiosulphate excreted in the urine is the best indicator of H_2S biosynthesis, together with sulphhaemoglobin determination in erythrocytes. H_2S is reported to act as a neuromodulator in the brain (Kamoun, 2004), increasing the responses mediated by NMDA receptors and facilitating the induction of long-term potentiation in the hippocampus. H_2S also acts as a vasodilator, acting directly on ATP-dependent potassium channels in vascular smooth muscle cells. H_2S and CH_3SH are thought to be rapidly bound and oxidized by red blood cells, so it is unlikely that high amounts would ever reach the lungs and thus could not be detected on alveolar air. However, dimethyl sulphide $(CH_3)_2S$, is much less reactive in blood (neutral molecule) and can be transported from the blood to alveolar air and be expired (Blom and Tangerman, 1988).

A list of VOCs and their origin and/or association with different medical conditions is shown in Table 6.4. A list of factors associated with breath malodour is described in Table 6.5. With regard to oral malodour, Table 6.6 describes some oral manifestations of different medical conditions and nutrient deficiencies.

6.4 Salivary flow, substrate and product clearance from the mouth

Whole saliva is the end product of secretions from three pairs of salivary glands. The large parotid glands produce a thick serous solution containing large amounts of salivary amylase. The sublingual and submandibular glands (located in the floor of the mouth) produce saliva containing large numbers of glycoproteins and mucin. At rest, the saliva mixture is approximately 25% parotid, 5% sublingual and 70% submandibular. The parotid secretion increases to approximately 50% at mealtimes and the flow rate increases up to 7 ml per minute. At rest, the pH is close to pH 6.7, shifting to around pH 7.5 when eating.

Whole saliva is composed of >99% water, plus an assortment of ions, buffers, waste products, metabolites, immunoglobulins and enzymes. The most important antibacterial enzymes in saliva are lysozyme and lactoperoxidase. Saliva is produced continually throughout the day, with higher production rates at mealtimes and very low production rates during the night. Approximately 1.5 litres of saliva is produced per day. Saliva flushes and lubricates the oral surfaces and controls the bacterial levels on oral biofilms. Mucins are responsible for the lubrication properties of saliva. During eating, salivary amylase (produced by the parotids) begins breaking down complex carbohydrates and mucin-containing saliva from the

Table 6.4 Different compounds identified in the oral cavity and their corresponding pathological and non-pathological conditions

Specific compounds	Odour description	Systemic pathology	Other conditions
Ketones (acetone, acetic acid, β-hydroxybutyrate, 2-pentanone, 2-butanone)	Sweet	Diabetes mellitus and diabetic ketoacidosis (ketonic breath)	Low fat, low carbohydrate diet for weight loss, Fasting
Dimethylamine, trimethylamine, ammonia	Fishy	Uraemia, kidney failure Trimethylaminuria	Menstruation. Preterm infants fed with a choline-containing food supplement. Alzheimer's disease, Huntington's disease treated with high doses of choline
Dimethyl sulphide, limonone, ethanethiol, C_2–C_5 aliphatic acids (acetic, propionic, butyric, isobutyric and isovaleric acids	Fetor hepaticus	Liver diseases	Sulpha drugs (e.g. disulphiram).
C_2–C_8 normal and branched organic acids		Upper respiratory or oropharyngeal carcinoma	
Acetone, methylketone, *n*-propanolol, 2-butanone, aniline, *o*-toluidine	Solvent-like smell	Lung carcinoma	
Hydrogen sulphide, methylmercaptan, dimethyl sulphide, dimethyldisulphide indole, skatole	Rotten eggs, faeces, moth balls	Periodontal disease, oral abscess, oropharyngeal infections, bronchus or lung (anaerobe) infection	Endogenous oral malodour (tongue biofilm)
Allyl methyl sulphide, methyl propyl sulphide	Garlic		Food: garlic/ onion/durian
Sotolone			Spices: fenugreek
Ethanol, D-limonene, menthol			Drinks
Dimethyl selenide	Garlic	Excessive selenium	

Table 6.5 Factors associated with oral malodour

Factors associated with oral malodour	Type or exemplar
Diet	Animal proteins (meat, fish, eggs), cereals, dairy products, vegetables, fruits, confiserie
Odiferous food	Garlic, onions, cabbage, cauliflower, leek, celery, broccoli, durian, bananas
Odiferous spices	Pepper, fenugreek, cumin
Odiferous drinks	Alcohol, coffee, tea, soda
Habits	Tobacco, chewing, snuff, drugs and alcohol
Physiological conditions	Morning breath, fasting, low fat, low carbohydrate diet, dehydration, menstruation
Medical conditions	Diabetes, renal failure, upper and lower respiratory tract infections and diseases, pulmonary abscess, bronchopulmonary neoplasia, trimethylaminuria, gastroeosophagal reflux disease, *H. pylori* infection
Oral conditions	Periodontitis, peri-implantitis, pericoronitis, glossitis, glossodynia, tonsillitis, presence of denture, bridges, orthodontic appliances, xerostomia (Sjorgen's disease), bucco-lingual neoplasia
Iatrogenic aetiology (xerostomia)	Antiacne, antianxiety, anorexiant, anticholinergic, anticonvulsiant, antidepressant, antiemetics, antihistamine, antihypertensive, antiparkinsonian, antipsychotic, bronchodilatator, diuretic, musculorelaxant, narcotic analgesic, sedative, choline medications

submandibular and sublingual glands lubricates the mouth and food bolus to facilitate chewing and swallowing. In order to taste food, taste chemicals must be in aqueous solution (i.e. dissolved in saliva) if the food is to stimulate the taste buds.

Salivary flow has an important influence on malodour. Xerostomia (dry mouth) is a condition whereby the flow rate of saliva is severely diminished (hyposialia) or non-existent (asialia). There are many causes. Transitory xerostomia may occur in the presence of anxiety (stage fright, fear or dehydration), whilst prolonged xerostomia is most often related to systemic disease (e.g. Sjorgen's disease), certain medicines, or to radiotherapy of the head or neck. As with the mucosal epithelium, the salivary glands are very sensitive to radiation and a radical course of radiotherapy will cause permanent loss of salivary function. The absence of saliva (xerostomia) has dramatic adverse effects including a rapid increase in oral bacterial loads on the tongue and teeth (which may be compounded by dietary supplements high in sugars to maintain nutrition) and recurring oral infections. Subjects often present with progressive erosion of the teeth and dental caries as well as periodontal disease. The loss of lubrication causes difficulty in eating and swallowing and certain foods provoke mucosal abrasions. Dry

Table 6.6 Oral manifestations of different medical conditions and nutrient deficiencies

Medical condition	Oral manifestations	Mechanism of damage
Diabetes particularly if poorly controlled Diabetes, medication (phenytoin, calcium channel blocker), pregnancy, nutrient deficiency	Gingivitis	Thickened blood vessels compromises nutrient flow and waste removal from body tissues weakening the gingivae and bone tissue making them more susceptible to infection. High levels of blood glucose favour the growth of certain bacteria responsible of gingivitis. Decrease in salivary flow
Advanced vitamin C deficiency	Spongy, abnormally red, bleeding gingivae	Diminution of resistance to infection
Malnutrition (low protein intake), poorly controlled diabetes, HIV Low intake of calcium and vitamin D (vegetarian, vegan diet)	Periodontal destruction Periodontal disease	Increased susceptibility of periodontum owing to compromised host defence mechanisms
Anaemia, diabetes, Candidiasis *H. pylori*	Burning mouth and tongue Burning mouth syndrome	Reduction in salivary flow, xerostomia Change in salivary flow and/or salivary content with increased susceptibility to infection
Iron deficiency, folate, B6, B12, niacin and or riboflavin, *H. pylori* infection, irritants: tobacco, alcohol, hot food and spices, abrasive foods such as nuts, crisps, hard sweets	Glossitis (red, raw, fissured)	Reduction in salivary flow, xerostomia Mucosal changes Increased susceptibility to infection
Chronic folate deficiency; aneamia by iron deficiency	Pale, atrophic tongue, smooth, slick (filiform papillar atrophy)	Reduction in salivary flow, Mucosal changes Connective tissue weakness Increased susceptibility to infection
Riboflavin deficiency	Magenta colour of tongue, atrophic, smooth, pale and painful	Reduction in salivary flow, Mucosal changes Connective tissue weakness Increased susceptibility to infection

mouth and reduced salivary flow have been correlated with halitosis (Koshimune *et al*., 2003). The antisialogogue, ammonium glycopyrrolate (Robinul), was used to reduce the salivary flow rate in healthy individuals with normal salivary function to determine whether the dry-mouth symptoms and reduced amounts and patterns of oral mucosal wetness found previously in hyposalivators could be induced by this means (Wolff and Kleinberg, 1999). Loss of volatile sulphur compounds into mouth air occurred progressively as the mouth became drier (Kleinberg *et al*., 2002).

Salivary flow rate varies through the day from lowest through sleep at night, to low between meals but high during and following food. Salivary flow also changes in anticipation of food. Typically, the rate is 0.3 ml per minute for unstimulated saliva, rising to over 7 ml per minute when stimulated (Dawes, 1987). Oral malodour is common on awakening (morning breath) and is transient and rarely of any special significance (Porter and Scully, 2006; Yaegaki and Coil, 2000), probably resulting from increased microbial metabolic activity during sleep aggravated by a physiological reduction in salivary flow, lack of nocturnal physiologic oral cleansing (e.g. movement of the facial and oral muscles) and variable oral hygiene procedures prior to sleep. Starvation can lead to a similar malodour.

Saliva both brings in substrates and removes products and shed cells (both mammalian and bacterial) from the mouth. For large molecules such as salivary mucins, their extent of breakdown depends on the residence time of the molecules. At fast flow rates, the residence time for all molecules that are not attached is much shorter in duration than at low salivary flow rates. With a longer residence time, the exposure and extent of mucin breakdown by sequential enzyme activities is more likely to progress beyond the deglycosylation phase into the peptidase phase to give a greater extent of total digestion.

Atropine, the main antagonist of the parasympathetic system, is present in many medicines used for pulmonary, ophthalmic or neurological purposes, potentially causing hyposialia. Similarly, imipramine antidepressants, phenothiazine neuroleptics, antihistamines and di-isopyramide predispose to the onset of hyposialia. It seems likely that a number of different types of food constituents (quinine, bitter flavours) may contribute to dry mouth and thus may indirectly affect malodour levels via their effects on salivary flow rate.

6.5 More complex model (quasi-steady states with perturbations)

Breath odour is likely to be a complex mix (or bouquet) of different odours from different sources (see summary Fig. 6.5). Moreover, it is likely to change character and intensity with time. If a person holds their breath or breathes in through their nose or mouth, but out through their nose, there

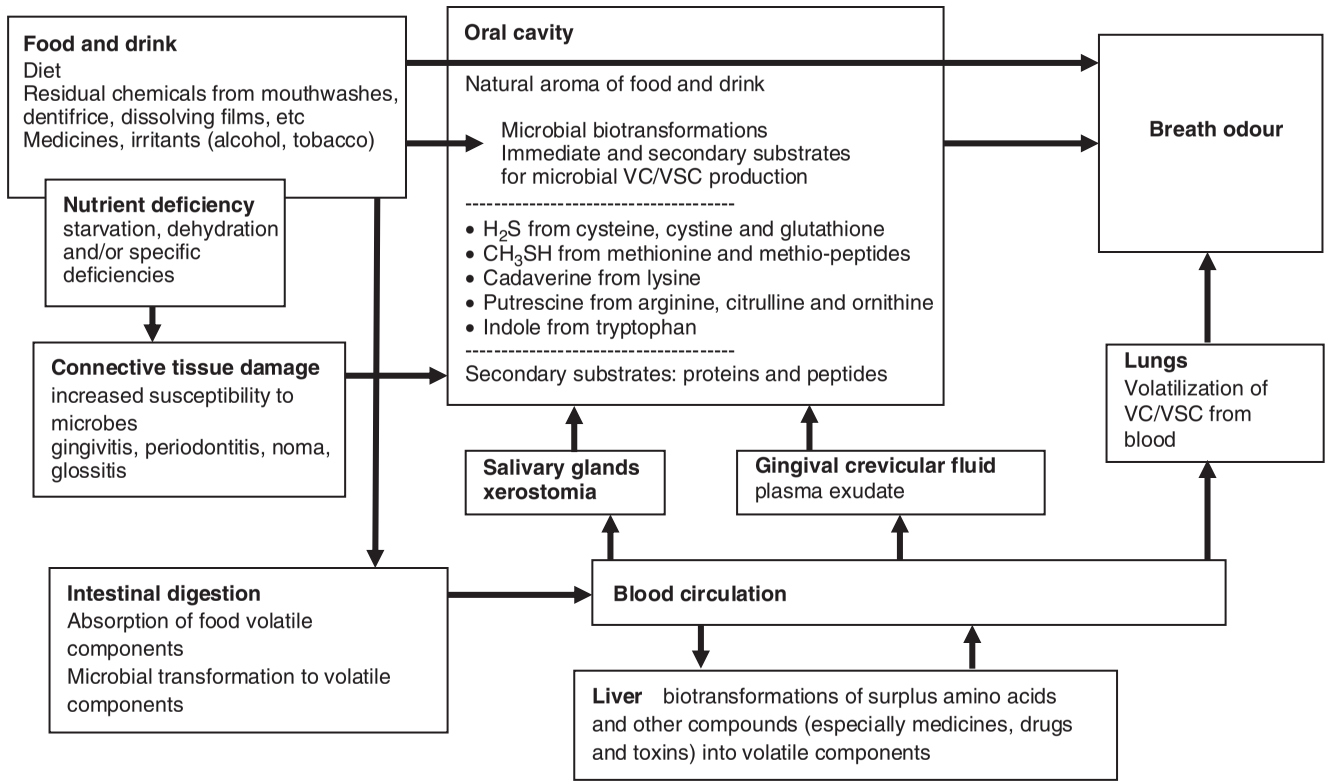

Fig. 6.5 Summary of mechanisms and pathways of bad breath volatile compounds.

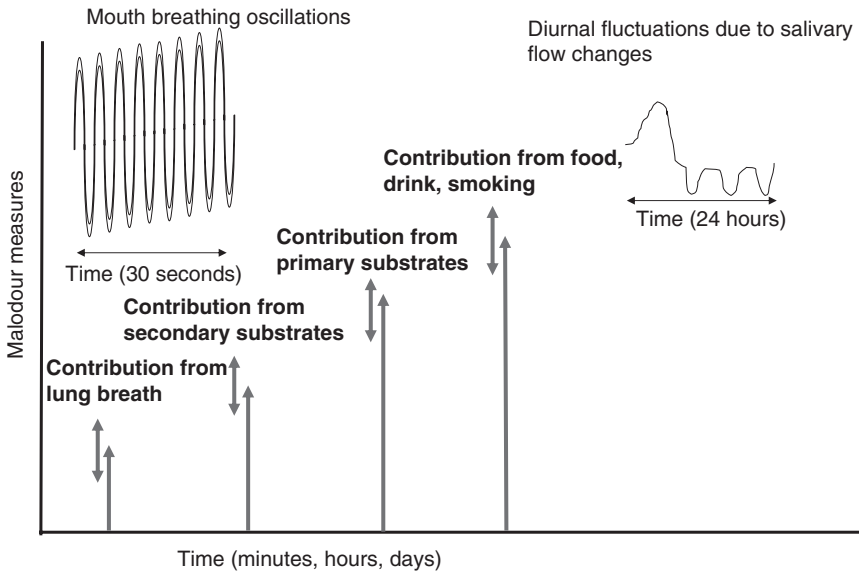

Fig. 6.6 Perturbations and oscillations in breath malodour.

will be little emission of oral malodour. However, if the mouth is opened, then this will give rise to oscillatory malodour with a short duration (breathing frequency). According to Niinimaa *et al.* (1981), up to 15% of the population are mouth breathers. During normal conversation by non-mouth breathers, more than half the inspired air passes through the mouth (Camner and Bakke, 1980). The short frequency oscillation of breathing is superimposed upon oral malodour (mouth origin), which in turn is superimposed upon the basal level of lung gas VOCs. An individual may have bloodborne halitosis and oral malodour. At mealtimes, the flavour and fragrances of food may then be additionally superimposed onto those of the breath. Through the day there may be increased and decreased salivary flow rate. This will give rise to further oscillations (diurnal rhythm and mealtimes) of the oral malodour component of the breath odour (see Fig. 6.6).

6.6 Interventions and management

Halitosis caused by ingestion of certain food and drinks such as spices, garlic, onion, durian, cabbage, cauliflower and radish, or by habits such as smoking tobacco or drinking alcohol is usually transient, often caused by sulphur-containing volatile agents and is considered to arise both from intra-oral (food debris) and extra-oral (respiratory) origins. Tobacco smoke contains VSCs, which are at least partly responsible for the oral malodour

of smokers (Stedman, 1968), but tobacco products also predispose to dry mouth and periodontal disease, further causes of malodour. Alcohol intake may be a predictor of oral malodour (Rosenberg *et al.*, 2007).

The management of halitosis depends largely on the cause. Avoidance of smoking, drugs and foods that might be responsible is sensible. In addition, chewing gum, parsley, mint, cloves or fennel seeds and the use of proprietary 'fresh breath' preparations may help, although most cosmetic non-pharmacological methods merely provide a competing and temporary smell that may simply mask the unfavourable odour. For the majority of subjects who suffer from oral malodour, reducing the accumulation of food debris and malodour-producing oral bacteria by physical removal of trapped substrates and microbial populations is an important and appropriate first step.

For malodour of microbial aetiology where the generation is the tongue dorsum (and oral cavity in general), then good oral hygiene, including soft brushing or soft scraping of the tongue will reduce odour levels, usually by about one-integer on the 0–5 Rosenberg scale. However, after a few hours, the levels of odour will again increase along with the populations of microbes and aerial density of the tongue biofilm, until oral hygiene is re-applied. An increase in the frequency of oral hygiene may be useful in these cases. Needless to say, flossing or other mechanical methods should be used to remove proteinaceous substrates in the form of impacted food from between the teeth or around prosthetic devices or false teeth.

Microbial populations can also be reduced by the use of medicated toothpastes, mouthwashes, dissolving thin films, chewing gum or lozenge, which are manufactured products designed to reduce malodour. However, in the majority of cases, the effectiveness of products has not been properly assessed. Some contain compounds that are primarily designed to kill microbes, whilst some contain compounds that react with sulphides or inhibit sulphur transformations. Some contain 'masking' aromas. Some products combine all three processes.

6.6.1 Management

This is usually achieved by treating oral/dental diseases, improving oral hygiene and reducing the tongue coating. A combination of treatments typically helps (Haas *et al.*, 2007; Porter and Scully, 2006; Scully and Porter, 2006). Regular meals are important, as is dental prophylaxis, and the patient should use appropriate regular oral hygiene procedures, which include regular tooth cleaning (brushing and interdental flossing) and use of antimicrobial toothpastes and/or mouthwashes. Generally, it is recommended that mouthwashes should be used two or three times daily for at least 30 seconds. A multitude of oral healthcare and pharmaceutical products is available over the counter, testimony to the extent of the perceived, or indeed real, problem of halitosis. The effectiveness of active ingredients in

oral healthcare products is dependent on their concentration and, above a certain concentration, the ingredients can have unpleasant side effects (van den Broek *et al*., 2007 and 2008). If odour is present on nose breath, and improved oral hygiene does not improve the condition, the clinician should consider infection of the pharynx, bronchioles or lungs, or a case of blood-borne halitosis and should suggest further specific diagnosis and provide appropriate treatment where possible.

Halitophobia almost always requires referral for clinical psychologist management. In extreme instances, patients become socially isolated, may have their teeth extracted and occasionally commit suicide. However, patients often refuse to acknowledge that they may have a psychological problem. Therefore, the involvement of a third party (e.g. a confidant such as close family member or trusted friend) in the management may provide the patient with additional psychological support to consider the problem in a more objective manner (Rosenberg, 1996).

6.7 Future trends

Our knowledge of bad breath and its aetiology has increased enormously over the last few years. By using a commonly agreed classification (see Table 6.1), it should now be possible to invoke 'best available' treatment regimes to tackle the various categories of halitosis. For oral malodour, there are many over-the-counter treatments that are claimed to tackle the problem of bad breath and bring relief. Although the majority of commercial products have not been tested for efficacy using well-designed (double-blind) clinical trials, an increasing number of products have been so tested. Those that prove useful will no doubt survive as commercial treatments, whilst those that have little or no effect will eventually be identified as such and, in the fullness of time, will be seen to be ineffectual and thus of lesser commercial value. However, there is a need for more independent research (and researchers) in this field and for more products to be robustly tested. With regard to dissemination of knowledge and best practice, there is scope for better education and training of dental students and dental hygienists in the problems and treatment of bad breath. It is hard to understand why more basic oral microbiology is not covered in the majority of undergraduate or postgraduate courses for dentistry, since most of the clinical problems that they will encounter (caries, periodontal disease, oral abscesses, oral malodour) all have a microbial aetiology.

Electronic noses (E-noses) have not yet fulfilled their promise in terms of analysing breath, but their development continues and it may be possible in the near future for hand-held E-noses to diagnose different types of bad breath. E-noses work in a way that is analogous to the human nose in that they use multiple sensors, each with a different sensitivity to the target molecules in question. The resultant output is combined into a 'pattern' that

is distinct for different VOCs and VOC mixtures which in turn are characteristic of different clinical conditions. Such E-noses may offer the dental and medical profession a quick method to diagnose differentially, say, physiological oral malodour from that caused by periodontal disease or to differentiate the likely sources of bloodborne halitosis (e.g. liver or kidney dysfunction). Such E-noses may also help in the diagnosis or early detection of cancer.

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7

Sugar alcohols and dental health

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Abstract: Polyols, commonly known as sugar alcohols, are a class of sweeteners commonly used as sugar substitutes. Polyols such as sorbitol, maltitol, mannitol, erythritol and xylitol are ubiquitous in sugar-free food and confectionery products with some claiming to have oral health benefits. Polyols as a class are widely held to be low- to non-aciduric and very low- to non-cariogenic. Xylitol, in particular, has unique properties that set it apart from its class. Xylitol actively promotes better oral health by reducing the levels of pathogenic mutans streptococci in plaque and saliva and by reducing tooth decay. The active protective effects are not limited to those who consume xylitol but also extend to the children of mothers who consume xylitol during the perinatal period. Some xylitol-containing products have been adequately studied and have the potential for use in specific or generalized public health programs to promote oral health and reduce tooth decay.

Key words: dental caries, oral health, polyols, public health, sorbitol, sugar alcohols, sweeteners, tooth decay, xylitol.

7.1 Introduction to sugars and sugar-free sweeteners

To most people, sugar refers to common household table sugar which, in scientific terms, is sucrose. Sucrose, like other naturally occurring sugars, is a carbohydrate, which are classified as monosaccharides (5–6 carbon simple sugars), disaccharides (two monosaccharide units), oligosaccharides (several monosaccharide units) or polysaccharides (dozens to thousands of monosaccharide units). Monosaccharides like glucose (grade sugar or corn sugar), fructose (fruit sugar) and galactose, and disaccharides including sucrose, lactose and maltose are ubiquitous in the human diet, particularly sucrose. These two classes of carbohydrates are highly acidogenic and readily fermentable by the oral bacteria that are implicated in the development of tooth decay. Frequent and excessive consumption of these carbohydrates,

particularly between meals, results in dental caries (Gustafsson *et al.*, 1954; Scheinin *et al.*, 1976; Zero, 2004).

The growing epidemics of tooth decay and health problems, particularly diabetes and obesity, associated with excessive consumption of these simple sugars (carbohydrates) have created a multi-billion dollar industry for sugar substitutes popularly known as sugar-free sweeteners and their use in dietary and confectionery products. Sugar-free sweeteners include sugar alcohols and intense sweeteners which are naturally occurring or artificially produced and grouped as nutritive (caloric) or non-nutritive (non-caloric).

Sugar alcohols commonly found in foodstuff today include sorbitol, maltitol, mannitol, erythritol and xylitol. These belong to the nutritive group, generally having one-third to two-thirds of the caloric content of sucrose and are less sweet than sucrose, with the exception of xylitol which has similar sweetness. Chemically, sugar alcohols are made up of monosaccharides containing hydroxyl groups. They are found naturally but can also be produced through chemical processes.

On the other hand, intense sweeteners such as aspartame, saccharin, sucralose and acesulfame are artificially produced and have sweetness many to several hundred times that of sucrose, while having negligible to no caloric value. These are classified as non-nutritive sweeteners and are non-cariogenic. Table 7.1 summarizes their properties.

7.2 Sugar alcohols and cariogenicity

Sugar alcohols as a class are considered to be low- to non-acidogenic and very low- to non-cariogenic (Van Loveren, 2004); that is, their consumption does not contribute to the development of tooth decay (Hayes, 2001). This is attributed to the low fermentability of sugar alcohols by oral mutans streptococci (Edwardsson *et al.*, 1977), specifically *Streptococcus mutans* and *Streptococcus sobrinus*, a primary group of bacteria implicated in the cause of human tooth decay (Loesche, 1986). These bacteria are capable of producing large quantities of lactic acid from fermentable carbohydrates in aciduric conditions. They also produce extracellular polysaccharides, which increases the adhesion of dental plaque to the tooth surface (Kandelman, 1997).

Among the sugar alcohols, sorbitol and xylitol have been extensively studied. Of particular interest for dental caries prevention, xylitol has been shown to have an active protective effect by reducing plaque and tooth decay. Xylitol reduces the levels of mutans streptococci in plaque and saliva and thereby limits the level of enamel-demineralizing lactic acid produced by these bacteria (Milgrom *et al.*, 2008). Similar active protective effects for other polyols are not well supported by clinical studies and are lacking in large clinic trials. Indeed, dental caries research involving polyols other than

Table 7.1 Properties of natural sugars and sugar substitutes (Ly *et al*., 2006b)

	Nutritive value (kcal g^{-1})	Cariogenic	'Sugar-free' label (non-cariogenic)	Sweetness[a]
Natural sugars				
Sucrose	4	Yes	No	1.0
Glucose	4	Yes	No	0.7
Fructose	4	Yes	No	1.5
Lactose	4	Yes	No	0.2
Sugar substitutes				
Sugar alcohols/polyols				
Xylitol	2.4	No	Yes	1.0
Sorbitol	2.6	No	Yes	0.6
Mannitol	1.6	No	Yes	0.5
Maltitol	2.1	No	Yes	0.9
Lactitol	0.02	No	Yes	0.4
Erythritol	0.02	No	Yes	0.8
Isomalt	2.0	No	Yes	0.5
Hydrogenated starch hydroxylate	3.0	No	Yes	0.4–0.9
Artificial sweeteners				
Aspartame[b] (NutriSweet®, Equal)	0.0	No	Yes	180
Saccharin (Sweet 'N Low)	0.0	No	Yes	300
Sucralose (SPLENDA®)	0.0	No	Yes	600
Acesulfame Potassium (Sunett)	0.0	No	Yes	200

[a] Sucrose (table sugar) is the standard for sweetness comparison and is given the sweetness value of '1'.
[b] Aspartame is technically a nutritive sweetener. Because of its intense sweetness, however, it is used in such small amounts that its nutritive value is negligible.

sorbitol or xylitol is sparse and has often involved the use of animal models or has employed a combination of polyols in the study products. Nevertheless, the literature overwhelmingly supports their low fermentation by mutans streptococci and thus low-acidogenic potential and low- to non-cariogenic property.

7.2.1 Xylitol and sorbitol and tooth decay

Sorbitol and xylitol are the most commonly studied polyols in dental caries research. The cariogenicity of sorbitol has been evaluated in short- and

long-term studies in humans, animal experiments and pH measurements in dental plaque *in vivo* and *in vitro* since the 1970s. By the mid-1980s, most of these studies indicated that sorbitol had low- to no-cariogenicity (Birkhed *et al.*, 1984; Birkhed and Bar, 1991). However, questions arose regarding the adaptation of oral mutans streptococci to prolonged exposure to sorbitol whereby these bacteria developed alternative mechanisms to ferment sorbitol. Adaptation has been demonstrated but only to a small degree such that the risks of caries were not increased in normal conditions (Hogg and Rugg-Gunn, 1991; Bowen, 1996).

Xylitol is superior to sorbitol in that it is completely non-acidogenic and non-cariogenic (Gales and Nguyen, 2000; Burt, 2006). It has been shown to actively reduce tooth decay by reducing the level of mutans streptococci in plaque and saliva (Milgrom *et al.*, 2008; Soderling, 2008). In studies where xylitol and sorbitol were included, independently or in combinations, the overall results showed that participants in groups consuming 100% xylitol had greater reductions in caries or mutans streptococci levels than participants in groups that consumed a combination of xylitol and sorbitol. In turn, the participants in this latter group experienced greater reductions in tooth decay than those in groups that consumed sorbitol alone (Ly *et al.*, 2008; Milgrom *et al.*, 2008). Some *in vitro* studies have also shown that, in the presence of xylitol, mutans streptococci are less capable of fermenting other cariogenic carbohydrates and polyols, thus producing less demineralizing acid (Waler *et al.*, 1984; Kakuta *et al.*, 2003). This suggests that, although polyol sweeteners used in combination can reduce caries, the amount of xylitol in the combination determines the degree of reduction observed and the presence of other polyol sweeteners may enhance, but does not reduce, the effectiveness of xylitol. Furthermore, the consumption of greater amounts of xylitol per day has been associated with a larger reduction in tooth decay.

7.3 Xylitol efficacy in the prevention of tooth decay

Xylitol has bacteriostatic effect on mutans streptococci in the oral cavity and actively prevents tooth decay. The degree of the effect is associated with amount and frequency of xylitol consumption where higher amounts and higher frequencies enhance efficacy. However, there appears to be a minimal amount and frequency of use for effectiveness as well as a plateau in effect at higher amounts of daily consumption.

7.3.1 Xylitol and mutans streptococci

Many oral bacteria, including mutans streptococci, do not readily metabolize xylitol into energy sources and its consumption has minimal effect on plaque pH. However, xylitol does get absorbed and accumulates intracel-

lularly in mutans streptococci. These bacteria absorb and metabolize xylitol through the same cell-wall fructose phosphotransferase system and the intracellular citric acid (Krebs) cycle used for sucrose and other simple carbohydrates. But, unlike the metabolism of the latter which produces energy for cellular growth and its resulting lactic acid by-products, mutans streptococci expend energy trying to break down the accumulated xylitol without yielding energy in return. Furthermore, the energy-producing intermediates are consumed and not reproduced. This has been termed the 'futile-cycle' of xylitol metabolism (Trahan *et al.*, 1985). This has been demonstrated *in vitro* and may contribute to a reduction of mutans streptococci levels in the plaque and saliva and a reduction in acid production (Trahan, 1995). Furthermore, some researchers suggest that long-term use or habitual consumption of xylitol leads to the selection for 'xylitol-tolerant' mutans streptococci strains that are less virulent (Trahan *et al.*, 1992; Trahan *et al.*, 1996; Roberts *et al.*, 2002). These strains do not accumulate xylitol-5-phosphate intracellularly like xylitol-sensitive strains, but they are depleted in the fructose phosphotransferase system (Benchabane *et al.*, 2002), which is needed in the process of carbohydrate uptake and production of extracellular polysaccharides that is used in plaque biofilm formation.

7.3.2 Xylitol dose and frequency of use for effectiveness

The effectiveness of xylitol in reducing caries is dependent on the daily amount and frequency of consumption. Currently, a dosing and frequency guideline for efficacious use of xylitol has not been put forth. However, there is growing agreement that xylitol is effective at a daily amount of 5 to 10 g divided into at least three consumption periods. Increasing the frequency of xylitol use is associated with greater effectiveness (Isokangas, 1987; Rekola, 1989; Ly *et al.*, 2006a). Xylitol consumption of less than 5 g/day has often been found to be no more effective than consumption of sorbitol or a placebo alone. There also appears to be a plateau effect for xylitol at higher doses (Milgrom *et al.*, 2006). Studies have found no added benefit in caries (DMFS–decayed, missing, filled teeth) reduction at doses greater than 10 g/day (Mäkinen *et al.*, 1998).

Xylitol and other sweeteners in the polyol class are safe for consumption and have been used in food products for several decades. Laxative symptoms such as bloating, loose stool or diarrhea are the main side effects when polyols are consumed in large amounts. The tolerance prior to laxation is more than 40 g per day for xylitol which is three to four times the quantity of xylitol needed for clinical efficacy. Still, it is recommended that xylitol should be introduced slowly to allow the body to acclimatize to a new product, especially in populations that are unfamiliar with xylitol. Furthermore, polyol sweetener use as a class is increasingly common in food and dietary products and consideration should be given to the cumulative dietary intake of polyols and potential laxative symptoms.

7.4 Xylitol clinical applications

The uses of polyols as sugar-free sweeteners in food and beverage products marketed for their dietary and oral health benefits are widespread. The most frequently used polyols in products include sorbitol, mannitol, and/or maltitol because they are less expensive. Nevertheless, evidence supporting the role of xylitol in reducing mutans streptococci in plaque and saliva and in reducing tooth decay is influencing the market. Xylitol is appearing in consumer products rapidly, sometimes purely as a sweetener with minimal potential for efficacy, while at other times the products are formulated to provide therapeutic effects. The most popular with regard to the latter is xylitol chewing gum, which has captured a majority stake in the chewing gum industry in many European and Asian countries. These xylitol-containing products, when used at efficacious levels by consumers or by well-planned dental public health programs for children at high risk for caries, may have a significant impact on reducing tooth decay.

7.4.1 Xylitol and children at high risk of caries

The stable primary caries preventive tools have been water fluoridation where possible, fluoridated toothpaste and topical fluorides. Beyond fluoride, there are few strategies available for the prevention and control of the high rates of caries in the primary dentition of high-risk children. For children with mixed dentition, sealants can be added. The use of polyol sweeteners in food and confectionery products may help to reduce exposure to cariogenic sugars. However, sucrose is still widely used in domestic and manufactured food preparations and is easily accessible to the general population because of its low cost.

Effective strategies to reduce risk by modifying the diet of children are not readily applicable, nor are they typically effective without significant effort. As such, the use of xylitol is particularly attractive because its action is not dependent upon reducing the amount of other sugars in the diet. Thus, xylitol can be added to the diet without having to make additional changes to dietary patterns. On the other hand, if dietary modifications are being attempted in order to reduce or replace high caloric and cariogenic sugars, the use of xylitol would enhance the benefits both in oral health and general health, the latter because of xylitol's reduced caloric content and lack of the spike in blood sugar level. Xylitol-containing products appropriately formulated for efficacious use in children have the potential to improve the success in controlling rampant decay in the primary dentition beyond current practices.

Studies conducted among schoolchildren of various ages have shown that consumption of xylitol chewing gum not only decreases the development of new caries but also reduces the extent of existing caries. Among schoolchildren in Belize, consumption of xylitol gum was associated with

the arrest of carious lesions and the number of lesions that rehardened ranged from 9% to 27% in all groups (Mäkinen *et al.*, 1995). This study is important because the children continued to consume very high levels of sucrose in their everyday diet. In Kuwait, daycare children who chewed xylitol gum three times a day during daycare hours had better oral health status than the group that brushed once after lunch with fluoridated toothpaste (0.05% NaF). All children brushed at home as they normally would (Kovari *et al.*, 2003). Another study in Europe showed that DMFS increment among groups of fifth graders who used xylitol chewing gum either for two years or for three years were no different from the group who received sealants at the end of the five-year study period (Alanen *et al.*, 2000a).

Finland implemented the 'Smart Habit' Xylitol campaign in 1992, aimed at increasing the consumption of xylitol chewing gum among 13-year-old school children, to promote their oral health. The campaign was conducted in elementary schools in the form of a quiz and lesson related to xylitol. Subsequently, xylitol oral health benefits were included in all dental health education programs and, by 1999, 44–69% of children ages 11 to 15 years used xylitol chewing gum daily (Nordblad *et al.*, 1995). A similar program geared at a uniquely high-risk adult population is the United States Army's 'Look For Xylitol First' initiative that was implemented in 2004. The program aimed to increase knowledge of the positive benefits of xylitol among their dental health providers and their troops and to encourage 'Look For Xylitol First' in purchasing consumer products to improve oral health (Richter and Chaffin, 2004). The US Army also began to include xylitol chewing gum in their Meals Ready-to-Eat (MRE) packages for deployed troops who, because of the field circumstances, often have very poor oral hygiene habits. However, chewing gum as a mode of xylitol delivery may be inappropriate in some countries such as the United States where it is classified as a risk for choking and is not considered safe for young children; its consumption is highly discouraged in daycare and school settings. Furthermore, prevention should begin as soon as possible, that is at 6 to 9 months of age when teeth first erupt and, for these children, products other than chewing gum are needed.

Other xylitol-containing products such as candy and lozenges have been shown to be effective in reducing tooth decay by 30–60% (Kandelman *et al.*, 1988; Alanen *et al.*, 2000b; Honkala *et al.*, 2006). More recently, one-year old children who consumed xylitol syrup at 8 g per day divided into two or three frequencies for 12 months was shown to have reduced the risk of tooth decay by one-half to one-third compared to controls. In the xylitol syrup 8 g per day groups 30–50% fewer children developed tooth decay compared to controls at the end of the study (Milgrom *et al.*, 2008). This suggests that these delivery modes may be as effective as chewing gum in caries prevention. In field tests, xylitol-containing popsicles, gummy bears, puddings, macaroons and sorbet were readily accepted by children and

produced no side effects (Lam *et al.*, 2000). Xylitol stick and pellet chewing gum, gummy bear, and syrup with a similar dose have similar oral bioavailability (Riedy *et al.*, 2008). However, each new xylitol-containing product needs to be tested to establish its effectiveness at significantly reducing mutans streptococci or in decay prevention. It remains uncertain if various types of food might have different delivery and release of xylitol in the oral cavity before being swallowed.

Xylitol is also found in several fluoride and non-fluoride toothpaste formulations which have been found to be 10–15% more effective in reducing DFS (decayed filled surfaces) and DFT (decayed filled teeth) than fluoride toothpaste alone (Sintes *et al.*, 1995; Sintes *et al.*, 2002). Xylitol toothpaste also reduces mutans streptococci in plaque by nine-fold and saliva by eight-fold (Jannesson *et al.*, 2002). These studies used a 10% xylitol formulation. Fluoride toothpaste with xylitol can be recommended as a substitute for regular fluoride toothpaste and other xylitol products can be recommended concurrently with fluoridated toothpaste, topical fluorides and sealants. Xylitol and fluoride can be used simultaneously as they have different mechanisms of action and a potentially synergistic effect.

7.4.2 Xylitol and perinatal women

A combination of good dental care, instruction to improve oral hygiene, fluoridated toothpaste, and chlorhexidine or other antimicrobial products among high caries risk pregnant women can lead to significant improvement in oral health and reductions in maternal mutans streptococci levels in plaque and saliva. This is important because tooth decay is an infectious disease where pathogenic mutans streptococci strains are passed vertically from mother to child. Common mutans streptococci strains have also been found among toddlers and their immediate family members or carers, particularly those who have the most contact time with the growing infant/toddler.

The use of xylitol chewing gum by women during the perinatal period can greatly reduce their mutans streptococci levels as well as the transmission of pathogenic bacteria to their offspring. Early investigation showed toddlers whose mothers had high mutans streptococci levels and chewed xylitol gum on average had lower levels of mutans streptococci than their peers whose mothers received chlorhexidine varnish or fluoride varnish pre- and post-partum (Soderling *et al.*, 2001). The percentage of children colonized by mutans streptococci at 2 years old was 10% compared to 29% and 49% in children with mothers in the xylitol, chlorhexidine and fluoride group, respectively. These children were followed up at 6 years old and were found still to have the lowest mutans streptococci levels, 52% were colonized in the xylitol group compared to 86% in the chlorhexidine and 84% in the fluoride groups. Furthermore, they also had 70% less dentinal caries than their peers in the study (Isokangas *et al.*, 2000; Soderling *et al.*, 2001).

Whether used alone or in combination with other antimicrobial therapies such as chlorhexidine, xylitol has an important role in the prevention of dental decay among children born to mothers with high levels of mutans streptococci. This is not only because of its effects on mutans streptococci levels and bacterial properties during the period of consumption, but also because the beneficial effect on decay reduction in these children appears to persist far beyond the period of consumption. Both chlorhexidine and xylitol may be used safely by pregnant women and nursing mothers (Wang and van Eys, 1981; Brambilla *et al.*, 1998). Currently available data suggest that twice daily use of a chlorhexidine gluconate rinse (0.12%) for two weeks followed by 5–10 g of xylitol via chewing gum per day chewed for five minutes each time, should lead to a major reduction in mutans streptococci levels and tooth decay in the mother as well as the child. In very high-risk individuals, periodic use of a chlorhexidine rinse may be beneficial.

7.5 Deciphering xylitol-containing products for potential efficacy

Food products containing xylitol, particularly chewing gums and mints, are currently available commercially worldwide in retail stores, through specialized manufacturers and online venues. In Europe, especially Nordic countries, and Asia such as Japan and Korea, these products are more available, marketed and recognized by consumers and their use in other countries in Asia, Europe and the Americas is rapidly growing. Tables 7.2 and 7.3 show examples of xylitol products available in the United States and online venues. The list is far from being exhaustive and serves as a sample of the rapidly proliferating number and type of xylitol products appearing in the market. For example, xylitol-containing flavored towelettes for cleaning infant and toddlers' teeth and gums, xylitol in vitamin supplements and even floss are now available. Many of these novel xylitol-containing products have not been subjected to rigorous research studies and should be considered with great caution, perhaps even skepticism. Aside from the adequate amount and frequency of xylitol needed for efficacy, it is critically important to appreciate that xylitol acts locally in the oral cavity and the contact time with plaque and saliva is paramount. Not all types of products are efficient at making the xylitol they contain available in the oral cavity and for an adequate contact time with saliva, and especially plaque, before being swallowed.

The challenge is for clinicians to recommend products that have been tested and shown to be effective and to have the potential to deliver the recommended 5–10 g per day divided into three or more frequencies of use per day. This requires a basic understanding of sugar substitutes and product labeling as the xylitol content is frequently not clearly labeled. Gums, mints

Table 7.2 Xylitol-containing gums and mints available in the United States' markets or online, their xylitol content, preventive potential and approximate cost[a] (Ly *et al.*, 2006b)

Products[b]	Xylitol (g per piece)	Pieces 6 (10) g/day	Preventive potential[b]	Approximate cost/10 pieces (US$)
Gums:				
Epic–xylitol gum (various flavors)	1.05	6 (10)	Yes	0.70–1.00 online
Clen-Dent/Xponent gum (various flavors)	0.67	10 (15)	Yes	1.00–1.20 online
Eco-Dent 'between dental gum' (various flavors)	0.70	9 (14)	Yes	1.05–1.40 online
Fennobon Oy 'XyliMax Gum'	1.00	6 (10)	Yes	0.80–1.00 online
Hershey 'Carefree Koolerz Gum' (various flavors)	1.50	4 (7)	Yes	0.95–1.50 retail
Lotte–xylitol gum (various flavors)	0.65	10 (15)	Yes	0.70–0.80 online
Omnii 'Theragum'	0.70	9 (14)	Yes	1.25–1.50 online
Spry xylitol gum (various flavors)	0.75	8 (13)	Yes	0.70–0.90 online
Tundra Trading 'XyliChew Gum'	0.80	8 (13)	Yes	0.95–1.30 online
Vitamin Research 'Unique Sweet Gum'	0.72	9 (14)	Yes	1.00 online
Altoids sugar-free chewing gum	1st of 3 polyols (1.0)	NC[c]	Maybe	1.80–2.00 retail
B-FRESH gums (various flavors)	1st of 2 polyols (1.0)	NC	Maybe	0.70 online
Arm & Hammer 'Dental Care Baking Soda Gum'	2nd of 3 polyols (1.0)	NC	No	0.80–1.00 retail
Arm & Hammer 'Advance White Icy Mint Gum'	2nd of 3 polyols (1.0)	NC	No	1.00–1.30 retail
Biotene 'Dental Gum' and 'Dry Mouth Gum'	2nd of 2 polyols (1.0)	NC	No	1.00–1.40 retail
Starbucks 'After Coffee Gum'	2nd of 4 polyols	NC	No	1.80–2.00 retail
Warner-Lambert 'Trident Gum with Xylitol'	2nd of 3 polyols (1.0)	NC	No	0.60–0.70 retail
Warner-Lambert 'Trident for Kids Gum'	3rd of 3 polyols (1.0)	NC	No	1.20–1.40 retail
Wrigley 'Orbit sugar-free gum'	3rd of 3 polyols (1.0)	NC	No	0.72–0.80 retail
Fennobon 'XyliDent gum'	NC	NC	NC	NC
Ford Gum 'Xtreme Xylitol gums'	NC	NC	NC	0.65–0.85 online
WellDent xylitol gum	67% weight	NC	NC	0.90 online
Wrigley 'Everest Mint Gum'	NC	NC	NC	0.45 retail, online

Table 7.2 *Continued*

Products[b]	Xylitol (g per piece)	Pieces 6 (10) g/day	Preventive potential[b]	Approximate cost/10 pieces (US$)
Mints:				
Clen-Dent/Xponent mints	0.67	9 (15)	Yes	0.62–0.70 online
Epic xylitol mints	0.50	12 (20)	Maybe	0.35–0.50 online
Spry mints	0.5	12 (20)	Maybe	0.38–0.49 online
Tundra Trading 'XyliChew Mints'	0.55	11 (18)	Maybe	0.32–0.40 online
VitaDent mints/ 'Unique Sweet Mints'	0.5	12 (20)	Maybe	0.62–0.65 online
SMINT mints	<0.20	30 (50)	No	0.35–0.40 retail
WellDent xylitol mints	94% weight	NC	NC	0.38 online
Brown & Haley 'Zingos Caffeinated Peppermints'	2nd of 2 polyols	NC	No	0.38–0.40 online
Oxyfresh 'Breath Mints'	2nd of 2 polyols	NC	No	0.35–0.40 online
Starbucks 'After Coffee Mints'	2nd of 2 polyols	NC	No	0.20 Starbucks
Omnii 'Theramints'	NC	NC	NC	0.45 online
Tic Tac 'Silvers'	NC	NC	No	0.23–0.25 retail
Xleardent mints	NC	NC	No	0.35 online

N/C = not certain. Information cannot be derived from internet vendor, or market packaging, or not successful in obtaining information from vendors' information representatives.

[a] Costs are in US dollars and vary based on retail, convenience stores and internet vendors. Stated costs are based on several Seattle, WA, USA retailers or internet vendors.

[b] 'Yes', 'No', or 'Maybe' is based on the willingness of a person to consume 2–3 pieces, 3–5 times per day to meet the effective dose range of 6–10 g per day. Products with potential for effectiveness but whose xylitol dose is either unknown or 0.5 g or less are assigned as 'Maybe'.

[c] Product list is not exhaustive. The xylitol market is rapidly changing and new xylitol-containing products appear frequently.

Table 7.3 Xylitol-containing diet, oral hygiene and healthcare products available in the United States' markets or online, and their xylitol content (Ly *et al*., 2006b)

Products*	Xylitol	Cost (US$) per unit	Availability
Energy bars and food:			
Buddha bars	4–5 g/bar	3.00/bar	online
E Enterprises 'E Bar'	14 g/bar	2.00/bar	online
Fran Gare's 'Decadent Desserts' Mix (various types)	15–25 g/30 g serving	7.00/canister	online
Jay Robb Enterprise 'Jaybar'	13 g/bar1	3.00/bar	online
Kraft Jell-O Pudding Sugar Free Chocolate	7 g/serving		
Biochem 'Ultimate LoCarb 2' bars	2nd of 2 polyols	2.00/bar	retail and online
Richardson Labs 'Carb Solutions' Creamy Chololate	3rd of 3 polyols (13 g)	1.50/bar	retail and online
Nature's Hollow sugar free jam (various flavors)	4.5 g/20 g serving	6.00/10 oz.	online
Nature's Hollow sugar free syrup (various flavors)	2.5 g/40 ml serving (7%)	5.40/8.5 oz.	online
Nature's Hollow sugar free ketchup	0.8 g/20 g serving (4%)	5.50/10 oz.	online
Nature's Hollow sugar free honey	1.2 g/20 g serving (8%)	5.50/10 oz.	online
Oral hygiene:			
Biotene dry mouth toothpaste (+/– Calcium)	10%	6.00–7.00/4.5 oz	retail and online
Crest 'Multicare Cool Mint toothpaste'	10%	3.50–4.50/8.0 oz	retail and online
Epic toothpaste	25% (no fluoride)	4.50–5.00/4.9 oz	online
Epic toothpaste with fluoride	35%	7.00–8.00/4.9 oz	online
Squigle 'Enamel Saver Toothpaste'	36% (0.24% sodium fluoride)	7.25–8.00/4.0 oz	online
Spry toothpaste 'MaxXylitol and Aloe'	N/C only polyol (no fluoride)	4.50–5.00/4.0 oz	online
Tom's of Maine baking soda toothpaste line	N/C (varies in ingredient list)	3.50–4.50/6.0 oz	retail & online
Tom's of Maine natural toothpaste line	N/C (varies in ingredient list)	3.50–4.50/6.0 oz	retail & online
Tom's of Maine sensitive toothpaste line	N/C (varies in ingredient list)	3.50–4.50/6.0 oz	retail & online
XyliWhite toothpaste (fluoride free)	25%	3.50/6.4 oz	online
Rembrandt toothpaste 'For Canker Sore'	only sugar (4th ingredient)	6.50–7.50/3 oz	retail & online

Table 7.3 *Continued*

Products*	Xylitol	Cost (US$) per unit	Availability
Biotene 'First Teeth' infant toothpaste	1st of 2 polyols	5.00–6.00/1.4 oz	retail and online
Gerber tooth & gum cleanser	2nd of 2 polyols (6th ingredient)	5.00–5.50/1.4 oz	retail & online
Spry Infant 'tooth gel'	N/C only polyol (no fluoride)	4.50–5.50/2.0 oz	online
Biotene 'Oral Balance' dry mouth gel	2nd of 2 polyols	5.00–6.00/1.5 oz	retail and online
Biotene mouthwash	1st of 2 polyols	6.00–7.00/16 oz	retail and online
Epic oral rinse	25%	7.50 0 8.50/16 oz	online
Oxyfresh mouthrinse	only sugar (2nd ingredient)	9.00–10.00/16 oz	online
Rembrandt 'Dazzling Breathdrops'	only sugar (2nd ingredient)	1.00–1.50/0.22 oz	retail & online
Spry oral rinse	1st of 2 polyols (no fluoride)	5.00–5.50/16 oz	online
Tom's of Maine natural mouthwash line	N/C (varies in ingredient list)	4.00–6.00/16.0 oz	retail & online
Healthcare:			
Bayer 'Flintstone' vitamins – complete			
Bayer one a day vitamins – complete			
Sundown 'Superman' complete vitamins			
Micro Spray vitamin sprays			
B&T 'Echina Spray'			
Dr. Ray's Products 'Spiffies Dental Wipes'			
Nicorette gum			
Xlear nasal wash			
Xylifloss pocket dental flosser			

[a] Product list is not exhaustive. The xylitol market is rapidly changing and new xylitol-containing products appear frequently.
[b] Cost varies based on retail and convenience stores. Stated cost based on a few Seattle area retailers.
[c] N/C = not certain. Information cannot be derived from market packaging and not successful in obtaining information from company information representative.

and other products labeled 'sugar-free' or 'does not promote tooth decay' may contain three or even four non-cariogenic sweeteners including artificial intense sweeteners with the total sugar alcohols (polyols) content listed by percent or weight in grams. Xylitol may not be the first sugar alcohol listed, although the packaging may highlight that it is present for marketing purposes. The amount of an ingredient in a product decreases with the order in which it appears. Furthermore, often the first several ingredients make up the bulk of the product.

Take this hypothetical mint, for example, the nutritional facts packaging indicates that each mint weighs 1.3 g and lists sugar alcohols content to be 1 g. The ingredients list shows that xylitol is the third of three sugar alcohols listed and is the fourth ingredient in the list. Therefore, the exact amount of xylitol in the product is unknown, but being the fourth ingredient indicates that only a small proportion of the weight of the mint is xylitol and being the third sugar alcohol indicates that xylitol does not make up the bulk of the sweetener in the gum. Thus, in all likelihood, only a small proportion, perhaps 0.1–0.2 g, of the 1 g of sugar alcohols in the gum is actually xylitol. Therefore, it is unlikely that consumption of this xylitol-containing mint would yield preventative benefits. As demonstrated, the task of deciphering the xylitol content from product packaging is quite taxing, inaccurate and makes effective use of available xylitol-containing products for preventive applications difficult. Manufacturers who indicate that their product is sweetened with 100% xylitol, list xylitol as the first ingredient, or indicate the number of grams of xylitol per piece, facilitate professional evaluation and consumer knowledge and choice in selecting products appropriate for preventive measures.

7.6 Key points and conclusions

Polyols are ubiquitous in food and confectionery products, particularly with those claiming to be sugar-free or to have oral health benefits. Polyols as a class are widely held to be low- to non-aciduric and very low- to non-cariogenic. Xylitol in particular has unique properties that set it apart from its class. Xylitol actively promotes better oral health by reducing the levels of pathogenic mutans streptococci in plaque and saliva and by reducing tooth decay. The active protective effects are not limited to those who consume xylitol but also extend to the children of mothers who consume xylitol during the perinatal period. The mechanism of action of xylitol is related to its disruption of carbohydrate metabolism by mutans streptococci thereby limiting the production of energy and intermediates for growth and replication and the polysaccharides that enhance adhesion of the bacteria to plaque and biofilms.

There is growing agreement among researchers that the minimum amount and frequency of xylitol consumption needed for efficacy in chewing

gum, lozenges and hard candies is 5–10 g per day divided into at least three frequencies of use. Numerous studies of these products and using these doses and frequencies of consumption provided the bases for this growing agreement. At lower doses and/or lower frequencies, the studies have reported mixed results, some efficacious, some not at all. Perhaps better designed and controlled studies in the future will help clarify these mixed results. Furthermore, it remains unclear if different xylitol-containing products would have varying results. Xylitol syrup was shown to be effective when consumed for 12 months at 8 g per day divided into two or three doses.

Numerous xylitol-containing products are available and may be marketed with oral health claims; however, many have not been rigorously tested, if at all. Thus, caution must be exercised when clinicians recommend the use of xylitol and awareness of products test-proven to be efficacious will greatly enhance the selection of appropriate product choices that provide oral health benefits. Some xylitol-containing products have been adequately studied and have the potential for use in specific or generalized public health programs to promote oral health and reduce tooth decay. The most well-tested and popular product among these is xylitol chewing gums. However, their use may not be appropriate for certain populations, particularly among very young children. Mints and hard candies may be appropriate for use in certain populations, but among children there is concern that these products may promote the use of candies in general, many of which are sweetened with fermentable sugars. Other xylitol-containing products, such as syrup and gummy bears are being tested for efficacy in tooth decay reduction with very promising results and may have great potential for public health application among young children if packaged appropriately, for example, packing gummy bears in a similar fashion to gummy bear vitamins where the daily dose and frequency of use is controlled (as recommended on user instructions).

Whether delivered by chewing gums, lozenges, syrups, or other modes currently under investigation, the required dose, 5–10 g/day, and frequency of xylitol use, at least two times per day for syrup and three times per day for chewing gum, lozenges and hard candies, for efficacy are significant barriers by themselves to successful public health intervention programs. Implementing such frequencies of xylitol consumption among children may be facilitated by structured settings such as daycare, pre-school and school, but the challenges and barriers that must be overcome can be taxing. Creative ways of maintaining interest and compliance in consuming the same xylitol product over several months can be challenging.

Future research to understand better how xylitol interacts with the plaque biofilm and how novel delivery methods such as slow-release technologies can be applied so that xylitol dose and frequencies of use can be reduced to the minimum would greatly enhance compliance in use and improve xylitol bioavailability and contact time with plaque for maximal

effect. With these achievements, xylitol applications have the potential dramatically to improve oral health status and reduce tooth decay among children, especially those at high risk.

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8

Dairy products and oral health

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Abstract: This chapter reviews the evidence from epidemiological studies for an effect of intake of dairy products on the risk of dental caries and chronic periodontitis, the two most prevalent oral diseases. There is considerable evidence from cross-sectional studies of an inverse association between intake of dairy products and the prevalence of caries in children and adolescents. However, few studies have evaluated the association in adults and evidence from longitudinal studies or intervention studies is scarce. More recently, cross-sectional studies from the USA and Japan have also reported inverse associations between intake of dairy products and chronic periodontitis; however, no longitudinal or intervention studies have been conducted. While there are promising data from cross-sectional studies that dairy products could have an important role in the prevention of the two most prevalent oral disease, longitudinal and intervention studies are needed to confirm these associations.

Key words: dental caries, dairy products, periodontitis.

8.1 Introduction

In this chapter, we will review the evidence from clinical and epidemiological studies for an association between the intake of dairy products and their constituents and the risk for caries and chronic periodontitis. Research studies into the role of dairy products and their components in caries in both humans and other animals date back over 50 years (McClure and Folk, 1953; Muhler, 1957; Prewitt, 1950; Shaw *et al.*, 1959). Hypotheses for the cariostatic effect of milk and cheese include: (i) adhesion of casein to the enamel which may protect the tooth from plaque acid (Weiss and Bibby,

1966), (ii) antimicrobial properties including inhibition of *Streptococcus mutans* (Bowen and Pearson, 1993; Jenkins and Ferguson, 1966), (iii) inhibition of demineralization and promotion of remineralization through the adsorption of casein and integration of calcium and phosphorus into the enamel surface (Bowen, 1998; Silva *et al.*, 1987; Silva *et al.*, 1986) and (iv) buffering action of saliva with concomitant reduction in plaque acidity and increase in salivary flow rate (Jensen and Wefel, 1990). The mechanisms underpinning the cariostatic effects of dairy products are discussed in more detail elsewhere in this volume. Also, studies that have investigated the effects of fluoridated milk on caries risk are not included here (Yeung *et al.*, 2005).

Periodontitis is characterized by the inflammatory resorption of alveolar bone. Low bone mineral density and osteoporosis have long been considered as risk factors for periodontitis (Wactawski-Wende *et al.*, 1996). Hence, calcium intake has been considered as a possible determinant of periodontitis risk for some time. More recently, epidemiological studies have also evaluated the association between dairy intake and periodontitis.

8.2 Intake of dairy products and risk of dental caries

Several animal studies and much basic science research into the mechanism of action have been conducted to investigate the effect of dairy products on dental caries. However, only a limited number of epidemiological studies have been conducted to investigate the cariostatic potential of dairy products.

8.2.1 Intake of dairy products and risk of dental caries in children and adolescents

Two of the earliest epidemiological studies on child nutrition including milk and dental caries were conducted by Brodsky in 1933 (Brodsky, 1933) and Read and Knowles in 1938 (Read and Knowles, 1938). In another early epidemiological study, Potgieter *et al.* (1956) collected dietary data from 864 Connecticut school children in grades 5 to 8 from a 7-day record of food intake, and dental caries were assessed by two public health dental hygienists. Dental examinations were conducted with a mirror, explorer and the use of good light. However, no radiographs were available. Potgieter *et al.* (1956) found a marked and continuous drop in DMFT (decayed, missing and filled teeth) score with increase in the number of cups of milk consumed, from an average DMFT of 10.65 for children consuming less than 1 cup of milk per day down to an average DMFT of 5.95 for those children consuming 4.1 to 5.0 cups of milk per day (Potgieter *et al.*, 1956).

In the late 1950s, Zita *et al.* (1956) conducted a study to investigate the relationship between dietary habits and dental caries experience in chil-

dren. The records of 200 children were randomly selected from the files of the pediatric dentistry department in the School of Dentistry at Indiana University (Zita *et al.*, 1959). A 7-day diet survey was collected from the parents of each child, a careful clinical examination was performed with mirror, explorer and adequate light, and a complete radiographic survey including bite-wings was available. A slight negative correlation (–0.08) was found between total milk intake and DMFS (number of decayed, missing and filled surfaces) score. The authors also found a weak positive correlation between total sugar intake and DMFS score (+0.10).

More recently, Petti conducted a cross-sectional study of 890 schoolchildren surveyed from two primary schools in Rome, Italy (Petti *et al.*, 1997). They examined the effect of milk and sucrose on caries development in 6 to 11-year old children. The children that were enrolled in the study lived in an area with low fluoride levels in the water. Parents filled out surveys on each child's fluoride use, a 24-hour dietary diary (including quantity of milk) and information to determine socioeconomic status. Oral exams included clinical observation and X-rays. Plaque indices and DFdef scores were calculated. The DFdef score was calculated as the sum of the number of decayed permanent teeth (D), the number of filled permanent teeth (F), the number of decayed primary teeth (d), the number of extracted primary teeth (e) and the number of filled primary teeth (f). Children who used fluoride, or those children with a plaque index of less than 1, were considered to be protected against caries by means other than dietary and were excluded from the study, resulting in a final sample of 439 children. Data on socioeconomic status were available for 322 of these children.

Analyses were performed on both the sample of 322 children with socioeconomic information as well as all 439 children. Analyses were also performed after stratifying the sample by sucrose intake: low (0–1 time/day), moderate (2–3 times/day) and high sucrose intake (4 or more times/day). In both samples, sucrose frequency, milk frequency, plaque index, age, gender and socioeconomic status were statistically significant predictors of having at least one DFdef tooth. Increased consumption of milk was associated with lower caries prevalence. After stratifying by sucrose level, the protective effect of milk consumption was more pronounced among children with high sucrose intake.

In a two-year longitudinal study, Rugg-Gunn (Rugg-Gunn *et al.*, 1984) recruited seventh and eighth grade children (mean age: 11.7 years) who were caries-free at the beginning of the study. Radiographs of proximal and fissure sites were examined for caries. Gingival index was used as an indicator of the amount of plaque. Tooth brushing frequency was also assessed and subjects were provided with fluoride toothpaste. As in an earlier study, all subjects completed a three-day dietary record at the start of the study and five more times over the study period. 405 children completed the study. Comparisons were made between children with no caries to children who were in the highest caries quartile. Milk consumption was highest in

the highest caries group (mean milk intake: 242 g for children with low caries versus 269 g for children with high caries). Correlations between milk intake and dental caries as well as between sugar intake and dental caries for this study were very weak. As noted by Ismail (Ismail, 1986), the weak correlations noted for the study by Rugg-Gunn *et al.* (1984) may have been the result of an overall low incidence of caries, underestimation of amounts and frequencies of consumption of certain foods and low overall sugar intake among the children enrolled in the study. Hence, the contradictory findings of this particular study are likely to be attributable to unique aspects of the study population rather than demonstrating the lack of cariostatic properties of milk.

In a cross-sectional study of 380 Greek adolescents between the ages of 12 and 17, Petridou found evidence that intake of milk products was associated with lower DMFT and DMFS scores, whereas sugar intake was not significantly associated with higher DMFT and DMFS scores (Petridou *et al.*, 1996). Subjects for the study were randomly selected from two rural high schools and two urban high schools. WHO criteria were used for dental examinations, interviews were conducted to assess oral hygiene habits and an interviewer-administered, semi-quantitative food frequency questionnaire was used to assess dietary patterns. Multiple regression models for predicting DMFT and DMFS were fitted to assess the effect of each of nine food groups while controlling for the core variables for gender, age, self-reported oral hygiene habits, residence type and income level and school performance. The food groups from the food frequency questionnaire that were assessed included: (1) cereals, (2) starch, (3) sugars, (4) legumes and nuts, (5) vegetables, (6) fruits, (7) meat, fish, eggs, (8) milk and dairy products and (9) oils and fats. The only food group found to be significantly associated with DMFT and DMFS scores was milk and dairy products. Intake of milk and dairy products was divided into quartiles for the statistical analysis. However, in the multiple regression models for predicting DMFT and DMFS, the variable for quartiles of milk and dairy consumption was treated as a continuous variable. Hence, based on the analysis presented for this study, it is not possible to determine if a minimum threshold of milk and dairy consumption is required to observe a protective effect for dental caries.

In a cross-sectional study conducted in Spain, Serra-Majem found skimmed milk to be protective against dental caries (Serra Majem *et al.*, 1993). A thousand schoolchildren between the ages of 5 and 14 were randomly selected from 2887 children participating in a community trial of water supply fluoridation in the cities of Girona and Figueres. In addition to complete dental assessment, information was gathered from a self-administered food-frequency questionnaire. Children were given the questionnaire on the day of the dental examination to be completed with the help of their parents that evening. It was returned to their teachers one week later. Completed food frequency questionnaires were only available for 893 (89.3%) of the original 1000 children selected for the study. In this

study, children were divided into quartiles according to skimmed milk consumption and consumption of various foods high in sugar content – ice cream, bakery products, pastries, sugared soft drinks, and so on. Multiple logistic regression models for predicting the prevalence of caries were fitted for quartiles of consumption of the various foods under investigation with adjustment for potential confounders, including age, gender, oral hygiene habits and dental visit frequency. For this logistic model, a threshold effect for skimmed milk consumption was observed with no statistically significant reduction in caries prevalence until the fourth (highest) quartile of skimmed milk consumption. In particular, Spanish children in the highest quartile of skimmed milk consumption had a 67% reduction in the prevalence of caries.

In a four-year longitudinal study of 608 children, aged 7–11 at baseline, Levine investigated the relationship of dietary patterns and toothbrushing habits to caries experience among schoolchildren in West Yorkshire, England (Levine *et al.*, 2007). Three-day, self-reported dietary data were gathered for these children and caries experience was monitored over four years using the diagnostic criteria of the British Association for the Study of Community Dentistry (BASCD). Moderate consumption of dairy products was found to be associated with significantly less caries among children aged 11–15 years old. Consumption of sugar-sweetened drinks and lack of regular toothbrushing were the risk factors most strongly linked to increased caries in this study.

Kolker recently conducted a study of 3–5-year-old African–American children in Detroit, Michigan from households that had incomes below 250% of the 2000 federal poverty level to assess the relationship between dietary patterns and severity of dental caries in primary teeth (Kolker *et al.*, 2007). Dietary intake was measured using the Block Kids Food Questionnaire, while severity of dental caries in primary teeth was measured by the International Caries Detection and Assessment System criteria. Among the 436 low-income African–American children enrolled in the study, increased consumption of milk was associated with a lower level of DMFS (number of decayed, missing and filled surfaces) in primary teeth among low-income, 3–5-year-old African–American children in Detroit.

Sheshah analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III) to investigate the association of milk intake with the prevalence of early childhood caries (ECC) among 2–5-year-old children (Sheshah, 2003). In a multiple logistic regression model to assess the association of total milk intake with prevalence of ECC, while adjusting for age, race/ethnicity, poverty income ratio, time since last dental visit and eating breakfast regularly, it was found that children who consumed three or more servings of milk per day were 42% less likely to have ECC compared to children who consumed less than two servings of milk per day.

Öhlund conducted a study of 124 four-year-old children whose parents agreed to participate in a follow-up survey within the Study of Infant Nutrition in Umeå, Sweden (SINUS project) (Ohlund *et al.*, 2007). Three hundred healthy infants less than 6 months of age were originally recruited from six well-baby clinics in the town of Umeå, Sweden between December 1995 and June 1998 for the baseline SINUS registrations. Of these, 234 children completed monthly food and medical registrations from 6 to 18 months of age and were invited to participate in the follow-up study that started in 2001. The parents of 124 children consented to the follow-up study. The purpose of the follow-up study was to evaluate the associations between dental caries, levels of *Streptococcus mutans* and lactobacilli in saliva; and diet, with a particular interest in the relationship between consumption of dairy products and fermentable carbohydrates to caries in young children living in a country with a low overall prevalence of caries. Based on multiple stepwise logistic regression, caries experience was negatively associated with frequency of cheese intake with each additional serving of cheese in a week reducing the risk of dental caries by a factor of 33% (odds ratio = 0.67, 95% confidence interval: 0.44 to 0.98, $p = 0.037$). In other words, among 4-year-old children in a low-caries risk country, an additional one serving of cheese per week reduces the risk of dental caries by 33%, an additional two servings of cheese per week reduces the risk of dental caries by 55%, an additional three servings of cheese per week reduces the risk of dental caries by 70%, and so forth.

8.2.2 Intake of dairy products and risk of dental caries in adults

Papas recruited 274 adult subjects to assess the relationship of various nutritional components to the prevalence of root caries (Papas *et al.*, 1995a). Each subject received a complete clinical examination including assessment of both coronal and root caries. Subjects completed the modified Block semi-quantitative food frequency survey with a subset of 50 subjects completing a 3-day food record. Subjects were divided into four groups: (1) group 1 (disease subjects) had recession with at least one active root lesion, (2) group 2 had recession but no active root lesion or restoration, (3) group 3 (mixed subjects) had at least one restoration with recession with no active root caries, and (4) group 4 (healthy subjects) had no recession or root caries. Subjects with healthy teeth were found to have consumed significantly more cheese than subjects with diseased teeth and somewhat more cheese than mixed subjects (mean servings of cheese per week: 'healthy' subjects: 3.22, 'diseased' subjects: 2.07, mixed subjects: 2.85, $p = 0.05$ for 'healthy' versus 'diseased' subjects). In addition to cheese, 'healthy' subjects tended to eat more other dairy products than 'diseased' subjects or mixed subjects, although the difference was not statistically significant (mean occurrence of dairy products per week: 'healthy' subjects: 8.18, 'diseased'

subjects: 6.62, mixed subjects: 7.99, $p = 0.52$ for ANOVA (analysis of variance) comparing 'healthy', 'diseased' and mixed subjects).

A stepwise logistic regression model found sugar and cheese intake to be the only significant dietary variables that were related to root caries, while milk did not achieve statistical significance. As cheese consumption increased, the prevalence of root caries decreased (odds ratios for cheese consumption measured in units per week compared with less than two occurrences per week: two occurrences per week = 0.74, three occurrences per week = 0.64, four occurrences per week = 0.55 and five occurrences per week = 0.47). Hence, based on the results of this study, eating cheese five or more times a week reduced the risk of root caries by 53% compared with eating no cheese or only eating cheese once a week.

In another study, Papas asked 141 healthy middle-aged and elderly adult healthy subjects with at least six teeth to complete a 3-day food diary and a food frequency questionnaire (Papas *et al.*, 1995b). Clinical dental examinations were also performed to determine dental decay on both coronal and root surfaces, although no radiographs were taken. They found that subjects aged 65 years and older with root caries ate half the amount of cheese as similarly aged adults without root caries (mean frequency of consumption of cheese ± SD: 4.7 ± 3.0 for subjects with root caries, 2.20 ± 2.00 for subjects without root caries).

8.3 Intake of dairy products and risk of periodontitis

As our understanding of the pathogenesis of periodontitis has progressed considerably over the past several decades, it has become clear that periodontitis is not simply a response to bacterial plaque that invariably occurs in all individuals, but occurs in individuals susceptible to periodontitis, largely determined by environmental and genetic host factors. Indeed, smoking and diabetes are now recognized as major periodontal risk factors, and over the past decade, several studies have investigated nutritional determinants of periodontitis risk, including dairy products. Nevertheless, bacterial plaque is considered a necessary cause of periodontitis and it is bacterial components and products that elicit the inflammatory response in the periodontal host tissues. Hence, dairy products may affect periodontitis risk through an effect on plaque quality and/or quantity as well as through modulation of the inflammatory response to periodontal pathogens.

However, the evidence for a role of dairy products in the pathogenesis of periodontitis is scarce and entirely from cross-sectional studies, in particular the Third National Health and Nutrition Examination Survey conducted in the USA from 1988 to 1994 (NHANES III). Dairy intake is a major determinant of calcium intake and low calcium intake has long been proposed as a possible risk factor for periodontitis (Henrikson, 1968).

More recently, data from NHANES III showed inverse associations between intake levels of calcium (Nishida *et al.*, 2000) and also dairy products (Al-Zahrani, 2006) with periodontitis prevalence in the USA. In addition, Krall and co-workers have reported a beneficial effect of calcium and vitamin D supplementation on tooth retention (Krall *et al.*, 2001) in a small observational study. However, a small randomized controlled clinical trial in patients with periodontitis comparing the effects of 1000 mg calcium supplementation for 180 days with a placebo showed no effect of calcium supplementation on periodontal disease parameters (Uhrbom and Jacobson, 1984).

Importantly, in the USA milk is fortified with vitamin D and increased vitamin D intake in individuals with higher milk consumption may be an alternative explanation for the inverse association between dairy intake and periodontitis seen in the USA. Indeed, lower periodontitis prevalence among subjects older than 50 years of age with higher serum concentrations of vitamin D (25-hydroxyvitamin D) has been reported (Dietrich *et al.*, 2004). In addition to its canonical effect on calcium metabolism and bone, vitamin D may reduce periodontitis susceptibility through its immunomodulatory functions. Indeed, a strong inverse association between vitamin D status and gingivitis prevalence has been reported in NHANES III (Dietrich *et al.*, 2005).

However, constituents of dairy products other than calcium or vitamin D may contribute to a beneficial effect of these foods on periodontitis risk. The association between different types of dairy products (milk, cheese and lactic acid foods) and periodontitis prevalence was investigated in a recent cross-sectional study of 942 subjects aged 40 to 79 years in Japan. The daily intake of lactic acid foods, but not milk, was inversely associated with periodontitis prevalence and severity, which the authors explain by a possible beneficial effect of 'probiotic' dairy products on the oral microflora (Shimazaki *et al.*, 2008).

8.4 Future trends

Overall, the bulk of the evidence for the cariostatic properties of milk, cheese and other dairy products can be found in animal studies and basic science research. Nevertheless, a number of epidemiological studies are consistent with the cariostatic properties of milk translating into lower caries prevalence in populations. However, the vast majority of available studies are cross-sectional studies in children or adolescents and much less epidemiological evidence is available to support the cariostatic properties of dairy products in adults.

Another important limitation of the available epidemiologic studies on caries prevalence and incidence is that validated standard instruments to assess nutritional intake do not capture patterns of consumption that are important for caries pathogenesis. For instance, Geddes reported that

eating a cariostatic food, such as milk or cheese, after eating a cariogenic food, such as calorie-dense foods high in extrinsic sugar content, has a protective effect for dental caries as opposed to eating high-sugar foods at the end of the meal (Geddes, 1994). In addition, the lactose in milk is a fermentable carbohydrate with cariogenic potential when teeth are exposed to prolonged contact. It has been argued that 'nursing bottle caries' can develop if children are put to bed with a bottle of milk, as milk pools in the infant's mouth over a prolonged period of time enabling plaque microorganisms continually to ferment the lactose in milk to acid. The result is an almost continuous exposure of the infant's teeth to these acids generated by plaque that accumulates on the teeth and the eventual progressive dissolution of the tooth enamel under the plaque (Bowen, 1998).

Clearly, further epidemiological and intervention studies need to be conducted to show conclusively the cariostatic properties of dairy products. Promising results from a limited number of cross-sectional studies suggest that dairy products may have an important role in the prevention of periodontitis. However, these associations need to be confirmed in longitudinal and, ultimately, in intervention studies. Furthermore, the mechanisms by which dairy products may reduce periodontitis risk are not well understood, and basic and mechanistic research in this area is needed.

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9

Impact of food sugars and polysaccharides on dental caries

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Abstract: Diet plays a key role in the development of dental caries. The impact of a specific food depends on a number of factors related to the product consumed and also to factors within the individual. Food sugars and starch may both contribute to this process. Frequent consumption of fermentable carbohydrates promotes a shift in the oral ecological system towards a more caries-promoting environment. The impact of diet has changed since the middle of the last century, particularly due to the increased use of fluoride, but there are still individuals in all age groups found to be at risk for caries. This chapter considers the effect of different aspects of food sugars and polysaccharides on dental caries in modern society.

Key words: dental caries, foods, starch, sucrose, sugars.

9.1 Fermentable carbohydrates: a key factor in the caries process

Dental caries can be referred to as a process of enamel and dentine demineralisation, which is caused by organic acids produced in the bacterial fermentation of sugars derived from the diet. In order for caries to develop, a fermentable carbohydrate substrate must be provided over a sufficient period of time. However, the interplay between the three key factors – tooth/host, bacteria and diet – is influenced by a large number of biological and socioeconomic factors of which saliva secretion rate and plaque composition are examples of the previous category and ethnic background and educational level can exemplify the latter (Keyes and Jordan, 1963; Selwitz *et al*., 2007). No variable can be looked upon separately without taking the others into account.

The impact of the consumption of a specific food is dependent on a number of factors related to the product itself, that is type of fermentable

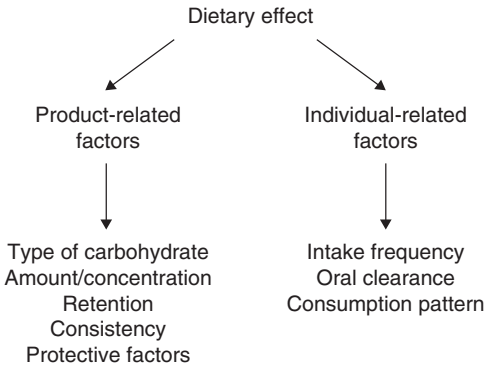

Fig. 9.1 The impact of the consumption of a specific food on caries is dependent on a number of product- and individual-related factors.

carbohydrate, concentration or amount, characteristics such as stickiness and retention, and the content of protective factors (Fig. 9.1). The ultimate effect of a food product is also related to a number of individual factors. Of these, intake frequency is the most important, but oral clearance and consumption pattern are also critical in order to determine the risk of disease occurring.

Dental caries will not occur if the interplay described above takes place only occasionally. Even if organic and inorganic acids formed during the fermentation process initiate the demineralisation of the hard tissues, a single attack does not cause caries at the clinical level. The early stages of demineralisation after a limited number of acid attacks can be remineralised by the action of saliva and the mechanical removal of bacterial plaque, but the process is further promoted by exposure to fluoride ions.

The chemical process needs to occur with a certain frequency in order for caries to develop. The demineralisation/remineralisation cycles will then result in increased mineral loss and a carious cavity is formed. The discussion about the impact of food sugars and polysaccharides on dental caries is complex and a large number of aspects need to be taken into account when assessing the risk of caries in relation to the ingestion of a certain food product. This chapter deals with the food itself and will only discuss the impact of other factors involved in the caries process to a limited degree.

9.2 The different carbohydrates in the diet

Dietary carbohydrates are macronutrients with a range of physical and physiological properties. Apart from being one of the three principal energy sources, they are currently also known both to possess health benefits and

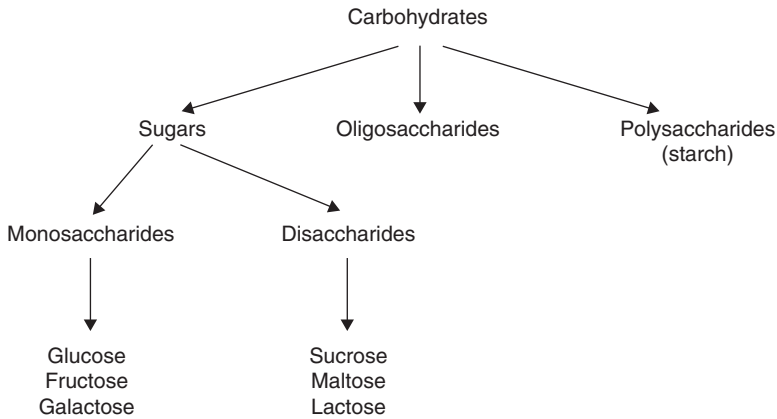

Fig. 9.2 Classification of carbohydrates.

to be associated with disease (Anon, 2003). Carbohydrates, including sugars, starches and fibres found in fruit, vegetables, grains and milk products, constitute an important part of a healthy diet. The nomenclature for carbohydrates in food may seem somewhat confusing and a number of terms are currently used. The primary classification is based on the chemical composition and this divides carbohydrates into three main categories: sugars, oligosaccharides and polysaccharides (Fig. 9.2). The sugars consist of carbohydrates with one or two units linked together, such as the monosaccharides, glucose, fructose and galactose, and the disaccharides, sucrose, maltose and lactose (Moynihan, 1998). Apart from the classification mentioned above, they can also be divided into fermentable and non-fermentable carbohydrates.

In the case of sugars, a distinction is often made between intrinsic sugars, that is sugars located naturally within the cellular structure of the product and extrinsic sugars, that is sugars located outside the cellular structure of the cell. The former are found in whole fruit and vegetables. The latter are free in the food, or added to it, and are often also referred to as free sugars. They can further be divided into milk sugars (almost entirely lactose), naturally present in milk and dairy foods, and non-milk extrinsic sugars, which are equivalent to all the sugar added by the manufacturer, cook or consumer, plus sugars in juices and honey (Moynihan and Petersen, 2004).

Starch is synthesised by plants and is the main component of most cereals and tubers (Guilbot and Mercier, 1985). It is a polymer of α-D-glucose, linked together into linear or branched chain structures, for example amylase and amylopectin (Würsch, 1989). Starch molecules are located within starch granules, which vary in size within any plant source, and even more between sources, and can assume a wide variety of shapes (Lineback, 1984). Most starch can be broken down and this results in a blood-glucose

response. Raw starch granules are insoluble in cold water. Treatment with heat, pressure or mechanical stress can result in the irreversible destruction of the crystalline structure, so-called gelatinisation, and this increases its enzymatic availability. Starches that do not undergo digestion in the small intestine are referred to as resistant starch. Fibres are polysaccharides made up most frequently of glucose units, but the monosaccharides are bonded to each other differently compared with starch. Human enzymes are unable to break these bonds and, consequently, the fibres cannot be utilised by the host.

9.3 Relative cariogenicity of different sugars

In order for a food product to be cariogenic, it must be able to undergo bacterial fermentation resulting in end products capable of initiating demineralisation. When such products are ingested, this results in a fall in dental plaque pH caused by the organic acids which then increases the solubility of calcium hydroxyapatite in the dental hard tissues and demineralisation occurs. The pH at which demineralisation occurs is referred to as the 'critical pH' and is lower for enamel (around pH 5.5–5.7) compared with dentine (around pH 6.2). The rate of demineralisation is affected by the concentration of hydrogen ions (i.e. pH) at the tooth surface and the frequency with which the plaque pH falls below the critical pH.

The carbohydrates which can be easily metabolised by oral microorganisms are sugars and starch. Of the major food sugars, there is a small difference in the acidogenic potential of glucose, fructose, maltose and sucrose, while lactose appears to induce lower acidogenicity (Neff, 1967).

Sucrose is the sugar that is given special consideration as a substrate for bacterial fermentation (Newbrun, 1969, 1982a; Tanzer, 1989). Many oral microorganisms, particularly mutans streptococci, have a unique ability to synthesise extracellular glucans (van Houte, 1994). The production of both water-soluble and water-insoluble glucans results in increased plaque production. The extracellular polysaccharides favour plaque growth and may thereby increase the cariogenicity of dental plaque (Carlsson and Egelberg, 1965; Rateitschak-Plüss and Guggenheim, 1982). There are indications that the increased cariogenicity seen in animals infected by *Streptococcus mutans* is strain specific rather than being related to different animal models. It has recently been suggested that the virulence of glucan may be related to an alteration in plaque ecology. An increase in plaque porosity results in the deeper penetration of dietary sugars and greater acid production adjacent to the tooth surface (Zero, 2004). Mutans streptococci can synthesise insoluble plaque matrix polymers. Mutans streptococcal invertase splits sucrose into glucose and fructose, which can be metabolised by most oral bacteria to produce mainly lactic acid but also other acids, including acetic and formic acids.

Fructose constitutes the majority of the sugars found in fruits, but glucose and sucrose are also present. Fruit and vegetables are generally regarded as beneficial from a general health perspective, but there is no general agreement regarding their impact on dental health. They may be consumed as fresh fruit, dried fruit or juices. The sugar content varies between different types of fruit, but, in the case of fresh fruit, it is often found to be between 10 and 15%, while the concentration in dried products is much higher and may very well be increased by up to 75%. Fruit has been found to be acidogenic, but this varies according to the sugar content and texture (Imfeld, 1983). Moreover, animal studies have demonstrated that fresh fruit causes caries when consumed in mashed form at high frequency (Imfeld *et al*., 1991). When consumed as dried fruit, the plaque pH indicates high cariogenic potential (Rugg-Gunn *et al*., 1978), which differs from that of fresh fruit. The acidogenic potential of different kinds of fruit may vary and, in order to determine the final cariogenic potential, it is necessary to take the consumption pattern and differences in texture into consideration. Apples have a high fibre content, which makes them self-cleansing. There is little information in the literature about the cariogenic potential of fresh vegetables, but the data indicate appreciable cariogenic potential (van Loveren, 2000).

Lactose is also known to be less cariogenic compared with other sugars (Rugg-Gunn, 1993). It constitutes the main sugar of milk and milk products. Milk also constitutes one of the main sugars in the diet of infants and, indeed, throughout life. The concentration of lactose varies between 3 and 8% in cow and human milk, with the former having a slightly higher concentration. Milk contains a large number of agents, such as calcium, phosphate, casein, other lipids and protein components, which are known to protect against caries. Cheese has also been evaluated for its caries-protective value; it is known to contain most of the protective components found in milk, but it is basically free from lactose (Kashket and DePaola, 2002). Postulated protective mechanisms suggested for both milk and cheese are buffering, salivary stimulation, the reduction of bacterial adhesion, the reduction of enamel demineralisation and/or the promotion of remineralisation. In the case of lactose, it is important to distinguish between the consumption of milk sugars and non-milk extrinsic sugars, as well as to consider the actual intake pattern and frequency (see also Section 9.10).

9.4 Starches and dental caries

Starches constitute major components of the human diet and, for many individuals, represent about 50% of the total carbohydrate intake (Rugg-Gunn *et al*., 1986; Burt and Szpunar, 1994).

Starch cannot be utilised directly after intake by oral microorganisms. The decomposition of starch in the oral cavity is initiated in saliva by host

and bacterial amylases (Fiehn and Moe, 1983). Following hydrolysis intra-orally into maltose, maltotriose and low-molecular-weight dextrins, starch can be used as a substrate for bacterial fermentation (Mörmann and Mühlemann, 1981). There is general agreement that starches can be broken down by oral microorganisms, resulting in acid production, but the impact starch may have in the caries process is still the subject of debate. In general, only the gelatinised starches that are susceptible to enzymatic breakdown, that is, they are bioavailable. The significance of the bioavailability factor has been clearly demonstrated systemically, where different starches or starchy foods have been shown to induce different responses in blood glucose and insulin.

The degree of cariogenicity of starchy foods is believed to depend on several factors, of which the process the product has undergone before consumption, that is the degree of gelatinisation, is believed to be the most important (Fig. 9.3). Raw starch is considered to have low cariogenic potential. With a higher degree of gelatinisation, caused by heat or mechanical stress, the starch can be more easily broken down by oral microorganisms (Würsch *et al*., 1986; Holm and Björck, 1988; Colonna *et al*., 1992). As a result, the acidogenic potential of dental plaque will increase (Lingström *et al*., 2000). In addition, starch consumption frequency and starch retentiveness are regarded as important factors affecting the final cariogenic potential of starch.

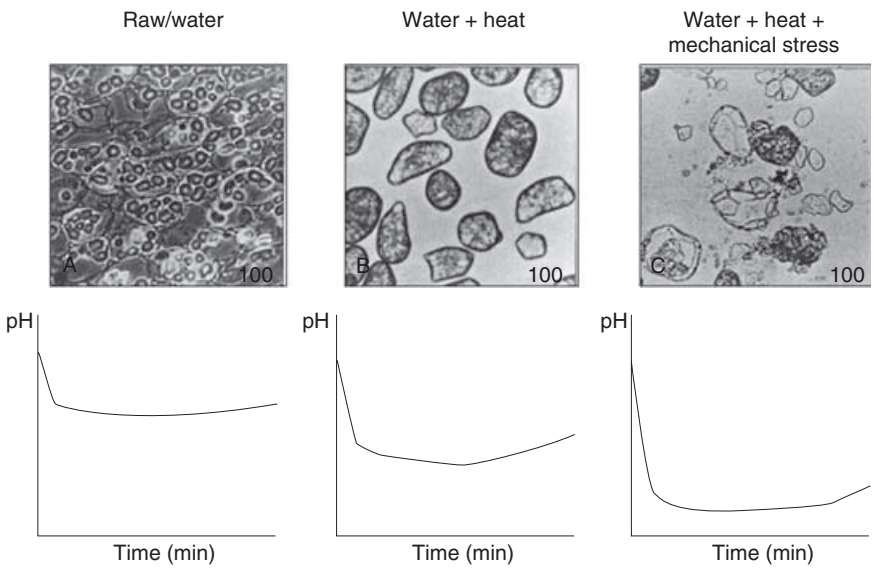

Fig. 9.3 Top: Difference in degree of gelatinisation in relation to various pretreatments (scale in μm; Würsch *et al*., 1986). Bottom: Subsequent effect of pretreatment on plaque acidogenicity after consumption.

The importance of starch in dental caries is believed to differ between populations, with the consumption primarily of 'pure' starchy foods with few sugars during a limited number of intakes per day in contrast to modern populations where high-sugar/high-starch diets are consumed (Lingström *et al*., 2000). Starch may influence the stickiness of products, which is an important factor for its cariogenic potential. Particles of foods containing high levels of starch have been found to be retained in larger amounts than foods that contain relatively little starch, but high levels of sucrose and other sugars (Kashket *et al*., 1991). Studies of the starch and plaque pH relationship in humans have shown that processed starch may induce significant pH falls that may be close to those obtained for sucrose (Bibby *et al*., 1986; Pollard, 1995; Lingström *et al*., 2000). The interaction between sugars and hydrolysable starch has been found to be important for cariogenicity and evidence to indicate that a combination of starch and sucrose may be more cariogenic than sucrose alone has been presented (Firestone *et al*., 1982; Lingström *et al*., 1993).

9.5 Impact on caries from a historical perspective

The evidence demonstrating the role of diet in the aetiology of dental caries is overwhelming (Zero, 2004). This evidence comes from many different types of investigation, including human studies (both observational and interventional), human plaque pH and demineralisation studies, animal studies and *in vitro* data. Taken together, all this information provides an overall picture of the cariogenic potential of different dietary carbohydrates. The strength of the evidence incriminating sugars in the aetiology of dental caries comes from the multiplicity of the studies rather than from the power of any one study alone.

Some of the data supporting the role of sugar in dental caries in humans are referred to as 'classic evidence' (Zero, 2004). For many of these studies, it is difficult to distinguish between the actual intake of different sugars and the consumption of refined carbohydrates and, as a result, it is difficult to distinguish between the effects of sugar and starch. Classical studies include data on ancient man, which show that caries was kept at a low level as long as sugar consumption was kept low (see Fig. 9.4) (Moore and Corbett, 1973). Sweet foods such as berries and honey have always been available, but the total sugar intake was low. When the spread of sugar cane and sugar beet began, sugar was a luxury product and was only available to a small, selected fraction of the population. Sugar consumption really took off following the commercial mass production of sugar about 150 years ago.

Historical data also show that caries prevalence can fall as the intake of sugar and refined carbohydrates is reduced (Toverud, 1957a; Toverud, 1957b; Takeuchi, 1961). A sugar reduction varying between 47 and 60% in some Scandinavian countries during the Second World War was followed

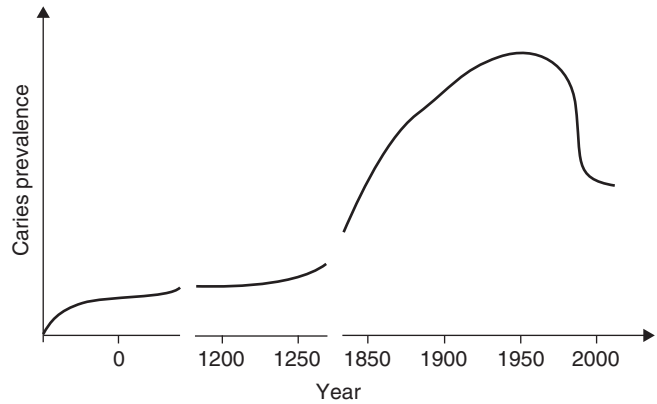

Fig. 9.4 Variation in caries prevalence from a historic perspective.

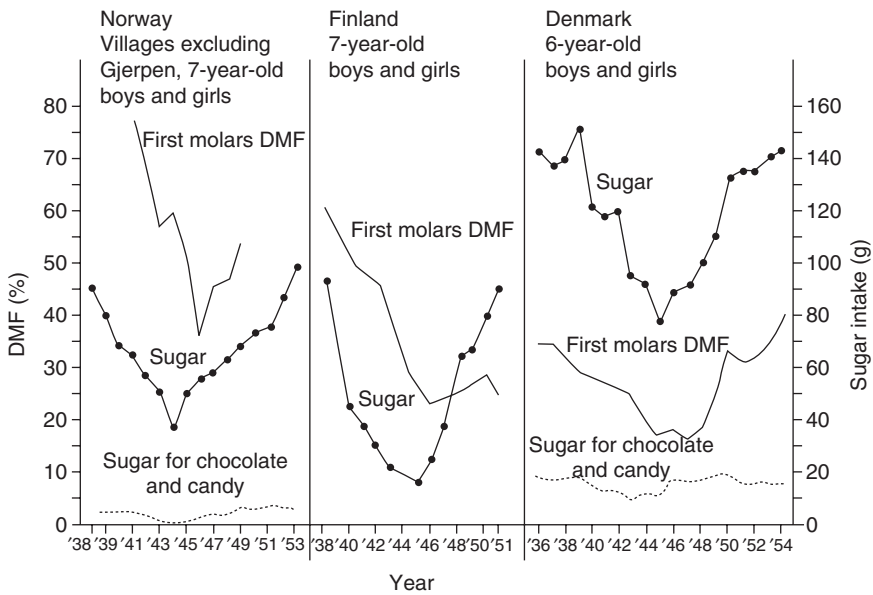

Fig. 9.5 Sugar availability (g) and decayed, missing and filled teeth (DMFT) (%) in 6- or 7-year old boys and girls in Norway, Finland and Denmark between 1936 and 1954 (Toverud, 1957a and b).

by a corresponding decline in caries of 34–62% (see Fig. 9.5) (Toverud, 1957a, b). The low caries prevalence found in children aged 6–13 in Hopewood House, Australia, living under conditions of a mainly lacto-vegetarian diet low in sugar and refined flour, in comparison to children with a modern diet, is also used as evidence of the relationship between sugars and caries (Harris, 1963).

The introduction of a modern diet including sugar and refined carbohydrates to isolated areas, such as Tristan da Cunha, has resulted in a great increase in caries prevalence (Fisher, 1968). The lower caries levels found in individuals suffering from hereditary fructose intolerance, making them unable to eat fructose and sucrose, have also been used as an example of the impact of sugars on high caries prevalence (Marthaler, 1967; Newbrun *et al*., 1980). A sugar intake of 2.5 g per day was found among the individuals with hereditary fructose intolerance in comparison to 48.2 g for the control group. The corresponding figures for caries prevalence were 2.1% and 14.3%, respectively (Newbrun *et al*., 1980). In the Turku sugar study, a diet sweetened with xylitol was evaluated in adult subjects over 25 months and compared with groups consuming either sucrose or fructose (Scheinin and Mäkinen, 1975; Scheinen *et al*., 1976). The caries reduction for the xylitol and fructose groups was 85% and 32%, respectively, when compared with the sucrose group. In a similar manner, the difference in caries increment between sucrose- or sorbitol-containing sweets has been evaluated in children aged three to 12 years in a three-year longitudinal study (Bánóczy *et al*., 1981). The final caries reduction for the sorbitol group was 45% in comparison to the children consuming sugar-containing sweets. However, it is important to remember that there was a high drop-out rate (52%) and that the children were living under special conditions.

The above data present indirect evidence of the impact of sugars in relation to dental caries; in other words they include the consequence of a reduction in intake. One of the few studies in which the effect of an increase in sugar consumption has been studied is the Vipeholm study (Gustafsson *et al*., 1954). It was conducted on a population of adult, mentally handicapped individuals in Sweden between 1946 and 1951. The subjects were divided into different groups and, during the five-year period, they consumed varying quantities and frequencies of different sugar-containing products (Fig. 9.6). The main conclusion was that sugar consumed between meals has a much greater caries potential than sugar consumed during a meal. It was also found that highly retentive (sticky) products resulted in the highest caries activity. The study has been seriously discussed, not least from an ethical perspective. It is also possible to argue that the study population is not representative of modern society and that the complex design may limit the interpretation of the data.

Only a few short-term experimental caries studies have been performed on humans. One such study was conducted on six dental students who rinsed with 10 ml of a 50% sucrose solution nine times a day (von der Fehr *et al*., 1970). After 23 days of rinsing, a higher number of early carious lesions were found in the test subjects compared with a control group. The study was then interrupted, after which a reversal of the lesions was seen. A chewing gum, sweetened with 60% sucrose and 20% glucose, was compared with no gum in children aged 7–11 years during a period of two years (Glass, 1981). The group chewing the sugar-gum developed significantly

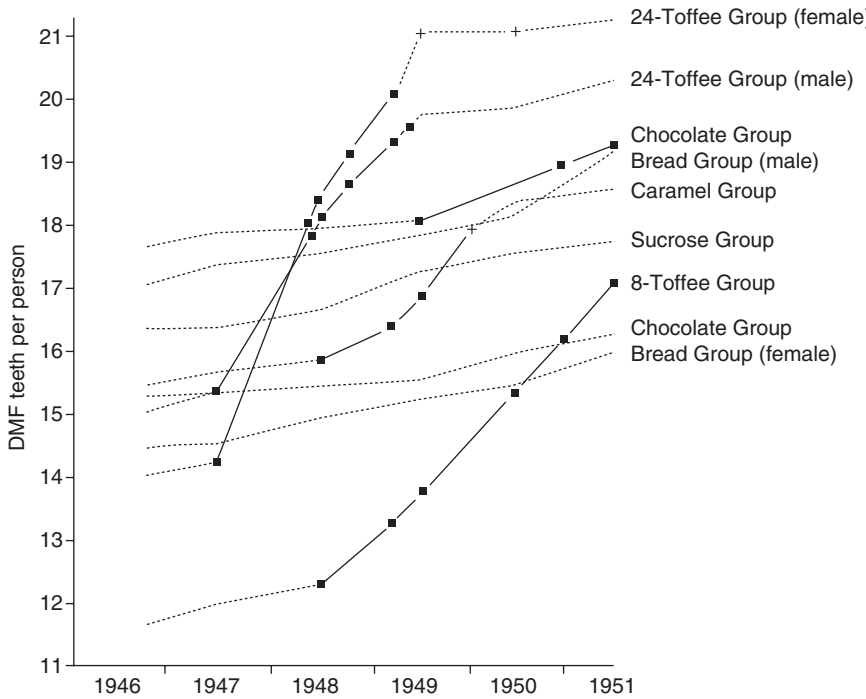

Fig. 9.6 DMFT per person relative to the type of various sugars and sugar-containing products consumed at meals or at and between meals in the Vipeholm study (Gustafsson *et al*., 1954). ---, sugar consumed at meals; – ■ –, sugar consumed at and between meals.

more carious lesions during the experimental period. Data from experimental animal studies support the data from humans (Shaw, 1983).

9.6 Impact in the modern era

Today, in many countries worldwide, there are great difficulties when it comes to detecting a strong correlation between total sugar consumption and caries prevalence. A decrease in caries figures has even been observed in many countries in spite of an increase in sugar consumption. It is difficult to apply the findings from studies performed during earlier periods to modern man. Not only has the dietary intake and pattern changed, additional factors such as fluoride use and the awareness of optimal oral hygiene have also changed the impact of diet. The overall increase in people's awareness of health issues is also believed to play an important role. Different reviews made during the 21st century of the relationship between diet/sugar and dental caries have produced widely varying results with

conclusions such as 'dental health problems do not require dietary recommendations other than those required for maintenance of general health' (König, 2000), 'if good oral hygiene is maintained and fluoride is supplied frequently, teeth will remain intact even if carbohydrate-containing food is frequently eaten' (van Loveren, 2000), 'sugars, particularly sucrose, are the most important dietary cause of caries' (Sheiham, 2001), 'no study was found evaluating the effect of information designed to reduce sugar intake/frequency as a single preventive measure' (Lingström *et al*., 2003) and 'measures to educate the public on the dangers of frequent sugar consumption, combined with recommendations for proper oral hygiene and fluoride use, are still warranted' (Zero, 2004).

Analyses of national data on caries prevalence and sugar consumption have been used in order to provide additional evidence of the role of sugars. A positive correlation has been demonstrated when dental caries and sugar intake have been compared at the country level for 47 countries and 12-year-olds, while no such strong correlation could be found for 6-year-old children in 23 nations (Sreebny, 1982). A linear relationship has also been demonstrated between the number of decayed, missing and filled teeth (DMFT) and sugar consumption in 90 nations (Woodward and Walker, 1994). In the UK, a positive correlation over time (50 years) has been found between caries experience and national sucrose availability (Downer, 1999).

During a more modern era, two distinct trends regarding caries prevalence can be seen. There has been a fall in the prevalence of caries in the developed countries, while an increase has been seen in a large number of developing countries (WHO, 2008). The main reason for the decrease in caries disease in the first group of countries is the increased use of preventive action, primarily the use of fluoridated toothpaste, while increased exposure to sugars underlies the change detected in developing countries. Despite the marked reduction in dental caries in many countries worldwide, it remains unacceptably high in others and constitutes a severe health problem. In many western countries, the decline in caries has come to a halt and this is perhaps partly explained by modern dietary habits (Poulsen, 1996). The trend towards the retention of dentition in older age, with an increased risk of both coronal and root caries in the elderly, means that, in the future, diet must be looked upon differently in the elderly compared with earlier generations.

9.7 Cariogenic risks of foods and beverages

Sugars and starches are consumed *per se* to a limited extent, while carbohydrates are included in different food products consumed at main meals or between meals. A large number of food products contain a mixture of different sugars. Although sucrose is still the sugar most frequently added

to different products, this sugar is also found naturally in a wide range of food. Fruit like apples and oranges contains sucrose and traces of sucrose can also be found in a large number of food items, such as ketchup, mustard and bread, many of which are not usually regarded as risk products for caries. The suggestion that sucrose should be replaced by monosaccharides in order to reduce proximal- and smooth-surface caries (Burt, 1993) is difficult to implement from a practical perspective as the diet often contains a mixture of sugars. When considering the risk of a certain food, the final risk of developing caries is determined by a large number of factors. The impact of the specific food item consumed is dependent on the product-related factors, but the final outcome for a food product is also associated in this respect with a number of individually related factors of which intake frequency is the most important.

It is important to distinguish between acidogenicity and cariogenicity, as no carbohydrate has a direct effect on the dental tissues. Sucrose has acidogenic potential similar to that displayed by fructose, glucose and maltose. The acidogenicity of food and food components is related to their cariogenic potential, but other factors may adjust this link. Different characteristics of host, bacteria and food product can greatly modify the cariogenicity of an acidogenic food. The synthesis of extracellular glucans is one such factor, which gives sucrose a special role in the caries process. Studies of plaque acidogenicity can provide information about the relative cariogenicity of foods, but they are unable to predict its true cariogenicity. Furthermore, dental caries is a disease which develops slowly and the time aspect will therefore be important. Only products with cariogenic potential that are consumed frequently over a long period of time will therefore be of importance. The original claim that 'sucrose is the arch criminal of dental caries' Newbrun (1969) has been modified over the years and it is now known that other sugars and starch can participate in the caries process.

9.8 Consumption frequency versus amount consumed

There is currently overwhelming evidence that intake frequency has a major impact on caries development and that this outweighs the amount consumed (Karlsbeek and Verrips, 1994). The higher the number of consumption episodes during which the pH falls below the resting level for dentine and/or enamel, the greater the total time for demineralisation to occur. This is especially true if sugars are present in the oral cavity frequently or over prolonged periods, so that the time for remineralisation is shortened concomitantly. For this reason, it is important to evaluate not only the number of intakes a day but also the length of each period when the pH is reduced. The concept of frequency as an important factor originates from the Vipeholm study (see Section 9.5), but it has also been evaluated in later studies. The effect on caries development of consuming between

zero and five or more pieces of confectionery and sugar-containing gum a day was evaluated in 14-year-old children in Hawaii (Hankin *et al*., 1973). The increased intake of sweets was followed by a corresponding increase in DMF scores. Furthermore, the *in situ* study by Duggal *et al*. (2001) points to the importance of intake frequency for caries development. They studied the degree of demineralisation in enamel specimens after a range of one to ten intakes a day during a two-week period. Animal studies, during which strict control regarding the intake pattern can be exerted, have also demonstrated a significant correlation between intake frequency and caries development (König *et al*., 1968).

A change in plaque ecology in relation to increased carbohydrate exposure has been suggested (van Houte, 1980, 1994). A gradual increase in carbohydrate consumption induces a gradual increase in the pH-lowering capacity of plaque. The effect of increased sucrose exposure on plaque acidogenicity has been evaluated *in situ* (Aranibar *et al*., 2003). For subjects with a regularly low number of mutans streptococci (MS), a stepwise increase in plaque acidogenicity in relation to an increased number of sucrose rinses was seen. The same pattern was not found for individuals with high MS counts, who already had a high capacity to reduce the pH of dental plaque without additional sucrose. Duggal *et al*. (2001) showed that the degree of demineralisation from sucrose exposure varied greatly depending on whether or not regular brushing with fluoridated toothpaste took place. Although frequency is regarded as an important factor for all caries risk individuals, it may be of particular importance in developing countries. These individuals run an increased risk of rapid caries manifestation in relation to frequent sugar consumption, which is also due to the lack of access to fluoride and poor oral hygiene.

Although frequency is thought to be of major importance, others have suggested that the amount of sugar consumed is more important (Burt *et al*., 1988; Rugg-Gunn, 1993; Szpunar *et al*., 1995). A strong correlation between the two factors exists (Rugg-Gunn, 1993) and an increase in one factor will often lead to a rise in the other. A consumption episode may vary in length and frequency and may reveal very little about the total impact of a certain product on caries development. Attempts have been made to determine the maximum amount of sugar that can be consumed without any risk of caries. One such suggestion is 60 g a day for teenagers and adults and proportionally less for younger children (Sheiham, 2001). The same author also suggested that an intake of extrinsic sugars more frequently than four times a day increases the risk of caries. It has also been recommended, when comparing national data on caries and sugar consumption, that 50 g of sugar per person per day is associated with dmft or DMFT scores of less than three (Sreebny, 1982).

In addition to intake frequency and amount consumed, there is a wide range of other factors that need to be taken into account when assessing the risk of caries associated with a specific product (Zero *et al*., 2008). These

factors include the physical consistency of the food product and the intake pattern. Sticky products will have a prolonged clearance rate in comparison to less adhesive products or those that promote chewing. Particularly high retention rates have been found for products such as sweet biscuits, crackers and potato chips (Kashket *et al*., 1991). Products that contain a combination of starch and sugar are of special interest from this angle, as the starch increases the cariogenicity, owing to enhanced retention. The sequence of eating a product during a meal or snack may also alter its cariogenic properties (Rugg-Gunn and Nunn, 1999). From a cariogenic point of view, the recommendation is to reduce both the intake frequency and the total amount of sugar.

9.9 Influence of fluoride on the sugars–caries relationship

There is no doubt that the increased use of fluoride has played a major role in the caries reduction that has been seen worldwide since the middle of the 20th century (Bratthall *et al*., 1996). It may affect the tooth both during development and after eruption. The post-eruptive effect is without doubt considered to be the most important and includes its ability to inhibit demineralisation and promote remineralisation. The mechanisms underlying the influence of fluoride on plaque acidogenicity have not been evaluated in detail. It has been suggested that fluoride may have antimicrobial properties and modify different enzymatic functions, influence the phosphotransferase system and alter the proton-motive force across the cell membrane of oral streptococci (Zero *et al*., 2008).

Fluoride has changed the impact of sugars and larger quantities of sugar can currently be tolerated before caries occurs compared with the pre-fluoride era (see Fig. 9.7) (Zero, 2004). It is not thought that there is a direct relationship between the intake of sugars and dental caries. A sigmoid pattern has instead been suggested (Sheiham, 1983). This has recently been demonstrated in a study by Duggal *et al*. (2001) when evaluating the sugar intake in relation to the use of fluoridated toothpaste in an *in situ* caries model. When using fluoridated toothpaste, demineralisation occurred during seven or more intakes of sugar per day, while the corresponding figure was three times per day when fluoride-free toothpaste was used.

These findings are supported by data from the British National Diet and Nutrition Survey in which the dietary, fluoride and oral hygiene habits of 1450 pre-school children were evaluated (Gibson and Williams, 1999). It was found that food and drink containing sugar were not associated with caries experience, unless children brushed less than once a day, and that toothbrushing with fluoridated toothpaste twice a day inhibited caries. Studies of children have found that the use of fluoride only partially dampened the positive relationship between the frequency of snacking and caries levels (Stecksén-Blicks *et al*., 1985; Hinds and Gregory, 1995). A recent

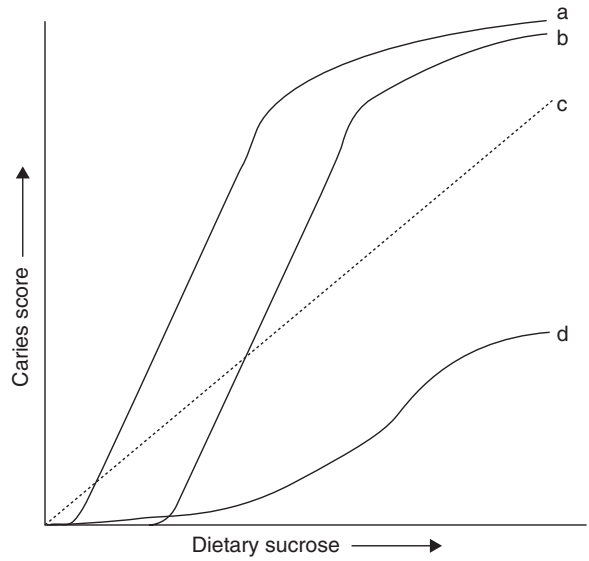

Fig. 9.7 Proposed relationships between sugar intake and caries as previously described by Zero (2004). (a) S-shaped relationship in the pre-fluoride era (Newbrun, 1982b), (b) S-shaped relationship shifted to the right in post-fluoride era (Newbrun, 1982b), (c) Linear relationship calculated from data in 90 countries (Woodward and Walker, 1994), (d) Individuals with good oral hygiene and regular fluoride exposure.

systematic review assessing 69 papers which meet the inclusion criteria evaluated the association between sugar intake and caries development in modern man (Burt and Pai, 2001). It was found that the control of sugar consumption is still an important factor when controlling caries but that the association was stronger in the pre-fluoride era. Even if the regular use of fluoride has managed to reduce caries rates, the disease has not been totally eliminated and both preventive actions should be employed together.

9.10 Individuals at increased risk

The development of caries requires fermentable carbohydrates, but the interaction between these two factors and the dental hard tissues is influenced by a large number of additional biological and socioeconomic factors. Even if caries is closely linked to less favourable dietary habits, there are some individuals in whom the disease develops as a result of a disturbed balance of attack and defence factors and not because of particularly poor dietary habits. Individuals with a higher sensitivity to disease can be found in all age groups and this is particularly related to diseases and/or the intake of medication. There are also certain periods in life during which caries

develops more easily. Teeth are most susceptible to dental caries during the period directly after eruption, which is why the risk of disease is particularly high during early childhood and adolescence. However, surfaces may also become exposed owing to gingival retraction later in life and as a consequence of periodontal treatment. For all these individuals and time points, it is important that the dietary habits are as good as they can possibly be from a cariological point of view. This means a low intake frequency and reduced total sugar intake.

Over time, a high sugar intake, resulting in a low pH caused by bacterial fermentation, will change the plaque ecology. A low pH of this kind is ideal for the bacteria associated with caries, that is streptococci, lactobacilli, actinomyces and bifido bacteria. They are more competitive at low pH than bacteria that are not associated with dental caries. More frequent carbohydrate exposure will result in more frequent plaque acidification. Consequently, a shift towards higher numbers of more acid-tolerant bacteria occurs and plaque acidogenesis at acidic pH increases. The increased reduction of the potential pH minimum in plaque will increase the probability of net mineral loss over time (van Houte, 1980, 1994). A shift in plaque acidogenicity after increased intake frequency and a change in plaque ecology have been demonstrated (Aranibar *et al*., 2003). The oral environment, including the dental biofilm, may undergo continuous changes in relation to dietary exposure – changes that may occur in both a positive and a negative direction. The general dietary habits, including frequent sugar intake, of individuals at high risk of caries promotes a shift in the oral ecological system towards a more caries-promoting environment. The question of whether the cariogenicity of starch varies between individuals in relation to their sugar intake has been discussed (Lingström *et al*., 2000). In individuals with a normally low sugar intake, as in ancient times, the cariogenic properties of the dental biofilm were low and the negative impact of starch was consequently limited (Table 9.1). In contrast, in modern man,

Table 9.1 Postulated effect of exposure to different dietary carbohydrates on the cariogenic properties of human dental plaque

	Consumption of diet containing		
Plaque property	Little or no carbohydrates	Moderate carbohydrate intake	High carbohydrate intake
pH-lowering capacity	Low to negligible	Higher	Highest
Number of acid-tolerant bacteria	Low	Higher	Highest
Volume/porosity	Low	Higher	Highest
Cariogenic capacity	Low to negligible	Higher	Highest

where sugars are eaten more regularly, the cariogenic properties of dental plaque are higher and the negative impact when consuming a starch product is therefore greater.

Several groups of individuals are regarded as running a greater risk of caries in relation to a high intake of fermentable carbohydrates (Zero *et al*., 2008; see Table 9.2). They include both infants and toddlers in relation to prolonged breast feeding and feeding by bottle at bedtime or during the night, children/adolescents in relation to the frequent intake of sweetened soft drinks and sweets and medically compromised subjects who may be given recommendations leading to increased intake frequency and dietary supplementation. There are also a large number of medicines, particularly those provided in the form of syrup for children, which contain up to 70% sugar. Medication, particularly that aimed at the elderly, is also known to contain fermentable carbohydrates. For example, a slow-release nitroglycerine tablet, recommended to be placed in the upper vestibulum, has been found to cause caries in the elderly (Lingström and Birkhed, 1994). Athletes may have an increased intake of sport-supplement drinks, which are often rich in carbohydrates. Modern dietary counselling often focuses on the reduction of fat intake and instead promotes the consumption of starch-rich food, fruit and vegetables. If followed, this advice may lead to an increase in the intake of carbohydrates. Moreover, different working environments may promote the increased consumption of sweets or change the intake frequency.

Table 9.2 Individuals with an increased risk of caries in relation to their dietary intake (Zero *et al*., 2008)

Infants and toddlers	Prolonged breast feeding Feeding with bottle at bedtime or night
Children, adolescents	Frequent intake of soft drinks, confectionery and sugary snacks
Medically compromised people	Increased intake frequency (gastrointestinal diseases, eating disorders, diabetes) Increased carbohydrate intake owing to total or partial dietary supplementation (Crohn's disease, chronic renal failure, chronically ill, malnutrition, failure to thrive) Prolonged clearance rate due to reduced salivary secretion rate (Sjögren's syndrome, irradiation, medication)
Athletes	Sport supplement drinks
In relation to work	Food sampling Work in confectionery industry, bakery Monotonous job, night shift
Drug abusers	Craving for sugar Prolonged clearance rate due to reduced salivary secretion rate

Many of the subjects described above not only have an unfavourable consumption pattern in relation to caries. It is often accompanied by less favourable oral hygiene and the less frequent intake of fluoride. As a result, caries prevention should include recommendations related to both diet and the use of fluoride.

9.11 Impact in the 21st century

With no risk of understatement, sugar was the dominant reason for the high caries prevalence seen in most countries at the beginning of the 20th century, but there has been a dramatic change in the impact of food sugars on dental caries since the middle of the last century. Although fluoride has changed the relationship between sugar and caries, the suggestion that there is no need to restrict sugar consumption in modern society (König, 2000; van Loveren, 2000) seems dangerous. The causal relationship between sugars and dental caries shown in the literature indicates that sugar will continue to play an important role in the future. Even fluoride has its limitations and there are individuals in whom fluoride either is not used or will not be able fully to compensate for the negative influence of sugars. The exact role of the different sugars and starch, alone or in combination, is still not fully understood. In the future, it is therefore thought to be important to focus on the role of fermentable carbohydrates in relation to caries both from a clinical perspective and in research.

Nutrition, diet, general and oral health are linked together for all age groups. Apart from being evidence based, dietary recommendations designed to reduce disease should be based on the individual and on the actual caries situation. Through valuable research and pioneering work, we have now reached a situation in which there is sufficient knowledge about both the different variables included in caries and the interplay between these factors. In order to succeed in fully preventing the disease, it is more a question of implementing the techniques and changing the behaviour of people. There are several questions that need to be addressed in order to succeed in changing dietary habits. In order fully to understand the complexity in the selection of food by a subject, it is necessary to ask not only what is consumed but also how often, when, how, why and where (Lingström and Fjellström, 2008).

To date, interest has largely focused on dietary sugars and highly refined starches, but in the future other fermentable carbohydrates, including fructose syrups, glucose polymers and synthetic oligosaccharides, will also require attention (Zero *et al.*, 2008). It is also important to focus on the effect of other components of the diet, the interaction between substances and the different protective components. Moreover, when giving dietary advice to future generations, it will be essential to consider not only oral health but also general health aspects. Recommendations to reduce the

intake of sugar in order to protect the teeth should, for example, not lead to an increase in fat content or other negative health patterns.

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10

Casein phosphopeptides in oral health

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Abstract: This chapter discusses the scientific evidence for the anticariogenic activity of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes. The chapter starts by introducing dental caries (tooth decay) and then presents the ultrastructure of the CPP-ACP nanocomplex. It then presents a detailed review of the scientific literature describing the anticariogenic activity of CPP-ACP and mechanisms of action. The chapter concludes with recommendations for clinical use of the CPP-ACP.

Key words: anticariogenic activity, casein phosphopeptide-amorphous calcium phosphate nanocomplexes, clinical uses, mechanism of action, scientific evidence.

10.1 Introduction

Food and oral health are intimately linked with a range of foodstuffs that have positive or negative effects on the oral environment. Dental caries is a highly prevalent diet-related disease and, although in most developed countries the prevalence of caries experience has declined, it remains a major public health problem (Selwitz *et al.*, 2007).

Dental caries is a pathological process that leads to localised destruction of tooth tissues by organic acids generated from microorganisms of dental plaque (Marsh, 1999). The non-shedding surfaces of the teeth are unique, allowing the development of a complex biofilm. If frequently supplied with sufficient quantities of fermentable carbohydrates, the microbial homeostasis of the biofilm can be disturbed, favouring the proliferation of acidogenic and aciduric microbiota that produce organic acids, overwhelming the host defences and buffering systems resulting in progressive mineral loss from the tooth.

Signs of the dental caries process cover a continuum from the first atomic changes in the crystals of the tooth, to a visible white spot lesion, through

to dentine involvement and eventual cavitation (Featherstone, 2004). Progression through these stages requires a continual imbalance between pathological and protective factors that result in the net loss of calcium, phosphate and other ions from the tooth. Simply, dental caries is an imbalance between demineralisation and remineralisation, where demineralisation is the loss of mineral caused by acid attack and remineralisation is the diffusion of calcium, phosphate and other ions back into the subsurface lesion to regrow the damaged crystals. A goal of modern dentistry is to arrest and repair the early stages of dental caries non-invasively through the development of novel remineralisation systems.

The white spot lesion is the first easily detectable sign of the dental caries process and the point at which intervention should be instituted to halt the progression of the lesion. The arrest of the lesion has been attributed to: (1) protein uptake into the lesion which acts to inhibit diffusion of ions, in particular, protons (H^+) into the lesion (Robinson *et al.*, 1990), (2) mineral deposition in the surface layer of the lesion thereby reducing the diffusion channels into the lesion (Thylstrup and Fejerskov, 1994) and (3) mineral deposition throughout the body and surface layer of the lesion to restrict inward diffusion and replace lost mineral to regress the lesion (Reynolds, 1998). Ultimately, the ideal treatment of an early lesion would be to restore it to sound enamel with the incorporation of fluorapatite to increase the enamel fluoride reservoir and to help resist future acid challenge.

Clinical evidence for the arrest or remineralisation of dental caries has been present for many years (Anderson, 1938; Backer Dirks, 1966). In these studies, once the carious lesion was free of plaque, the calcium and phosphate ions in saliva could diffuse into the lesion to repair some of the damaged crystallites. Since the process of remineralisation was first observed, it has been explored in order to create non-invasive treatments of dental caries that have the potential to be a major advance in the management of the disease.

The clinical use of calcium and phosphate ions for remineralisation has not been successful in the past owing to the low solubility of calcium phosphates, particularly in the presence of fluoride ions. Insoluble calcium phosphates are not easily applied, do not localise effectively at the tooth surface and require acid for solubility to produce ions capable of diffusing into enamel subsurface lesions. On the other hand, soluble calcium and phosphate ions can only be used at very low concentrations owing to the intrinsic insolubility of the calcium phosphates, in particular the calcium fluoride phosphates. Soluble calcium and phosphate ions do not incorporate into dental plaque or localise at the tooth surface sufficiently to produce effective concentration gradients to drive diffusion into the subsurface enamel.

Fluoride has become the cornerstone of prevention and early treatment of caries but depends on calcium and phosphate ions for its effect. Fluoride

ions promote the formation of fluorapatite in enamel in the presence of calcium and phosphate ions produced during enamel demineralisation by plaque bacterial organic acids (ten Cate, 1999). This is now believed to be the major mechanism of the action of the fluoride ion in preventing enamel demineralisation (Lynch *et al.*, 2004; ten Cate, 1999). Fluoride ions can also drive the remineralisation of previously demineralised enamel if enough salivary or plaque calcium and phosphate ions are available when the fluoride is applied. For every two fluoride ions, ten calcium ions and six phosphate ions are required to form one unit cell of fluorapatite [$Ca_{10}(PO_4)_6F_2$]. Hence, on topical application of fluoride ions, the availability of calcium and phosphate ions can be the limiting factor for net enamel remineralisation to occur and this is highly exacerbated under xerostomic conditions.

One of the food groups most frequently linked with good oral health is dairy products, as they contain high concentrations of calcium and phosphate ions and they have been shown to have anticariogenic effects (Reynolds and Johnson, 1981; Harper *et al.*, 1986; Rosen *et al.*, 1984; Krobicka *et al.*, 1987). Most frequently this effect has been attributed to calcium, phosphate and casein (Harper *et al.*, 1986; Silva *et al.*, 1987).

Casein is one of two major proteins in milk and accounts for approximately 80% of the total protein (Aimutis, 2004). There are four major variants of the casein protein – αs1-, αs2-, β- and κ-caseins. In milk, casein exists in micelles that stabilise calcium and phosphate ions. Casein has been incorporated into drinking water and chocolate in two separate studies which confirmed the anticariogenic properties of the casein (Reynolds and del Rio, 1984; Reynolds and Black, 1987). Both of these studies showed a marked reduction in dental caries between the control rats and those exposed to casein. Unfortunately, the palatability of the 16% (w/w) casein containing chocolate was deemed unacceptable (Reynolds and Black, 1987) and lower, more palatable concentrations were not significantly effective (Reynolds and Black, 1989). When undigested and tryptic-digested αs1-casein were compared, it was found that the tryptic digest retained the ability of the full protein to prevent demineralisation in an *in situ* model (Reynolds, 1987). This resulted in a further study that identified a number of highly phosphorylated peptides that could be extracted from casein and were effective in reducing demineralisation and enhancing remineralisation (Reynolds, 1998).

This discovery has led to the development of a new anticariogenic remineralisation technology based on casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes [Recaldent CASRN691364-49-5]. These complexes have been incorporated into commercial sugar-free chewing gums [Trident Xtra Care (Americas), Recaldent (Japan)] and dental crèmes [Tooth Mousse (Europe and Australia), MI (minimal intervention) Paste (USA)].

10.2 Casein phosphopeptide stabilised amorphous calcium phosphate nanocomplexes (CPP-ACP) structure

The casein phosphopeptides (CPP) are approximately 10% (w/w) of the protein casein (Swaisgood, 1982). They are tasteless (Swaisgood, 1982), have low antigenicity (Park and Allen, 2000) and can be extracted from casein by enzymic digestion and ultrafiltration (Reynolds, 1998). It was first shown in 1958 by Reeves and Latour that casein peptides were able to prevent precipitation of calcium phosphate from solution (Reeves and Latour, 1958). Four major CPP containing the sequence –Ser(P)–Ser(P)–Ser(P)–Glu–Glu– have been recently shown to stabilise high concentrations of calcium and phosphate ions in metastable supersaturated solutions with respect to a number of calcium phosphate phases (Cross *et al.*, 2005).

Extensive modelling and experimentation with these peptides and with a variety of analogues and homologues have been conducted to determine the mechanism of calcium phosphate stabilisation (Cross *et al.*, 2004; Cross *et al.*, 2005). Calcium on the surface of the calcium and phosphate ion nanoclusters primarily interacts with the CPP through the negatively charged residues of the peptides (Cross *et al.*, 2005). Cross *et al.* (2005) have shown that the CPP bind more calcium and phosphate ions than can be attributed to just the calcium-binding motif –Ser(P)–Ser(P)–Ser(P)–Glu–Glu– indicating that other acidic residues of the phosphopeptide sequence contributed to the stabilisation of calcium phosphate.

Calcium and phosphate ion binding studies have demonstrated that the casein phosphopeptide αs1-casein X-5P (f59–79) and β-casein X-4P (f1–25) can maximally bind 21 and 24 Ca^{2+} and 14 and16 Pi (inorganic phosphate) per molecule, respectively, producing a metastable, colloidal solution (Cross *et al.*, 2005). The CPP-ACP as a dried powder does not diffract X-rays and is therefore designated amorphous, with the calcium phosphate phase corresponding to an alkaline or acidic amorphous calcium phosphate depending on the pH or mode of preparation (Cross *et al.*, 2005). In solution, the peptides tend to form complexes of multimers with the unit formula $[\alpha s1\text{-casein}(f59–79)(ACP)_8]^n$ to stabilise clusters of calcium and phosphate ions (where $n > 1$ and $n = 6$ are likely to be the predominant form) (Cross *et al.*, 2007b) (Fig. 10.1). This allows high levels of calcium phosphate stabilisation. A 1% CPP solution at pH 7.0 can stabilise 60 mM calcium and 36 mM phosphate (Reynolds, 1997; Reynolds *et al.*, 1995). These CPP-ACP nanocomplexes prevent growth of the calcium and phosphate ion nanoclusters to the critical size required for nucleation and phase transformations (Cross *et al.*, 2005). The CPP have been shown to stabilise solutions that are highly saturated with respect to a number of solid calcium and phosphate phases at acidic and basic pH (Reynolds, 1997; Cochrane *et al.*, 2008).

In solution, the CPP forms an equilibrium between free and CPP-bound calcium and phosphate ions. This equilibrium is pH dependent as this determines the charge on the phosphopeptides. The dissociation constants char-

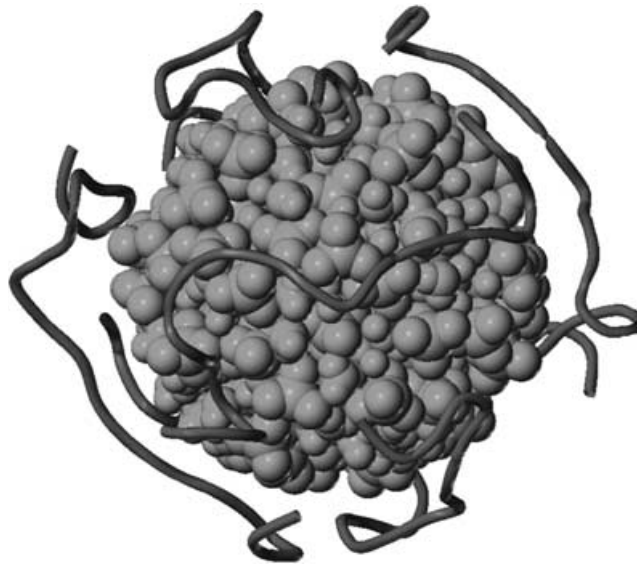

Fig. 10.1 Representation of a proposed CPP-ACP nanocomplex. The central space-filling spheres represent calcium, phosphate and water forming the ACP core and the ribbons represent the peptide backbones of six CPP molecules covering the ACP core. Reproduced from Cross *et al.* (2007b).

acterising the binding of calcium ions and calcium and phosphate ions to the CPP have been determined to be in the millimolar range (Park *et al.*, 1998; Meisel and Olieman, 1998; Rose, 2000a; Cross *et al.*, 2005). Binding affinities of this magnitude indicate that the CPP only weakly bind calcium and phosphate ions thus allowing a dynamic equilibrium between CPP-free and CPP-bound ions in solution.

In summary, the CPP weakly bind (mM kD) high concentrations of calcium and phosphate ions, stabilising them as amorphous nanocomplexes. In solution, an equilibrium between free and CPP-bound calcium and phosphate ions ultimately occurs based on the conditions of the environment, thereby providing a reservoir of bioavailable ions for inhibition of demineralisation and promotion of remineralisation.

10.3 Scientific evidence for casein phosphopeptide-amorphous calcium phosphate activity in preventing demineralisation and promoting remineralisation

The primary claims for CPP-ACP technology are that the nanocomplexes prevent demineralisation and promote remineralisation. There is now a large body of scientific evidence to support these claims. CPP-ACP technol-

ogy has been studied in a variety of vehicles in laboratory, animal and human *in situ* experiments as well as in randomised, controlled clinical trials. CPP-ACP has also been shown to be localised in dental plaque, to deposit acid-resistant mineral in subsurface enamel lesions and to interact with fluoride to enhance fluoride activity.

10.3.1 *In vitro* studies

Inhibition of demineralisation in vitro

CPP-ACP has been shown to inhibit demineralisation *in vitro* when incorporated in commercial crème preparations (Yamaguchi *et al.*, 2006; Yamaguchi *et al.*, 2007; Oshiro *et al.*, 2007; Rees *et al.*, 2007; Sudjalim *et al.*, 2007; Rahiotis and Vougiouklakis, 2007; Nasab *et al.*, 2007), experimental sports drinks (Ramalingam *et al.*, 2005), experimental glass ionomer cement (GIC) filling materials (Mazzaoui *et al.*, 2003) and solutions (Roberts, 1995). These studies, their method of CPP-ACP delivery, the substrate tested, the treatment groups and conclusions are summarised in Table 10.1. In all of these *in vitro* models, use of the CPP-ACP-containing product resulted in less demineralisation than the control product.

CPP-ACP included in the dental crème Tooth Mousse or MI Paste has been shown to inhibit demineralisation of dentine and enamel. Yamaguchi *et al.* tested the ability of Tooth Mousse to prevent demineralisation of enamel and dentine compared with a placebo paste not containing CPP-ACP (Yamaguchi *et al.*, 2007; Yamaguchi *et al.*, 2006; Oshiro *et al.*, 2007). The pastes were diluted 1:10 with water and exposed to bovine enamel or dentine for ten minutes prior to a ten-minute demineralisation challenge twice a day for ten weeks; at all other times the samples were stored in artificial saliva. Demineralisation was measured using an ultrasonic device and ultrastructure observed using field emission scanning electron microscopy (FE-SEM). As can be seen in Fig. 10.2 and Fig. 10.3, the ten-times diluted Tooth Mousse was able to inhibit the demineralisation of enamel and dentine. Both the placebo Tooth Mousse with no CPP-ACP and the no-treatment control did not prevent demineralisation of the enamel and dentine surfaces. Rahiotis and Vougiouklakis (2007) also examined the ability of Tooth Mousse to prevent dentine demineralisation *in vitro*. It was found that a five-minute application of Tooth Mousse prior to immersion in the demineralising solution significantly reduced the amount of demineralisation compared with the water control.

In vitro studies assessing the potential for CPP-ACP to be used as a preventive agent during the course of orthodontic treatment to prevent demineralisation occurring around brackets has been examined by Sudjalim *et al.* (2007) and Nasab *et al.* (2007). Sudjalim *et al.* found that Tooth Mousse, sodium fluoride (9000 ppm) and Tooth Mousse plus sodium fluoride all significantly reduced enamel demineralisation when composite resin

Table 10.1 Summary of *in vitro* demineralisation assays conducted using CPP-ACP

Study	Delivery vehicle	Substrate	Method	Groups	Conclusion
(Yamaguchi *et al.*, 2006)	10% CPP-ACP crème Tooth Mousse	Bovine enamel	4-week cyclic de- and remineralisation 2 × 10 min treatment per day Measurement: ultrasonic device and FE-SEM $n = 6$ per group	Tooth Mousse Placebo Tooth Mousse Demineralisation challenge Artificial saliva alone, no demineralisation	Tooth Mousse inhibited demineralisation of enamel
(Yamaguchi *et al.*, 2007)	10% CPP-ACP crème Tooth Mousse	Bovine dentine	4-week cyclic de- and remineralisation 2 × 10 min treatment per day Measurement: ultrasonic device and FE-SEM $n = 6$ per group	Tooth Mousse Placebo Tooth Mousse Demineralisation challenge Artificial saliva alone, no demineralisation	Tooth Mousse inhibited demineralisation of dentine
(Rahiotis and Vougiouklakis, 2007)	10% CPP-ACP crème Tooth Mousse	Human dentine	7-day demineralisation challenge 1 × 5 min application at start Measurement: Fourier transformance micro multiple internal reflectance infrared spectroscopy $n = 10$ per group	Tooth Mousse No Tooth Mousse	Significantly less demineralisation in Tooth Mousse treated group
(Sudjalim *et al.*, 2007)	10% CPP-ACP crème Tooth Mousse	Human enamel	4-day demineralisation challenge 4-hourly treatment Measurement: QLF $n = 5$ per group	No treatment Tooth Mousse 9000 ppm NaF 50% Tooth Mousse + 50% 9000 ppm NaF	All treatment groups significantly different to no treatment group when brackets were bonded with composite resin

Table 10.1 *Continued*

Study	Delivery vehicle	Substrate	Method	Groups	Conclusion
(Nasab *et al.*, 2007)	Crude CPP-ACP crème Topacal C-5	Human enamel	31-day cyclic de- and remineralisation 1 daily treatment post demineralisation Measurement: polarised light microscopy $n = 24$ per group	No treatment Topacal C-5	Topacal C-5 reduced the depth of demineralisation
(Roberts, 1995)	0.5% CPP solution	Bovine enamel	18-hour cyclic demineralisation model 5-min exposure to test solution, 4 × 1 min water rinses then 1 hour acid exposure, 8 cycles completed Measurement: solution calcium concentrations	CPP-Na CPP-Ca CPP-ACP Water 1000 ppm F	CPP reduced demineralisation. Approx one-third the efficacy of 1000 ppm F regardless of the counter ion
(Rees *et al.*, 2007)	10% CPP-ACP crème Tooth Mousse	Human enamel	Citric acid erosion challenge 1 treatment for 15 min at start Measurement: profilometry $n = 10$ per group	Tooth Mousse ProNamel Water	Tooth Mousse and ProNamel both offer a degree of protection from erosive drinks
(Ramalingam *et al.*, 2005)	0.063%, 0.09%, 0.125% and 0.25% CPP-ACP added to Powerade	Human enamel	30 min exposure to 50 mL of beverage Measurement: profilometry and SEM $n = 3$ per group	Powerade Deionised water Powerade with various concentrations of CPP-ACP	Addition of 0.09 to 0.25% CPP-ACP eliminated erosive step
(Mazzaoui *et al.*, 2003)	1.56% CPP-ACP added to Fugi IX GP GIC	Human dentine	4-day demin challenge Measurement: polarised light microscopy $n = 12$ per group	GIC containing CPP-ACP GIC without CPP-ACP	Significantly less demineralisation around GIC with CPP-ACP compared to GIC without

FESEM = field emission scanning electron microscopy. QLF = quantitative light-induced fluorescence.

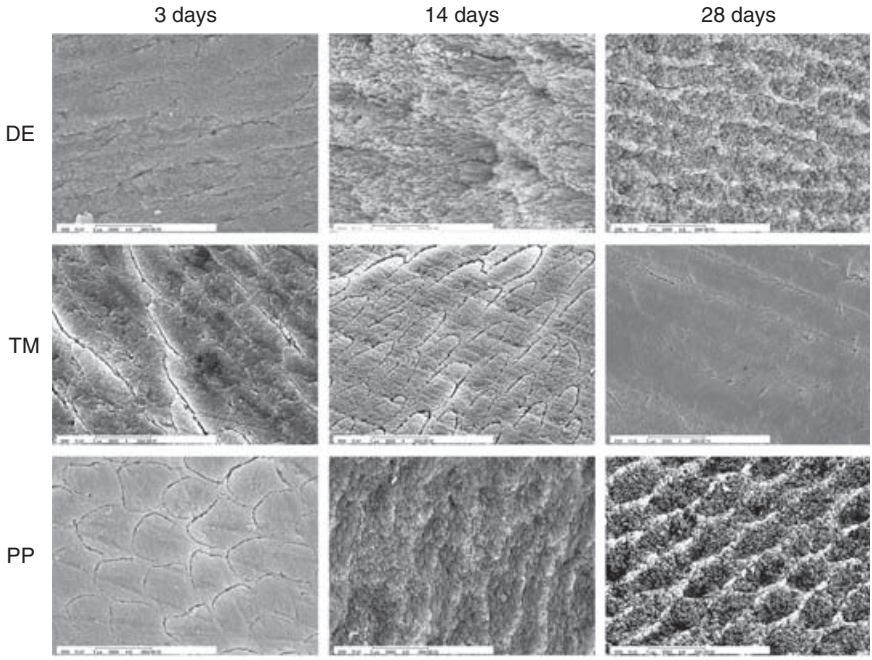

Fig. 10.2 FE-SEM micrograms of enamel surfaces exposed to a demineralisation assay without treatment (DE), with prior exposure to Tooth Mousse (TM) or a Tooth Mousse placebo paste (PP). Reproduced from Oshiro *et al.*(2007).

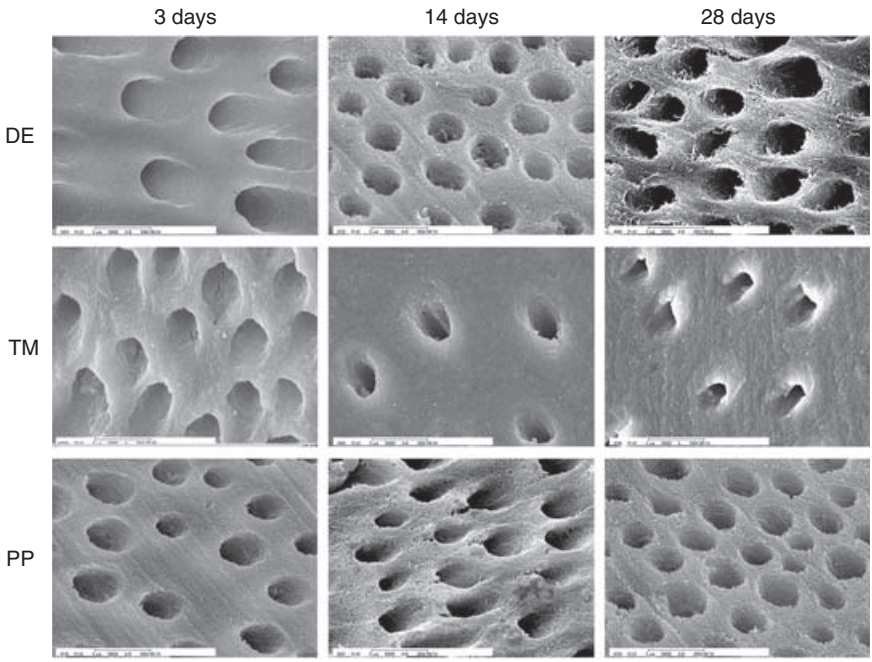

Fig. 10.3 FE-SEM micrograms of dentine surfaces exposed to a demineralisation assay without treatment (DE), with prior exposure to Tooth Mousse (TM) or a Tooth Mousse placebo paste (PP). Reproduced from Yamaguchi *et al.* (2007).

was used as the bracket bonding agent (Sudjalim *et al.*, 2007). Nasab *et al.* found a similar result using a crude crème preparation of CPP-ACP in a cyclic de- and remineralisation model. They found a significant reduction in the depth of demineralisation compared to the no CPP-ACP treatment control (Nasab *et al.*, 2007).

Rees *et al.* (2007) tested the ability of Tooth Mousse compared with Sensodyne ProNamel toothpaste (0.15 w/v NaF) to prevent erosion of human enamel caused by exposure to 0.2% citric acid. Compared with the water control, both products significantly reduced erosion with no significant differences between the Tooth Mousse and the ProNamel toothpaste (Rees *et al.*, 2007). Powerade alone was shown to produce erosion of enamel but the addition of 0.09% to 0.25% CPP-ACP prevented this erosion. Inclusion of up to 0.125% CPP-ACP did not affect the taste or colour of the beverage (Ramalingam *et al.*, 2005). These *in vitro* studies demonstrate that the use of Tooth Mousse prior to an erosive challenge provides the tooth surface with some protection against the challenge and that CPP-ACP as an additive to a beverage can reduce its erosivity.

Roberts (1995) found that crude CPP preparations at 0.5%, irrespective of the counter ion (sodium, calcium or calcium and phosphate), reduced enamel demineralisation by approximately 20%. The 0.5% CPP solution was shown to have approximately one-third the efficacy of the 1000 ppm fluoride solution in preventing enamel demineralisation.

The incorporation of 1.56% (w/w) CPP-ACP into Fugi IX GP GIC restorative material was found significantly to enhance its release of calcium, phosphate and fluoride at neutral and acidic pH. When the CPP-ACP modified GIC was placed adjacent to dentine and exposed to an *in vitro* acid challenge, the CPP-ACP modified GIC decreased the area of demineralisation by approximately 57% compared to the control GIC (Mazzaoui *et al.*, 2003).

Promotion of remineralisation in vitro

In vitro remineralisation of enamel (Reynolds, 1997; Cochrane *et al.*, 2008; Tantbirojn *et al.*, 2007) and dentine (Rahiotis and Vougiouklakis, 2007) has been demonstrated using CPP-ACP solutions and crèmes. These studies have been summarised in Table 10.2.

High levels of remineralisation have been demonstrated by exposing enamel subsurface lesions to CPP-ACP *in vitro* (Reynolds, 1997; Cochrane *et al.*, 2008). It has been shown that, as the concentration of CPP-ACP increased, the percentage of remineralisation also increased in a dose-dependent fashion (Reynolds, 1997). The pH of the remineralisation solutions was also shown to play an important role in remineralisation by Cochrane *et al.* (2008) and Reynolds (1997). Maximal remineralisation was found to occur at pH 5.5 (Cochrane *et al.*, 2008). This was explained by the high activity of the neutral ion pair $CaHPO_4^0$ at this pH which would allow unimpeded diffusion into the subsurface lesion relative to charged ions. The

Table 10.2 Summary of *in vitro* remineralisation assays conducted using CPP-ACP

Study	Delivery vehicle	Substrate	Method	Groups	Conclusion
(Reynolds, 1997)	CPP-ACP solutions of various concentration and pH	Demineralised human enamel	Remineralisation of subsurface enamel lesions 10-day 24-hour a day exposure to solution Measurement: TMR $n = 10$	0.1, 0.5, 1.0% CPP-ACP 0.5% CPP-ACP at pH 7.0, 7.5, 8.0, 8.5, 9.0	All solutions resulted in remineralisation of the subsurface lesions. Maximum remineralisation at 1% conc at pH 7.0
(Cochrane *et al.*, 2008)	CPP-ACP and CPP-ACFP solutions at various pH	Demineralised human enamel	Remineralisation of subsurface enamel lesions 10-day 24-hour a day exposure to solution Measurement: TMR $n = 5$	2% CPP-ACP and 2% CPP-ACFP solutions at pH 7.0, 6.5, 6.0, 5.5, 5.0, 4.5	All solutions resulted in remineralisation of subsurface lesions. Maximum at pH 5.5.
(Rahiotis and Vougiouklakis, 2007)	10% CPP-ACP crème Tooth Mousse	Demineralised human dentine	7 day remineralisation in artificial saliva 1×5 min application at start Measurement: Fourier transformance micro multiple internal reflectance infrared spectroscopy $n = 10$ per group	Tooth Mousse No Tooth Mousse	Significantly more remineralisation in Tooth Mousse treated group
(Tantbirojn *et al.*, 2007)	10% CPP-ACP crème MI Paste	Bovine enamel	8-min Cola demineralisation challenge 48-hour remineralisation 4×3 min applications Measurement: microhardness $n = 5$ per group	MI Paste No MI Paste	MI Paste treatment significantly rehardened Cola softened enamel compared to artificial saliva only treatment

TMR = transverse microradiography.

deposition of mineral into enamel subsurface lesions is shown in the microradiographs presented in Fig. 10.4. Treatment with CPP-ACP resulted in mineral deposition throughout the body of the lesion, whereas fluoride had predominantly a surface effect.

Dentine has also been shown to remineralise following treatment with a CPP-ACP crème (Rahiotis and Vougiouklakis, 2007). The demineralised dentine exposed to CPP-ACP remineralised by 7.2 ± 3.0%, which was 74% greater than that of the control treatment.

The white appearance of early dental caries lesions has been attributed to the scattering of light in the demineralised enamel. Water or air fills these porosities and the differences in refractive index result in substantial light scattering. Remineralisation with solutions of CPP-ACP has been shown to decrease the white opaque appearance of demineralised enamel when high levels of remineralisation are achieved (Reynolds and Walsh, 2005).

Erosion has been shown to produce a layer of surface softening that can be rehardened by saliva (Koulourides *et al.*, 1965). The ability of a CPP-ACP crème to reharden enamel eroded by an 8-minute exposure to a Cola drink was tested *in vitro* by Tantbirojn *et al.* (2007). Eroded bovine enamel was exposed to 3-minute applications of a CPP-ACP crème four times over 48 hours with a saliva substitute with or without 1 ppm fluoride dripping on the samples at all other times. It was found that the 10% CPP-ACP crème significantly increased the enamel surface hardness compared to the control, irrespective of whether the artificial saliva contained fluoride. The authors concluded that the ions from the CPP-ACP readily diffused through the porous lesion in order to deposit on the partially demineralised crystallites and to remineralise the enamel.

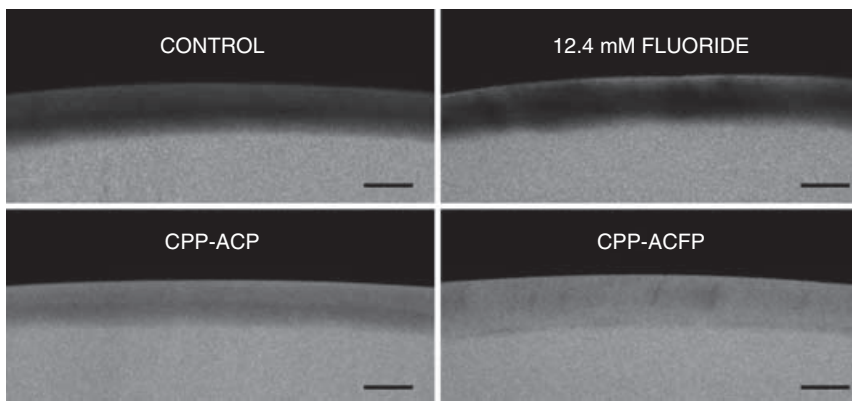

Fig. 10.4 Microradiographs of demineralised enamel (control) and enamel subsurface lesions remineralised by exposure to 12.4 mM fluoride, 2% CPP-ACP and 2% CPP-ACFP at pH 5.5. The scale bars indicate 100 μm. Reproduced from Cochrane *et al.* (2008).

10.3.2 Animal studies

The CPP-ACP nanocomplexes have been shown to reduce caries activity in the rat caries model (Reynolds *et al.*, 1995; Guggenheim *et al.*, 1994). In the Reynolds study (Reynolds *et al.*, 1995), solutions (100 μL) containing different concentrations of CPP-ACP were applied to the animals' molar teeth twice daily. Other groups of animals received 100 μL of either 500 ppm fluoride ions (positive control) or distilled water (negative control). The animals consumed a highly cariogenic sucrose/gluten diet that did not contain dairy products. The results of this study are presented in Fig. 10.5. The CPP-ACP significantly reduced caries activity in a dose–response fashion with 0.1% (w/v) CPP-ACP producing a 14% reduction and 1.0 % (w/v) CPP-ACP a 55% reduction relative to the distilled water control. CPP-ACP at 0.5% (w/v) produced a reduction in caries activity similar to that of 500 ppm fluoride. A solution containing both 0.5% (w/v) CPP-ACP and 500 ppm fluoride produced a significantly greater reduction in caries activity than either CPP-ACP or fluoride alone at the same concentrations.

10.3.3 *In situ* studies

The first *in situ* studies conducted to determine the effect of CPP-ACP on de- and remineralisation involved the testing of solutions of these nanocomplexes (Reynolds, 1987). Since then, CPP-ACP has been incorporated into numerous delivery vehicles including chewing gum, lozenges, milk, dentifrice and dental crème. These CPP-ACP vehicles have been tested in a range of *in situ* remineralisation studies (summarised in Tables 10.3 and 10.4).

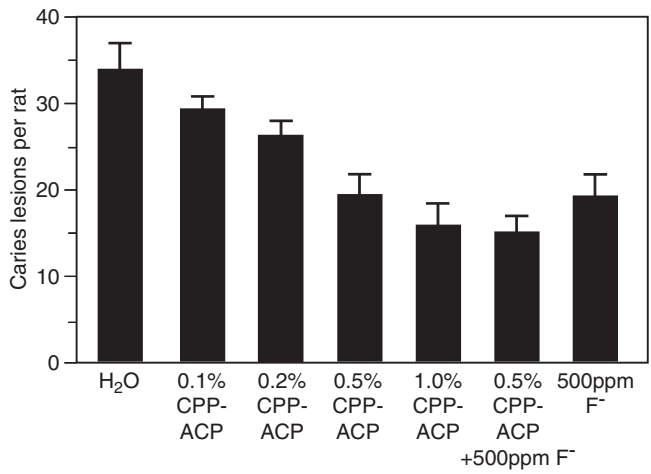

Fig. 10.5 Number of carious lesions per rat following twice daily treatment of their molar teeth with various solutions. Figure prepared from the data presented in Reynolds *et al.* (1995).

Table 10.3 Summary of *in situ* studies conducted on sugar-free chewing gums containing CPP-ACP

Study	Delivery vehicle	Substrate	Model	Groups	Conclusion
(Shen *et al.*, 2001)	Sugar-free chewing gum	Human enamel	Remineralisation assay: TMR Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 6 enamel half-slabs/ appliance palatally 4 × 20 min chews/day for 14 days Appliances remained *in situ* for 20 min post chew 10 subjects	No gum chewing Gum no CPP-ACP 0.19 mg CPP-ACP 10 mg CPP-ACP 18.8 mg CPP-ACP 56.4 mg CPP-ACP	Dose response relationship between CPP-ACP level in sugar-free gum and enamel subsurface remineralisation
(Reynolds *et al.*, 2003)	Pellet sugar-free chewing gum	Human enamel	Remineralisation assay: TMR Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 4 enamel half-slabs/ appliance palatally 4 × 20 min chews/day for 14 days 30 subjects	Lotte Xylitol gum Lotte Xylitol gum with calcium hydrogen phosphate and Funoran 18.8 mg CPP-ACP	Significantly more remineralisation with CPP-ACP containing gum
(Reynolds *et al.*, 2003)	Slab sugar-free chewing gum	Human enamel	Remineralisation assay: TMR Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 4 enamel half-slabs/ appliance palatally 7 × 5 min chews/day for 7 days 30 subjects	Xylish crystal mint Lotte Xylitol +2 18.8 mg CPP-ACP	Significantly more remineralisation with CPP-ACP containing gum
(Iijima *et al.*, 2004)	Sugar-free chewing gum	Human enamel	Remineralisation assay: TMR + acid challenge Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 4 enamel half-slabs/ appliance palatally 4 × 20 min chews/day for 14 days 10 subjects	Gum no CPP-ACP 18.8 mg CPP-ACP	CPP-ACP containing gum produced significantly more remineralisation than control and deposited mineral that was more resistant to subsequent acid challenges.

(Cai *et al.*, 2007)	Sugar-free chewing gum	Human enamel	Remineralisation assay: TMR + acid challenge Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 4 enamel half-slabs/ appliance palatally 4 × 20 min chews/day for 14 days 10 subjects	Gum no CPP-ACP no citric acid 18.8 mg citric acid 18.8 mg CPP-ACP + 18 mg citric acid	Citric acid containing sugar-free gum reduced the remineralisation effects of saliva. Addition of CPP-ACP negated the effect of citric acid and produced more remineralisation than the neutral citric acid free gum.
(Itthagarun *et al.*, 2005)	Sugar-free chewing gum	Human enamel	Remineralisation assay: lesion depth and TMR Lower appliance (Creanor *et al.*, 1986) containing 4 enamel sections covered in Dacron gauze lingually 21 days for 24-hours a day 5 × 20 min chews/day 2 cariogenic snacks supplied/day 12 subjects	No calcium phosphate + 30 mg urea 25 mg dicalcium phosphate dehydrate + 30 mg urea 47 mg Phoscal (crude CPP-ACP) + 30 mg urea	Lesion depth decreased in subjects chewing dicalcium phosphate dihydrate and crude CPP-ACP containing gums.
(Schirrmeister *et al.*, 2007)	Sugar-free chewing gum	Bovine enamel	Remineralisation assay: TMR Lower appliance (Koulourides *et al.*, 1974) containing 4 enamel specimens bucally 4 × 20 min chews/day for 14 days Appliances remained *in situ* for 20 min post chew 15 subjects	No treatment Gum no calcium 0.7% CPP-ACP + $CaCO_3$ 3.9% Dicalcium phosphate + other calcium forms 3.9% Dicalcium phosphate + other calcium forms + zinc citrate	No significant differences in mineral loss or lesion depth between any of the treatment or no treatment groups
(Manton *et al.*, 2008)	Sugar-free chewing gum	Human enamel	Remineralisation assay: TMR Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 4 enamel half-slabs/ appliance palatally 4 × 20 min chews/day for 14 days 10 subjects	Orbit gum Trident white containing 10 mg CPP-ACP per 2 pellets Orbit professional gum	The sugar-free gum containing CPP-ACP produced significantly more remineralisation than those gums not containing CPP-ACP.

Table 10.4 Summary of *in situ* studies conducted on solutions, lozenges, milks and dentifrices containing CPP-ACP

Study	Delivery vehicle	Substrate	Model	Groups	Conclusion
(Reynolds, 1987)	Solution	Bovine enamel	Demineralisation assay: TMR and microhardness Removable lower Co/Cr appliance (Ostrom and Koulourides, 1976) containing 4 enamel slabs bucally 8 × 20 min exposures to various solutions/day for 10 days	RHS–6 exposures/day to sugar solution and 2 exposures/day to 2% tryptic digested casein LHS–6 exposures/day to sugar solution and 2 exposures/day to $CaCl_2$ + salt solution	Twice daily exposure to a solution of tryptically digested casein prevented enamel subsurface demineralization.
(Cai *et al.*, 2003)	Lozenge	Human enamel	Remineralisation assay: TMR Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 4 enamel half-slabs/appliance palatally, 4 × 20 min exposures/day for 14 days 10 subjects	No treatment Lozenge no CPP-ACP 18.8 mg CPP-ACP 56.4 mg CPP-ACP	Dose–response relationship between enamel subsurface lesion remineralisation and level of CPP-ACP incorporation
(Walker *et al.*, 2006)	Milk	Human enamel	Remineralisation assay: TMR Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 4 enamel half-slabs/appliance palatally, one exposure/day for 14 days 10 subjects	Milk no added CPP-ACP 2 g L^{-1} CPP-ACP 5 g L^{-1} CPP-ACP	CPP-ACP incorporation into milk increased its remineralisation potential.

(Roberts, 1995)	Dentifrice	Bovine enamel	Demineralisation assay: Knoop hardness Partial denture (Vernon *et al.*, 1992) containing gauze wrapped enamel pieces worn 24 hours per day 2 enamel pieces per subject 3 × 5 min 5% sucrose soaks per day for three weeks	Paste no CPP-ACP 5% crude CPP-ACP	Significantly less surface softening in enamel exposed to the toothpaste containing 5% crude CPP-ACP
(Reynolds *et al.*, 2008)	Dentifrice	Human enamel	Remineralisation assay: TMR + acid challenge Mid-palatal acrylic appliance (Shen *et al.*, 2001) containing 4 enamel half-slabs/appliance palatally, 4 × 20 min exposures/day for 14 days 14 subjects	Paste no CPP-ACP 1100 ppm F 2800 ppm F 2% CPP-ACP 2% CPP-ACP + 1100 ppm F	2% CPP-ACP toothpaste produced similar level of remineralisation to 2800 ppm F. 2% CPP-ACP + 1100 ppm F produced significantly more remineralisation than all other treatments.

RHS = right hand side, LHS = left hand side.

The first CPP-ACP *in situ* study was based on the intra-oral appliance described by Ostrom and Koulourides (1976). It was a mandibular cobalt chrome denture with buccal flanges that contained two bovine enamel slabs set to simulate an approximal area. This was used in a demineralisation assay where sound bovine enamel was mounted in the appliance, worn and removed eight times throughout the day to be exposed to different solutions for 20-minute periods (Reynolds, 1987). Six exposures daily were to a sucrose solution and the other two were either a tryptic digest of casein in a calcium salt solution (on the right, RHS) or the calcium salt solution (on the left, LHS). After ten days, the enamel slabs were examined by microradiography and microhardness and it was found that the solution containing the calcium casein peptides prevented enamel subsurface demineralisation (Reynolds, 1987).

As an additive to sugar-free chewing gum, CPP-ACP has been shown to remineralise enamel subsurface lesions in several randomised, controlled, double-blind *in situ* clinical studies (Iijima *et al.*, 2004; Reynolds *et al.*, 2003; Shen *et al.*, 2001; Manton *et al.*, 2008) utilising a maxillary mid-palatal acrylic appliance containing human enamel slabs with subsurface lesions formed by the Carbopol method of White (1987). The sugar-free gums (control and CPP-ACP containing gums) were chewed for either 20-minute periods, four times a day or for 5-minute periods, seven times a day. Microradiography and computer-assisted densitometric image analysis demonstrated that, independent of gum type and chewing duration, the CPP-ACP nanocomplexes produced a dose-related remineralisation of enamel subsurface lesions *in situ* (Shen *et al.*, 2001). Gum containing 18.8 mg and 56.4 mg of the CPP-ACP nanocomplexes per serving, chewed for 20 minutes, four times per day for 14 days, increased enamel subsurface remineralisation by 102% and 152%, respectively, relative to the control sugar-free gum (Shen *et al.*, 2001). Manton *et al.* (2008) and Reynolds *et al.* (2003) tested a range of commercially available sugar-free chewing gums and found that those containing CPP-ACP produced superior remineralisation to the non-CPP-ACP containing gums, including some with much higher levels of calcium.

The same *in situ* model and method described by Shen *et al.* (2001) was used to test the efficacy of lozenges containing CPP-ACP nanocomplexes. A similar result to the sugar-free chewing gum studies was found with 18.8 mg and 56.4 mg CPP-ACP containing lozenges producing a 78% and 176%, respectively, increase in remineralisation compared to a control sugar-free lozenge (Cai *et al.*, 2003). Microradiographs from the chewing gum and lozenge studies of the enamel lesions before and after remineralisation showed that the CPP-ACP nanocomplexes promoted remineralisation throughout the body of the lesion.

Itthagarun *et al.* (2005) used a mandibular appliance to study the effect of sugar-free, urea-containing chewing gums with either added crude CPP-ACP or dicalcium phosphate dihydrate. It was found that the depths of the enamel subsurface lesions decreased significantly after the crude CPP-ACP

and dicalcium phosphate dihydrate gum treatments compared with the control, urea-alone gum.

An *in situ* chewing gum study conducted by Schirrmeister *et al.* (2007) sought to determine the effect of four different calcium phosphate containing gums on remineralisation. One of these gums contained 0.7% CPP-ACP. In contrast to *in situ* studies (Leach *et al.*, 1989; Shen *et al.*, 2001; Reynolds *et al.*, 2003) and clinical trials (Beiswanger *et al.*, 1998; Kandelman and Gagnon, 1990; Makinen *et al.*, 1995) reporting differences in remineralisation and caries incidence between chewing sugar-free gum compared with no-chewing, Schirrmeister *et al.* (2007) were unable to show a difference between their no-chewing and chewing controls. Additionally, they could not find any significant differences between any of the treatment gums as they had large standard deviations for mineral gain and reduction of lesion depth. Therefore, caution must be used in drawing conclusions from this study.

Food additives may have negative consequences for oral health. Cai *et al.* (2007) examined the effect of chewing sugar free-chewing gum that contained 20 mg of citric acid compared with a neutral gum not containing citric acid on remineralisation of enamel subsurface lesions. It was found that the citric acid chelated salivary calcium and reduced the remineralisation potential of the chewing gum-stimulated saliva from 9.4% to 2.6%. The addition of 18 mg of CPP-ACP to the 20 mg citric acid-containing sugar-free chewing gum negated the effect of the citric acid and produced a higher level of remineralisation than the neutral sugar-free gum without citric acid (Cai *et al.*, 2007).

Some foods, such as milk and dairy products, contain intrinsic remineralisation potential as they contain high concentrations of bioavailable calcium and phosphate ions, although the majority of these are bound in casein micelles. The addition of 0.2% or 0.5% (w/v) of CPP-ACP to milk was found to enhance further the remineralisation potential of milk (Walker *et al.*, 2006). This was attributed to the ability of CPP-ACP to localise at the enamel surface and increase the free concentrations of calcium and phosphate ions. The relationship was again dose-dependent with 0.2% and 0.5% (w/v) CPP-ACP producing a 70% and 148% increase in remineralisation respectively compared with the milk without CPP-ACP (Walker *et al.*, 2006).

Dentifrice is a common delivery vehicle for anti-caries and desensitising agents. A crude CPP-ACP preparation has been added to a dentifrice formulation and tested in an *in situ* demineralisation model (Roberts, 1995). Furthermore, a commercial preparation of the CPP-ACP nanocomplexes has been tested in a dentifrice formulation using an *in situ* remineralisation model (Reynolds *et al.*, 2008). Roberts (1995) found the test dentifrice containing a crude CPP-ACP preparation reduced surface softening by 20% compared with a CPP-ACP free dentifrice. Reynolds *et al.* (2008) tested five dentifrice formulations in an *in situ* remineralisation model. The

dentifrice containing 2% CPP-ACP produced 13.5 ± 1.5% remineralisation in a 14-day period compared with 8.2 ± 2.0% by a 1100 ppm F paste and 3.1 ± 1.6% by use of the placebo paste for the same period.

The level of remineralisation achieved by the daily use of a professional crème containing 10% CPP-ACP (Tooth Mousse / MI Paste) was tested compared with a placebo paste not containing CPP-ACP in the *in situ* model described by Shen *et al.* (2001). In this randomised, crossed-over, double-blind study, the pastes were diluted 1:5 with water and rinsed as a slurry four times per day. At the end of the 10-day treatment period, the 10% CPP-ACP crème produced 24.2 ± 2.3% remineralisation compared with 3.7 ± 2.1% for the placebo paste. This study concluded that, by supplying additional bioavailable calcium and phosphate, substantially more remineralisation could be produced than by the intrinsic calcium and phosphate in saliva alone (unpublished).

These *in situ* studies have shown that CPP-ACP can be added to a variety of vehicles for intra-oral delivery to produce positive dental health effects. Evidence exists to support CPP-ACP use in:

- chewing gums or lozenges to promote remineralisation of enamel subsurface lesions
- chewing gums to negate the effect of citric acid and promote remineralisation
- milks to enhance remineralisation
- dentifrices to inhibit demineralisation and promote remineralisation
- crèmes to promote remineralisation.

10.3.4 Acid resistance of casein phosphopeptide-amorphous calcium phosphate-induced enamel mineral

Enamel is a calcium-deficient carbonated apatite (LeGeros, 1991). An important question regarding CPP-ACP remineralisation is what phase of mineral is deposited in the lesion. Calcium and phosphate ions can combine to form a variety of solid phases with different solubilities and behaviours. To elucidate the nature of the mineral deposited, CPP-ACP treated lesions have been acid-challenged to determine their relative solubility (Cai *et al.*, 2007; Iijima *et al.*, 2004; Reynolds *et al.*, 2008) and analysed by electron microprobe to determine atomic composition (Cochrane *et al.*, 2008; Reynolds, 1997).

Iijima *et al.* (2004) tested the acid resistance of enamel subsurface lesions that had been remineralised *in situ* following chewing sugar-free gum containing 18.8 mg CPP-ACP compared with chewing a control sugar-free gum not containing CPP-ACP. Following treatment, the remineralised lesions were acid-challenged *in vitro* by exposure to a Carbopol demineralisation buffer for 8 or 16 hours. Chewing the CPP-ACP gum resulted in 17.9 ± 1.0% remineralisation compared with 9.0 ± 0.7% for the control gum.

Following acid challenge, the net remineralisation of the CPP-ACP treated lesions decreased by 30.5% and 41.8% after 8 and 16 hour exposure, respectively, to the demineralisation buffer. The net remineralisation of the non-CPP-ACP treated lesions decreased by 65.4% and 88.0%, respectively. The net mineral content of the CPP-ACP treated lesions was four-fold and ten-fold higher after an 8- and 16-hour acid challenge, respectively (Iijima *et al.*, 2004). These findings of more acid-resistant mineral deposited following exposure to CPP-ACP have been supported by studies conducted by Reynolds *et al.* (2008) and Cai *et al.* (2007).

Microradiographs of the remineralised and acid-challenged lesions can be seen in Fig. 10.6. The pattern of demineralisation was different between the two treatment groups. The CPP-ACP treated lesions tended to lose mineral below the remineralised zone compared with the control lesions that lost mineral throughout the lesion. This is consistent with the deposition of a more stable mineral phase that has a lower solubility than a calcium-deficient carbonated apatite (Iijima *et al.*, 2004).

Electron microprobe wavelength dispersive spectrometric analyses of sections remineralised by exposure to CPP-ACP indicated that the mineral deposited was hydroxyapatite with a Ca:P ratio of sound apatite (Reynolds, 1997; Cochrane *et al.*, 2008). This accords with the acid resistance data presented by Iijima *et al.* (2004), whereby the secondary demineralisation tended to occur below the remineralised zone, being consistent with the hypothesis that the more soluble calcium-deficient apatite preferentially dissolves compared with the more stable hydroxyapatite that had been deposited in the surface layer and body of the lesion following CPP-ACP treatment.

10.3.5 Caries clinical trials

Three randomised, controlled caries clinical trials of CPP-ACP have now been conducted. One assessed the impact of sugar-free gum containing CPP-ACP in preventing demineralisation and promoting remineralisation and the other two assessed the ability of a dental crème containing CPP-ACP to regress white spot lesions in patients following orthodontic treatment.

A randomised, controlled caries clinical trial of sugar-free chewing gum containing CPP-ACP demonstrated that the CPP-ACP gum significantly slowed progression and enhanced regression of caries compared with the control sugar-free gum (Morgan *et al.*, 2008). In the 24-month study, 2720 school children were randomly assigned to either a test or control sugar-free gum. All subjects received accepted preventive procedures, including fluoridated water, fluoridated dentifrice and access to professional care. Subjects were instructed to chew their assigned gum for ten minutes three times per day, with one session supervised on school days. Standardised digital radiographs were taken at the baseline and at the completion of the trial.

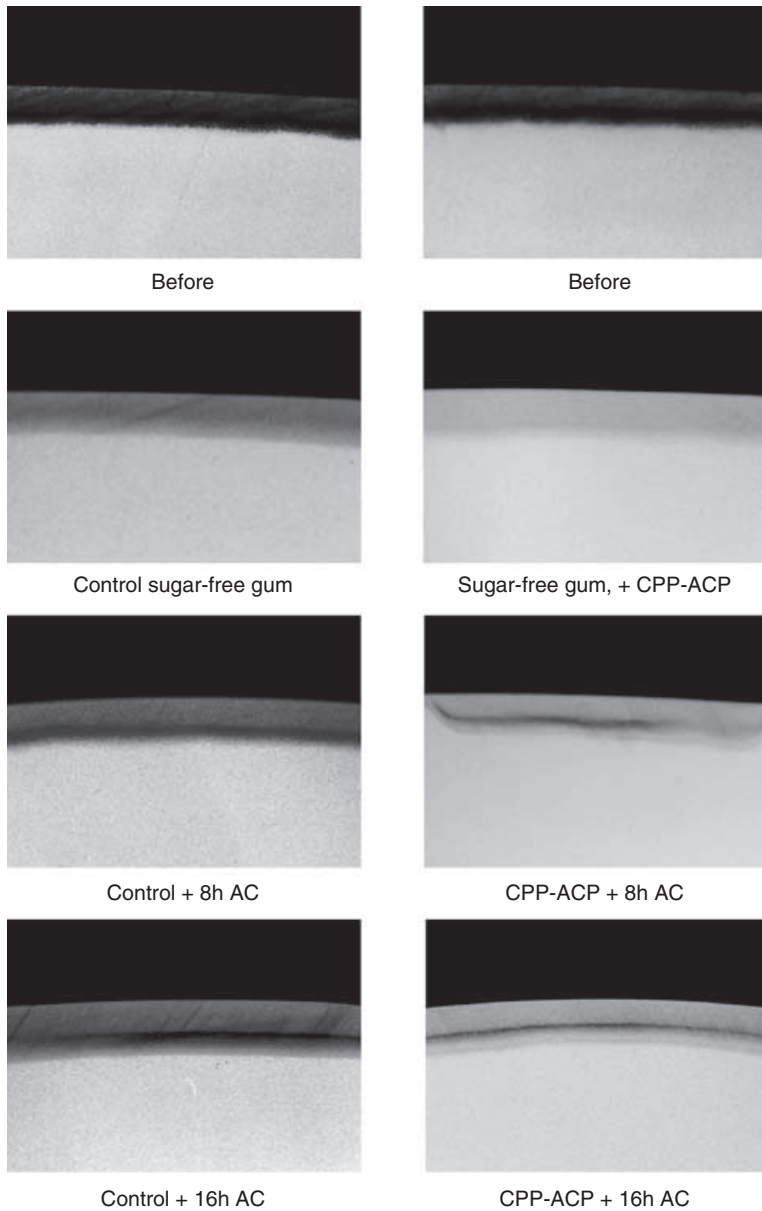

Fig. 10.6 Densiometric images of enamel subsurface lesions before and after *in situ* exposure to the control and sugar-free chewing gums containing CPP-ACP and subsequent *in vitro* acid challenge (AC). Reproduced from Iijima *et al.* (2004).

The radiographs, scored by a single examiner, were assessed for approximal caries at both the enamel and dentine level. Analysis of caries progression or regression was undertaken using a transition matrix. The CPP-ACP gum effected an 18% reduction in caries progression after 24 months at the subject level, with a 53% greater regression (remineralisation) of baseline lesions when compared with the control gum (Morgan *et al.*, 2008).

Two randomised, controlled clinical trials of post-orthodontic white spot lesion regression by a CPP-ACP dental crème have been reported by Andersson *et al.* (2007) and Bailey *et al.* (2008). The study by Andersson involved 26 subjects with 152 visible white spot lesions on 60 incisors and canines immediately after orthodontic debonding. After bracket removal, professional tooth cleaning and drying, a visual scoring (0–4) and laser fluorescence assessment were performed. The subjects were randomly assigned to two different treatment protocols with the aim of remineralising the lesions. One treatment was a daily topical application of a dental crème containing crude CPP-ACP for 3 months followed by a 3-month period of daily toothbrushing with a fluoride dentifrice. The other treatment protocol was daily topical application of a 0.05% sodium fluoride mouthwash combined with use of a fluoride dentifrice for 6 months. Clinical examinations were repeated after 1, 3, 6 and 12 months and data were compared with baseline measurements. The study showed that 63% of white spots regressed in the CPP-ACP group compared with 25% in the fluoride group which was significantly different ($p < 0.01$). The authors commented that the visual assessment indicated a more favourable outcome with CPP-ACP treatment.

The study by Bailey *et al.* (2008) examined 45 subjects with 408 white spot lesions immediately after orthodontic therapy who were randomly assigned to either a Tooth Mousse containing 10% CPP-ACP treatment or a placebo crème treatment. Subjects were instructed to apply the products twice daily for 12 weeks after normal oral hygiene procedures (subjects were supplied with toothpaste containing 1000 ppm F as NaF). Following initial assessment, lesions were assessed at 4, 8 and 12 weeks. The lesions were scored for lesion severity and activity using the ICDAS II criteria (ICDAS, 2008). At 12 weeks, 31% more of the ICDAS code 2 (white spot visible when wet) and 3 (loss of enamel surface integrity) lesions had regressed with Tooth Mousse compared with the placebo control (odds ratio = 2.3, $p = 0.04$). In both treatment groups, active lesions were more likely to regress than inactive lesions (odds ratio = 5.07, $p < 0.001$). It was concluded that significantly more post-orthodontic white spot lesions regressed with Tooth Mousse compared with a placebo control over a 12-week period (Bailey *et al.*, 2008).

10.3.6 Localisation in plaque

The CPP-ACP technology has been demonstrated to bind onto the tooth surface and into supragingival plaque significantly to increase bioavailable

calcium and phosphate ions at the tooth surface (Reynolds *et al.*, 2003). Reynolds *et al.* (2003) conducted a randomised, double-blind, cross-over *in situ* study to determine the calcium and phosphate incorporation into plaque after five days of rinsing with either water, 2% CPP-ACP, 6% CPP-ACP or unstabilised calcium and phosphate. The plaque calcium and phosphate levels following rinsing with the control the unstabilised calcium and phosphate rinses were similar, whereas both CPP-ACP rinses resulted in significantly higher incorporation of calcium and phosphate. When the plaque CPP levels were determined using competitive ELISA (enzyne-linked immuno sorbent assay), it was found that three hours' post-exposure to CPP-ACP there remained a 4.6-fold higher peptide content than prior to exposure. Electron microscopic analysis of immunocytochemically stained thin sections of supragingival plaque samples (Reynolds *et al.*, 2003) showed that the CPP-ACP nanocomplexes were localised in the plaque matrix and on the surface of bacterial cells (Fig. 10.7), confirming the work of Rose (2000a, 2000b) who showed the CPP-ACP nanocomplexes bound tightly to *Streptococcus mutans* and model plaque producing a reservoir of bioavailable calcium ions.

The method of binding into plaque has been hypothesized to be due to calcium cross-linking (Rose, 2000b; Rose, 2000a; Reynolds *et al.*, 2003) and/or hydrophobic and hydrogen-bond mediated interactions (Reynolds *et al.*, 2003). Using acid and alkali extraction, to help distinguish between these mechanisms of binding, it was found that the majority of the bonds localising CPP in the plaque were hydrophobic and/or hydrogen-bond mediated interactions between the CPP and bacterial cell/pellicle surfaces as the peptides were released by alkaline extraction and not by acid extraction (Reynolds *et al.*, 2003). These results are consistent with the proposed

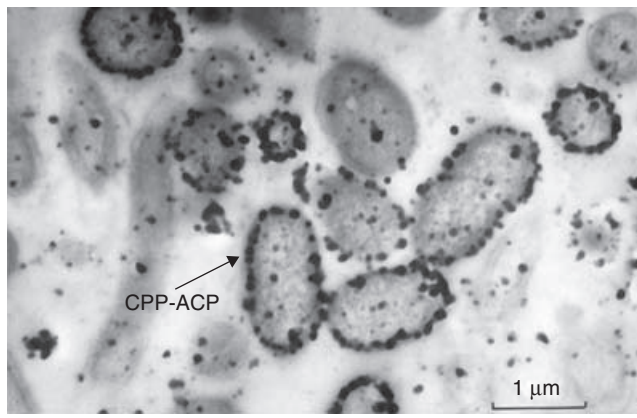

Fig. 10.7 Electron micrograph of supragingival plaque showing CPP-ACP as electron-dense particles associated with the surface of bacteria and the intercellular matrix. Reproduced from Reynolds *et al.* (2003).

three-dimensional structure of the CPP-ACP nanocomplexes (Cross *et al.*, 2007b) which shows a hydrophobic surface patch on the nanocomplex that may be responsible for the binding and localisation of the nanocomplexes at the tooth surface.

The ability of CPP-ACP to bind onto the tooth surface and into dental plaque make it an ideal delivery vehicle for calcium and phosphate ions as it is localised adjacent to the enamel surface where the ions are needed to prevent demineralisation and to promote remineralisation.

10.3.7 Interactions with fluoride

CPP-ACP nanocomplexes have been found to interact with fluoride ions to produce a novel amorphous calcium fluoride phosphate (ACFP) phase (Cross *et al.*, 2004; Reynolds *et al.*, 2008; Cochrane *et al.*, 2008). The identification of this novel ACFP phase is consistent with the observed additive anticariogenic effect of the CPP-ACP nanocomplexes and fluoride (Reynolds *et al.*, 1995; Reynolds *et al.*, 2008). The anticariogenic mechanism of fluoride is the localisation of the fluoride ion at the tooth surface, particularly in plaque, in the presence of calcium and phosphate ions. This localisation increases the degree of saturation with respect to fluorapatite (FA), thus promoting remineralisation of enamel with FA during an acid challenge. It is clear that, for the formation of FA [$Ca_{10}(PO_4)_6F_2$], calcium and phosphate ions must also be present with the fluoride ions. The reported additive anticariogenic effect of the CPP-ACP nanocomplexes and fluoride, therefore, may be attributable to the localisation of ACFP at the tooth surface by the CPP, which co-localises calcium, phosphate and fluoride as bioavailable ions in the correct molar ratio to form fluorapatite.

Cochrane *et al.* (2008) studied remineralisation of enamel subsurface lesions at acidic pH with CPP-ACP and CPP-ACFP. It was found that, at pH 5.5 and below, CPP-ACFP was superior to CPP-ACP, producing significantly more remineralisation. This was attributed to the presence of fluoride, which at low pH would form the neutral ion pair HF^0 penetrating deeply into the lesion and thus promoting fluorapatite formation and driving remineralisation. The localisation of fluoride in enamel following remineralisation was determined using electron microprobe analysis. Following CPP-ACFP treatment, it was found that fluoride was localised throughout the body of the lesion at significantly higher levels than the fluoride alone treatment. The calcium, phosphorus and fluorine contents of the remineralised lesions, were consistent with the deposition of fluorapatite and hydroxyapatite in the CPP-ACFP and CPP-ACP treated lesions, respectively. The level of remineralisation achieved by CPP-ACFP at pH 5.5 was sufficient to restore the optical properties of the subsurface lesions towards that of sound enamel (Fig. 10.8) (Cochrane *et al.*, 2008).

In a randomised, controlled, mouth rinse trial, a rinse containing 2.0% CPP-ACP nanocomplexes plus 450 ppm fluoride significantly increased

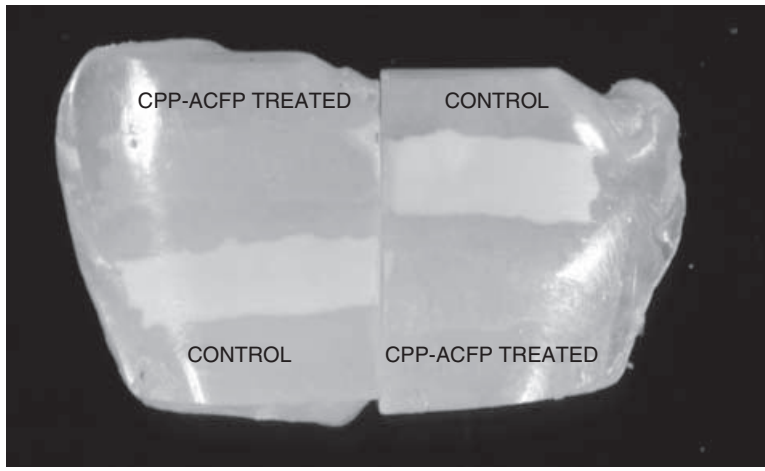

Fig. 10.8 Enamel subsurface lesions treated with a 2% CPP-ACFP solution at pH 5.5. The lesions labelled control were untreated, subsurface enamel lesions. Reproduced from Cochrane *et al.* (2008).

supragingival plaque fluoride ion content to 33.0 ± 17.6 nmol F/mg dry wt of plaque when compared with 14.4 ± 6.7 nmol F/mg dry wt of plaque attained by use of a rinse containing the equivalent concentration of fluoride ions as sodium fluoride (Reynolds *et al.*, 2008). Although marked increases in plaque calcium, phosphate and fluoride were found, calculus was not observed in any of the subjects, indicating that the plaque calcium, fluoride and phosphate remained stabilised at the tooth surface by the CPP as bioavailable ions and did not transform into a crystalline phase. These results suggest that the CPP are an excellent delivery vehicle for co-localising bioavailable calcium, fluoride and phosphate ions at the tooth surface in a slow release form with superior clinical efficacy (Reynolds *et al.*, 2008).

In a randomised, controlled, double-blind *in situ* clinical study, five dentifrice formulations were tested to compare their capacity to remineralise enamel subsurface lesions. The dentifrices containing 0, 1100 and 2800 ppm fluoride produced levels of remineralisation that followed a dose–response. The dentifrice containing 2% CPP-ACP nanocomplexes plus 1100 ppm fluoride was shown to be superior to all other formulations tested and was 2.6 times more effective than a dentifrice containing only 1100 ppm fluoride in remineralisation of enamel subsurface lesions with mineral that was more resistant to acid challenge (Reynolds *et al.*, 2008). The 2% CPP-ACP nanocomplexes plus 1100 ppm fluoride dentifrice resulted in significantly greater incorporation of fluoride into the subsurface enamel as fluorapatite as shown by electron microprobe wavelength dispersive spectrometry (Reynolds *et al.*, 2008).

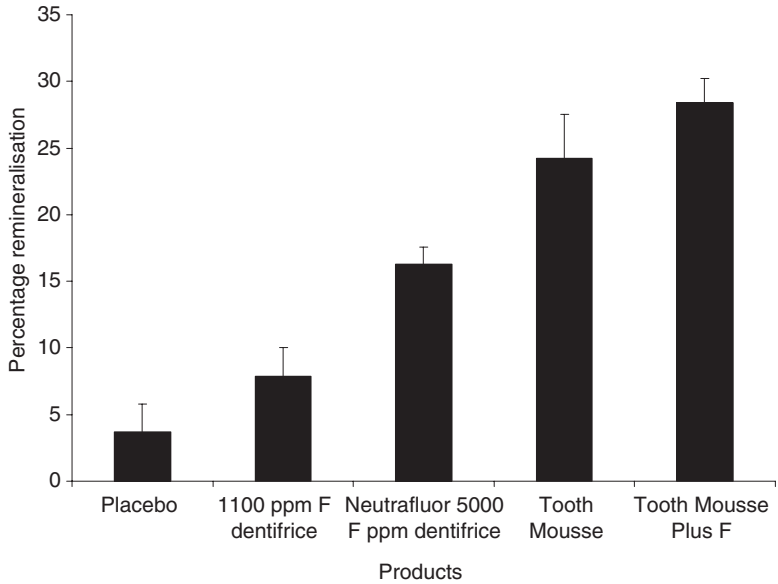

Fig. 10.9 Remineralisation *in situ* by fluoride dentifrices and CPP-ACP and CPP-ACP plus fluoride crèmes (unpublished).

A randomised double-blind *in situ* study of remineralisation produced by fluoride dentifrices, Tooth Mousse (10% CPP-ACP) and Tooth Mousse Plus (10% CPP-ACP plus 900 ppm fluoride) found that the highest levels of remineralisation were produced by the combination of CPP-ACP and fluoride-containing crème (Fig. 10.9) (unpublished).

These results indicate that fluoride alone treatments can be limited by the availability of calcium and phosphate ions, whereas CPP-ACFP or CPP-ACP plus fluoride deliver calcium, phosphate and fluoride ions to promote significantly greater remineralisation than either fluoride or CPP-ACP alone.

10.4 Mechanism of action

The published results are consistent with the proposed anticariogenic mechanism of the CPP-ACP technology being the inhibition of enamel demineralisation and enhancement of remineralisation through the localisation of bioavailable calcium and phosphate ions that are released at the tooth surface. The localisation and release of these ions at the tooth surface is critical, as without localisation the majority of the calcium and phosphate ions would be lost via salivary clearance as shown by Reynolds *et al.* (2003) with poor plaque retention of unstabilised calcium and phosphate. Once

localised and released at the tooth surface, the bioavailable calcium and phosphate ions can both inhibit demineralisation and promote remineralisation.

10.4.1 Release of calcium and phosphate ions from the casein phosphopeptide-amorphous calcium phosphate nanocomplex

The release of calcium and phosphate ions from the CPP-ACP nanocomplexes can be considered under four potential mechanisms.

(1) Equilibrium-driven release: An equilibrium will be maintained between the CPP-complexed and non-complexed calcium and phosphate ions. If the non-complexed concentrations of these ions decrease, then this equilibrium would help to maintain those concentrations.
(2) Conformational release: The CPP has binding affinities for a number of structures present in the mouth – apatite (Cross *et al.*, 2007a), calcium (Rose, 2000a; Cross *et al.*, 2005), pellicle (Ung *et al.*, 2004; Schupbach *et al.*, 1996), mucin (Ung *et al.*, 2004), proline-rich proteins (Ung *et al.*, 2004) and bacteria (Rose, 2000a; Reynolds *et al.*, 2003). Binding to apatite, pellicle or bacteria will induce a conformational change in the CPP that will release calcium and phosphate ions from the nanocomplex.
(3) pH-dependent release: As the pH decreases, the phosphorylated groups of the peptide become protonated, thereby decreasing the net negative charge that binds the calcium ions. This releases calcium and phosphate ions from the complexes (Cochrane *et al.*, 2008; Reynolds, 1997).
(4) Enzymic release (peptidase/ phosphatase): Intra-orally there are peptidases and phosphatases that can cleave the phosphopeptides. The dephosphorylation of the phosphoserines of the CPP by phosphatases substantially reduces the ability of the peptides to bind calcium and phosphate ions. From the immunolocalisation time course study of CPP in plaque by Reynolds *et al.* (2003), the half-life of CPP in plaque can be calculated ($t_{0.5} = \ln(2) \times$ mean lifetime) to give a mean value of 124.8 minutes. Furthermore, casein has been shown to be hydrolysed by salivary sediment bacteria (Reynolds and Riley, 1989) in a similar timeframe, hence the decrease in detection of the CPP in plaque is likely to be attributable to the enzymic digestion of the peptides to fragments not recognised by the antibodies used for the assay (Reynolds *et al.*, 2003). Cross *et al.* (2005) have shown that the full-length CPPs are required for maximal stabilisation of calcium and phosphate ions, therefore enzymic hydrolysis of the CPP would be expected to mediate release of the calcium and phosphate ions. It should be noted that the enzymic breakdown of the CPP has been shown to produce a plaque pH rise through the production of ammonia (Reynolds and Riley, 1989). Hence, this process also may contribute to the inhibition of demineralisation observed *in situ*/*in vivo* by the CPP-ACP.

10.4.2 Inhibition of demineralisation

The inhibition of demineralisation by CPP-ACP has been attributed to four major effects:

(1) Direct surface protection
(2) Acid buffering
(3) Maintenance of apatite ion activity product
(4) Alteration of plaque microbial composition to a less cariogenic form

Direct surface protection

CPP and CPP-ACP have been shown to bind to hydroxyapatite and saliva-coated hydroxyapatite (Reynolds *et al.*, 1982; Cross *et al.*, 2007a). Demineralisation assays using CPP and CPP-ACP have shown reductions in enamel demineralisation upon CPP/CPP-ACP treatment (Reynolds *et al.*, 1982; Roberts, 1995). This effect has, in part, been attributed to a surface effect of the CPP or CPP-ACP acting as a physical barrier to dissolution by binding to an apatite crystal face and blocking active sites of dissolution such as steps, edges (the result of a screw dislocation) and kinks. By binding to the apatite crystal surface, it is proposed that the CPP provides a physical barrier to dissolution (Reynolds *et al.*, 1982), restricting the release of calcium and phosphate ions from the lattice into the hydration layer. This would be particularly relevant to the prevention of erosive demineralisation.

Acid buffering

It has been proposed that CPP and the phosphate ions it stabilises can buffer pH changes in a cariogenic plaque (Reynolds, 1987; Reynolds, 1998). CPP is composed of a number of amino acid residues that contain R groups such as phosphoseryl, histidyl and glutamyl residues that can buffer acid at certain pH values. The relevant p*K*a value of the phosphoseryl group is 6.4, the histidyl group 6.6 and the glutamyl group 4.9 (Swaisgood, 1982), hence CPP incorporated into plaque will help buffer in the pH range 7 to 5 (Reynolds, 1987). Additionally, it has been shown that, when casein is degraded by bacteria contained in salivary sediment, then arginyl, asparaginyl and glutaminyl residues can be broken down to produce ammonia and consume protons, thereby increasing the pH (Reynolds and Riley, 1989). As the CPP contains arginyl, asparaginyl and glutaminyl residues, this pH rise mechanism will also apply to their catabolism in plaque. The phosphate ions being predominantly of the form PO_4^{3-} and HPO_4^{2-} will accept protons, particularly at pH values 7 and below. Therefore, the CPP and phosphate ions can both act to buffer acid between pH 7 and 5.

Maintenance of apatite ion activity product

The most frequently reported mechanism to explain the ability of CPP-ACP to prevent demineralisation is by increasing the calcium and phos-

phate ion concentrations in plaque, thereby maintaining the apatite ion activity product (IAP_{HA}) at a state of saturation with respect to dental apatite. For apatite to dissolve, the fluid surrounding it must be undersaturated with respect to the apatite phase (crystal). The degree of saturation is dependent on the activities of the ions in solution around the solid phase. For example, the degree of saturation with respect to hydroxyapatite is dependent on the product of the activities of calcium, phosphate and hydroxide ions.

$$IAP_{HA} = (Ca^{2+})^{10} (PO_4^{3-})^6 (OH^-)^2$$

Saturation is defined as the condition where the $IAP_{HA} \geq Ksp_{HA}$ (the apatite solubility product).

The ability of CPP-ACP to localise in plaque increases the bioavailable calcium and phosphate ion activities, thereby increasing the apatite ion activity product (IAP_{HA}). This increases the degree of saturation with respect to apatite thereby depressing demineralisation.

Alteration of plaque microbial composition

It has been proposed that CPP may cause alterations to the bacterial composition of dental plaque by preventing the adherence and colonisation of specific cariogenic bacteria. This inhibition of bacterial adherence may be mediated by a number of proposed mechanisms: (i) masking specific receptors (Schupbach *et al.*, 1996), (ii) competitively binding calcium to prevent calcium bridging of bacterial cells (Rose, 2000a) or (iii) binding to the surface of bacteria to cause electrostatic repulsion (Reynolds and Wong, 1983; Neeser *et al.*, 1994).

Schupbach *et al.* (1996) found that CPP incorporated into pellicle *in vitro* in exchange for albumin and, once incorporated, significantly inhibited the adherence of *S. sobrinus* and *S. mutans* to salivary-coated apatite discs by 75% and 83%, respectively ($p < 0.001$). Nesser *et al.* (1994) found that the acidic casein derivatives were able to inhibit streptococcal adhesion *in vitro* whereas binding of oral *Actinomyces* spp. was not affected. The authors concluded that this may explain part of the anticariogenic mechanism of CPP by inhibiting streptococcal adhesion and favouring the establishment of a less cariogenic plaque (Neeser *et al.*, 1994; Schupbach *et al.*, 1996). Rose (2000a) also proposed that CPP, by stabilising high extracellular concentrations of free calcium, may have bacteriocidal or bacteriostatic mechanisms. A recent *in situ* study conducted by Rahiotis *et al.* (2008) examining plaque formation on germanium crystals mounted in an intra-oral appliance, has shown that pretreatment with a CPP-ACP containing dental crème (Tooth Mousse) substantially delayed biofilm formation compared with the untreated control being consistent with the proposed antiplaque mechanisms.

10.4.3 Promotion of remineralisation

The CPP-ACPs when localised in plaque or directly onto the tooth surface, adjacent to a carious lesion, provide a reservoir of ions that can promote remineralisation. The proposed mechanisms for this activity are:

(1) release of ions, particularly during acid challenge
(2) formation of neutral ion pairs ($CaHPO_4^0$ and HF^0)
(3) diffusion of ions into the subsurface lesion
(4) increasing the degree of saturation in the lesion
(5) formation of fluorapatite or hydroxyapatite mineral.

The CPP-ACPs adjacent to an early caries lesion, either bound onto the surface pellicle or bound into plaque, provide a reservoir of bioavailable calcium and phosphate ions. The calcium and phosphate ions may be released from the nanocomplexes by the mechanisms described, for example equilibrium driven, conformational change, pH and enzymic. The free calcium and phosphate ions will then participate in a variety of equilibria to form a range of calcium phosphate species, depending on the pH and fluoride availability as described by Cochrane *et al.* (2008). The process of diffusion into a subsurface lesion must be overall an electroneutral process. Therefore, diffusion potential into an enamel subsurface lesion is best characterised by the activity gradient of the neutral ion pair, $CaHPO_4^0$, into the lesion (Reynolds, 1997; Cochrane *et al.*, 2008). As the neutral ion pair diffuses down its activity gradient into the lesion, unimpeded by the charges in plaque, pellicle and the enamel, it will dissociate along its diffusion path to produce calcium and phosphate ions, increasing the degree of saturation with respect to apatite in the lesion promoting crystal growth. In the case of CPP-ACP plus fluoride, fluoride would also be provided that would allow the formation of the neutral species $CaH_2FPO_4^0$ and HF^0 to diffuse down activity gradients dissociating and accelerating crystal growth with fluorapatite (Cochrane *et al.*, 2008). When CPP-ACP is provided with a low background of fluoride, electron microprobe analysis has shown that the mineral deposited is consistent with hydroxyapatite and, when fluoride is present, the mineral is consistent with fluorapatite (Cochrane *et al.*, 2008).

10.5 Recommendations for clinical use

CPP-ACP technology has been incorporated into commercially available crèmes and chewing gum. The topical crème is called Tooth Mousse (Europe, Australia and Japan) or MI Paste (USA) and contains 10% (w/w) CPP-ACP nanocomplexes in a water/glycerol base. Tooth Mousse Plus or MI Paste Plus is also available which contains 10% CPP-ACP plus 900 ppm fluoride. These products have been used clinically for the successful non-invasive treatment of mild to moderate fluorotic lesions (Ng and Manton, 2007;

Reynolds and Walsh, 2005), aesthetic improvement of hypomineralised enamel (Milnar, 2007), reversal of early caries lesions (Ardu *et al.*, 2007) and for caries stabilisation (Reynolds and Walsh, 2005; Guzman-Armstrong and Warren, 2007; Vlacic *et al.*, 2007). The chewing gums containing CPP-ACP are Trident Xtra Care (Americas) and Recaldent gums (Japan).

10.5.1 Tooth Mousse/MI Paste/Tooth Mousse Plus/MI Paste Plus

Tooth Mousse or MI Paste is a formulation of CPP-ACP that is particularly useful for young children with high caries risk as it does not contain fluoride and therefore does not have the associated risks of fluorosis. Tooth Mousse Plus has been shown to produce higher mean levels of remineralisation compared to Tooth Mousse owing to the fluoride accelerating the deposition of mineral (unpublished). Therefore, Tooth Mousse Plus is an ideal product for adults with a high caries risk or when trying to actively remineralise early caries lesions. The crèmes are contraindicated in patients with a milk casein allergy.

The evidence-based disease management protocol for dental caries designed in the United States, called caries management by risk assessment (CAMBRA), recommends calcium phosphate topical supplementation as an optional addition for the management of high caries risk individuals and as a required treatment for those patients in an extreme caries risk categories (Jenson *et al.*, 2007).

The clinical recommendations regarding caries stabilisation protocols utilising CPP-ACP containing crèmes are to apply them after brushing the teeth once or up to several times a day, dependent on the patient's risk. It can be applied in the morning without rinsing so that throughout the day, as cariogenic challenges occur, the CPP-ACP is localised in the plaque and can inhibit demineralisation, or it can be applied at bed time without rinsing to boost the acid buffering and calcium and phosphate ion concentrations at the tooth surface as salivary flow decreases during sleep. The recommended method of application is to use a finger or a cotton tip to apply the crème to individual or multiple teeth. If applying to the whole arch at home, a tray can be used to reduce the amount expressed per application but the tray should be removed after 5 minutes with the excess moved around the teeth with the tongue. The patient should not rinse or expectorate following the application of the crème. The paste has a water/glycerol base and requires interaction with the water present in saliva to allow the CPP-ACP to diffuse into and around the teeth for maximal results.

When using the crème actively to remineralise either hypomineralised, fluorotic or early caries lesions, a number of factors need to be considered. The lesion should be assessed for its: (1) activity and (2) location.

These are important considerations because, to remineralise a lesion, the ions are required in the enamel subsurface. An active lesion has a higher surface layer porosity so that the ions released by CPP-ACP will have a

greater chance of diffusing into the lesion. This was shown by Bailey *et al.* (2008), whereby active lesions responded better to remineralisation treatments than inactive ones. An old inactive lesion with a heavily remineralised surface layer, particularly if it has been remineralised by topical fluoride applications, will have poor diffusion channels that limit the movement of ions into the subsurface. Therefore, the approach to treating each of these two lesions will be different.

For active lesions, the aim should be to try and replace as much mineral as possible into the subsurface via remineralisation. The traditional approach of applying high concentration fluoride without any supplemental calcium and phosphate ions promotes surface deposition and may produce a scarred lesion with a thick surface layer that will no longer respond to remineralisation therapy. If this lesion is in a location that does not present an aesthetic concern, then producing a thick surface layer may be sufficient to arrest the disease. However, the aim should be to incorporate as much mineral, preferably fluorapatite, as possible to help reduce the risk of future disease. It is recommended that Tooth Mousse Plus be the preferred treatment rather than high concentrations of fluoride without additional calcium and phosphate as the fluoride and saliva alone will favour surface deposition only, thereby restricting the extent that the lesion can be remineralised with fluorapatite.

If the lesion is inactive, a decision needs to be made about whether the lesion requires treatment. This is where the location of the lesion is important. If it is in a site that can easily be kept clean, away from high occlusal forces that may fracture the lesion, or if it is not in an aesthetically concerning site, then a decision not to treat the lesion may be made. However, if the lesion is an aesthetic concern, or in a site that may be subjected to future cariogenic challenges, then modification of the inactive lesion may be indicated. Remineralisation with lesion modification should be the first choice in cases where the patient may be considering more destructive techniques to improve aesthetics, such as a restoration or veneer, as remineralisation is a much more conservative approach. If modification is necessary, then the clinician has a number of options. Access to the subsurface can be achieved by:

- microabrasion (Ardu *et al.*, 2007)
- etching (Meyer-Lueckel *et al.*, 2007; Flaitz and Hicks, 1994)
- bleaching (Ng and Manton, 2007; Robinson *et al.*, 1990)
- or a combination of the above – bleaching and etching (Milnar, 2007).

Once the surface layer has been modified, a CPP-ACP or CPP-ACFP treatment regime can be commenced to supply the ions necessary for remineralisation. The patient should be instructed to apply the crème numerous times per day to the lesion particularly in the first week following the lesion modification. Treatment of the lesion may take several months until no more improvement is noted. Revisiting the modification process may be

necessary if access has not been gained via the initial modification procedure.

10.5.2 Chewing gum

The use of CPP–ACP-containing sugar-free chewing gums is a useful adjunct to other preventive strategies in high and extreme caries risk patients. The clinical recommendations for chewing sugar-free gums containing CPP-ACP are to chew three to four times a day. This advice is based on a randomised clinical trial that found chewing CPP-ACP-containing gum three times a day reduced the progression and increased the regression of dental caries (Morgan *et al.*, 2008). This product is ideal for patients with a high caries risk as it leads to the incorporation of CPP-ACP into the plaque to increase the calcium and phosphate ion reservoirs but also stimulates saliva to aid in salivary clearance and buffering.

10.6 Future trends

CPP-ACP nanocomplex technology has been explored for use in a number of other applications. These new applications may provide dental care providers with a new range of products to contribute to the armamentarium for the prevention and treatment of disease of the dental hard tissues.

10.6.1 New products

Studies have indicated that there is an increase in the prevalence and incidence of dental erosion (Dugmore and Rock, 2004). The modification of erosive beverages with CPP-ACP has been shown to eliminate their erosivity without having an impact on the organoleptic properties of the beverage (Ramalingam *et al.*, 2005). CPP-ACP could be added to erosive drinks to reduce their erosivity in the future.

The anticariogenic properties of CPP-ACP have also been included in a range of restorative materials and foodstuffs to increase their anticariogencity (Mazzaoui *et al.*, 2003; Walker *et al.*, 2006). In the future, dental products and foodstuffs containing CPP-ACP may become available.

10.6.2 Dentine hypersensitivity

Anecdotally, many clinicians have had success with the crème containing CPP-ACP as a treatment for dentine hypersensitivity. Walsh *et al.* (2006) conducted a 6-week clinical trial comparing the effectiveness of Tooth Mousse with KNO_3. Both groups had a statistically similar level of self-reported symptom relief whether using the Tooth Mousse or KNO_3. Kowalczyk *et al.* (2006) also examined the ability of Tooth Mousse to reduce

dentine hypersensitivity. Prior to application, people reported medium to unbearable pain on a cold air stimulus of 80 of 101 teeth. Immediately after application of the Tooth Mousse this decreased to 38 of the 101 teeth with all others being symptom-free or only mildly painful. Further research to elucidate the mechanism of action of Tooth Mousse in densensitising hypersensitive dentine is necessary, but early results look promising.

10.7 Conclusions

A goal for dentistry is the treatment of dental diseases in a non-invasive manner. Surgical excision of diseased hard tissue should be substituted where possible, with the chemical treatment and restoration of the damaged tissue with new mineral, ideally fluorapatite. In those at-risk individuals, measures should be instituted to prevent the disease from developing. In those where disease is already evident, the lesions should be treated as non-cavitated lesions with the intention of repairing the damaged crystallites to narrow diffusion channels through the entirety of the lesion, restore the structural strength and aesthetic appearance of the lesion towards that of sound tooth tissue and to incorporate fluoride into the lesion as fluorapatite to increase the resistance of the lesion to future acid challenge. CPP-ACP technology is a food derivative that moves dentistry in the direction of avoiding operative treatment for early lesions as scientific research has demonstrated that CPP-ACP can inhibit the demineralisation process and enhance remineralisation of subsurface lesions by providing bioavailable calcium and phosphate ions at the tooth surface.

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11

Antioxidants and periodontal disease

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Abstract: This chapter reviews the nature, catagorization and function of antioxidants and their role in the pathogenesis and prevention of periodontal diseases. The evidence for oxidative stress as a cause of periodontal tissue damage is discussed alongside the evidence base for antioxidant depletion being a feature of periodontal pathology. Sources of antioxidants and potential causes of their depletion in periodontitis are discussed. The chapter concludes with a look into the future potential uses of antioxidants in the management of periodontitis.

Key words: antioxidants, micronutrients, oxidative stress, periodontitis, reactive oxygen species.

11.1 The nature of antioxidants

11.1.1 What are antioxidants?

To define an antioxidant it is necessary first to define an oxidant, as an antioxidant would be its antithesis. Thus traditional terminology describes an oxidant as a chemical that can oxidize another, or more simply, that can remove an electron from the substrate molecule. Antioxidants are molecules that equate to reductants, that is those that donate an electron to a substrate molecule. The term also encompasses molecules that appear to counteract the effects of oxidants. They can be defined as 'those substances which when present at low concentrations, compared to those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate' (Halliwell and Gutteridge 1989). Whilst *in vitro* antioxidants appear to react directly with oxidants, there is also some evidence that antioxidants *in vivo* act upon whole body systems, organs or cells to elicit effects that appear, at least on the surface, to be the same; for example decreasing biomarkers of oxidation (Aldred *et al.*, 2006). Thus a consensus view has emerged that the same chemicals that are termed 'antioxidants' in simple *in vitro* experiments

should be called 'micronutrients' in more complex *in vivo* situations (Azzi *et al*., 2004).

Within the body, chemicals called 'reactive oxygen species' (ROS) are continually generated and this broad term includes molecules that contain:

- oxidizing compounds containing stable paired electrons, such as hydrogen peroxide (H_2O_2)
- unstable paired electrons, such as singlet oxygen ($^1O_2$)
- true free radicals such as the hydroxyl anion radical ($OH^{\bullet -}$)

These naturally occurring oxidants serve as useful agents within the body involved in immune responses and in signalling.

11.1.2 How are antioxidants categorized?

Antioxidants can be categorized in various ways: by chemical properties, location of action or by source (an extensive review is available in Chapple and Matthews 2007). Within all of these categories are small chemicals, such as the well-known antioxidants ascorbate (vitamin C) and tocopherol (vitamin E), as well as proteins, such as the 'antioxidant enzymes', such as the superoxide dismutases and catalase, which catalyse the removal of the oxidants superoxide and hydrogen peroxide, respectively, and thiol containing proteins and peptides which break the free radical generation cascade, such as albumin and reduced glutathione (GSH).

11.1.3 What are the functions of antioxidants?

The efficacy of all the antioxidant species hinges on their localisation and the nature of the oxidative challenge. Other important factors are cooperative interactions with other antioxidant species and environmental conditions that may deem the activity of the antioxidant irrelevant (Fig. 11.1). Antioxidants are naturally present in the body to prevent untoward oxidative damage, keeping the natural production of oxidants in check and maintaining a homeostatic redox balance. It is only when this balance between oxidants and antioxidants is disturbed that oxidative stress results, specifically from oxidant activity outweighing antioxidant defence systems and oxidative damage may arise (Fig. 11.2).

11.2 Antioxidants and periodontitis

11.2.1 Sources of antioxidants

Antioxidants are either synthesized within the body or are taken in through the diet. Dietary antioxidants are generally small chemicals readily taken up from food, which are often phytochemicals; the larger proteinacious species are synthesized in the body.

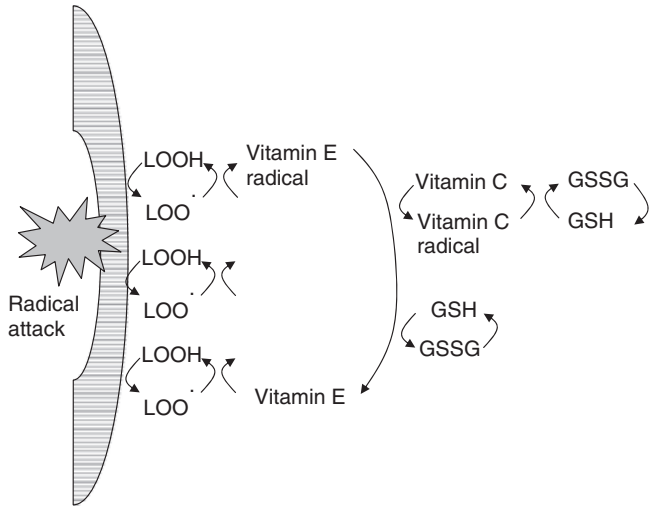

Fig. 11.1 An example of the cooperative nature of antioxidants in repairing lipid peroxidation damage via vitamin E, vitamin C and reduced glutathione (GSH) cycles. Glutathione breaks the chain of radical formation by forming a stable oxidation product (GSSG).

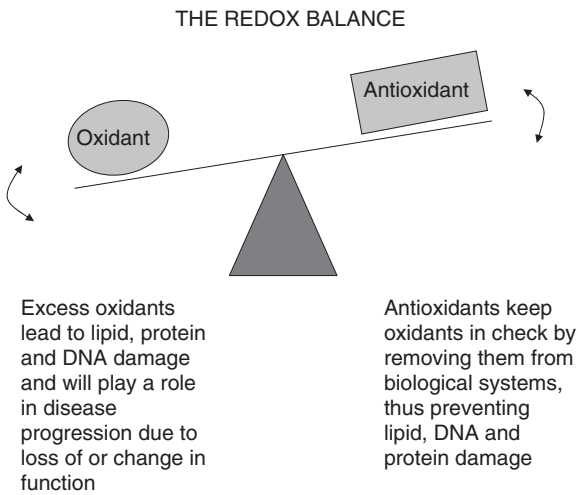

Fig. 11.2 A simplified schematic representing the redox balance in biological systems.

Unlike many mammals, humans cannot synthesize vitamin C, and therefore its sole source is dietary, for example from citrus fruits. Many vitamins have been identified as being vital for health, for instance the involvement of vitamin C in collagen synthesis is apparent when ascorbutic individuals present with scurvy, uncommon now but highly prevalent in previous

centuries. This is just one example of vitamin C's non-antioxidant involvement in cellular metabolism but is also an example of vitamins in general being utilized by the body in non-antioxidant functions. Other phytochemical micronutrients, such as the flavanoids, have been proposed as important antioxidants which are available from balanced diets containing fruits and vegetables. Questions still hang over their mechanism of action within the body and whether it is the parent compound or a metabolite which provides benefits either through direct antioxidant action or through modulation of cellular processes (Azzi *et al*., 2004).

11.2.2 Evidence-base for oxidative stress in periodontitis tissues

Free radicals and ROS have very short half lives (10^{-6} to 10^{-9} s) and are thus difficult to measure *in vivo*; hence the assessment of surrogate markers allows the estimation of levels of *in vivo* oxidative stress:

- For lipid damage, lipid peroxidation products such as conjugated dienes and isoprostanes are measured,
- For damaged DNA, fragmented DNA can be measured as an overall marker (the 'Comet Assay') and individually damaged bases such as 8-hydroxydeoxyguanosine (8-OHdG) can also be measured.
- For protein damage, loss of protein function can be assessed as well as damage to individual amino acids or to the protein backbone as protein carbonyls.

To make a robust case for ROS playing a role in periodontal tissue damage they must be found at the site of injury, at the same time as the injury takes place, and inhibition of their formation should decrease tissue damage. In addition, in experimental models direct application of ROS over a similar time course and at a similar concentration should elicit similar tissue damage to that seen in diseased sites.

It has been suggested that periodontal disease arises in individuals with irregular inflammatory and immune responses to microbial plaque. Neutrophils are the most abundant inflammatory cell within the gingival tissues and they can be a major source of ROS, since they produce superoxide and hypochlorous acid as part of their killing arsenal and indirectly, therefore, also H_2O_2 and $OH^{\bullet -}$. During circulation, ROS are only produced in very small quantities, but at the site of injury neutrophils can either phagocytose microorganisms or degranulate extracellularly, thereby exposing the surrounding tissues to oxidative stress and injury. Interestingly, it has been suggested recently that, in individuals predisposed to periodontitis, circulating neutrophils in these patients have a higher resting ROS output as well as higher stimulated ROS activity (Matthews *et al*., 2007a, b). This higher background activity may be due to priming of the neutrophils by serum born factors such as cytokines or other messenger molecules (Dias *et al*., 2008).

Many studies have assessed the association between neutrophil function and periodontal disease; however, few of these have used periodontally associated plaque bacteria. Whyte *et al.* (1989) demonstrated an increase in ROS production by neutrophils isolated from both young and old chronic periodontitis sufferers in comparison to age-matched controls in response to *Fusobacterium nucleatum*. Similarly, Åsman and Bergstrom (1992) showed an increase in ROS production following *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans* stimulation in neutrophils from juvenile periodontitis sufferers. Other studies have shown a variety of responses of neutrophils isolated from periodontitis patients to either *Staphylococcus aureus* stimulation or to isolated microbial components. Zymosan (a yeast polysaccharide) stimulation appears to be ineffective at neutrophil stimulation in the majority of experiments reported (Mouynet *et al.*, 1994; Biasi *et al.*, 1999; Zafiropoulos *et al.*, 1991), whereas whole bacteria, either opsonized or non-opsonized, demonstrated an increase in ROS production (Åsman and Bergstrom 1992; Gustafsson *et al.*, 1997; Whyte *et al.*, 1989). Taken together, the data demonstrate the potential for increased oxidative stress induced by neutrophils in periodontitis patients in comparison to healthy controls.

To assess whether increased oxidative stress occurs in periodontitis, the markers of oxidative stress need to be measured at the site of disease. To assess oxidative damage, samples need to be obtained in proximity to the site of disease. Thus in periodontitis the fluid of choice would be gingival crevicular fluid (GCF) as this flows directly through the area of host–microbial conflict. GCF is a serum transudate in health, comprising serum-derived fluid and blood cells and similar in composition, therefore, to serum. However, in disease it resembles more a tissue exudate, where products of the plaque biofilm and the host response to immunostimulatory components of that biofilm are added and change its basic composition. However, GCF contains relatively few eukaryotic cells compared with whole tissue samples and thus measurement of DNA damage is difficult as cellular material rich in DNA is needed to provide sufficient sensitivity for such measures of oxidative stress. Koichi Ito's group in Tokyo have used saliva as a source of oxidative stress biomarkers in periodontitis patients showing increased levels of 8-OHdG (Takane *et al.*, 2002; Sawamoto *et al.*, 2005). Only one study to date (Sugano *et al.*, 2000) has investigated DNA damage in gingival tissues using polymerase chain reaction (PCR) and demonstrated deletions in mitochondrial DNA of tissues from periodontitis patients, consistent with chronic inflammatory disease. In a recent paper by Akalin *et al.* (2007), the non-specific marker for lipid peroxidation, malondialdehyde (MDA), was demonstrated to be higher in GCF and saliva in periodontitis patients compared to controls; whilst in another Turkish group (Kurtis *et al.*, 2007) thiobarbituric acid reactive substances (TBARS), another non-specific marker of lipid peroxidation, showed a non-significant decrease after treatment. Panjamurthy *et al.* (2005) also demonstrated that TBARS were sig-

nificantly raised in the plasma of periodontitis patients. Protein oxidation has been reported as salivary carbonyl levels. Scully and Langley-Evans (2003) found increased carbonyl levels in unstimulated saliva from periodontitis patients. Taken as a whole, these data suggest an increase in oxidative stress in periodontitis; the true situation is not clear cut, perhaps owing to the non-specificity of some of the biomarkers selected (TBARS, MDA) and limited sample volumes available, particularly for GCF.

11.2.3 Evidence for antioxidant depletion in periodontitis

Increased levels of oxidative stress biomarkers in periodontitis may indicate a depletion of antioxidants in response to excessive ROS release. This may reflect an underlying genetic predisposition in individual patients which allows for the initiation of the disease or facilitates its progression, or it may be a consequence of the disease process, where the release of ROS during inflammatory responses consumes radical quenching species faster than they can be replenished. A third alternative is that there may be defects in the protein antioxidant response, for instance, polymorphisms in key enzymes or receptors that mediate a decrease in antioxidant capacity by decreasing the removal of oxidant species.

When assessing the presence and prevalence of antioxidant depletion, different tissue or fluid compartments can be utilized. The most representative tissues or fluids will be those adjacent to the site of disease and thus either a gingival biopsy or GCF could be considered the best. Other fluids more readily available are saliva and plasma or serum. Saliva, whilst having the advantage of being an orally derived fluid, only bathes the 'outside' of the periodontal tissues and not those in contact with the crevice and the supposed site of disease. Indeed, the functional role, mode of synthesis, release and composition of saliva, render it at best a surrogate fluid for the smaller volumes of GCF which enter into it, and which better represents the periodontal tissues themselves. Plasma or serum, which contribute a large proportion of GCF volume and composition, lack the additional components released by the host and periodontal organisms at the site of disease. Brock *et al*. (2004) argue that GCF, saliva, plasma and serum are completely different compartments for the assessment of antioxidant capacity. In GCF the main antioxidant is reduced glutathione (Chapple *et al*., 2002), in saliva it is uric acid, ascorbate and albumin (Moore *et al*., 1994) and in plasma (or serum) it is albumin. However, chemicals in lower concentration may also contribute to an overall total antioxidant capacity.

Despite these arguments, many studies have used the aforementioned compartments. Diab-Ladki *et al*. (2003) demonstrated that saliva from periodontitis patients had a lower antioxidant scavenging capacity than that of unaffected controls despite levels of ascorbate, urate and albumin being unaffected by disease status, whereas Moore *et al*. (1994) demonstrated no

difference between periodontitis patients and healthy controls. In 2003 Scully and Langley-Evans found reduced saliva antioxidant status and concomitant increases in protein carbonyls in unstimulated saliva. One of the problems with interpreting data from saliva analysis is the confounding influence of salivary flow rates upon the concentration of key antioxidant components (Chapple *et al*., 1997). To date, the picture in saliva remains unclear in terms of how its antioxidant composition is affected in periodontitis.

Over three decades ago Slade *et al*. (1976) failed to show any difference between serum vitamin E levels in periodontitis patients and controls. Several groups have investigated vitamin C levels in periodontitis. Amaliya *et al*. (2007) and Amarasena *et al*. (2005) found an inverse relationship between serum vitamin C levels and the severity of periodontal disease measured by clinical attachment loss. The same group also published work on serum albumin levels (Ogawa *et al*., 2006). However, epidemiological studies have demonstrated that higher than RDA doses of vitamin C are not associated with better periodontal health (Ismail *et al*., 1983) and an early analysis of the NHANES III database only showed a weak association with periodontitis and vitamin C intake (Nishida *et al*., 2000). However, Leggott *et al*. (1986) supplemented 11 healthy men and demonstrated ascorbate plasma changes in response to that supplementation, but lingual and salivary ascorbate levels did not change. Only on dietary depletion to 5 mg/day did an increase in propensity for bleeding on probing occur; this was not evident at 65 mg/day, the recommended daily allowance, or at 605 mg/day, a supplemented dosage (Jacob *et al*., 1987).

Later in 1991, Leggott *et al*. investigated how a similar regime affected the composition of the subgingival plaque microflora. Interestingly, in 2003 Pussinen *et al*. showed that only *P. gingivalis* seropositivity was associated with a lower plasma vitamin C level. This confused image of vitamin C is also compounded by its essential involvement in collagen biosynthesis.

Does the underlying lower vitamin C level assist the 'invasion' of periodontal tissue destructive pathogens or does their invasion into the tissues cause depletion of vitamin C via oxidative stress or a combination of the two? The answer, in part, may be explained by the fact that these early studies utilized non-periodontitis patients to evaluate antioxidant levels and effects and vitamin C was assessed by 24-hour dietary recall or food frequency questionnaires, which do not reflect serum biochemistry (Gregory *et al*., 1990; Knutsen *et al*., 2001). Chapple *et al*. (2007a) addressed this aspect in an NHANES III analysis of 11 480 adults and demonstrated that plasma vitamin C concentrations were inversely associated with prevalence of periodontitis. Higher serum vitamin C concentrations were associated with lower odds ratios for severe periodontitis and in a subanalysis of never-smokers, the protective effect was more pronounced. We concluded that increased serum antioxidant concentrations were associated with a reduced relative risk of periodontitis even in never-smokers.

We have also demonstrated recently, in a smaller case-control study, a borderline difference in plasma total antioxidant status but a clearly significant difference in GCF antioxidant status between patients and controls (Chapple *et al*., 2007b). Moreover, reduced glutathione levels were lower in GCF samples of periodontitis patients than in controls (Chapple *et al*., 2002), whereas Guarnieri *et al*. (1991) failed to show a difference between periodontitis and control subject GCF total antioxidant capacity. In the same NHANES analysis as cited for serum vitamin C levels (Chapple *et al*., 2007a), we found that serum total antioxidant concentrations were associated with lower odds ratios for severe periodontitis, in the subpopulation of never-smokers.

Within the gingival tissues, cellular components can be used to measure cell antioxidant status, such as SOD and catalase, two key antioxidant enzymes for the removal of superoxide and hydrogen peroxide, respectively. Ellis *et al*. (1998) found the activity of these enzymes decreased as pocket depth increased (>6 mm), and Akalin *et al*. (2005, 2008) and Baltacioğlu *et al*. (2006) showed lower superoxide dismutase (SOD) activity in periodontitis patients than in control groups. They also demonstrated it was lower in GCF but in a previous study no differences in GCF SOD activity were found (Akalin *et al*., 2005). Glutathione peroxidase, an enzyme involved in the reduction of lipid hydroperoxides, is also negatively correlated with pocket depth and attachment loss in periodontitis patients and activity of this enzyme increased upon treatment (Huang *et al*., 2000).

It could be argued that the measurement of cytokines which attract ROS-producing leukocytes such as neutrophils within GCF, may be a marker of potential oxidative stress in the periodontium. A correlation study in periodontitis between oxidative stress markers, antioxidants and cytokines in GCF has not yet been performed. However, several studies determining levels of cytokines in GCF have been published in the last 10 years. IL-8, a neutrophil chemoattractant, has been found in the GCF of both healthy and periodontitis patients, although more is found in periodontitis (Gamonal *et al*., 2000; Lee *et al*., 2003). Mathur *et al*. (1996) found higher total amounts of IL-8, but lower concentrations owing to increased flow of GCF in periodontitis. IL-1beta is also found to be increased (Goutoudi *et al*., 2004; Zhong *et al*., 2007; Mogi *et al*., 1999) as is IL-6 (Mogi *et al*., 1999; Lin *et al*., 2005). Receptor activator of nuclear factor kappa B ligand (RANKL) has also been found to be increased in GCF (Vernal *et al*., 2004) as has TNFα (tumour necrosis factor α). No studies have yet been published that show that cytokine levels respond to antioxidant status in periodontitis.

Polymorphisms in key enzymes or receptors may change antioxidant capacity, for instance vitamin D receptor polymorphisms. Although reports on this protein are mixed (Machado de Souza *et al*., 2007; Naito *et al*., 2007; Park *et al*., 2006; Zhang *et al*., 2005; de Brito Júnior *et al*., 2004; Tachi *et al*.,

2003; Yoshihara *et al*., 2001; Hennig *et al*., 1999) concerning their association with periodontitis, it may be that, in particular ethnic subgroups, vitamin D receptor polymorphisms may play a role in determining the severity of particular types of periodontitis. The complexity of this receptor and its associations warrants its own review and is only mentioned briefly here. Glutathione-S-transferase M1 and cytochrome P450 1A1 are enzymes involved in the detoxification of tobacco-derived substances. Polymorphisms in the genes of these proteins were demonstrated to have a significant risk of association with periodontitis (Kim *et al*., 2004).

11.3 Micronutrients in future therapeutic approaches to periodontitis

11.3.1 Use of antioxidants in managing periodontal disease

To date, only a limited number of studies have reported on the use of antioxidants as an intervention for periodontal disease. Neiva *et al*. (2005) used a vitamin B complex supplement (5 B vitamins 50 mg/day plus biotin, folate and cobalamin) after periodontal therapy to assess effects on periodontal wound healing. They found changes in clinical attachment loss were more favourable in supplemented individuals compared with controls. Previously, Muñoz *et al*. (2001) used a nutritional supplement containing seven active ingredients over a 60-day period and demonstrated that only in the deepest pockets (>4 mm) was there an improvement in gingival index and probing pocket depth with supplementation. Woolfe *et al*. (1984) took 10 non-deficient individuals and supplemented them with 250 mg vitamin C/day. They found no significant clinical benefit; however, this is likely to be due to the subjects already being replete with vitamin C. Vogel *et al*. (1986) managed to increase plasma vitamin C by supplementation but no differences in neutrophil chemotaxis or in experimental gingivitis induction could be demonstrated. Staudte *et al*. (2005) used grapefruit consumption to increase plasma vitamin C, from a below normal range to a normal range, and demonstrated improved sulcus bleeding scores, though this could have been associated with an increase in collagen synthesis.

Topical application of coenzyme Q10 in chronic periodontitis improved the gingival index and bleeding on probing at treatment sites (Hanioka *et al*., 1994). However, a vitamin E gel (800 mg/12 ml) showed no significant effect on plaque or gingival indices (Cohen *et al*., 1991). Lastly, *in vitro* toothpaste antioxidant actives containing ascorbyl phosphate demonstrated protection against DNA fragmentation by hydrogen peroxide treatment in cultures of keratinocytes (Battino *et al*., 2005). One of the problems of intervention studies involving antioxidants is that phytonutrients work in concert with endogenous antioxidant systems in complex cascades (Fig. 11.2). The ingestion of individual vitamins or 'nutraceuticals' (synthetic supplements) provides incomplete nutrition and it is difficult to determine

whether negative results from such intervention studies are genuine or caused by a failure to provide the essential co-factors for optimal micronutrient absorption and/or function.

11.4 Sources of further information and advice

Information gathered from studying other diseases in which oxidative stress is implicated such as cancer, atherosclerosis, diabetes and neurodegenerative disorders (Rodrigo *et al*., 2007), may shed light upon micronutrient mechanisms involved in periodontitis. Inflammatory diseases such as rheumatoid arthritis which involves a similar neutrophil influx to that in periodontitis may be of particular interest. Evidence from studies in rheumatoid arthritis suggests that vitamin E and omega-3 fish oils decrease proinflammatory cytokines and lipid mediators such as the eicosanoids (Tidow-Kebritchi and Mobarhan, 2001). However, more long-term randomized studies are required to prove the proposed benefits of specific nutritional supplementation (Rennie *et al*., 2003). A systematic review of antioxidant treatment of rheumatoid arthritis (Canter *et al*., 2007) concluded that there is no convincing evidence yet of vitamins A or C or selenium, either singly or in combination, being effective, although there was weak evidence for vitamin E.

11.5 Future trends

As in other fields, examining the efficacy of antioxidants in treating disease requires future plans to incorporate large, well-designed, randomized controlled trials. With improvements in the sensitivity of assays to determine levels of markers of oxidative stress and cytokines, such as multiplexed high throughput immunofluorescent assays and advanced mass spectral techniques, better understanding of how periodontitis is modulated by antioxidant therapy or supplementation will be gained. Lastly, focussing more on directly relevant samples will also increase our knowledge of the disease processes. In conclusion, recently conducted larger-scale association studies support the contention that periodontitis is associated with more subtle, but nevertheless significant decreases in antioxidant micronutrient status and that oxidative stress is a feature of periodontitis. However, longitudinal intervention studies of disease initiation and progression, as well as post-therapy stability, are required, ideally using phytonutrients rather than individual supplements. Such studies are complex to design and costly, but offer the best opportunity of determining whether antioxidant micronutrition is beneficial in preventing or treating inflammatory periodontal diseases.

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12

Anticaries and antiadhesive properties of food constituents and plant extracts and implications for oral health

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Abstract: The aim of this chapter is to review the knowledge acquired on those food constituents and plant extracts that exert anticaries and/or antiplaque activity by inhibition of cell growth, adhesion and virulence factors of either mutans streptococci or periodontopathogenic bacteria. The role of the phenolic components is emphasized. The potentially important implications of these studies for human oral health are then discussed together with the possible mode of administration of the compounds.

Key words: adhesion of oral bacteria, caries, food polyphenols, gingivitis/periodontitis, prevention of caries and periodontitis.

12.1 Introduction

Food and drink have long been considered the main factors responsible, along with poor oral hygiene, for caries and periodontitis, the two main oral pathologies whose aetiological agents are to be found among the bacterial inhabitants of the oral cavity. This was shown to be true especially after the discovery of the synergistic role of dietary sucrose and *Streptococcus mutans* in caries initiation and progression (Loesche, 1986). It is generally thought that the sugar (and protein) content of food and drink stimulates the over-proliferation of oral bacteria, organized in a biofilm structure (Kolenbrander and London, 1993), to such an extent as to be responsible for dental and gum damage in the host. More recently, the role of food and drink has been reassessed, in that beneficial compounds for oral health have been detected

and characterized. This is by no means new, however, because the folk medicine of populations around the world has used herbs, plants and derived beverages for thousands of years to cure human diseases and maintain oral hygiene.

The most emblematic example worthy of mention is the Japanese popular notion that green tea 'makes the mouth clean' and, more specifically, 'there is an old tradition that those who drink large amounts of tea have less tooth decay' (Hamilton-Miller, 2001). Based on these observations, tea (in the form of tea-leaves from *Camelia sinensis*) was one of the first natural products studied for its anticaries and antiplaque activity. Several additional types of food and drink of vegetable origin were later studied in a certain amount of detail (e.g. cranberries, wine, coffee, cocoa) together with sporadic reports of dozens of herbs and plants that possess intrinsic anticaries and/or antiplaque activity. During the 1980s the phenolic component of vegetables was indicated as the one endowed with biological activity. These studies are of great potential interest in that the availability of natural products for daily oral hygiene goes some way towards overcoming the increasing mistrust of 'man-made' chemicals that has been reported among the general public, while increasing support is emerging for naturally occurring compounds.

The aim of this chapter is to review the knowledge acquired in this field of research, in an attempt to identify specific active compounds and analyse their anticaries and antiplaque activity. The important repercussions of these studies for human oral health will be discussed together with the mode of administration of the compounds.

12.2 Plaque-dependent oral pathologies

Dental plaque is a microbial biofilm composed of hundreds of different bacterial species enveloped by, and tightly adherent to the tooth surface via, exopolysaccharides (EPSs) of bacterial origin (see Chapter 1). The main oral pathologies resulting from plaque accumulation are caries and gingivitis/periodontitis (Marsh and Martin, 1999). The aetiological agents responsible for caries are mainly *Streptococcus mutans* and *Streptococcus sobrinus*, both belonging to the oral streptococcal species (Loesche, 1986). The initial adhesion of bacteria to the tooth surface is mediated by a variety of bacterial surface molecules that recognize specific glycoproteins of the acquired pellicle, an oral biofilm of salivary origin. Bivalent cations are mainly involved in this mechanism of early adhesion. Later, tight adhesion to the tooth surface is mediated by the synthesis of a sucrose-dependent exopolysaccharide, a water-insoluble glucan called mutan. The third phase of the pathogenic pathway is the production of lactic acid, together with the ability to tolerate it by these microorganisms to such an extent as to solubilize hydroxyapatite of the tooth enamel, resulting in the initiation of the carious lesion and its progression over time. Although mutans streptococci play the main role in

caries initiation and progression, other microorganisms such as lactobacilli have been considered as secondary invaders of the caries lesion. *Actinomyces* species, on the contrary, are involved in root surface caries, as the result of gum retraction. Periodontal disease is the commonest cause of tooth loss in adults. As opposed to dental caries, in which a single odontopathogenic bacterium plays the main role, the aetiology of periodontal disease is more complex and a bacterial consortium (mainly composed of anaerobic bacteria) has been suggested as the trigger (Socransky *et al.*, 1998). Bacterial toxins produced by this mix of bacteria are responsible for the tissue damage observed with detachment of the gum and production of so-called periodontal pockets. The depth of the pockets is proportional to the severity of the disease, which ultimately results in tooth loss. Clinical and laboratory evidence shows that, as in the case of caries, regular oral hygiene, by lowering bacterial counts, allows us to block or reduce disease progression.

12.3 Classic approaches for the prevention of tooth and gum pathologies

Dental caries is a multifactorial pathology in which four elements should be present simultaneously: (i) host susceptibility, (ii) dental plaque (i.e. the odontopathogen), (iii) dietary habits of the host (i.e. the availability of sucrose in the oral cavity) and (iv) time to produce the caries lesion (Loesche, 1986). Preventive approaches are based on the removal of one of the risk factors and include: (i) fluoride administration which enhances the stability of enamel and dentine to acid dissolution, (ii) substitution of dietary sucrose by less cariogenic sugars (e.g. xylitol) and/or a more rationale daily intake of sucrose and, finally, (iii) removal of odontopathogenic bacteria by mechanical methods, by oral disinfection or by vaccination. The search for natural compounds endowed with either antimicrobial or anti-adhesive activity is part of the process of odontopathogen removal. Prevention of gingivitis and periodontitis, on the other hand, is more complex owing to the higher degree of complexity of the diseases. Removal or reduction of the dental plaque, however, plays a crucial role.

12.4 Phenolic content of food and plant extracts

Polyphenols are a broad family of chemicals widely distributed in vegetables. Their antioxidant activity has been found to be associated with beneficial effects on health. The daily intake of polyphenols has been calculated as 0.5–1 g. Polyphenols can be classified as phenolic acids and flavonoids.

Phenolic acids are simple molecules universally distributed in plants and include hydroxybenzoic (e.g. *p*-hydroxybenzoic, gallic and ellagic acids) and hydroxycinnamic acids (e.g. *p*-coumaric, caffeic and ferulic acids). Further-

more, phenolic acids occur naturally as esters or glycoside conjugated with other natural compounds such as flavonoids, alcohols, fatty acids, sterols and glucosides. Flavonoids are widely distributed, albeit not uniformly, and thus specific vegetables are rich sources of one of more subclasses of these polyphenols. They include: (i) anthocyanins and anthocyanidins, which are large water-soluble coloured molecules responsible for the brilliant colour of flowers, fruits and vegetables; (ii) catechins (or flavonols) especially found in tea, grape seeds and cocoa beans, including the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin and epicatechin 3-*O*-gallate; (iii) flavones (e.g. apigenin); (iv) isoflavones; (v) lignins; (vi) proanthocyanidins present mainly in red wines; (vii) procyanidins (oligomeric catechins) found in red wine, grapes and grape seeds, cranberries, cocoa and apples; (viii) stilbenes (resveratrol); (ix) tannins, which are large molecules contained in red wine, tea and nuts, including hydrolysable tannins and condensed tannins. The phenolic composition of several natural foods and plant extracts has been determined. We report a few representative examples below.

12.4.1 Tea

Three different kinds of tea are commercially available: green, black and oolong tea. They are obtained by different treatments of *Camelia sinensis* leaves resulting in different chemical compositions. Green tea is obtained by steaming or drying fresh tea leaves, thus maintaining the original chemical composition. Polyphenols may account for up to 30% of the dry weight and include flavonols, flavonoids and phenolic acids. Flavonols (catechins, molecular mass (MM) <450 Da) are the main green tea polyphenols and include: (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC), (+)-gallocatechin (GC) and (+)catechin (C). Alkaloids such as caffeine, theobromine and theophylline account for 4% of green tea, with phenolic acids such as gallic acids making up another 4%. A cup of green tea (250 ml) contains about 300 mg of catechins. Black tea is the result of extensive oxidation and condensation of catechins during the fermentation process to produce larger, dark-coloured molecules including theaflavines (500–1000 Da) and thearubigins (1000 Da). Thearubigins represent about 20–30% of the dry weight of black tea. Simple catechins, however, are still present. A cup of black tea contains about one-third the polyphenols found in green tea. Oolong tea is a partially oxidized tea and contains monomeric catechins, theaflavins and thearubigins.

12.4.2 Cranberries

Cranberry (*Vaccinium macrocarpon*) juice and fruits are reported to be health foods endowed with potent antioxidant and anticancer activity, with

vasorelaxing effects as well as inhibitory effects on bacterial adhesion in the urinary tract and biofilm formation (Reid *et al*., 1992). The biological effects have been linked to a large variety of different compounds including sugars (40%), organic acids (30%), low MM phenolic acids (1.5%), flavonol glycosides (2.5%), proanthocyanidins (5.5%) and anthocyanins (galactoside and arabinoside conjugates of cyanidin and peonidin, 1.2%) which confer the characteristic red colour to cranberries (Seeram *et al*., 2004). Total polyphenols amount to 10–12% of the dry weight. Several biological activities have been attributed to a non-dialysable material (NDM) consisting of a high MM cranberry fraction mainly composed of proanthocyanidins and only a small amount of anthocyanins but devoid of sugars and acids (Labrecque *et al*., 2006).

12.4.3 Wine and grapes

Wines, grapes and grape pomace have a chemical composition that varies considerably depending on the variety of the grapes, and on environmental and seasonal factors, vintage and vinification technology. In addition, the phenolic of red and white wines is different, red wine having higher concentrations (up to 10 times higher). Differences in phenolic concentration have been adduced to explain why white wine is endowed with a lower antioxidant activity in comparison with red wine (Vinson and Hontz, 1995). Phenolic compounds in red wine are derived from the grape skin, seeds, stems and pulp, all of which are important sources of flavanols which are transferred during contact with grape juice in the course of fermentation. In contrast, white wines are produced by fermenting grape juice without (or with very brief) contact with grape skins. In addition to the presence of ethanol, red and white wines contain organic acids (acetic, citric, lactic, malic, succinic and tartaric acid), monomeric and oligomeric flavan-3-ols, anthocyanins, proanthocyanidins, and low- and high-degree-polymerization condensed tannins (Daglia *et al*., 2007a). Grape pomace is characterized by a high phenol content owing to poor extraction during vinification. Anthocyanins, cathechins, flavonol glycosides and phenolic acids are the main components of grape pomace (Kammerer *et al*., 2004).

12.4.4 Coffee

Coffee brew, produced with roasted coffee powder, is one of the most widely consumed beverages in the world, partly owing to its putative health benefits. Natural green coffee beans contain higher amounts of caffeine, trigonelline, nicotinic and chlorogenic acids than the roasted beans and their antioxidant activity has been linked to the presence of these compounds (Daglia *et al*., 2000). The roasting process allows the production of several new compounds (not included among the polyphenols) as the result of the Maillard reaction, carbohydrate caramelization and pyrolysis of

organic compounds (Daglia *et al*., 2000). A significant decrease in chlorogenic acid content (up to 90%) and the formation of high MM brown compounds (melanoidins) and heterocyclic compounds such as furan, pyrroles and maltol are observed during the roasting process (Fuster *et al*., 2000; Daglia *et al*., 2000). Melanoidins are endowed with potent antioxidant activity. The formation of high MM brown melanoidins has also been described in the roasting process of the barley, from which a brew, called barley coffee, is obtained. This kind of coffee is considered to be coffee substitute, especially for children.

12.5 Main foods and plant extracts with anticaries and antiplaque activity

Screening in PubMed performed in December 2007 yielded more than 350 hits about this subject. The food or plant extract with the highest reference scores was tea cited in about 110 publications; other foods of interest were cranberries, cocoa, propolis, mushrooms, red and white wine, coffee and apples. To these publications regarding specific foods and beverages, we must add about 100 additional single or sporadic articles evaluating a total of about 130 plant extracts.

12.5.1 Tea

The salutary effect of tea was recognized thousands of years ago. Green tea has long been considered by traditional Chinese medicine as a healthful beverage. Several studies suggest that tea may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer, as well as to the promotion of oral health and other physiological functions such as antihypertensive effects, body weight control, solar ultraviolet protection, increased bone mineral density, anti-fibrotic properties and neuroprotective potency (Lotito, 2006). The observation of such important properties has led to the designation of tea as first in the group of beverages endowed with functional properties. The first scientific approaches aimed at proving the effects of tea on oral health date back to the early 1950s. The first hypothesis postulated the high fluorine content of tea as the main anticariogenic activity (Gershon-Cohen and McClendon, 1954; McClendon and Gershon-Cohen, 1957) and in the 1980s the specific effect of tea polyphenols was demonstrated (Onisi *et al*., 1981a; Kashket *et al*., 1985). The effects of tea polyphenols on oral bacteria are listed in Table 12.1.

Antimicrobial activity (both inhibitory and bactericidal) was determined for green and black tea extracts and catechins on mutans streptococci. Minimal inhibitory concentrations (MICs) range from 50 to 500 mg l^{-1} (Sakanaka *et al*., 1989; Sasaki *et al*., 2004), but, in general, there is a consensus of opinion that the polyphenol concentration in a cup of tea is enough

Table 12.1 Anticaries and antigingivitis activity of common foods and plant extracts

Foods and plant extracts	Anticaries activities (against mutans streptococci)	Antigingivitis activity
Tea	Antimicrobial Antiadhesion Inhibition of acid production Inhibition of GTFs Antiplaque (*in vitro* and *in vivo*) Animal model (rat, hamster) Epidemiological studies (Tibetans, Israeli Arabs, Philippines schoolchildren, Senegalese, Dallas schoolchildren, Japanese schoolchildren, English schoolchildren) Clinical trials	Inhibition of growth and adhesion of *P. gingivalis* Inhibition of cysteine proteinase of *P. gingivalis* Inhibition of toxic end metabolites of *P. gingivalis* Inhibition of tyrosine phosphatase of *P. intermedia*
Cranberries	Antimicrobial Antiadhesion (HA) Antibiofilm Inhibition of glucan synthesis by GTFs Reduction of *S. mutans* counts in saliva	Antimicrobial (*P. gingivalis*) Antiadhesion (*P. gingivalis*) Antibiofilm (*P. gingivalis*) Antipathogenicity factor (cysteine protease of *P. gingivalis*) Anti-inflammatory activity
Cocoa	Antimicrobial Antibiofilm *in vitro*/*in vivo* Inhibition of GTFs Cariostatic in an animal model (hamster)	Inhibits collagenase of *P. gingivalis*
Edible fungi	Antimicrobial Inhibition of glucan synthesis by GTFs Antibiofilm Animal model (rat)	Antimicrobial (*Porphyromonas* spp. and *Prevotella* spp.)
Coffee	Antiadhesion (HA)	NE
Wine and grapes	Antimicrobial Antiadhesion Antiplaque Inhibition of GTFs	NE
Propolis	Antimicrobial Antiadhesion Animal model (rat)	NE

HA, adhesion to hydroxyapatite beads; NE, not evaluated.

to exert an inhibitory and often bactericidal action. The mode of antibacterial action, however, has yet to be studied. Interestingly, it has recently been shown that EGCG possesses antimycotic activity against *Candida albicans*, a yeast often involved in oral pathologies (Hirasawa and Takada, 2004). Inhibition of the adhesion of *S. mutans* to saliva-coated hydroxyapatite (HA) by simple catechins extracted from green tea was determined by Otake *et al*. (1991). A substantial decrease in adhesion was determined at a concentration lower than 100 mg l^{-1} which corresponds to a concentration of one cup of tea. Similar results were also obtained for oolong tea extract together with the observation of a reduction in surface hydrophobicity (Matsumoto *et al*., 1999).

Glucosyltransferases (GTFs), the enzymes of mutans streptococci involved in the production of water-insoluble glucan (mutan), are inhibited by polyphenolic compounds of both green and oolong tea. In detail, Otake *et al*. (1991) have shown that EGCG and ECG from green tea are more active than other catechins; Nakahara *et al*. (1993) report that polymeric polyphenols from oolong tea with a MM of about 2000 Da greatly inhibit GTFs, while low MM polyphenols and catechins do not. In addition, the strongest inhibition is observed in water-insoluble glucan synthesis. More recently, by molecular analysis of the effects on *S. mutans* recombinant GTFs it has been shown that the reduction in glucan synthesis is the consequence of inhibition of the glucan-binding domain of the GTFB (Matsumoto *et al*., 2003).

Several studies have been conducted in order to evaluate the *in vivo* anticaries effect of tea polyphenols in animal models (rat and hamster). A 25–50% decrease (statistically significant) in caries scores in different experiments (Ooshima *et al*., 1993; Kempler *et al*., 1997) or a significant reduction in the number of caries lesions was observed (Onisi *et al*., 1981a; Rosen *et al*., 1984). Furthermore, a greater activity of oligomeric catechins from oolong tea was observed in comparison with green tea (Ooshima *et al*., 1998). As far as research in humans is concerned, a few studies have been conducted in order to evaluate decayed, missing, filled teeth (DMFT) and/or plaque score in tea drinkers versus non-tea drinkers. In general, interesting results have been obtained, but no clear conclusion has yet been reached (Onisi *et al*., 1981b; Elvin-Lewis and Steelman, 1986; Jones *et al*., 1999; Ooshima *et al*., 1994). Recently, a significant reduction in saliva counts of mutans streptococci and lactobacilli has been observed, together with a reduced plaque score in adult tea drinkers versus water drinkers (Signoretto *et al*., 2006).

The effect of tea has also been evaluated on periodontopathogenic bacteria such as *Porphyromonas gingivalis* and *Prevotella intermedia*. Inhibition of growth and adhesion of *P. gingivalis* to buccal epithelial cells was observed with EGCG isolated from green tea at a concentration of 250–500 mg l^{-1}. A similar behaviour pattern was seen for ECG, GC and GCG. In contrast, C, EC, GC and EGC had little effect (Sakanaka *et al*., 1996).

The same pattern of inhibition by the different green tea polyphenols was observed for cysteine proteinase and for the toxic end metabolites of *P. gingivalis* (Sakanaka and Okada, 2004; Okamoto *et al*., 2004). EGCG was also shown to inhibit tyrosine phosphatase in *P. intermedia* (Okamoto *et al*., 2003).

12.5.2 Cranberries

Cranberries are a rich source of polyphenols and are biologically active against *S. mutans* (Table 12.1). A high MM, NDM from cranberries inhibits the adhesion of a number of bacterial species (including *S. mutans* to saliva-coated HA) and prevents the co-aggregation of many oral bacterial pairs (Weiss *et al*., 1998, 2002, 2004). In addition, inhibition of GTF activity has been observed (Yamanaka *et al*., 2004; Gregoire *et al*., 2007). More recently, starting from the observation that cranberry juice disrupts the accumulation and acidogenicity of the *S. mutans* biofilm *in vitro* (Duarte *et al*., 2005), the effects of flavonols (FLAV), anthocyanins (A) and proanthocyanidins (PAC) from cranberries have been evaluated on *S. mutans* biofilm development and acidogenicity (Duarte *et al*., 2006; Gregoire *et al*., 2007). PAC (500 mg l^{-1}) and FLAV (125 mg l^{-1}), alone or in combination, inhibit *S. mutans* GTFs and the proton-translocating F-ATPase activities, and acid production by *S. mutans* biofilms. Anthocyanins are devoid of any significant biological effect. It is worth noting that anthocyanins, which give cranberries their red colour, are mainly composed of galactoside and arabinoside conjugates of cyanidin and peonidin.

A clinical trial has been conducted in an attempt to evaluate the efficacy of NDM from cranberries as a mouthwash on salivary bacterial counts in 60 healthy volunteers from the student population of Tel-Aviv University (Weiss *et al*., 2004). Total salivary bacteria and mutans streptococci counts were evaluated over the 42-day study. On day 42, the bacterial counts in the whole saliva of 30 volunteers using an NDM mouthwash showed a marked reduction in both mutans streptococci and total bacteria compared with those in the placebo mouthwash group (30 volunteers). Gingival and plaque indices, in contrast, showed no differences. This apparent discrepancy is explained by the fact that NDM, a potent antiadhesion compound, but one endowed with no antimicrobial activity, acts exclusively via detachment of oral bacteria. The observation that cranberry NDM promotes *S. sobrinus* detachment from artificial biofilm strongly supports this suggestion (Steinberg *et al*., 2005).

Effects of cranberries have also been observed on the periodontopathogen *P. gingivalis* (Table 12.1). The above-mentioned NDM from cranberries has been shown strongly to inhibit *in vitro* biofilm formation and attachment of *P. gingivalis* to mammalian proteins immobilized on a plastic surface. No effect, however, is observed on the growth and viability of bacteria (Labrecque *et al*., 2006). Yamanaka *et al*. (2007) have shown that PC

and FLAV inhibit the synergistic biofilm formation of *P. gingivalis* and *Fusobacterium nucleatum* and, in addition, Arg-gingipain and Lys-gingipain are significantly inhibited at concentrations starting from 1 mg l^{-1}. Anti-inflammatory activity has also been described for cranberry NDM on macrophages stimulated by lipopolysaccharides of different periodonto-pathogenic bacteria (Bodet *et al*., 2006).

12.5.3 Cocoa

The anticaries activity of whole cocoa was demonstrated during the 1960s in the hamster *in vivo* model (Stralfors, 1966 a,b; Stralfors, 1967). The same author also showed that the anticaries activity was associated with the water–alcohol extract (polyphenolic fraction). In recent years, the anticaries activity of cocoa has been evaluated in more detail. Ooshima *et al*. (2000 a,b) have shown that cocoa mass extract has no antimicrobial activity, while cocoa bean husk extract does and it reduces acid production by oral streptococci. Both cocoa preparations drastically reduce the surface hydrophobicity of *S. mutans* and the synthesis of insoluble glucan by the GTFs of both *S. mutans* and *S. sobrinus*. In addition, cocoa bean husk preparations result in a significant reduction in caries development and dental plaque deposition in rats infected with the two cariogenic streptococci. The inhibitory activity of cocoa bean husk extract has been described for collagenase produced by *P. gingivalis* (Osawa *et al*., 1990).

12.5.4 Fungi

Lentinus edodes (shiitake, an edible mushroom very popular in Japan) contains components endowed with an inhibitory effect on water-insoluble glucan formation from sucrose by crude GTF of both *S. mutans* and *S. sobrinus*, together with antiplaque activity (Table 12.1). These laboratory results have been confirmed in an *in vivo* model by the observation of lower caries scores in pathogen-free rats infected with *S. mutans* and fed with a cariogenic diet containing 0.25% shiitake extract compared with rats fed only with the cariogenic diet (Shouji *et al*., 2000). Antibacterial activity has also been reported for different shiitake extracts. Hirasawa *et al*. (1999) have shown that three different extracts (chloroform, ethyl-acetate and water) from shiitake mushrooms display efficient antibacterial activities against oral streptococci, *Actinomyces* spp., *Lactobacillus* spp., *Prevotella* spp. and *Porphyromonas* spp. Interestingly, other medically important bacteria which are not oral inhabitants are relatively resistant. Only the chloroform extract displays bactericidal activity. The data obtained with shiitake extracts therefore indicate dual antimicrobial and antiplaque activity.

Mutastein is a high MM protein produced by *Aspergillus terreus,* which is endowed with anticaries activity in that it is capable of blocking

water-insoluble glucan synthesis by GTFs (Endo *et al*., 1983) and the formation of the artificial dental plaque of *S. mutans* (Nakano *et al*., 1987).

12.5.5 Coffee

The beverage is obtained after the green coffee roasting process, which condenses (i.e. polymerizes) monomeric compounds and produces hundreds of chemicals. The anticaries activities of coffee are listed in Table 12.1. As far as antimicrobial action is concerned, green coffee does not possess any antibacterial activity (Daglia *et al*., 1994), but the roasted coffee exerts activity against enterobacteria (Almeida *et al*., 2006) and *S. mutans* (Daglia *et al*., 2007b). The discrepancy between the two kinds of coffee has been explained in terms of differences in their chemical composition. Naturally occurring coffee compounds, such as chlorogenic acids and caffeine, cannot be responsible for the significant antibacterial activity exerted by the coffee beverage. Daglia *et al*. (2007b) have demonstrated that the antimicrobial activity of roasted coffee is attributable to α-dicarbonyl compounds (glyoxal, methylglyoxal and diacetyl compounds) formed during the roasting process. Caffeine acts synergistically with these compounds.

Green and roasted coffee have also been evaluated for their ability to inhibit the *S. mutans* sucrose-independent adsorption to saliva-coated HA. All coffee solutions exhibit substantial antiadhesive properties (Daglia *et al*., 2002). Inhibition of *S. mutans* adsorption to HA beads was observed both when coffee was present in the absorption mixture and when it was used to pretreat the beads, suggesting that the active molecules present in the coffee may adsorb to the host dental surface preventing tooth receptors from interacting with bacteria. Antiadhesive properties have been observed in natural occurring coffee components, such as trigonelline and chlorogenic acid, and in roasted coffee components such as nicotinic acid and a fraction with an MM ranging from 1000 to 3500 Da, commonly considered to be low MM coffee melanoidins.

Barley coffee, a beverage made with roasted barley, is generally considered to be a coffee substitute, especially for children. In view of the high incidence of caries in young people, studies evaluating the anticaries activity of this beverage would seem to be at least as relevant as studies of the effects of tea. Antimicrobial activity against oral streptococci has been observed only at high barley coffee concentrations [>400 mg of roasted barley powder (RBP) per ml], but, in contrast, has been shown to be very active at a concentration of 30 mg of RBP per ml against the sucrose-dependent and sucrose-independent adhesion of *S. mutans* to HA beads pretreated with saliva (Papetti *et al*., 2007). Antiadhesive activity has been detected in both the low MM (<1000 Da) fraction containing polyphenols, zinc and fluoride ions, and in the >1000 Da fraction mainly consisting of melanoidins, a product formed during the roasting process.

12.5.6 Wine and grapes

Several studies suggest that moderate wine consumption (especially red wine) has a beneficial effect on human health (German and Walzem, 2000). Wines and grapes are very rich sources of flavonols, anthocyanins, proanthocyanidins, catechins and other phenolic compounds. The total polyphenol content is higher in red than in white wine owing to the different mode of production, red wine being mainly obtained after fermentation of grape juice in the presence of grape skin, seeds and stems, all of which are important sources of phenolic compounds (Fuhrman *et al*., 2001). The anticaries activities of wine and grapes are summarized in Table 12.1. Antimicrobial activity against oral streptococci has been demonstrated in both red and white wine with no major differences between the two (Daglia *et al*., 2007a). The compounds responsible for this activity are succinic, malic, lactic, tartaric, citric and acetic acid. Wine polyphenols, in contrast, are completely devoid of activity against oral streptococci (Daglia *et al*., 2007a). The effect of red wine has also been studied on sucrose-dependent and sucrose-independent adhesion and biofilm formation of *S. mutans*. Potent inhibition of both adhesion and biofilm formation has been observed for the red wine fraction with an MM >1000 Da. The fraction with MM <1000 Da was ineffective as far as the inhibition of adhesion is concerned, but very active in inhibiting biofilm growth. This latter result may be explained by the antimicrobial activity of organic acids (Canepari *et al*., unpublished results).

Not only are components of red and white wine of interest for oral hygiene, but also grapes and pomace, a waste product composed mainly of grape skins and seeds obtained by pressing fermented solids during wine making. Polyphenols are very rich in this solid material (Kammerer *et al*., 2004). Thimothe *et al*. (2007) evaluated the effects of the chemical composition and biological characteristics of phenolic extracts prepared from several red grape varieties and the resulting fermented pomaces during wine making on a number of virulence properties of *S. mutans*. Anthocyanin and flavonol contents vary greatly as a function of grape variety and type of extract (whole fruits versus fermented pomaces), but all grape phenolic extracts induce 70–85% inhibition of GTFs B and C at a concentration <62.5 mg l^{-1}. In addition, *S. mutans* acid production is greatly inhibited by the grape extracts without affecting bacterial viability. This effect may be attributed to 30–65% inhibition of F-ATPase activity at a concentration of 125 mg l^{-1}. Thus, these results suggest the possibility of using what today is considered a waste product as an economic source of polyphenols for medical use or as functional food ingredients.

12.5.7 Propolis

Propolis is the mixture of resinous substances collected by *Apis mellifera* bees from various plants and secreted bees waxes and is used by bees for the construction, maintenance and protection of their hives. This natural

product has a complex chemical composition which varies widely according to climate, season, location and year, and possesses several pharmaceutical properties. Many components have been identified in propolis, belonging to the flavonoid aglycone, cinnamic acid derivative and terpenoid groups (Bonhevi *et al*., 1994). Flavonoids are the most biologically active compounds of propolis (Table 12.1). Antimicrobial activity as a function of the chemical components of propolis has been determined. Koo *et al*. (2002) report that flavonones, a number of dihydroflavonols and a sesquiterpene (*tt*-farnesol) inhibit *S. mutans* and *S. sobrinus* growth (MICs of 14 and 28 μg ml^{-1} for *S. sobrinus* and *S. mutans*, respectively). Uzel *et al*. (2005) also indicate that flavonoids are the most active compounds, with MICs of 2 and 4 μg ml^{-1} for *S. sobrinus* and *S. mutans*, respectively. Interestingly, the same authors reported that the MIC for *C. albicans* is 4 μg ml^{-1}.

As far as inhibition of GTFs is concerned, flavones and flavonols are potent inhibitors of these mutans streptococci enzymes, the best being apigenin, a 4′,5,7-trihydroxyflavone (90–95% inhibition at 135 μg ml^{-1}) (Koo *et al*., 2002). More recently, Koo *et al*. (2006) have shown that apigenin modulates the expression of *S. mutans gtfB*, *gtfC* and *gtfD* genes. Apigenin significantly decreases expression of *gtfB* and *gtfC* mRNA, but enhances expression of *gtfD* in *S. mutans* growing as planktonic cells. This means that apigenin is a potentially unique therapeutic compound that affects both the activity of GTFs and the expression of the codifying genes. Animal tests have also been performed in rats with encouraging results (Ikeno *et al*., 1991).

12.5.8 Miscellanea

Apples and hop bract are worthy of separate note. Apples are consumed on a large scale and may be associated with improved dental health. Apple condensed tannins, a mixture of procyanidins, strongly inhibit GTFs. The 50% inhibitory doses are 1.5 and 5 μg ml^{-1} for *S. sobrinus* and *S. mutans*, respectively. Furthermore, inhibition of *S. sobrinus* sucrose-dependent adhesion has been observed (Yanagida *et al*., 2000). Similarly, hop bract polyphenols, extracted from the bract part of hops (*Humulus lupulus* L.) have been shown to be active against caries. The high molecular weight polyphenols (separated by ultrafiltration) potently inhibit the adherence of *S. mutans* and *S. sobrinus* to glass surfaces and inhibit GTFs, but are not endowed with any antimicrobial activity (Tagashira *et al*., 1997). This hop bract fraction, mainly composed of anthocyanidines, has recently been experimented with as a mouth rinse in a clinical trial in Japan in which 29 volunteers were enrolled (Shinada *et al*., 2007). The clinical study was a parallel-group, randomized, double-blind, crossover design. Plaque score, gingival index and bacterial count in plaque samples were used to evaluate the efficacy of the fraction tested. The results showed that plaque scores and the number of mutans streptococci in plaque samples after the

volunteers used the mouth rinse were significantly lower than those when volunteers used the placebo.

Dozens of single reports regarding plants and herbs endowed with anticaries and antiplaque activity should be added to the selected foods and beverages of natural origin mentioned above, for which many studies have been conducted often by different research groups. Interesting results in terms of antimicrobial activity, inhibition of adhesion, inhibition of GTFs or plaque formation of cariogenic streptococci have been described for the following vegetables: *Perilla* seeds, khat, *Kola nitida* (ginseng), *Acacia aroma*, *Sanitaria sagittifolia*, *Cyperus rotundus*, rhizome of *Curcuma xanthorhiza*, seeds of *Psoralea corylifolia* (bakuchiol), *Ceanothus americanus*, *Juglandaceae regia*, *Helichrysum italicum*, *Swartzia polyphylla*, rhizomes of *Hydrastis canadensis*, *Streblus asper*, *Terminalia chebula*, *Zizyphi fructus*, *Hypericum triquetrifolium*, oat hulls, *Chelidonium majus*, *Varthemia iphionoides*, miswak, garlic, onions, *Juglandaceae regia*, *Saussurea lappa*, *Azadirachta indica* (neem), *Asarum sieboldii*, *Psidium guajava* (Guaijaverin), *Areca* nuts, cloves, *Myristica fragrans*, *Roselle calyx* (hibiscus), *Momordica grosvenori*, *Mikana* genus, liquorice and glycyrrhizine, *Galla chinensis*, seaweed, betel nuts, *Polygonum cuspidatum* (bamboo), *Rheum undulatum*, *Rabdosia trichocarpa*, *Cratoxylum formosum*, *Areca catechu*, *Morus alba* (root bark), sugar cane molasses (phenolic compounds), *Erythrina variegata*, oat glume, *Paullinia cupiana*, *Diopyros lycioides* (Namibian chewing stick), several Nigerian chewing sticks (*Vitellaria paradoxa* root, *Bridellia ferruginea* stem and twigs, *Garcinia cola* stem, *Terminalia glaucescens* root, *Morinda lucida* root, *Serindeia warnekei* bark), *Cnestis ferruginea* fruit, Asian chewing sticks (*Acacia arabica* and *Salvadora persica*). It is worth noting that the World Health Organization has recommended and encouraged the use of chewing sticks used in several developing countries of both Africa and Asia as an effective tool for oral hygiene (Al lafi and Ababneh, 1995).

12.6 Effects of bacterial target inhibition by food and beverage components

The virulence of mutans streptococci is mainly due to the expression of three classes of factors including: (i) sucrose-independent colonization, (ii) sucrose-dependent adhesion and (iii) acid production and tolerance. Sucrose-independent colonization allows *S. mutans* cells to achieve early colonization of the tooth enamel surface covered by the acquired pellicle through specific bacterial surface ligands. The main *S. mutans* surface molecules involved are proteinaceous antigen I/II, antigen III, and peritrichous fibrils, as well as lipoteichoic acid (Handley *et al*., 1999). Sucrose-dependent adhesion is mediated by the synthesis of glucans from sucrose by the action of GTFs. *S. mutans* produces three types of GTF – GTFB, GTFC and GTFD

– and their cooperative action is essential for bacterial adhesion. GTFB and GTFC, which mainly synthesize water-insoluble glucans, are located on the *S. mutans* cell surface, while GTFD, which synthesizes water-soluble glucans, is released into the culture medium (Kuramitsu, 1993). Furthermore, *S. mutans* effects glycolysis of multiple carbohydrates efficiently with lactic acid production.

Acidity cannot be buffered by saliva owing to the presence of the poorly permeable exopolysaccharide matrix of the dental plaque. The low pH values contribute to the demineralization of the tooth enamel and to the selection of aciduric microorganisms such as mutans streptococci and later lactobacilli. *S. mutans* is capable of tolerating acidification by increasing proton-translocating F-ATPase activity in response to low pH. This enzymatic activity transports protons out of the cell, maintaining an intracellular pH higher than that of the extracellular environment (Sturr and Marquis, 1992). Based on this knowledge, several useful targets can therefore be identified in *S. mutans* for chemotherapeutic intervention using natural food compounds.

Antimicrobial activity is displayed by several phenolic components of practically all foods and beverages of natural origin evaluated to date. Although little information is available regarding the mode of antimicrobial action, it is worth noting that EGCG and EC disrupt reconstituted bacterial membranes in an *in vitro* system (Ikigai *et al*., 1993). More recently, Navarro-Martinez *et al*. (2006) have shown that EGCG acts in *C. albicans* as an antifolate compound, disturbing its folic acid metabolism and, secondarily, inhibits ergosterol production.

Inhibition of the sucrose-independent adhesion of *S. mutans* has been displayed by tea, cranberries, coffee, wine and propolis. Inhibition of GTF enzymatic activity (as measured by glucan synthesis) has been identified in tea, cranberries, edible fungi, wine, apples and hop bract components. Inhibition of acid production has been demonstrated in tea and inhibition of F-ATPase activity by wine polyphenols. No studies devoted to the analysis of the mode of inhibition of the activity of the above-mentioned proteins have yet been published, but, since catechins are known to have affinity for proteins, it could be speculated that bacterial protein binding produces changes in spatial protein conformation, thus resulting in loss of enzymatic activity or lack of further binding of a host cell receptor. Finally, it should be recalled that the studies conducted to date indicate that more than one target could be simultaneously bound by the different phenolic compounds of a certain food or drink, thus indicating a potential synergistic action against caries or other dental pathologies.

As far as periodontopathogenic bacteria are concerned, little information is available at present about the effects of natural phenolic compounds on their virulence traits. *P. gingivalis* and *P. intermedia* are the two oral pathogens for which inhibition of growth, adhesion, biofilm formation and virulence traits (gingipains) have been evaluated. Inhibitory activity has

been detected in phenolic compounds of tea, cranberries, cocoa and edible fungi. A mode of action very similar to that hypothesized for cariogenic bacteria is to be expected.

12.7 Mode of delivery of polyphenols to the oral cavity

The choice of an appropriate system for delivering food polyphenols to the oral cavity is crucial for developing the reliable prevention of oral pathologies. In order to achieve this objective, several elements must be taken into consideration: (i) facility and feasibility of administration, (ii) compliance of individuals, (iii) release of active compound(s) in the oral cavity, (iv) persistence of active compound(s) in the oral cavity, (v) degradation/modification of active compound(s) by human or bacterial enzymes.

The simplest method for the administration of suitable compounds consists of encouraging the consumption of food and drink containing them. Special attention should, however, be paid to those foods containing sucrose, the main cariogenic sugar. In some cases this may not be easy, especially when the targets of delivery are children. An interesting study regarding the delivery of tea polyphenols to the oral cavity as an oral cancer preventive and/or anticariogenic substance has recently been conducted by Lee *et al*. (2004). Recruited volunteers (aged 25–50 years) were asked to hold in their mouths or chew 2 g of green or black tea leaves for 5 min and then rinse out their mouths thoroughly with water. Saliva was then collected over time to evaluate the concentration of polyphenols by high performance liquid chromatography (HPLC). Initially, the authors hypothesized that chewing green tea leaves would result in a higher salivary level of catechins compared with holding the leaves in the mouth without chewing them. This was not true for all subjects enrolled in the study. A second peak was observed later in the saliva of some subjects but it is unclear whether this was due to the reduction of saliva produced after lengthy chewing of tea leaves or whether tea leaves were trapped between teeth with prolonged release of polyphenols. The results of the same experiment performed with black tea-leaves were hard to interpret because the black tea leaves were too brittle and thus small fragments persist longer in the oral cavity, distorting the experimental findings. Holding pure theaflavins in the mouth resulted in a higher salivary peak than that obtained with black tea extract containing the same concentration. This suggests that additional components of black tea interfere with transfer of theaflavins from the solution into saliva. Finally, several extra compounds of unknown composition were detected by HPLC after holding tea leaves in the mouth, suggesting the creation of metabolites in the oral milieu by either host or bacterial enzymes. In practice, however, chewing or holding black or green tea leaves in the mouth probably results in more effective and economically favourable delivery of these compounds to the oral cavity.

Additional modes of administration should be considered; chewing-gum that slowly releases the active compounds seems to be more acceptable when eating the food or holding it for several minutes is hard to achieve. Mouthwashes and toothpastes are the simplest form of topical application of naturally occurring food compounds for daily oral hygiene. This may be a valid alternative to current mouthwashes and toothpastes containing chemical antimicrobial agents. Moreover, the consumers' demand for new products containing natural components instead of components resulting from a process of chemical synthesis is of by no means secondary importance. Alternatively, new approaches consist of enriching foods with healthy compounds (functional foods) either by adding the desired compound(s) during food processing or by selective breeding of the parent plant to increase the active compound content.

12.8 Implications of using natural food components for oral health

Caries and gingivitis/periodontitis are the most prevalent infectious diseases of humans and result from the accumulation of dental plaque on the tooth surface and in the gingival crevice. These are the only infectious diseases in which the valid preventive approach consists of daily prophylactic measures such as tooth brushing, flossing and mouthwashes. However, the widespread prevalence of oral diseases indicates the inability of most individuals to achieve a level of plaque control consistent with good oral health. In addition, the poorer members of society are often incapable of bearing the costs of regular oral hygiene and thus are affected by these diseases to a greater extent than other people. Professional treatment is therefore required to remove accumulated plaque and remove or restore teeth affected by caries and to treat periodontitis.

In the year 2000 the total costs for oral healthcare in the 28 member and accession states of the European Community/European Economic Area amounted to approximately 54 billion Euros. Hence, what is needed are innovative approaches to oral healthcare intrinsically endowed with simplicity of use; these must be user-friendly (possibly containing natural compounds much appreciated by the consumers), applicable to both children and adults, available at low cost, thus promoting a more generalized use of effective healthcare products with a consequent increase in the overall welfare of the population at large. The research devoted to the identification of natural compounds present in widely consumed food and drink is fully consistent with this proposed approach. Great sensitivity towards this issue has been demonstrated by the European Community, who, within the 6th Framework Programme (FP6), supports, over the years 2006–2009, a more than 2 million Euro research project entitled 'Towards functional foods for oral healthcare – isolation, identification and evaluation of beverage and food components with anti-caries and/or anti-gingivitis activities'.

The aims of this 'NUTRIDENT' project are to: (i) identify constituents of beverages or foods that are able to protect against caries and/or gingivitis, (ii) evaluate the effectiveness of such constituents in clinical trials, and (iii) produce functional foods, toothpaste and mouthwashes containing appropriate active constituents (see the NUTRIDENT web site at http://www.ucl.ac.uk/eastman/nutrident/index.php).

Of by no means secondary importance are the repercussions of oral health on the overall welfare of an individual. The relationship between oral and general health has been increasingly recognized over the past two decades (Li *et al.*, 2000). Oral diseases are also recognized, on the other hand, as an important problem for patients suffering from chronic diseases such as cancer or AIDS as the result of severe immunodepression (Rautemaa 2007).

Oral mucosal lesions may provide an additional portal of entry for systemic life-threatening infections. In view of the fact that oral infection may constitute a concrete risk factor for systemic diseases and vice versa in some instances, the control of oral diseases is crucial for the prevention and/or management of these systemic pathologies. A large number of clinical studies have been conducted in order to establish the efficacy of the use of topical antimicrobial agents such as chlorhexidine or triclosan in the prevention and control of oral diseases (Gilbert *et al.*, 2007). In the light of these observations, the availability of a new, innovative approach for plaque control such as that proposed in this chapter will provide us with a new and possibly potent weapon to combat infectious diseases and their consequences.

Finally, the possibility of producing functional foods and, possibly in the near future, genetically modified plants with increased contents of naturally occurring healthcare components would obviate the need for the use of cosmetic products for daily oral health on that section of the population that is unable to bear such expenses, especially in the developing countries.

12.9 Future trends

The review of the literature presented above has clearly shown that several widely consumed food and plant extracts contain substances belonging to the complex polyphenol family which are endowed with intrinsic positive health effects for humans. This broad range of beneficial properties includes anticaries and antigingivitis activity. Thus, these natural components are promising candidates for extensive future use in oral health. Before any practical application of this knowledge can be achieved, several aspects need to be studied in depth. These include: (i) identification of the precise active component(s) for each food and beverage, (ii) identification of food component(s) which could affect the biological activity of the active compound, (iii) definition of the oral delivery systems for the active compound(s) in terms of lasting biological activity, (iv) validation of the active compound(s)

in large clinical trials. In addition to the implementation of these essential steps, special attention should also be paid to the identification of broad spectrum active compounds (e.g. components simultaneously active against cariogenic and periodontopathogenic bacteria or capable of inhibiting more than one bacterial target), also taking into consideration the possibility of using combinations of different polyphenols endowed with synergistic activity.

There is currently a lack of knowledge about the mode of action of phenolic compounds in terms of inhibition of bacterial growth and killing, if observed, inhibition of bacterial adhesive properties, and inhibition of GTFs. More information on these essential aspects would allow us to select new compounds endowed with a more potent action in the near future.

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13

Food preservatives and dental caries

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Abstract: The use of food preservatives has increased enormously over the past several decades. Intuitively, constant ingestion of food preservatives (antimicrobials) could have a profound effect on the oral flora. Preservatives used range from antibiotics to the more commonly used weak acids, for example benzoates, propionates and sorbates. Weak acids reduce the acid tolerance of microorganisms, damage the properties of their cell membranes and exert their antimicrobial effect in a manner similar to that of fluoride. Results from laboratory- and animal-based investigations support the concept that food preservatives alone or in combination with fluoride could reduce the prevalence of dental caries and possibly other plaque related diseases of the mouth.

Key words: acid tolerance, dental caries, fluoride, glucosyltransferase, weak acids.

13.1 Introduction

Methods for the preservation of food go back almost to the beginning of time. The approaches used in earlier times were to a large extent restricted to physical methods such as drying, salting, heating, cooling and even high concentrations of honey. In this chapter, observations will be confined to commenting on chemicals used in food preservation and their possible effects on oral health and the environment of the mouth.

The regulations governing the use of chemicals for food preservation in the USA have a fascinating history. Initial toxicity studies were carried out in a small group of humans recruited from the US Department of Agriculture. These volunteers ate all of their meals containing various additives including, for example, borax, copper sulfate, sodium benzoate, salicylic acid, sulfuric acid and even formaldehyde! The group became known as the Poison Squad. The result of their contributions was the Pure Food and Drug Act of 1906 which laid the foundation some 30 years later for the Federal Food, Drug and Cosmetic Act which in the main governs the use

of preservatives in foods in the USA. It is of interest that the first national food law was passed in 1860.

The chemical and physical reactions involving salt and smoking have been used in the preservation of food for thousands of years. These methods have included, for example, drying, heating, freezing, fermentation and of course use of chemicals. Introduction of chemical preservatives may appear to be comparatively recent; however, high concentrations of salt have been used for centuries. Smoking has also been extensively used and many spices are recognized for their antimicrobial properties (White, 2002).

13.2 Spices

The antimicrobial effects of spices have received considerable attention, although clinical evidence of their dental effects in the mouth is lacking (Anonymous, 2007). Many oral microorganisms are killed rapidly when exposed to low concentrations of garlic extract (Bakri and Douglas, 2005). The active ingredient is allicin, which is an oxygenated sulfur compound which exerts its inhibitory effects by reacting with free thiol groups. Allicin is also known to penetrate cell membranes. *Porphyromonas gingivalis* protease activity has been shown to be inhibited by garlic extracts containing allicin.

13.3 Clove

Clove oil is not used widely as a food preservative but is often included as a flavor enhancer. The principal ingredient is eugenol, which has potent antimicrobial effects and which of course is still used extensively in dentistry (Burt, 2004).

13.4 Antibiotics

Nisin is an antibiotic which has 34 amino acid residues and is extensively used, particularly in cheeses, as a food preservative (Lubelski *et al.*, 2008). Nisin belongs to the lantibiotics, so named because they contain an unusual amino acid, lanthionine. Nisin is commonly produced by fermentation of natural products such as milk by *Lactococcus lactis* and is active against a range of Gram-positive organisms, including spores, but shows little effect on Gram-negative bacteria, yeasts and fungi (Zottola *et al.*, 1994). Nisin exerts its bactericidal effect by targeting cell wall synthesis. It is important to note that nisin is positively charged and therefore can bind readily to negatively charged plasma membranes. The net result is dissipation of the transmembrane potential because of the creation of pores in the cell wall

(Bonev *et al.*, 2004). Based on its mechanism of action, it might be expected that fluoride and nisin would have an additive antibacterial action. Nisin is active in the pH range 3–3.5 and activity falls off considerably at pH 1.0 and pH > 0. Levels of nisin ranging from 6.0 to 37 mg l^{-1} may be found in processed foods. Feeding primates low levels of nisin in their diet was without effect on caries incidence (Bowen, unpublished).

13.5 Chelation agents

Chelators such as ethylene diamine tetraacetic acid (EDTA) and citrate are often included as aids in the preservation of foods. They act through several possible mechanisms, for example enhancing the permeability of bacterial cell walls and chelating essential ions for bacterial enzymes (Rasooly, 2005). Both agents can dissolve enamel at the appropriate pH (Zipkin, 1953).

13.6 Weak acids

Most of the commonly used food preservatives, for example benzoic acid, its salts and derivatives, belong to a category of chemicals termed 'weak acids'. Weak acids become disassociated at low pH values and do not ionize fully when dissolved in water. They can enter cells readily where they dissociate, can acidify the cytoplasm and disrupt the proton motive force of the cell membrane.

In an extensive review, Marquis (1995) has shown that many enzymes which are essential for cell metabolism are extremely sensitive to acidification of the cytoplasm. NADH oxidases are particularly sensitive. Many microorganisms can survive at low pH values because of their ability to control the pH of their cytoplasm by pumping protons through their cell wall. This is accomplished through ATPases, many of which have optima at very acidic pH values (Sutton and Marquis, 1987). Weak acids can cause enhanced proton permeability of cells resulting in disruption of pH. Some weak acids in combination with aluminium additions are also effective inhibitors of ATPase (Salmond *et al.*, 1984).

Several groups of microorganisms survive at low pH values by altering the pH of their environment. For example, species of streptococci can utilize arginine through the arginine deiminase system to generate CO_2 and NH_3. The system can be inhibited, for example, by parabens (paraminobenzoic acid) (Bender *et al.*, 1986).

13.6.1 Effects of weak acids and fluoride on oral microorganisms

The protective effect of fluoride against development of carious lesions has focused almost exclusively on remineralization of early carious lesions. The

effects of fluoride on the pathogenesis of the disease process has received scant attention, fluoride in aqueous solutions behaves as a weak acid just as most food preservatives. Fluoride like HF can diffuse into bacterial cells where it becomes ionized and in combination with aluminium, inhibits ATPases, which renders the organism less tolerant to cytoplasmic acidification. Furthermore, at low pH values fluoride inhibits enolase; fluoride enhances the proton permeability of cells (Sturr and Marquis, 1990). Fluoride may also affect other virulence traits of oral microorganisms. For example, the production of glucosyl transferase by microorganisms is reduced in the presence of fluoride. The amount of enzyme synthesizing insoluble glucan has been found to be reduced more than the soluble enzyme (Bowen and Hewitt, 1974).

Although food preservative agents are used to treat food, it is frequently forgotten that large amounts of these agents are ingested. The amounts of preservatives that may be added to a particular food is, in general, tightly controlled; the amounts of foods and the variety ingested remain the choice of the consumer. The total amount of preservative ingested in any one day may exceed what might be regarded as prudent. Many of the most used preservatives occur in nature, for example benzoates, sorbates and propionates. Indeed, some fruits, for example dried berries, harbor more benzoate than would be permitted if it was added by a food processor (White, 2002).

13.6.2 Effects of weak acids (food preservatives) and fluoride on the incidence of dental caries

Since the 1970s, there has been a significant decline in the prevalence of dental caries in the Western world and in some segments of the population (Stephen, 1993). It is generally agreed that the observed reduction is attributable to exposure to fluoride, even though sources of fluoride differ from one region to another. In large measure, the decline in the prevalence of caries followed the widespread introduction of fluoridated toothpastes; however, the dimension of the reduction far exceeded that observed in most clinical trials and has raised questions (Scheie, 1992; Haugyorden, 1996) about whether additional factors were also influencing caries prevalence.

Suggested additional influences include exposure to antibiotics (see Nisin), changes in eating patterns including reduction in the consumption of sugars and increase in exposure to food preservatives, such as benzoate (and its derivatives such as parabens), sorbate, propionate salicylate, and non-steroidal anti-inflammatory agents such as ketoprofen (see later). However, there has been a large increase in the use of food preservatives over the last few decades. For example, benzoate use in the USA has increased from 1.2 M pounds (2.64 Mkg) in 1960 to 25.6 M pounds (56.23 Mkg) in 1995. It is estimated that average ingestion of benzoate in the USA is currently 2.3 mg kg^{-1} per person per day compared with

0.18 mg kg^{-1} in Japan. Increase in the use of sorbate is equally dramatic. Similar increases in consumption of sorbate have been observed. Manufactured foods and soft drinks are the primary sources of preservatives that are ingested. Yearly consumption of soft drinks is estimated at 600 12 oz. (336 g) servings per person in the USA. Many toothpastes and mouthwashes contain benzoate, although it is not listed as an active ingredient.

It is clear that over the same time period when the incidence of carious lesions was declining there was a progressive increase in the ingestion of food preservatives. Could this increase in the use of preservatives have contributed to the observed reduction? Direct clinical evidence of an effect is lacking; nevertheless, it is noteworthy that the mechanism of action of many preservatives together with *in vivo* data from animals makes the hypothesis plausible and certainly worthy of more complete investigations. Furthermore, over the same time period, it has been noted that there was a decline in mortality from heart disease which is attributed in part to increasing consumption of salicylates in foods (Ingster and Feinleib, 1997).

13.6.3 Pathogenesis of dental caries

It is essential to explore the virulence properties of dental plaque and some cariogenic microorganisms to facilitate comprehension of how preservatives may affect oral health and dental caries in particular. One of the major features of cariogenic plaque is its ability to lower the pH value of a solution rapidly to a value of 4 or lower (with consequent dissolution of enamel). Clearly, microorganisms present in such an acid milieu have an extraordinary tolerance of acid or they would be unable to survive (Marquis, 1990, 1995). Mutans streptococci, and not *Streptococcus mutans* in particular, and lactobacilli display a remarkable level of acid tolerance. For example, *S. mutans* can continue to carry out glycolysis even when the environmental pH is as low as 4.0. The ability of *S. mutans* and other microorganisms to grow at acid pH values is dependent on their ability to extrude protons through membrane ATPases (see later). Clearly, any substance that can discharge the pH gradient will short circuit the proton barrier function of the membrane and reduce the acid tolerance of the microorganisms. Microorganisms that reside in an acidogenic environment become increasingly tolerant of their hostile environment, thus the acidogenic virulence of bacteria can be enhanced. This enhanced tolerance may be quickly lost if the organism is cultured at neutral pH values. When the ability of microorganisms to extrude protons is compromised, they become susceptible to acid killing. Thus multiple effects may arise when microorganisms are exposed to agents that disrupt the export of protons from the microbial cytoplasm.

The ability to form extracellular polysaccharide (glucan) from sucrose is an additional proven virulence property of *S. mutans*. Up to 20% or more of the dry weight of dental plaque can be made up of glucan. Glucan plays

a critical role in the formation of dental plaque through promoting adherence of microorganisms to tooth surfaces, thereby contributing to the bulk of plaque, affecting the diffusion of substances into and out of plaque and protecting organisms from inimical influences (Kopec *et al*., 1997). The production of glucan by microorganisms is adversely affected in the presence of food preservatives such as benzoate and sorbate (see later).

The effectiveness of several weak acids (e.g. some food preservatives) alone or in combination with fluoride in reducing acid tolerance in *S. mutans* has been explored extensively by Belli *et al*. (1995). The effect was measured by determining the amount of agent required to halt the effect of glucose on a bacterial suspension at pH 4.0 instead of 3.5. The relative effective concentrations of some agents are shown in Table 13.1 (from Belli *et al.*, 1995). Combining agents had additive effects. Clearly, as pointed out by Belli *et al.* (1995), molecules do not have to pass into the cytoplasm but perhaps can bind to the cell membrane and pass protons back and forth through cell membrane.

The production of glucosyltransferases by microorganisms is influenced by the pH of the medium and by the ability of the organism to export the enzyme. It is clear that weak acids (including fluoride) have profound effects on the properties of cell membranes. The ratio of soluble to insoluble glucan produced by *S. mutans* is affected by the presence of sorbate and/or benzoate in the growth medium (Vacca Smith *et al.*, 2001; Vacca Smith *et al*., 2002). In general, more water-soluble glucan was formed in the presence of the preservatives and furthermore, the structure of the glucan formed was affected based on an enhanced susceptibility to dextranase. It is noteworthy that few microorganisms adhere to glucan formed on a hydroxyapatite surface in the presence of sorbate and benzoate.

Based on the mode of action of weak acids and their demonstrated additive effects on acid tolerance and permeability of bacterial cell walls, it might be anticipated that combinations of weak acids such as food preservatives and non-steroidal anti-inflammatory agents and fluoride would have an additive effect in the prevention of dental caries. Using a rodent model

Table 13.1 Effect of weak acids in reducing acid tolerance of *S. mutans*

	Molecular weight	Relative effective concentration (mM)
Benzoate	22	8.0
Cinnamate	148	4.0
Fluoride	19	0.15
Ketoprofen	254	0.50
Salicylate	138	2.0
Sorbate	112	3.0

(desalivated or intact), it has been demonstrated that benzoate at concentrations as low as 0.1% in the animals' drinking water had a profound effect in reducing the incidence of caries in desalivated animals (32%). Combinations of fluoride (15 ppmF) and benzoate (0.2%) had dramatic effects on the incidence of caries and clearly demonstrated an additive effect. Animals exposed to 15 ppmF had 30% fewer lesions than controls, whereas animals exposed to 0.2% benzoate and 15 ppmF had 46% fewer lesions. The populations of mutans streptococci were also significantly reduced in animals exposed to the combination of benzoate (0.2%) and 15 ppm in water (Davis *et al*., 2001). Comparable but less dramatic effects were noted in rats fed up to 0.3% sorbate (Bowen and Pearson, 2000).

In the same vein, support for the concept that combinations of weak acids and fluoride have an additive anti-caries effect can be found in the observation that a combination of ketoprofen and fluoride is more cariostatic than either alone (Bowen *et al*., 2000). Animals treated topically with a paste containing 3% (S)-ketoprofen and 0.1% F had 47% fewer smooth surface lesions than animals treated with F paste alone. Sulcal surface lesions were significantly less severe in the animals treated with the combination compared with those observed in the control animals. These observations are completely consistent with the reported effects of ketoprofen and fluoride on acid tolerance *in vitro* (Belli *et al*., 1995).

Results of studies conducted in humans show that sucrose containing 0.4% benzoate lowered the pH of plaque significantly less than controls. Solutions containing 2% potassium sorbate had an even greater acid inhibiting effect. Overall, the evidence suggests strongly that the food preservatives alone or in combination could have a profound effect on oral health and particularly on the incidence and severity of dental caries. Unfortunately direct proof is lacking. Given the current and enhanced interest in the effects of additives on plaque ecology (Leikanger *et al*.; 1992; Rogers, 1986), it appears that the influence of food preservatives on this environment is likely to attract increasing interest.

13.7 Sulfites

Sulfites are used extensively in the wine industry to prevent spoilage by bacteria and to preserve color. They are also used in the preservation of fruit. Sulfur dioxide inhibits a large range of oxidative enzymes. Lactic acid bacteria are sensitive to free and, to a lesser extent, bound SO_2; the sensitivity of microorganisms to sulfites is also influenced by environmental pH values. Given the potency and relative low toxicity, it is surprising that sulfites have not been explored extensively as caries-preventive and antiplaque agents. Inclusion of sodium metabisulfite at concentrations of 0.9%, 0.3% and 0.15% in the diet fed to rats leads to 32% to 77% reductions in caries incidence compared with controls. The growth of several groups of oral

microorganisms was inhibited (Jordan *et al*., 1960). Clinical studies appear not to have been carried out.

13.8 Hypersensitivity

Several food preservatives have been linked to hypersensitivity in humans. This may be displayed as rashes in and around the oral cavity and skin lesions. Parabens and cinnaminic acid derivatives have been cited as causative agents (Sasseville, 2004; Steinmann *et al*., 1993).

13.9 Conclusions

The total amount of food preservatives ingested from natural sources and prepared foods is largely unknown. Although the concentration of preservatives permitted in foods is well controlled, the total amount ingested by consumers has not been determined. Based on data derived from *in vitro* and *in vivo* investigations, it appears highly probable that frequent ingestion of agents such as benzoates, parabens and propionates could have an effect on the bacterial populations and biofilms formed in the mouth. Ready access to the mouth appears to offer a unique opportunity to explore the ecological effects of widespread exposure to food preservatives.

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14

Food, nutrition and oral cancer

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Abstract: Oral cancer is increasing in incidence in most industrialized countries and is potentially one of the most preventable cancers. The major causes of this neoplasm are smoking and alcohol misuse. Many promising avenues have been proposed as chemopreventive for cancer and a growing number of these agents are being tested as primary, secondary and tertiary prevention strategies. This chapter begins by presenting the evidence from epidemiological studies that suggest fresh vegetables and fruits provide protection against oral cancer. Relevant laboratory studies are also presented. It then reviews the mechanisms by which these plant foods may reduce risk and also outlines plant foods and drinks that may be carcinogenic to humans. Attempts to modify the development of oral cancer have included the use of nutritional supplements such as vitamin A (retinol), β-carotene and vitamin E. While many of these agents have undesirable side effects, low-dose β-carotene supplementation has borderline effects on premalignant oral lesions. The message – to eat at least 5 portions (400 g) of a variety of fruit and vegetables each day – is consistent with dietary recommendations to prevent oral cancer.

Key words: antioxidants, carotenoids, chemoprevention, foods and drinks, oral cancer, phytochemicals, polyphenols, retinoids.

14.1 Introduction

Malignant neoplasms – what we mostly mean by the lay term 'cancer' – are today the second most common cause of deaths in industrialized countries, exceeded only by diseases of the heart and circulatory system. One in three persons in the West will contract cancer by the age of 75. In developing countries, infections and diseases of under-nutrition are more common causes of death, but cancer is still common; indeed over 70% of the world's population live in such countries and oral and oropharyngeal cancers are more common in so-called developing countries (IARC, 2002;

Warnakulasuriya, 2009). In all parts of the globe, an individual's diet and nutrition are fundamental to his or her susceptibility to cancer.

The most common body cancers are of epithelial origin, that is carcinomas of some kind. In the orofacial region, skin cancer (the most common cancer in white Caucasoid populations) are melanomas or carcinomas. In the head and neck, most neoplasms are carcinomas of some kind and this is true of the upper aerodigestive passages. Squamous cell carcinoma of the oral cavity and continuous mucosae represents more than 80% of orofacial malignancy, and oral and pharyngeal cancers are now reported in about 500 000 people each year, around the world (Ferlay *et al*., 2004). The lips, mouth and tongue and oropharynx are at the most anterior part of the upper air and food passages and the causes and prevention of epithelial cancers in this tract have much in common. This chapter, therefore, focuses on squamous cell carcinomas of the lips, oral cavity (mouth and tongue) and the oropharynx.

Strong evidence has accrued for most of the industrialized world over the past five years that cancers of the oral cavity (particularly the tongue) and oropharynx are on the increase. The effect is at present most obvious in young adult males, suggesting the possibility of an increased incidence of oral cancer as these cohorts reach the higher risk age groups (Llewellyn *et al*., 2001).

14.2 The causes of cancer

In a very real sense, the cause of all cancers is genetic – alterations in the structure and behaviour of a gene or genes (oncogenes, tumour suppressor genes and DNA repair genes) which control the normal homeostatic mechanisms of cell proliferation, differentiation and maturation. These genetic mutations, under-expressions and over-expressions of gene products (see review; Warnakulasuriya, 2002) are predisposed to, or precipitated by, inherited polymorphisms and a variety of environmental agents, notably tobacco, betel quid and alcohol, and some viruses. Many of these may act in synergy to produce adverse effects on our genome which become compounded by dietary deficiencies and nutritional state will be considered in this chapter.

14.3 Food and the risk of cancer

Most foods that are protective against cancer of the mouth and pharynx are of plant origin. Based on the recent evaluation published by the World Cancer Research Fund (WCRF, 2007), there is convincing evidence that a higher consumption of non-starchy vegetables (raw vegetables including cruciferous and green, leafy vegetables) and non-starchy tubers (e.g. carrots)

may protect against cancers of the upper aerodigestive tract, including the mouth and pharynx (summary odds ratio OR = 0.72, 95% confidence interval CI: 0.63–0.82). There is published evidence that fruits (particularly citrus fruits) may also provide protection (Summary OR = 0.72, 95% CI: 0.59–0.87; per 100 g/day). Fruit juices have different nutritional properties from whole fruits, but separate studies are meagre.

14.3.1 Epidemiological evidence

Several epidemiological studies have reported that increased consumption of vegetables and fruits modifies the risk of mouth and pharyngeal cancer. Evidence has accrued from two cohort studies, three ecological studies and numerous case-control studies. Data from several case-control studies (Notani and Jayant, 1987; Franco *et al*., 1989; Oreggia *et al*., 1991; Franceschi *et al*., 1991, 1992, 1994, 1999; Zheng *et al*., 1992, 1993; De Stefani *et al*., 1994, 1999, 2000, 2005; Kune *et al*., 1993; Takezaki *et al*., 1996; Levi *et al*., 1998; Garrote *et al*., 2001; Rajkumar *et al*., 2003; Marchioni *et al*., 2002; Gaudet *et al*., 2004; Lissowska *et al*., 2003; Sanchez *et al*., 2003; Tavani *et al*., 2001; Pisa and Barbone, 2002; Lee *et al*., 1985; La Vecchia *et al*., 1991; Fioretti *et al*., 1999; Gridley *et al*., 1990; Mclaughlin *et al*., 1988; Llewellyn *et al*., 2004a) are presented below. These have involved asking people with (oral) cancer (cases) and those without (controls) – usually matched for age and gender at least – what vegetables/fruits they have normally eaten in previous years, and in what quantities. The majority of these studies are adjusted for smoking and alcohol consumption. From this the relative risk of cancer in people with low, as opposed to high intake of a particular nutrient can be estimated. The available data can be grouped under the following categories of vegetables: non-starchy vegetables, raw vegetables, cruciform vegetables, green leafy vegetables and carrots and tomatoes.

14.3.2 Total (non-starchy) vegetables

Most studies reported decreased risk with increased intake of non-starchy vegetables. Nineteen case-control studies have been reported so far worldwide comparing high against low intake. Of these, 16 studies have confirmed a decreased risk of mouth and pharyngeal cancer for the highest intake group. In 11 of them, the observed reduction was statistically significant. Three of the studies showed an increased risk, but the results were not significant. A dose–response effect is apparent in three case control studies, suggesting the greatest effect appears to be in the first increment (50 g/day), suggesting any increase above the lowest level of vegetable consumption confers a protective effect. Some studies separately reported data on raw vegetables, cruciferous vegetables (e.g. the cabbage family: broccoli and watercress) and green, leafy vegetables (e.g. spinach and lettuce). These data are summarized below.

14.3.3 Raw vegetables

Sixteen case-control studies separately reported risk estimates for raw vegetable consumption, of which reduced risks were noted in most, except the Cuban study, and among them 12 were statistically significant. No studies reported that increased intake of raw vegetables caused cancer of the mouth or pharynx. These results are consistent with data from total vegetable estimates reported above.

14.3.4 Cruciferous vegetables

Twelve case-control studies separately analysed the effect of cruciferous vegetables of which four showed a statistically significant decreased risk with increased intake, either overall or in specific subgroups of cruciferous vegetables, while nine showed inconsistent or non-significant associations. One study related to pickled cabbage and this is separately reported below.

14.3.5 Green, leafy vegetables

Six case-control studies reported separate relative risk estimates for green, leafy vegetable consumption. All studies showed decreased risk with increased intake which was statistically significant in one.

14.3.6 Carrots

Fourteen case-control studies have investigated the effects of consumption of non-starchy root vegetables on the risk of oral and pharyngeal cancer. Data collection was somewhat different in these 14 studies, some inquiring about 'tubers and carrots', or 'non-starchy root vegetables' or 'yellow/orange vegetables'. The majority of studies analysed carrots as a separate exposure. Twelve reported estimates of reduced risk with increased intake, half of these were statistically significant.

14.3.7 Tomatoes

Eleven case control studies examined the effect of tomatoes on mouth and pharynx cancer and all except one reported reduced risks, four of which were statistically significant. Two of the studies that reported increased risks did not adjust for smoking and alcohol drinking.

14.3.8 Fruits

Fruits have been shown to protect against mouth and pharyngeal cancers. Most case-control studies reported that a decreased risk was associated with a higher frequency of fruit intake, which was statistically significant in 10 studies. No study reported statistically significant increased risk. Three

studies conducted in a similar way, and all adjusted for smoking and alcohol consumption, were significant for their protective effect. Citrus fruits were the most common category among fruits that were inquired in most studies.

14.3.9 Vegetables and fruits

Three case-control studies that examined associations of vegetables and fruits in combination reported reduced risks which were statistically significant in two.

The magnitude of the decreased risk with high consumption appears moderate, estimated at about one-quarter of those who are low consumers (with wide confidence intervals, in some studies not reaching statistical significance). Nevertheless, the protective effect of raw vegetables and fresh fruits is clear, and that of fruits appears to be greater than that of vegetables, possibly because of destruction of the latter during cooking. A more recent meta-analysis, focussing on oral cancer alone, which examined 15 case-control studies and one cohort study providing diet data from nearly 5000 subjects estimated that each portion of fruit or vegetables consumed per day reduced the risk of oral cancer by around 50% (Pavia *et al.*, 2006). Carotene-rich (such as broccoli and capsicums) or green-leafy vegetables appear to provide greater protection than vegetables lacking in β-carotene. Most studies reported to date have been conducted in the USA or in western Europe where serum levels of these micronutrients among cancer subjects may be not be far below population means. It is likely that the true protective effects of these are amongst those who infrequently consume raw/fresh fruits and vegetables and who may be relatively undernourished.

14.3.10 Other micronutrients: iron

Iron deficiency is the most common and widespread nutritional disorder in the world, particularly in developing countries. The association of iron deficiency with cancer of the upper aerodigestive tract was noticed by early workers (Ahlbolm, 1936) and led to the description of Plummer–Vinson (or Paterson–Kelly) Syndrome. Good case-control studies are lacking, however. Atrophy of the mucosa in iron-deficient states (Ranasinghe *et al.*, 1983) is thought to be a predisposing factor in the development of oral cancer (Warnakulasuriya and Prabhu, 1992). There is also preliminary evidence to suggest that low concentrations of zinc and selenium may predispose to oral cancer (Rogers *et al.*, 1991).

14.4 Vegetarian diet and oral cancer

Whilst strict vegetarian sects exist in the Indian subcontinent – an area of high oral cancer incidence – reliable dietary information about oral cancer

cases and controls from such population groups is meagre. Asian dietary patterns have not been investigated for cancer risks, except Japanese cohort studies relating to stomach cancer. One study from India (Notani and Jayant, 1987) documented the relative risk associated with low/infrequent consumption of vegetables among oral cancer cases as 2.39 (CI: 1.4–4.0) compared to 1 for population controls. Surprisingly, no differences were observed for fruit intake. Similar data were reported in a more recent study by Rajkumar *et al*. (2003). Among a population in Tamil Nadu, India a non-vegetarian diet was found to increase oral cancer risk (OR 6.1) (Subapriya *et al*., 2007).

Dietary information on subjects who develop oral cancer despite their low-risk status, attributable to the absence of major risk-factors, that is non-smokers or non-chewers of tobacco and abstainers from alcohol, has not been documented. It is known that, among Seventh-Day Adventists (Lemon *et al*., 1964), deaths from buccal and pharyngeal cancer are significantly lower than in the population at large, but this could be attributable to many protective elements in their lifestyle in addition to their pro-vegetarian Seventh-Day Adventist diet. Examining risk factors in young people who develop oral and oral oropharyngeal cancer, Llewellyn *et al*. (2004a, b) reported that 25% of these young people may not be exposed to major risk factors (tobacco and alcohol). Young females who consumed at least three portions of fresh fruits and vegetables a day had a significant risk reduction (OR = 0.08, 95% CI: 0.01–0.8) (Lewellyn *et al*., 2004a). In a second study on young people, the long-term consumption of three or more potions of fresh fruits and vegetables per day in the diet appeared protective for both males and females (OR = 0.5, 95% CI: 0.3–0.8: $P < 0.01$) (Lewellyn *et al*., 2004b). A non-significant 40% decrease in the risk of oral cancer was shown for those reporting a vegetarian diet (OR = 0.6, 95% CI: 0.3–1.4) after adjusting for smoking and alcohol (Lewellyn *et al*., 2004b).

14.5 Animal studies

The epidemiological evidence reviewed above is strongly supported by numerous animal studies. Pioneering work by Shklar and his co-workers has shown that many forms of vitamin A – both naturally occurring and synthetic – as well as β-carotenoids, have inhibitory effects on experimental oral carcinogenesis in rodent models, whether administered topically (Suda *et al*., 1986) injected locally (Schwartz *et al*., 1988), or administered systemically (Shklar *et al*., 1980). Other groups (Gillmore and Giunta, 1981), however, have failed to alter carcinogenesis using 13-*cis* retinoic acid. Topical and systemic administration of vitamin E has also been shown to inhibit experimental hamster cheek pouch carcinogenesis (Odukoya *et al*., 1984) by immunostimulation (Shklar *et al*., 1990).

An experimental study on oral carcinogenesis in rats (Prime *et al*., 1982) has shown that tumour development occurs significantly earlier in iron-deficient animals compared to those who are maintained on an iron-sufficient diet. Experimental iron deficiency results in reduced cell production and oxidative metabolism in hamster cheek pouch mucosa, a process which probably contributes to atrophy and increased permeability to topical carcinogens (Ranasinghe *et al*., 1983, 1987). Dietary restriction enhances experimental oral carcinogenesis in hamster cheek pouch (Andreou and Morgan, 1981).

Dietary turmeric (*Curcuma longe*) reduced oral cavity tumours in hamsters in an oral experimental carcinogenesis model (Azuine and Bhide, 1994).

14.6 Anti-cancer agents in plant foods: mechanisms of action

The reason why fruit and vegetables are so beneficial is because of their array of compounds. As well as vitamins and minerals, fruit and vegetables also contain many complex plant components, particularly carotenoids and phytochemicals, including flavonoids, glucosinolates and phyto-oestrogens. Some of the vitamins and phytochemicals are also antioxidants, destroying free radicals in the body. These free radicals are known to have a role in causing cancer as well as having other harmful effects.

Retinoids include vitamin A (retinol) and are known to have potent hormone-like effects (Peto, 1983), being transported to target sites in plasma, bound to proteins. The main sources of provitamin A are carotenoids, such as β-carotene. Their actions, via retinoid receptors (RAR and RXR), by altering gene expression can control epithelial differentiation (Sporn and Roberts, 1983) and also induction of apoptosis which can, in turn, inhibit neoplastic transformation. In addition, retinol has a variety of effects on cell membranes involving glycoprotein synthesis and changes in membrane receptors. They also antagonize known tumour promotors such as phorbal esters (De Vet, 1989). β-carotenoids have antioxidant effects that are known to deactivate free radicals and excited oxygen, a process known as quenching singlet (to triplet) oxygen (Krinsky and Deneke, 1982), and they inhibit lipid peroxidation (Mobarhan *et al*., 1990), all of which have been implicated in carcinogenesis. β-carotenoids are metabolically converted to retinoid – a precursor of vitamin A – and could therefore act either via their own scavenging properties or after being metabolized. Their oxidized products, however, may facilitate carcinogenesis, particularly in a free radical-rich environment in the lungs of smokers. Other carotenoids such as canthaxanthium, which do not convert to retinal, are considered protective because of their scavenger effects. Lycopene, the carotenoid that gives the ripe tomato its bright red colour, is a very effective antioxidant and a quencher of free radicals. Carotenoids appear to

enhance the immune system, particularly activating cytotoxic T cells (Malter *et al*., 1989) and may be a factor contributing to lower cancer risk in vegetarians. Changes in levels of oncoproteins (p53 and bcl-2) have been demonstrated after treatment with vitamin A for three months in patients with oral premalignant lesions (Varma *et al*., 2007). Carotenoids can suppress proliferating cell nuclear antigen and cyclin D1 (both are over-expressed in oral cancer) in oral carcinogenic models *in vivo* and *in vitro* (Cheng *et al*., 2007).

All plant products contain a number of phytochemical compounds in variable quantities. Phytochemicals extracted from avocado fruit have been shown to inhibit growth and induce apoptosis in oral precancerous cell lines (Ding *et al*., 2007). Polyphenols are the largest class of phytochemicals and these molecules are responsible for the bitter and astringent properties commonly associated with certain foods. Polyphenols have a chemical structure that is ideal for absorbing free radicals. Some foods that are rich in polyphenols include cabbage, broccoli, wild berries, grapes and green tea. Over 4000 polyphenols are known and the largest class of polyphenols is the flavonoids. Risk of oral and pharyngeal cancer is lower in those reporting high consumption of flavonoids (Rossi *et al*., 2007); the odds ratios for the highest versus the lowest quintile was 0.51 (95% CI: 0.37–0.71).

Cruciferous vegetables also contain high levels of glucosinolates which, via hydrolysis, release indoles and isothiocynates endowed with anticarcinogenic properties. For example, they are capable of favourably modifying carcinogen metabolism via inhibition of phase 1 enzymes and/or induction of phase 2 enzymes (Hecht, 1999) and also by triggering apoptosis. Garlic, a species of *Allium*, which may reduce stomach cancer, is high in sulfur-containing phytochemical compounds, but no direct evidence exists of its protection again oral cancer. Phyto-oestrogens are known to reduce breast cancer risk in premenopausal women and, again, there is no evidence of its effectiveness against oral cancer. The anticancer properties of grapes, grape and cranberry juice and red wine are due to their high content of resveratrol, which is also a phytochemical. The anticancer properties of resveratrol are largely due to the induction of apoptosis by upregulation of molecules such as Bax and Bak and the downregulation of expression of Bcl-2 and survivin (Shankar *et al*., 2007). It may also contribute to inhibition of cyclooxygenase-2 activity. Curcumin is a natural agent present in turmeric (*Curcuma longa*) and it has promising anticancer properties. The active molecule binds to a variety of proteins thereby modulating the activity of various transcription factors in cancer cell lines *in vitro* (Duvoix *et al*., 2005; Goel *et al*., 2007). Curcumin has been shown to inhibit growth and DNA synthesis in an oral cancer cell line (SCC-25), but cisplatin is five-fold more potent than curcumin (Elattar and Virji, 2000). Curcumin specifically inhibits COX-2 expression, and may have a chemopreventive role in the prevention of colon and other aerodigestive tract cancers (Goel *et al*., 2001). A reduction of Cox 2 expression has also been achieved in oral leukoplakia treated with

black raspberry gel (Mallery *et al*., 2008). Tomatoes, ketchup and paste are rich in lycopene, its anticancer effect in prostrate is proven and there is limited evidence of its benefits for the prevention of mouth cancer (Singh *et al*., 2004).

14.7 Carcinogens in foods: additives and preparation

Food itself, as well as the additives included by food producers, may contain carcinogens, cocarcinogens and promoters. One study has shown a statistically significant increased risk of mouth cancer associated with eating kimchi or pickled cabbage in Korea (Lee *et al*., 1985).

Areca nut (betel nut), the endosperm of the areca fruit, is not a food but is a masticatory substance used by over 600 million people around the globe, mostly in south Asia. Traditionally, areca nut was used mixed with betel (pan) and used as a quid but now freeze-dried preparations are available (pan masala) in commercially packaged form for chewing. The International Agency for Research on Cancer in its evaluation has confirmed that areca nut is carcinogenic to humans, based on available epidemiological and animal data (IARC, 2004).

Food consumed raw or uncooked may contain carcinogens derived from microbial metabolism, for example fungal contamination, and from fertilizers and pesticides. For example, Aflatoxin B1, formed in peanuts and other foods contaminated by the mould *Aspergillus favus*, is a highly potent carcinogen. Hydrazines occur in several types of mushroom and some of their derivates are carcinogens and mutagens. There is no current evidence that these agents cause cancer of the mouth or pharynx. The processes of cooking, including frying, grilling, barbecuing and roasting may result in measurable levels of the products of pyrolysis of fats and amino acids and could create high levels of carcinogens. The type of wood used may also be an important factor. Charcoal-grilled meat has been shown to be a significant risk factor for oral cancer in Brazil (Franco *et al*., 1989).

Substances used to preserve food, including nitrates and nitrites, may result in the formation of nitrosamines, as for instance in the curing of meats. Nitrites are present in cured meats and baker goods (Creasey, 1985). Pure nitrosamines, albeit in high doses, are powerful carcinogens in experimental animals, including for oral cancer, whether administered topically or systemically (IARC, 2007).

14.8 Drinks and the risk of cancer

14.8.1 Alcohol

Ethanol is classified by the International Agency for Research on Cancer as a human carcinogen. The strength of the evidence for alcoholic drinks is

convincing for cancers of the mouth and pharynx (IARC, 1988; World Cancer Research Fund, 2007). The probable linkage between alcohol consumption and human cancer has been extensively studied. The results of 89 case-control studies have been published in which the reported intake of alcohol in patients with cancer of the mouth or pharynx has been compared with that in controls. In the majority of these, a clear correlation between long-term alcohol intake and cancer of the mouth, tongue and pharynx was shown.

A meta-analysis showed strong trends for the risk of oral cancer with alcohol drinking (Bagnardi *et al*., 2001). Significant increases in the age-standardized mortality rate (ASMR) for every litre of pure ethanol (0.15 per 100 000; 95% CI: 0.01–0.29) and spirits (0.26 per 100 000; 95% CI, 0.03–0.49) have also been reported (Petti and Scully, 2005). The risk of cancer in subjects who both smoke and drink alcohol is much higher than would be expected from consideration of the factors separately. Alcohol synergizes the carcinogenic effects of tobacco in a multiplicative fashion, increasing the risk of oral cancer in heavy smokers and heavy drinkers by factors of up to 70 times (Brugere *et al*., 1986; Blot *et al*., 1994).

Laboratory evidence (from animal studies) for a direct carcinogenic effect of alcohol is lacking, however. The evidence of the epidemiological association between oral cancer and alcohol consumption cited above, however, suggests direct action. Alcohol may act as a solvent, by removing the lipid content of cell membranes thereby enhancing the penetration of other carcinogenic molecules, particularly tobacco metabolites, across the oral mucosa (Squier *et al*., 1986). Alcoholic drinks often contain small amounts of nitrosamines and other carcinogens such as benzanthracene and phenanthrene (MacSween, 1982). There is recent evidence that reactive metabolites of alcohol, such as acetaldehyde (a known carcinogen), may be found in the oral mucosal cells of heavy drinkers (Warnakulasuriya *et al*., 2008). In addition to the local production of carcinogenic acetaldehyde, the effects of alcohol may be mediated through the production of prostaglandins, lipid peroxidation and the generation of free-radical oxygen species. Finally, patients suffering from alcoholism commonly suffer from loss of appetite, chronic gastritis and severely impaired intake of the elements of a satisfactory diet including minerals and vitamins, and are not infrequently malnourished (Harris *et al*., 1997) making tissues susceptible to carcinogens. Low body mass index (BMI) contributes to an increased risk of oral cancer (OR = 3.64, 95% CI: 2.18–6.05) among long-term drinkers (Nieto *et al*., 2003).

14.8.2 Mate

Mate, a herbal infusion, is a traditional hot drink in some countries. Regular consumption of mate, as drunk in the traditional style in South America (chimarro) is probably carcinogenic to the mouth and pharynx (Franco *et*

al., 1989). Four other case-control studies (Oreggia *et al*., 1991; Pintos *et al*., 1994; Nishimoto *et al*., 2002; Toporcov *et al*., 2004) investigated mate drinking and cancers of the mouth and pharynx. All five studies showed moderately increased risk in heavy consumers, in four studies the data being significant. It is probable that the cause of cancer is not the herb but the thermal effect on the lining of the oral cavity. The evidence has been reviewed by Goldenberg *et al*. (2002, 2003).

14.8.3 Cachaca (sugarcane)

Hard liquor distilled from sugarcane has been implicated as a risk factor for oral cancer in Brazil (Franco *et al*., 1989). Oral cancer risk is significantly elevated (OR = 4.4, 95% CI, 1.4–13.6) among sugarcane workers in Puerto Rico and is described as an occupational risk among farm workers (Coble *et al*., 2003).

14.8.4 Tea

Green tea is a popular beverage consumed mostly by the Japanese and Chinese and contains polyphenols (see Section 14.6), chemicals that act as antioxidants. One cohort study in Japan (mean follow-up period = 10.3 years) did not find a significant inverse association of green tea consumption with oral cancer, but there was tendency for a reduced risk in women (Ide *et al*., 2007). No other data are available for the protection offered by green tea for oral or pharyngeal cancer.

14.9 Chemoprevention

Chemoprevention attempts to reduce cancer incidence by prescribing pharmacological agents or dietary supplementation using vitamins, minerals, trace elements and other bioactive substances. Encouraged by the findings from epidemiological and experimental studies, the concept of chemoprevention for oral cancer is receiving increasing attention by oral physicians and oncologists.

Retinoids that are available so far for clinical use are tretinoin (all-*trans*-retinoic acid), isotretinoin (13-*cis*-retinoid acid) and etretinate (ethylester of retinoid). These appear to be promising cancer prevention agents in many clinical trials (Anderson *et al*., 2001). Vitamin A and retinoids (e.g 13-*cis* retinoid acid, RA) and their analogues have been used topically and systemically in the treatment of oral leukoplakia (Silverman *et al*., 1963, 1965; Stich *et al*., 1988a; Sankaranarayanan *et al*., 1997; Koch, 1978, 1981; Cordero *et al*., 1981; Chiesa *et al*., 1992; Shah *et al*., 1983; Hong *et al*., 1986; Toma *et al*., 1992a; Lippman *et al*., 1990, 1993). These initial studies suffered from excessive toxicity and recurrence of some oral lesions on withdrawal

of these supplements. Epstein and Gorsky (1999) reported limited effects from the use of topical application of vitamin A (tretinoin) acid gel on oral leukoplakia, with 27% complete remission but with 40% recurrence.

Unfortunately, toxicity is reported in many agents used in chemoprevention (Scully and Boyle, 1992). These include headache, alopecia, itchiyosis, carotenodermia, chelitis, facial erythema, desquamation, conjunctivitis, photophobia, hypertriglyeridaemia and liver damage.

Vitamin A plays an essential role in the normal differentiation of epithelial tissues. Retinoids, analogues of vitamin A, are potential chemopreventive agents in squamous cell carcinomas. The synthetic retinoid most often used in leukoplakia trials (Table 14.1) has been 13-*cis*-retinoic acid. This compound is toxic even at low doses (0.1 mg kg^{-1}/day), particularly when given over several months. Although effective, it cannot be advocated in the prevention of oral cancer. Topical treatment of leukoplakia with retinoic acid may also help to control persistent and recurrent lesions (Gorkyand Epstein, 2002).

In 1981, Peto *et al*. hypothesized that high dietary intake of carotenoids may reduce human cancer. Observational studies of diet suggest that its role in cancer prevention effects is related to β-carotene (IARC, 1998). Low β-carotene intake has been shown to be a risk factor for oral leukoplakia in Japanese subjects (Nagao *et al*., 2000). β-carotene (a vitamin A precursor) is a naturally occurring, non-toxic carotenoid which serves as an important source of vitamin A. Though less effective in its antioxidant effects, β-carotene is used as a nutritional supplement to correct vitamin A deficiency and it is safer to use with fewer side effects. Recently, more impressive treatment outcomes have been reported for β-carotene, on its own or in combination with other antioxidants (vitamins C and E) (Stich *et al*., 1988b; Garewal *et al*., 1990, 1994, 1999; Toma *et al*., 1992b; Lippman *et al*., 1990; Malakar *et al*., 1991; Sankaranarayanan *et al*., 1997). Evidence from these studies is too preliminary to find clinical applications outside controlled trials.

β-carotene could be effective in cancer prevention in many ways (Van Poppel and Goldbohm, 1995). Its main action is by conversion to vitamin A and retinoids and this conversion has been reported to take place at a tissue level. It has antioxidant potential to scavenge free radical species. It also has immune enhancing effects, enhances cell-to-cell communication, induces programmed cell death and influences the activity of carcinogen-detoxification enzymes.

β-carotene has been shown to be efficacious against oral leukoplakia with an overall response rate ranging from 15–71% (Table 14.1). The reported dosages of β-carotene used in the chemoprevention of oral cancer range from 30 to 90 mg per day, mostly given over a 12-month period. Furthermore, it has been shown that effective supplementation with β-carotenoids can be measured in exfoliated buccal cells (Cameron *et al*., 1989) by monitoring micronuclei formation. In a large-scale population study conducted in Southern India, Stich *et al*. (1991) demonstrated

Table 14.1 Reported chemoprevention studies on oral leukoplakia

Supplement	Agent	Number	Population	Author	Overall response (%)
Vitamin A	Retinol (local)	16	USA	Silverman *et al*., 1963	44
	Retinol	21	India	Stich *et al*., 1988a	57
	Retinyl acetate	42	India	Sankaranarayanan, 1997	52
Retinoids	Etretinate	24	Germany	Koch, 1978	87
	Tretinoin	27			
	Etretinate	45	Germany	Koch, 1981	71
	Etretinate	3		Cordero *et al*., 1981	100
	Fenretinide	115	Italy	Chiesa *et al*., 1992	95
	Tretinoin (local)	26	USA	Epstein and Gorsky, 1999	27
13-*cis* RA	Isotretinoin (local)	11	USA	Shah *et al*., 1983	82
	Isotretinoin	24	USA	Hong *et al*., 1986	67
	Isotretinoin	16	Italy	Toma *et al*., 1992a	36
	Isotretinoin	26	USA	Lippmann *et al*., 1990	92
Carotenoid	β-carotene	27	India	Stich *et al*., 1988b	15
	β-carotene	24	USA	Garewal *et al*., 1990	71
	β-carotene	18	Italy	Toma *et al*., 1992b	44
	β-carotene	56	USA	Lippman *et al*., 1990	45
	β-carotene	56	Canada	Malaker *et al*., 1991	50
	β-carotene	46	India	Sankaranarayanan, 1997	33
	β-carotene	54	USA	Garewal *et al*., 1999	52
Vitamin E	α-tocopherol	43	USA	Benner *et al*., 1993	46
Combinations	β-carotene + α tocopherol + vitamin C	79	USA	Kaugars *et al*., 1996	56
	β-carotene + α tocopherol + vitamin C	24	Germany	Zoller, 1995	98
	β-carotene + vitamin C	16	Japan	Nagao *et al*., 2004	23.5

that micronuclei formation in (exfoliated) buccal cells can be reversed by β-carotene supplementation (180 mg per week) administered as capsules.

Two chemoprevention trials (ATBC and CARET) have shown some adverse effects of β-carotene when taken in excess of 20 mg/day, particularly among smokers (ATBC, 1994; Omenn *et al*., 1996). The adverse effects

reported in these two studies mainly related to an increased incidence of lung cancer. This was explained by the pro-oxidant effects of β-carotene owing to increased oxygen tension in the lung (Palozza, 1998), confirmed by *in vitro* studies that illustrate the oxidized products of β-carotene may facilitate carcinogenesis (Wang and Russell, 1999). Considering the adverse effects of β-carotene among smokers when taken in excess of 20 mg/day (ATBC and CARET trials) and the health benefits reported in observational diet studies where β-carotene is prescribed usually not in excess of 10 mg/day, supplement dosages should be selected cautiously (Lovas *et al*., 1996; European Commission, 2000).

As the oxidized products of β-carotene may facilitate carcinogenesis, particularly in a free radical-rich environment, supplementation of low doses of β-carotene together with a reducing agent such as vitamin C may provide a novel chemopreventive approach. Such a randomized control, multi-centre trial is currently on-going in Japan to evaluate the effects of β-carotene given daily (10 mg) with vitamin C (500 mg) (Nagao *et al*., 2004).

When micronutrients are deficient, chemoprevention with β-carotene (Blot *et al*., 1993) and other micronutrients may have a beneficial effect (Maher *et al*., 1997). In a study during 1984/85, which evaluated several vitamins including vitamins A, B, C and E in four areas of the USA, vitamin E was the only supplement that remained associated with a significantly reduced cancer risk. The adjusted odds ratio of oral and pharyngeal cancer for 'regular use' of vitamin E was 0.5 (95% confidence interval 0.4–0.6) (Gridley *et al*., 1992).

In phase 1 trials, it has been demonstrated that curcumin is not toxic to humans up to 8000 mg/day when taken by mouth for three months. The biological effect of curcumin in the chemoprevention of cancer, however, is not proven (Cheng *et al*., 2001).

A critical review of published chemoprevention studies of oral leukoplakia suggests numerous inadequacies in the trial designs (Kaugars *et al*., 1996; Scheer *et al*., 2004). The dosage consideration of new studies should be based on available knowledge to maximize benefits and to reduce harm. Most reported studies have not been randomized, typically involved small number of subjects, lacked controls and lacked widely accepted criteria for defining leukoplakia (Lodi and Porter, 2008). Recurrence after discontinuation of supplements has not been extensively studied. Improvements to study design were considered in a review by Garewal (1994). Even aggressive treatment combinations are not so far effective in reversing advanced premalignant lesions of the oral cavity and oropharynx, suggesting an urgent need for innovative approaches (O'Shaughnessy *et al*., 2002).

Many people now take dietary supplements – up to 35% in the UK and around 50% in the USA – but carefully designed multicentred intervention trials with prolonged observations, involving sufficient numbers of subjects with high risk oral premalignant lesions would provide the answer to the critical question of whether chemoprevention is a worthwhile approach to

control oral cancer. Timing and the optimal dose intensity required need further examination as we still do not know whether it is 50 g, 100 g or 200 g of carrots per day which will provide effective protection.

14.10 Prevention of second primary tumours

Daily treatment with high doses (50–100 mg/m^2/d) of isotretinoin (13 cRA, a synthetic retinoid, RA) is claimed to prevent second primary tumours of the head and neck **(**Hong *et al*., 1990), although the toxic effects caused by the high doses used in the study led to non-compliance. This is the only agent shown to prevent second primary tumours (Anderson *et al*., 2001). A further trial using a lower dose regimen (30 mg/day for three years) also showed significant reduction in former- and never-smokers (Khuri *et al*., 2001). The EUROSCAN study that included a 2-year supplementation with vitamin A (retinyl palmitate) did not provide a significant benefit to prevent second primary tumours of head and neck (van Zandwijk *et al*., 2000).

14.11 Summary and conclusions

It is clear that cancers of the mouth and pharynx, and probably also the wider range of malignant neoplasms that affect the aerodigestive tract, can be prevented by consuming a diet rich in antioxidants, notably fresh vegetables and fruits. This should be coupled with a reduction in the levels of consumption of processed, preserved and smoked foodstuffs as well as roasted and grilled foods. Moderation of alcohol consumption to within recommended guidelines (limiting units of alcoholic drinks to two for men and one for women a day) will also reduce the risk of cancers of the mouth and pharynx. Current recommendations by the Department of Health (UK) are that everyone should eat at least five portions of a variety of fruit and vegetables each day, to reduce the risks of cancer and many other chronic diseases (http://www.dh.gov.uk/en/Publichealth/Healthimprovement/FiveADay). Yet average fruit and vegetable consumption among the population in England is less than three portions a day. Consumption tends to be lower among children and people on low incomes. The message at the heart of the '5 A DAY' programme – to eat at least five portions (400 g/day) of a variety of fruit and vegetables each day – is consistent with dietary recommendations around the world, including those from the World Health Organization (2003). As well as eating '5 A DAY', in order to protect against other disorders, the diet should include whole grains (e.g. brown rice, wholemeal bread and pasta) and/or pulses with every meal. Under such circumstances, vitamin or mineral supplementation should not be necessary but the role of supplementation needs to be researched among high-risk subjects.

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15

Vitamin supplements and oral health

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Abstract: Vitamins are essential micronutrients whose deficiencies are widespread particularly among the underprivileged. They play key roles in human metabolism. Prevention of vitamin deficits is best solved by ingesting a wide range of good quality foods, or through use of appropriately fortified foods. Vitamin supplementation is an option which must be used cautiously to minimize risks of toxicity and other adverse effects resulting from interactions with medications, health status, age, life style factors and complications emanating from genetic background. Ideally, clinical evaluation of a subject should precede judicious use of vitamin supplements.

Key words: adverse reactions to supplements, vitamin deficiencies, vitamin supplement, vitamin toxicities.

15.1 Introduction

The essential organic micronutrients in the human diet include 13 vitamins which are usually required at minimum levels to protect against specific nutritional diseases (Della Penna, 1999). They constitute chemically and biologically disparate groups of substances, derived from foods, and are essential for the regulation of cellular functions, human growth and maintenance of good health (Ames, 2004). The first vitamin (thiamine) was characterized almost a century ago and over the last eight decades, particularly during the period 1928 to 1964, 12 Nobel Prizes in Medicine were awarded for vitamin discoveries, thus underscoring the importance of these nutrients to human health and well-being (Vina *et al.*, 2007).

Classic deficiency diseases historically linked to the vitamins included scurvy (vitamin C deficit), beriberi (thiamine deficiency) and pellagra (niacin deficiency), among others. Because these diseases are now rare, particularly in the affluent developed countries, there is the incorrect assumption in many quarters that deficiencies of these vitamins have been

eradicated. Low or marginal vitamin status is still encountered in large sectors of developed countries, particularly in pregnant and lactating mothers, elderly citizens, smokers, chronic abusers of alcohol and drugs and in individuals with restricted food intake for various reasons (Webb, 2007; Wintergerst *et al*., 2007).

Ames (2004) has underscored the fact that severe, chronic metabolic damage can result from minimal vitamin consumption levels that protect against acute deficiencies and extended this to the widely accepted recommended dietary allowances (RDAs). It is now well accepted that scurvy, for example, is not a first symptom of compromised ascorbic acid status but rather represents the end result of a series of adverse biological events. The scientific evidence which underpins the establishment of recommended intake levels of most micronutrients by humans has undergone significant revision in recent years (Ames *et al*., 2002; Wintergerst *et al*., 2007). With advances in nutrigenomics, the recommended dietary allowances should be adequate for promotion of genomic stability (Fenech, 2001, 2007). There is widespread global use of dietary supplements, particularly vitamins, in health promotion and disease prevention (Shenkin, 2006; Eberhardie, 2007; Rock, 2007; Rosenberg, 2007). Quite often, the supplements are ingested in high dosages and for prolonged periods for pharmaceutical rather than nutritional reasons (Webb, 2007). This practice ignores an important age-long dictum which states that 'All substances are poisonous; there is none which is not a poison. The right dose differentiates a poison and a remedy' Paracelsus, 1493–1541 (Touger-Decker, 2007; Webb, 2007).

This report is an attempt to update the scientific basis for the widespread, escalating use of vitamin supplements by more than one-half the population of the Western world (Mason, 2007; Rosenberg, 2007) and to examine the efficacy of the health claims made for them as used.

15.2 Classification of vitamins

The vitamins are broadly classified into two groups, namely, the fat-soluble and the water-soluble (Tables 15.1 and 15.2). The former group includes vitamin A (retinol, and its major plant precursor, β-carotene), vitamin D (ergocalciferol (vitamin D2), and cholecalciferol (vitamin D3)), vitamin K (vitamin K1 or phylloquinone in plants, and vitamin K_2 or menaquinone in the intestinal microbiota), and vitamin E (consists of four tocotrienols and four tocopherols, of which α-tocopherol has the highest biological activity). The retinol activity equivalent for β-carotene is 1:12, which is much higher than the previously used 1:6 conversion factor. As will be shown in subsequent sections of this report, both vitamins A and D behave essentially like hormones. Vitamin K is the only fat-soluble vitamin with a coenzyme function (Champe and Harvey, 2005).

Table 15.1 Fat-soluble vitamins

Nutrient	Good food sources
Vitamin A, retinol includes provitamin A carotenoids that are precursors of retinol.	Liver, fish, dairy products, darkly colored fruits and leafy vegetables
Vitamin D, ergocalciferol (vitamin D2) found in plants; cholecalciferol (vitamin D3) found in animal tissues; endogenous vitamin D precursor (7-dehydrocholesterol).	Fish liver oils, flesh of fatty fish, liver from seals and polar bears, artificially fortified foods such as milk, cereals, eggs from hens fed vitamin D fortified products
Vitamin E, consists of eight naturally occurring tocopherols, of which α-tocopherol is the most active.	Vegetable and seed oils, meats
Vitamin K, phyloquinone (vitamin K_1 in plants), menaquinone (vitamin K_2 intestinal bacteria), menadione (synthetic derivative of vitamin K).	Green leafy vegetables, plant oils, egg yolk, margarine

Table 15.2 Water-soluble vitamins

Vitamins	Good food sources
Thiamin (vitamin B1)	Seeds, nuts, wheat germ, whole grain
Riboflavin (vitamin B2)	Organ meats, milk, fortified cereals
Niacin (vitamin B3); includes nicotinic acid amide	Meats, fish, poultry, enriched grains
Pantothenic acid	Chicken, beef, yeast, egg yolk, potatoes
Biotin	Liver, tomatoes, yeast, soybeans
Pyridoxine (vitamin B6); pyridoxine, pyridoxal, pyridoxamine	Organ meats, wheat germ, nuts, beans, fortified cereals
Folic acid, folate, folacin, pteroylpolyglutamates	Dark leafy vegetables, liver, enriched cereal grains
Vitamin B12, cobalamin	Fortified cereals, meat, fish, poultry
Ascorbic acid, vitamin C	Citrus fruits, vegetables, strawberries, tomatoes, tomato juice

The water-soluble vitamins include thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), biotin, pantothenic acid, the hematopoietic subgroup (folic acid and cobalamin), vitamin B6 (pyridoxine, pyridoxal and pyridoxamine), and the non-B-complex vitamin (ascorbic acid, vitamin C). Most water-soluble vitamins function as coenzymes in intermediary metabolism or as cosubstrates in enzymatic reactions. Some of the important food sources of the vitamins and their biologically active forms are fully described in recent reports (The National Academies, 2001, accessed via www.nap.edu; Champe and Harvey, 2005).

15.3 Recommended dietary intakes

In the United States of America, recommended intakes of nutrients, including vitamins, are set by the Food and Nutrition Board (FNB) of the Institute of Medicine (IOM), National Academy of Sciences. Similar authorities exist in the United Kingdom, Canada and many other countries. As shown in Table 15.3, four reference values, namely the EAR, RDA, AI and UL, constitute the dietary reference intakes (DRIs) which have replaced the RDAs and the recommended nutrient intakes (RNIs) previously published in the

Table 15.3 Definitions of components of the dietary reference intakes (DRIs)*

- Recommended dietary allowance (RDA): This is the average daily dietary intake level sufficient to meet the nutrient requirement of nearly all (97–98%) healthy individuals in a life stage and gender group.
- Estimated average requirement (EAR): This is the average daily nutrient intake value estimated to cover the requirement of one-half of the healthy people in a life stage and gender group. If requirement for the nutrient is normally distributed, the RDA is set at two standard deviations above the EAR.
- Adequate intake (AI): A recommended intake value used when there is insufficient scientific data to calculate RDA. It is based on observed or experimentally deduced estimates of nutrient intake by a group (or groups) of healthy people that are assumed to be adequate. For example, AI for young infants is based on the estimated daily mean nutrient intake supplied by human milk for exclusively breast-fed, healthy full-term infants.
- Tolerable upper intake level (UL): This is the highest level of average daily nutrient intake unlikely to pose risk of adverse health effects to almost all individuals in the general population. The potential risk of adverse effects increases with nutrient intake above the UL.

* Sources: Institute of Medicine of the National Academies, Food and Nutrition Board (2006); Touger Decker (2007).

Professional resource websites:

- National Library of Medicine, Medline Plus. Vitamins. www.nlm.nih.gov/medlineplus/vitamins.html
- American Heart Association. Vitamin and mineral supplements: AHA scientific position. www.americanheart.org/presenter.jhtml?identifier=4788
- Office of Dietary Supplements. Vitamin and mineral supplement fact sheets. http://dietary-supplements.info.nih.gov/Health_Information/Vitamin_and_Mineral_Supplemnt_Fact_sheets.aspx
- American Dietetic Association. www.eatright.org
- Institute of Medicine of the National Academies. Dietary reference intakes tables: The complete set. 'www.iom.edu/CMS/8788/21370.aspx'
- U.S. Food and Drug Administration, Center for food Safety and Applied Nutrition. Dietary supplements: Questions. www.cfsan.fda.gov/-dms/ds-faq.html
- United States Pharmacopoeia. USP verified dietary supplements. www.usp.org/USPVertified/dietarySupplements/
- Agency for Healthcare Quality and Research. Evidence report/technology assessment. Number 139: Multivitamin/mineral supplements and prevention of chronic disease. www.ahrq.gov/downloads/pub/evidence/pdf/multivit/mulitvit.pdf

List of online resources from Touger-Decker (2007).

USA and Canada, respectively (Mason, 2007; IOM, Dietary Reference Intakes: vitamins, 1997, 1998, 2000, 2001, www.nap.edu). The DRIs, unlike the RDA values, were established with decreased risk of chronic diseases as end points (Lupton, 2005) and cover 22 distinct life stages and sex groups (Kennedy and Meyers, 2005). The life stages include infants (birth to 6 m; 7–12 m), toddlers (1 to 3 years), early childhood (4 to 8 years), puberty (9 to 13 years), adolescence (14 to 18 years), young adulthood (19 to 30 years), middle ages (1 to 50 years), adulthood (51 to 70 years), older adults (70 years and above), pregnancy (≤18 years, 19–30 years, 31–50 years) and lactation (≤18 years, 19–30 years, 31–50 years). The DRIs specify appropriate body size, namely, body mass indices (BMI) of 24.4 and 22.8 for 19 to 30-year-old males and females, respectively. The latter specification may be irrelevant in the USA where the majority of adults have average BMI well above the reference body size (Kennedy and Meyers, 2005). This also applies to some urban communities in the developing world presently experiencing nutrition transition and increasing incidence of obesity (Popkin, 2006).

Where toxicity data exist, the DRIs include the UL which is not a desirable, recommended level of intake. As shown in Table 15.4, there are marked differences in the ULs proposed by various authorities and the key reasons for the variations are explained in a recent report (Mason, 2007). It should also be emphasized that UL values established for relatively healthy communities in the Western world do not necessarily apply to Third World countries where severe malnutrition and infectious diseases are endemic.

15.3.1 Some groups with special dietary needs

Like the RDA, the DRI values do not cover all groups, especially individuals with genetic disorders. For example, a recessive allele of the methylene tetrahydrofolate (MTHFR) gene, with a C to T transition mutation at position 677 (677T) causes an alanine to valine substitution in the encoded protein. This mutation reduces and impairs the activity of MTHFR (EC1.7.99.5). Individuals with the C677T genotype or the T/T genotype (homozygous) may constitute 5–15% of the population and have increased requirements for folic acid and riboflavin which are not provided for in the DRI (Simopoulos, 1999). Persons with the TT genotype have MTHFR that is particularly sensitive to riboflavin status (McNulty *et al*., 2002). A good number of all mutations in a gene increase the Michaelis constant (K_m) of the corresponding enzyme for a coenzyme. Enzymatic activity may be partially restored by supplementation with the B-vitamin component of the corresponding coenzyme (Ames, 2004; Ames *et al*., 2002).

Additional to the C677T/alanine 222 valine methylenetetrahydrofolate reductase (NADPH) and the co-factor FAD, others include the C609T/Pro 187 Ser mutation in NAD (P): quinine oxidoreductase 1 and FAD (in relation to cancer); the C131G/Ala 44 Gly mutation in glucose-6-phosphate-1-dehydrogenase and NADP (in relation to favism and hemolytic anemia);

Table 15.4 Upper safety limits for vitamin intakes set by various authorities[a]

Vitamin	Authorities				
	CRN/EHPM	EVM UK	FNB USA	SCF EU	AUS/NZ
Vitamin A (retinol equivalent; μg)	2300	1500[b]	3000	3600	3000
β-Carotene (mg)	20	7[c]	–	20	–
Vitamin D (cholecalciferol; μg)	10	25[b]	50	50	80
Vitamin E (tocopherol; mg)	800	727[c]	1000	300	300
Vitamin K (μg)	–	1000[b]	–	–	–
Thiamine (mg)	100	100[b]	–	–	–
Riboflavin (mg)	200	100[b]	–	–	–
Vitamin B_6 (pyridoxine; mg)	100	10[c]	100	25	50
Vitamin B_{12} (cobalamin; μg)	3000	1000[b]	–	–	–
Niacin (mg)	150	–	35	–	–
Nicotinamide (mg)	900	500[b]	–	900	900
Nicotinic acid (mg)	10	17[b]	–	10	35
Folic acid (μg)	400	1000[b]	1000	1000	1000
Biotin (μg)	2500	970[b]	–	–	–
Pantothenic acid (mg)	1000	200[b]	–	–	–
Vitamin C (ascorbic acid; mg)	2000	1000[b]	2000	–	–

CRN/EHPM, upper safe level defined by the European Federation of Health Product Manufacturers Association and the UK Council for Responsible Nutrition as daily intakes from supplements that could be consumed on a long-term basis; EVM UK, values produced by the UK Expert Vitamin and Mineral Group; FNB USA, tolerable upper intake levels defined by the Food and Nutrition Board of the US National Academy of Sciences as the highest total level of a nutrient (diet plus supplements) that can be safely consumed on a daily basis that is unlikely to cause adverse health effects to almost all individuals in the general population; SCF EU, tolerable upper intake levels defined by effects on human subjects; AUS/NZ, upper levels of intake for vitamins in adult men and women (Australia, New Zealand).

[a] Source: Adapted from Mason (2007); IOM/FNB (1999, 2000a, 2000b, 2002); WHO/FAO (2006);

[b] Probable safe total daily intake from supplements alone;

[c] Safe upper levels from supplements alone.

See footnotes to Table 15.3 for professional and on-line resources websites.

and the Glu 487 Lys mutation (present in about 50% of Asians) in aldehyde-dehydrogenase and NAD (in relation to alcohol intolerance and cancer) (Ames, 2004). There are also important but rare disorders resulting from inborn errors of cobalamin cofactor synthesis that may influence

active folate status (Coelho *et al*., 2008). With respect to ascorbic acid needs, haptoglobin (Hp), an acute phase protein with hemoglobin-binding capacity, is characterized by a genetic polymorphism. There are two different alleles (Hp 1 and Hp 2) with three main types (Hp 1-1, Hp 2-1 and Hp 2-2). Studies in healthy Belgians suggest that Hp 2-2 carriers, with less protection against hemoglobin–iron driven peroxidation, may have the highest requirement for vitamin C than the Hp 1-1 and Hp 2-1 subjects (Langlois *et al*., 1997). From these few examples, it is clear that accurate recommendations for individualized dietary vitamin supplementation may require information on genetic and functional differences in metabolism, transport proteins and other relevant biological mechanisms.

15.3.2 Global dimensions of vitamin deficiencies

It is estimated that more than two billion people worldwide, particularly pregnant and lactating mothers, as well as young children, are at risk of deficiencies of vitamin A, folate and the B-complex vitamins, with the prevalence especially high in southeast Asia and sub-Saharan Africa (Ramakrishnan, 2002). The rural families in India rely mainly on cereal-based monotonous diets which supply only 20–90% of the RDA, and 8–30% of the families are vitamin A deficient (Demment *et al*., 2003). Similarly, Adelekan (2003) reported that 30–67% of African children are vitamin A deficient, and this is confirmed in a more recent study (Mariya-Dixon *et al*., 2006). Dietary thiamine (vitamin B1) deficiency is still seen in communities with habitual consumption of polished rice as staple food or foods containing thiaminases. The dietary intake levels of vitamins B6 and B12 in young children and in women of reproductive age in rural Kenya are below 66% of the RDA (Demment *et al*., 2003). Stabler and Allen (2004) have reported that dietary deficiency of vitamin B12 is severe in the Indian subcontinent, Mexico, parts of Africa, central and south America, and that this may be due in part to the increasing prevalence of vegetarianism. Using a serum level of 25-hydroxycholecalciferol as a marker, it is estimated that one billion people worldwide are vitamin D deficient (Holick, 2007).

Similar to findings of significant poverty-driven vitamin malnutrition in the developing countries, there are pockets of marginal to severe vitamin deficiencies in the affluent countries (Wakimoto and Block, 2001; Ames, 2004). Table 15.5 summarizes some of the findings in the USA. In several studies of vitamin D status, it was shown that 52% of Hispanic and black adolescents (Gordon *et al*., 2004), 48% of white preadolescent girls (Sullivan *et al*., 2005), 42% of 15–49-year-old black girls and women (Nesby-O'Dell *et al*., 2002) and 32% of supposedly healthy students and physicians (Tangpricha *et al*., 2002), among other groups (Holick, 2007), were potentially at risk of vitamin D deficiency. The situation is not very different in Europe where studies in British populations revealed that 20% of adolescent girls and 12% of boys had vitamin A intakes below the lower reference

Table 15.5 Vitamin deficiencies in US subjects[1]

Vitamin	Population group	Current RDA	Consuming <RDA (%)	Consuming <0.5 RDA (%)
Folate[2]	Women 20+years	400 µg	75	50
	Men 20+years	400 µg	75	25
B-6	Women 20+years	1.5 mg	50	10
	Men 20+years	1.7 mg	50	10
B-12	Women 20+years	2.4 µg	25	10
	Men 20+years	2.4 µg	10	5
C	Women 20+years	75 mg	50	25
	Men 20+years	90 mg	50	25

[1] Data adapted from Wakinoto and Block (2001). [2] Folate intake before US fortification in 1998.
See footnotes to Table 15.3 for professional and on-line resources websites.

nutrient intake (LRNI); biochemical evidence of poor vitamin D status in 13% of 11-to-18-year-olds, with higher numbers in the winter months; insufficient vitamin D status in 37% of elderly adults residing in institutions; biochemically deficient folate status in 30–40% elderly adults, and avitaminosis C in 14% of free-living elderly and in 40% of institutionalized elderly (Webb, 2007).

15.4 Nutritional supplements

A cost-effective strategy for improving the micronutrient status of populations is by food fortification with the limiting nutrients, but some groups may be inadvertently excluded because of geographical isolation, age and other factors (Dary and Mora, 2002). Individual biological differences may also militate against appropriate use of fortification (Ames, 2004). For example, menstruating women need more iron than do males and older post-menopausal women. Similarly, vitamin B12 deficiency is quite common among elderly citizens (Hin *et al*., 2006) and concern has been expressed about adverse neurologic function in such individuals if habitually fed folic acid-fortified grains as mandated by the US Department of Agriculture in 1998 (Clarke, 2006; Pfeiffer *et al*., 2005).

Nevertheless, food fortification with micronutrients such as iodine, vitamin D, niacin and vitamin B2 has been quite successful and sustainable in the USA and other parts of the developed world because of the presence of centralized food industries (Dary and Mora, 2002; West *et al*., 2002). These industries have adequate facilities for monitoring distribution of the packaged and labeled foods, and informed consumers have sufficient purchasing power. Such facilities are relatively lacking in the developing countries, but successful attempts have been made to fortify several products,

for example, sugar, cereal flours and oil with vitamin A (Nestel, 1993). Other products, often fortified with multiple micronutrients, are now available in school feeding programs in several developing countries. One of the major prerequisites for successful food fortification programs in the developing world is identification of an indigenous food/commodity that is produced in a few sites and widely eaten in measured amounts by the target population (Dary and Mora, 2002).

15.5 Use of vitamin supplements

Public Law 103-417, the Dietary Supplement and Health Education Act (DSHEA) passed in the US Congress in 1994, discontinued premarket safety evaluation of ingredients in dietary supplements by the Regulatory Agencies (Neuhouser, 2003). This act has led to a significant increase in the number and varieties of supplements in use. By law, the dietary supplements are supposedly marketed devoid of medicinal claims relating to treatment and/or prevention of specific diseases, but in practice are often used for pharmaceutical instead of nutritional reasons (Webb, 2007). Vitamins are included among the products defined as supplements by the DSHEA and still not much is known about the risks and benefits of their long-term use (Eberhardie, 2007; Mason, 2007; Rock, 2007; Webb, 2007).

Vitamin supplements are among the most commonly used dietary supplements in the USA (Radimer *et al*., 2004), with the B-complex vitamins and ascorbic acid at the top of the list (Touger-Decker, 2007). No less than one-third of dietary supplement users specifically ingest vitamin C supplement daily, and about 5–10% of them consume more than 1000 mg of the vitamin per day (Johnston, 1999). Although the UL for total intake of ascorbic acid from all sources including supplements in adults is set at 2000 mg/day, supplementation at the level of 1000 mg/day may increase risk of kidney stones (Massey *et al*., 2005) and other health complications for individuals who are heterozygous for hemochromatosis (Werneke, 2007).

With respect to vitamin D, the optimal dose is likely to be influenced by calcium intake/status (Weaver and Fleet, 2004). The current UL for vitamin D which is 2000 IU (50 μg)/day may need to be re-examined since it has been shown that serum 25-hydroxycholecalciferol concentration plateaus at about 96 nmol l^{-1} with no signs of hypercalcemia or hypercalciuria in healthy men and women, ages 23–56 years, fed 4000 IU of vitamin D3 daily for 2–5 months (Veith *et al*., 2001), The IOM presently recommends 200 IU/day from birth through age 50 years, 400 IU/day for 51–70 years, and 600 IU/day for those >70 years (IOM, 1997), although some experts consider values closer to 1000 IU/day as optimal amounts (Veith *et al*., 2007). The UL for vitamin D consumption is about 10 000 IU/day, a dose administered to adult men for 20 weeks with no significant alterations in serum calcium

levels (Heaney *et al*., 2003). In a recent clinical trial registered at www.clinicaltrials.gov as #NCT00473239, a single large dose of cholecalciferol (100 000 IU) was shown to be a safe, effective and simple way to increase serum calcidiol concentration, with dosing interval about ≤2 months to ensure continuous serum calcidiol level above the baseline (Ilahi *et al*., 2008).

The current RDA (μg/day) for vitamin A varies from 300–400 in children, 1–8 years, to 600–900 (for males, ages 9 to >70 years) and 600–700 (for females, ages 9 to >70 years). For pregnant and lactating women, the recommended RDAs are 750–770 and 1200–1300 μg/day, respectively. The UL for adults is about 3000 μg/day (Gerster, 1997; Allen and Haskell, 2002). In industrialized countries, daily supplements provide 1500 μg of retinol activity equivalents (RAE), that is, 5000 IU of retinol palmitate daily for adults and 750 μg of RAE for children 2–5 years (Allen and Haskell, 2002). These supplements are barely available in developing countries where no less than between 5 and 10 million young children develop xerophthalmia every year.

In such vitamin A-deficient communities, a high dose of vitamin A supplement is promoted. The International Vitamin A Consultation Group 2002 recommended that women in such communities be given vitamin A supplement, 200 000 IU (60 mg) at delivery, plus another 200 000 IU before 8 weeks postpartum or a single dose not exceeding 10 000 IU (3 mg) daily or 25 000 IU (7.5 mg) once a week at any time postpartum (Ross, 2002). The revised recommendation for infants aged 0–5 months involves three doses of 50 000 IU (15 mg) at least 1 month apart, (6, 10 and 14 weeks). A single dose of 100 000 IU (30 mg) is recommended at 6–11 months, followed by 200 000 IU every 6 months between 12 and 59 months of age (Allen and Haskell, 2002). Recent reports have raised some serious concerns about the scientific wisdom of replacing the current low-dose vitamin regime (Sommer, 1995), with the new high dose regime (Benn *et al*., 2005; Prentice *et al*., 2007).

The RDA for folate varies from 150 μg/day in children to 600 μg/day for pregnant females ≤18 years, 31–50 years (IOM, 1998). The safe upper limit for intake is about 1 mg/day and there is lack of consensus regarding the safe upper blood concentration (Smith *et al*., 2008). Potentially serious health issues have been identified with respect to high consumption of folic acid from fortified foods or dietary supplements (Smith *et al*., 2008). These may include diminished efficacy of several antifolates (e.g. methotrexate, trimethoprin, etc) widely used in the treatment of health conditions such as acute leukemia in children, malaria, psoriasis and rheumatoid arthritis (Symmons, 2005; Chattopadhyay *et al*., 2007). Equally disturbing are reported observations that high folate intake has both protective and facilitatory effects on some cancer sites (Kim, 2007; Hultdin *et al*., 2005; Ericson *et al*., 2007). There is also the concern that, in individuals with compromised vitamin B12 status, a chronically elevated blood folate level may promote

adverse neurological effects. A recent report has presented detailed evidence to the effect that a high folic acid intake may be harmful for many individuals (Smith *et al*., 2008).

The AI levels for vitamin K are 55–75 μg/day for children 4–18 years and 90–120 μg/day for adults (Kalkwarf *et al*., 2004). A sensitive marker of vitamin K nutritional status is the serum concentration of under-gamma-carboxylated osteocalcin (uc OC), which is a predictor of bone mineral density (IOM, 1997) and also an indicator of hip fracture (Vergnaud *et al*., 1997). In the prospective cohort studies involving 72 327 women, vitamin K_1 intakes of <109 μg/day were associated with increased risk of hip fracture (Feskanich *et al*., 1999). A similar observation was made in elderly men and women in the Framingham Heart Study (Cashman, 2007). The serum concentration of uc OC dropped rapidly in response to supplementation with 80 μg to 1.0 mg of phylloquinone (Binkley *et al*., 2000). Many vitamin K_1 supplementation studies showing an increase in bone density, used pharmacological doses (Cashman, 2007), and at times concurrently with supplements of calcium, magnesium, zinc, and vitamin D (Braam *et al*., 2003).

The RDA for vitamin E varies from about 6 mg/day in young children ages 1–3 years to 15 mg/day for adult males and females, with an additional 4 mg/day for lactating women (IOM, 2000). The normal plasma level of vitamin E is 23.2 $\mu mol\,l^{-1}$ (1 $mg\,dl^{-1}$), with a range of 11.6–30.8 $\mu mol\,l^{-1}$ (0.5–1.6 $mg\,dl^{-1}$). Requirement for vitamin E increases with a high intake of polyunsaturated fatty acids (PUFA). RRR-α-tocopherol (RRR-AT) occurs naturally in foods and is both an antioxidant and an anti-inflammatory agent (Brigelius-Flohe and Traber, 1999; Devaraj *et al*., 2007). Several studies have shown that RRR-AT supplementation in humans lowers urinary F_2-isoprostanes, but may function as a pro-oxidant in cigarette smokers consuming a high-PUFA diet (Singh *et al*., 2005). A high dose of RRT-AT, about 1200 IU/d for 2 years, is reported to be safe and effective in reducing plasma biomarkers of oxidative stress and inflammation (Devaraj *et al*., 2007). High levels of intake may antagonize vitamin K-dependent clotting factors resulting in increased risk of bleeding (Werneke, 2007). The UL for vitamin E is 200–300 mg/day in children and increases to 1000 mg/day for adults (IOM, 2000).

Demographics and lifestyles of dietary supplement users

Vitamin supplement use, often in combination with minerals, contributes a significant proportion of micronutrient intakes in the USA and in the other developed economies, with the potential for risk of excessive intakes (Mason, 2007; Rock, 2007; Smith *et al*., 2008). More women than men, older age groups, more educated individuals with higher physical activity levels and lower body mass index, use dietary supplements. In the USA, more non-Hispanic whites than non-Hispanic blacks or Mexican-Americans use vitamin supplements and the users are generally better nourished than the non-users. The reasons advanced by users in support of micronutrient

supplementation are varied and include insurance of nutrient adequacy, compensation for perceived increased requirements, prophylaxis and treatment of illnesses, and improvement of physical performance, among others (Webb, 2007).

15.6 Brief review of the biological functions of vitamins

Recent scientific advances in the biological functions of vitamins offer important clues to their potential roles in the promotion of optimal oral health and prevention of diseases.

15.6.1 Vitamin A

This fat-soluble vitamin is an anti-infective micronutrient that plays key roles in vision, cellular differentiation, reproduction and growth (Champe and Harvey, 2005). Retinoic acid, derived from oxidation of dietary retinol found in animal tissues as a retinyl ester, mediates most of the actions of vitamin A except for vision, which is dependent on retinol. The detailed fundamental biological roles of retinol in vision and of retinoic acid (RA) in the regulation of gene transcription have been reviewed (Ross, 2002). All epithelial surfaces, including the skin, mouth, salivary glands, eye and the gastrointestinal tract suffer mucosal damage in vitamin A deficiency. The epithelial damage in vitamin A deficient subjects following viral infections offers a portal of entry for bacteria, resulting in secondary infections and contributing to severe viral infections (Ross and Stephensen, 1996; McCullough *et al*., 1999).

Vitamin A is stored as retinyl esters (REs) in the liver and in several cells, including oral mucosa cells (Biesalski and Nohr, 2004). Topically applied retinyl esters may reverse the morphological changes of the epithelium in human tissues that are vitamin A deficient and potentially correct cellular deficiencies and function (Sobeck *et al*., 2003). Inflammation, prenatal developmental events and several systemic problems, which are known to influence oral health and diseases, are conditions that require optimal vitamin A status. The leukotrienes are involved in several inflammation-driven diseases and it has been demonstrated that inactivation of leukotriene signaling is an anti-inflammatory property of therapeutic retinoids, effected through the CYP4F gene products expressed by epidermal keratinocytes (Kalsotra *et al*., 2008).

15.6.2 Vitamin D

The metabolic product of vitamin D, 1,25-dihydroxycholecalciferol (1,25 OHVitD3), is a pleiotropic secosteroid hormone that targets more than 200 human genes whose actions are associated with calcium homeostasis,

immune responses, cellular growth, differentiation and apoptosis (Cannell *et al*., 2008; Omdahl *et al*., 2002). The serum concentration of its precursor, 25 OHVitD3, which reflects both dietary intake and cutaneous synthesis, is considered a reliable, integrative measure of vitamin D status, and the acceptable normal range is 75–125 $nmol l^{-1}$ (Hollis, 2005). Cutaneous production of the vitamin is influenced by increase in age, melanin pigmentation, and the time of day, season and latitude. Additionally, there may be some sequestration of vitamin D in central body fat in obese individuals with a body mass index of >40 (Aasheim *et al*., 2008). More than 7% of adult USA women have a BMI greater than 40.

Calciotropic function of vitamin D

This is fully described in relatively recent reports (Holick, 2007; De Luca, 2004). The physiological effects are mediated through the vitamin D receptor (VDR), whose heterodimeric partner is the retinoid X receptor (RXR). Its physiological effects involve calcium and the parathyroid hormone in the maintenance of blood calcium levels. Calcitriol (1,25OHVitD3) induces proteins which promote active gastrointestinal absorption of calcium and phosphorus, mobilizes calcium from bone through stimulation of osteoblasts to produce receptor activation nuclear factor-$_k$B ligand (RANKL), which then stimulates osteoclastogenesis and activation of resting osteoclasts for bone resorption (De Luca, 2004).

Non-calciotropic effects of vitamin D

The enzyme 25D1 alpha-hydroxylase (CYP27B1), which converts 25OHVitD3 to the active hormone, is expressed at barrier sites in many extrarenal tissues, including the macrophages and normal cells involved in immune surveillance, such as dendritic cells and key epithelial cells (Hewison *et al*., 2004). Levels of CYP27B1 are increased in wounds and induced in keratinocytes in response to transforming growth factor (TGF-beta1). There are suggestions that the activity of this enzyme has an important impact on membrane integrity (Hewison *et al*., 2004).

Within the gastrointestinal tract, vitamin D hormone stimulates tight junction, adherens junction and gap-junction genes 43 (Gniadecki *et al*., 1997; Palmer *et al*., 2001). This hormone also induces expression of antimicrobial peptide gene in isolated human monocytes, neutrophils, keratinocytes and human cell lines, and in conjunction with lipopolysaccharide, synergistically elicits expression in neutrophils of cathelicidin antimicrobial peptide (camp), as well as activity against pathogens such as *Pseudomonas aeruginosa* (Wang *et al*., 2004).

Experimental animal studies suggest that epidermal keratinocytes surrounding a wound can elicit expression of the genes that code for the microbial pattern recognition receptors CD14 and Toll-like receptor (TLR)2, promoting an increase in camp expression (Schauber *et al*., 2007). The Th1 and Th2 cells are direct targets of vitamin D hormone which

promotes Th1 to Th2 cytokine production shift (Cantorna *et al*., 2004). Vitamin D deficiency is widespread (Holick, 2007) and there are suggestions that the marked differences in ability to produce vitamin D encountered in various human populations may partially explain the differences in susceptibility to infections such as tuberculosis (Liu *et al*., 2006). Vitamin D supplementation is particularly essential for certain special groups such as individuals with inflammatory bowel disease (Andreassen *et al*., 1997) and cystic fibrosis (Rovner *et al*., 2007), and in kidney transplant patients (Ewers *et al*., 2008).

15.6.3 Vitamin E

The mechanisms of action of vitamin E are still poorly understood. It is both an antioxidant and an anti-inflammatory nutrient. There are reports of prominent reduction in plasma biomarkers of oxidative stress and inflammation if a high dose of RRR-α-tocopherol (1200 IU/day for 2 years) supplementation is given to a patient with stable coronary artery disease (Devaraj *et al*., 2007). Apparent vitamin E deficiency is rare in the Western world but occurs in individuals with malabsorption syndromes. In patients suffering from a cardiomyopathy resulting from selenium deficiency, vitamin E status may be marginal (Beck, 2007). Deficiencies in selenium and/or vitamin E promote increased viral pathogenicity and altered immune responses, as well as viral mutations (Beck, 2007). Vitamin E supplementation may be necessary in patients with sickle cell anemia (Meydani, 1995) and may also reduce risk of some cancers, particularly in non-smokers. Several clinical trials employing vitamin E supplementation have, however, yielded controversial, often disappointing, results. A very high supplemental dose of vitamin E can antagonize vitamin-K-dependent clotting factors, resulting in increased risk of bleeding (Werneke, 2007).

15.6.4 Vitamin K

The principal function of vitamin K is the post-translational modification of blood clotting factors. Vitamin K-dependent (VKD) carboxylase functions to convert glutamyl residues (Glus) to carboxylated Glus (Glas) in VKD proteins, thereby activating them to participate in hemostasis, bone development, apoptosis, signal transduction, growth control and other physiological parameters (Berkner, 2005). Vitamin K is suggested to have a poorly understood role in bone metabolism. Inadequate intake increases the risk of osteoporotic fracture (Kalkwarf *et al*., 2004). The vitamin K-dependent proteins found in bone are osteocalcin which is the most abundant, matrix Gla protein and protein S (Vermeer *et al*., 1995). The current belief is that these proteins are required for bone mineralization, increased osteoblastogenesis and reduced osteoclastogenesis (Vermeer *et al*., 1995; Koshihara *et al*., 2003). The circulating concentration of under-

γ-carboxylated osteocalcin, a sensitive marker of vitamin K nutritional status, is considered to be an indicator of hip fracture (Cashman, 2007; Sokoll and Sadowski, 1996).

Current recommended adequate intakes are 55–75 μg/day for children 4–18 years, and 90–120 μg/day for adults (Kalkwarf *et al*., 2004). Findings in two large prospective cohort studies, the Nurses Health Study (Feskanich *et al*., 1999) and the Framingham Heart Study (Booth *et al*., 2000) confirm the association between the relative risk of hip fracture and vitamin K intake. Deficiency of vitamin K is uncommon except when the bacterial population in the gastrointestinal tract is markedly decreased by misuse of antibiotics, and also in individuals using certain second generation cephalosporins which have a warfarin-like action (Champe and Harvey, 2005). All newborns are vitamin K deficient and do require a single intramuscular dose of the vitamin to protect against hemorrhagic disease.

15.6.5 Vitamin C

Excellent reviews that deal with the functions of ascorbic acid are available (Johnston, 1999; Li and Schellhorn, 2007). This report focuses on new perspectives that relate closely to oral health. Vitamin C is a cofactor in diverse biological processes including collagen synthesis, neuromodulation, immune responses, scavenging of damaging free radicals and other functions (Li and Schellhorn, 2007). The ascorbic acid content of human tissues varies widely, with the highest concentrations found in the pituitary and adrenal glands (Jacob, 1996). It is also found in millimolar concentrations in circulating human neutrophils (Wang *et al*., 1997).

Of special interest is the well-documented observation that human keratinocytes possess efficient systems for the maintenance of high intracellular levels of vitamin C (Catani *et al*., 2005). The oral epithelium consists mainly of keratinocytes which adhere to one another by desmosomes and adherens junctions and, via hemidesmosomes, to an epithelial basement membrane, and ultimately to the mesenchyme of the lamina propria/dermis (Scully *et al*., 2005). Catani and her colleagues (2005) have reviewed the roles played by vitamin C in skin keratinocytes. The relevance of some of the findings to oral epithelial keratinocytes deserves to be evaluated. The ability of skin keratinocytes to accumulate high levels of vitamin C is due to the need to counter the effects of ultraviolet light exposure and also to influence differentiation of the keratinocytes (Catani *et al*., 2005). This vitamin modulates the transcription factors, nuclear factor kB (NF-kB) and the activator protein-1 (AP-1) which are both redox sensitive. In the presence of vitamin C, AP-1 complexes contain the Fra-1 protein, a member of the Fos family and a negative regulator of AP-1. Vitamin C interferes with the c-Jun N-terminal kinase (JNK)/AP-1 signaling pathway to mediate the cellular response against stress-induced damage (Catani *et al*., 2005). While AP-1 plays a key role in keratinocyte cell death, NF-kB is activated in

stressed cells and vitamin C enhances its activation (Tebbe *et al*., 2001; Shang *et al*., 2002).

Other important roles of vitamin C
Vitamin C, although an antioxidant, does also act as a mild pro-oxidant to promote the baseline oxidative stress required to initiate signal transduction which ultimately induces detoxification and/or antioxidant enzyme systems (Lee *et al*., 2003). It also prevents hydrogen peroxide disruption of gap junctions. Vitamin C is believed to possess anti-inflammatory potential (Ford *et al*., 2003; Wannamethee *et al*., 2006), but this view is disputed by some workers (Jialal and Singh, 2006).

Dietary vitamin C has been shown to downregulate inflammatory gene expression in apoE4 smokers (Majewicz *et al*., 2005). It is also suggested that the increased 'capillary fragility' associated with vitamin C deficiency is due to histaminemia (Clemetson, 1980). Ascorbic acid is essential for the metabolism of histamine (Chatterjee *et al*., 1975) and studies in humans have shown that the blood histamine level rises very significantly ($P < 0.001$) when plasma ascorbic acid concentration drops below 0.7 mg/100 ml (Clemetson, 1980). Using ascorbic acid supplementation (2000 mg/day) to increase plasma vitamin C to 1.11 ± 0.05 mg, the blood histamine level drops significantly from 52.8 ± 3.7 to 33.0 ± 1.6 mg ml^{-1} (Johnston, 1999). Additional evidence in support of an anti-inflammatory role for vitamin C is the observation that in scurvy-prone ODS rats (genotype od/od with a hereditary defect in ascorbate biosynthesis), the serum concentration of interleukin-6, an inflammatory mediator that stimulates gene expression of some acute phase proteins, is significantly higher in the animals subjected to ascorbic acid deficiency for 14 days than in well fed controls (Ikeda *et al*., 1998).

Other rich tissue reservoirs of vitamin C are the neutrophils (Jacob, 1996; Wang *et al*., 1997) and the major salivary glands (Hornig, 1975; von Zastrow *et al*., 1984; Sawiris *et al*., 1995). Vitamin C stimulates neutrophilic chemotaxis, oxidative metabolism and neutralization of histamine, among other functions (Alvares, 1996). Manifestations of neutrophil dysfunction may include infections of the oral mucosa and severe breakdown of periodontal tissues (Van Dyke and Hoop, 1990). The Sicca syndrome was one of the first observations in all five subjects subjected to vitamin C deficiency (Hodges *et al*., 1971). The features included loss of secretion of salivary and lacrimal glands and swelling of the parotid and submaxillary glands and arthritis with limitation of motion. Refeeding with mega doses of vitamin C resulted in rapid recovery (Hodges, 1971).

15.6.6 The B-complex vitamins

Folate participates in one-carbon reactions in the synthesis of purines and pyrimidine (thymine), the metabolism of amino acids and the formation of S-adenosylmethionine (SAM), which is the primary methylating agent in

tissues (Bailey and Gregory III, 1999). The pteroylmonoglutamate known as folic acid, which is used commercially for food fortification and in dietary supplements, has to be converted to the reduced tetrahydrofolates (THF) to function as coenzymes and regulatory molecules in the body (Bailey *et al*., 2003). The detailed biochemical roles of natural folates are discussed in a very recent report by Smith *et al*. (2008), which also points out that folic acid has a much higher bioavailability than do the natural folates and may compete with the latter for binding with enzymes, carrier proteins and binding proteins. This underscores the fact that folic acid supplement must be reduced to tetrahydrofolate through dihydrofolate before it can enter the folate cycle (Wright *et al*., 2007).

Adequate folate status is essential for cell division and homeostasis. The inverse association between dietary folate intake and vascular disease risk is due to the role of this vitamin, along with others like vitamins B6 and B12, in regulating the plasma homocysteine level. Folate deficiency causes megaloblastic anemia and impairs cell division in tissues, particularly in those with rapid turnover rates such as the mucosal cells of the gastrointestinal tract and the oral cavity. Studies several decades ago showed that adults fed a pharmacologic dose of folic acid for 30 days exhibited significantly increased resistance to dental plaque and diminished gingival inflammation (Vogel *et al*., 1976).

Vitamin B12 is required in humans for metabolism of homocysteine to methionine, for the isomerization of methylmalonyl CoA produced during degradation of fatty acids containing odd numbers of carbon atoms, and some amino acids. Except in individuals with partial or total gastrectomy, which compromises the availability of the intrinsic factor necessary for cobalamin absorption, it takes several years to deplete the body's pool of this water-soluble vitamin. Deficiency of vitamin B12 results in pernicious anemia and increases circulating levels of homocysteine and methylmalonic acid, among other features.

Thiamine is rapidly phosphorylated to diphosphate ester (thiamine diphosphate; TDP). TDP may undergo additional phosphorylation to thiamine pyrophosphate (TPP) by TDP phosphoryltransferase enzyme which is expressed in some tissues, for example the brain, kidney, heart and liver (Butterworth, 2006). TDP is an essential cofactor for enzymes participating in the metabolism of glucose and amino acids, namely transketolase, pyruvate dehydrogenase complex and α-ketoglutamate dehydrogenase. Causes of thiamine deficiency may include alcohol abuse, malabsorption and the increased requirement in AIDS, among other diseases.

Niacin: Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) are the biologically active forms of niacin. They serve as coenzymes in oxidation–reduction reactions in which the coenzymes promote reduction of the pyridine ring by accepting a hydride ion (Champe and Harvey, 2005). The reduced forms are NADH

and NADPH and deficiency of the vitamin causes pellagra, a disease that affects the skin, central nervous system and the gastrointestinal tract. Pellagra can also be caused by deficiencies of vitamin B2, vitamin B6, copper and iron which are involved in the biotransformation, although inefficiently, of tryptophan to nicotinic acid. Niacin, but not nicotinamide, in pharmacologic doses (1–3 g/day), is antilipidemic (Champe and Harvey, 2005; Bourgeois *et al*., 2006). It is also proposed that deficiency of this vitamin promotes a cancer risk in view of the influence on genomic stability of some NAD-dependent enzymes such as the poly (ADP-ribosyl) polymerases (PARPs) and Sir2-like NAD-dependent deacetylases (SIRTs) (Bourgeois *et al*., 2006).

Riboflavin: The two biologically active forms are flavin mononucleotide (FMN) and the more complex coenzyme, flavinadenine dinucleotide (FAD). Deficiency of riboflavin is seen mainly in women and children in developing countries, and particularly in children suffering from protein energy malnutrition (PEM) who have poor intake as well as diminished absorption (McCormick, 2006). Lactose maldisgestion may be an important risk factor.

Pantothenic acid: Deficiency of this vitamin, a component of coenzyme A, is rare, and most likely occurs concurrently with deficiencies of other vitamins (Trumbo, 2006). No adverse effects of overconsumption of this vitamin have been reported.

Pyridoxine: The biologically active form is pyridoxal phosphate which functions as a coenzyme in many reactions involving the metabolism of amino acids, one-carbon units, lipids, heme synthesis, as well as the biosynthesis of neurotransmitters (Mackey *et al*., 2006). Several disease states are associated with vitamin B6 status, and in many of these, there is no clarity regarding a cause–effect relationship. Alcohol abuse decreases pyridoxal phosphate status. The availability of the vitamin is also compromised by extensive interactions with drugs which covalently bind to its carbonyl group. These drugs include isoniazid, L-dopa, cycloserine and gentomicin.

Biotin participates in classical carboxylation reactions as an essential cofactor for five mammalian carboxylases (Mock, 2006). Biotin also functions in cell signaling and gene expression, with more than 2000 biotin-dependent genes so far identified in human tissues (Zempleni, 2005). Post-translational biotinylation of histones (basic proteins associated with DNA in nucleosomes) increases in response to cell proliferation in human lymphocytes (Stanley *et al*., 2001). Histones are involved in DNA replication and repair and thus related to cell proliferation and differentiation. Biotin deficiency is rare, but mild deficiency in pregnant women may cause teratogenicity in the offspring (Mock, 2006). In normal human pregnancies, the fetal to maternal biotin ratio increases during the second trimester (Zempleni, 2005).

15.7 Oral manifestations of vitamin deficiencies

Table 15.6 summarizes some of the oral signs and symptoms suggestive of deficiencies of the vitamins. Several of these signs are non-specific and can be caused by deficiencies of other micronutrients such as zinc and iron, as well as non-nutritional factors (Enwonwu, 1992; FDI, 1994). Increased carriage of *Candida* species, and often candidiasis, characterize deficiencies of folic acid, vitamin A or cobalamin (Samaranayake, 1986). Single micronutrient deficiencies are rare in the human. Multiple deficiencies are the rule and they elicit a wide spectrum of clinical manifestations and functional impairments. Vitamin deficiencies downregulate immune responsiveness, affecting both innate and acquired immunity. This was recently reviewed by Maggini *et al*. (2007) and Wintergerst *et al*. (2007) (Tables 15.7 and 15.8).

The epithelial barrier system, cell mediated immunity (CMI) and antibody production, are adversely affected by deficiencies of the vitamins. It is commonly believed that supplementation with the missing/deficient vitamins will correct the immunological defects (Maggini *et al*., 2007). The supplementation calls for caution since a massive amount of an antioxidant like ascorbic acid may end up protecting both pathogens and the host tissues from oxidants (Wang *et al*., 1997). Additionally, excessive antioxidant supplementation may adversely compromise some important physiological processes which depend on free radicals (Sardesai, 1995).

Table 15.6 Common clinical oral lesions associated with specific vitamin deficiencies

Deficiency	Manifestations
Vitamin A	Hyperkeratinization of oral mucosa, impaired healing, impaired bone and tooth formation, blockage of salivary duct/reduced saliva flow, mucocutaneous candidiasis, potentiates existing inflammation
Vitamin D	Delayed tooth eruption/development, enamel hypoplasia, rickets, osteoporosis and low alveolar bone density, increased gingival bleeding; increased susceptibility to dental caries
Vitamin K	Impaired blood clotting; gingival bleeding
Vitamin E	Rare; anemia
Folic acid	Anemia; glossitis, cheilitis, increased carriage of *Candida*
Vitamin C	Gingival bleeding, impaired wound healing, impaired mucosal membrane integrity; salivary gland hypofunction
Thiamine	Stomatitis, 'burning' mouth syndrome
Riboflavin	Angular cheilitis, burning oral mucosa, ulceration
Niacin	Angular stomatitis, papillary atrophy of tongue
Vitamin B12	Pernicious anemia, redness and 'burning' of tongue, tongue-fissuring, nuclear enlargement of oral mucosal cells, epithelial dysplasia
Pyridoxine	Glossitis, angular stomatitis, cheilitis

Adapted in part from Robbins (2005).

Table 15.7 Role of fat-soluble vitamins in the immune system

Vitamin A	Vitamin A and related retinoids are essential for the normal differentiation of epithelial tissue and are involved in gene expression.
	Plays an important role both in humoral antibody response and cell-mediated immunity supporting a Th2 anti-inflammatory response.
	Deficiency impairs innate immunity by hindering regeneration of epithelial barrier damaged by inflammation.
Vitamin D	Potent immune system modulator when metabolized to $1,25(OH)_2D_3$;
	Involved in cell proliferation and differentiation;
	Most cells of the immune system except B cells express vitamin D receptors;
	Enhances innate immunity by increasing the differentiation of monocytes to macrophage.
Vitamin E	Most important fat-soluble antioxidant; protection of membrane lipids from oxidative damage;
	Reduced production of immune suppressive factors such as PGE_2 in macrophages;
	Optimizes and enhances immune response (Th1 response).

Adapted in part from Maggini *et al.* (2007) and Wintergest *et al*. (2007).

Table 15.8 Role of water-soluble vitamins in the immune system

Vitamin B_6	Interferes with immune function through its involvement in nucleic acid and protein biosynthesis in concert with vitamin B_{12} and folate.
	Adequate intake maintains a Th1 immune response.
Folate	Participates in immune function through its involvement in nucleic acid and protein biosynthesis in concert with vitamin B_{12} and vitamin B_6.
	Maintain innate immunity (NK cell activity).
Vitamin B_{12}	Interferes with immune function through its involvement in nucleic acid and protein biosynthesis in concert with vitamin B_6 and folate.
	May act as an immunomodulator for cellular immunity, especially with effects on cytotoxic cells (NK; CD8+ T-lymphocytes).
Vitamin C	Effective antioxidant contributing to the maintenance of the redox integrity of cells and protection against reactive oxygen species generated during respiratory burst and inflammatory response.
	Regenerates other antioxidants (e.g. vitamin E, glutathione).
	Stimulates leukocyte functions (neutrophil, monocyte movement).
	Role in antimicrobial and NK cell activities, lymphocyte proliferation, chemotaxis and DTH response.
	Involved in metabolism of histamine.

Adapted in part from Maggini *et al*. (2007) and Wintergest *et al*. (2007).

15.7.1 Role of supplements in prevention and/or management of oral diseases

Chronic periodontitis, an inflammatory-immune response to specific bacteria, is linked to systemic inflammatory diseases and a dysmetabolic status (Chapple *et al*., 2007; Nibali *et al*., 2007). A major systemic effect of inflammation is the acute phase response (APR), resulting in increased production of the acute phase proteins such as the C-reactive protein and serum amyloid A, and decreased availability of micronutrients like retinol, iron, zinc and others (Enwonwu and Ritchie, 2007). It is estimated that the approximate negative changes on plasma vitamins induced by APR are retinol (–50%), riboflavin (–60%), pyridoxine (–50%) and ascorbate (–70%) (Prentice *et al*., 2007) and they are likely to be temporary survival adaptations. It is not clear whether these depleted vitamins should be fully replenished prior to correction of the cause of the inflammation/disease. There is, so far, no reliable information on the effect of inflammation on the plasma level of 25-cholecalciferol.

Vitamin D, through its anti-inflammatory effect and calciotrophic effect on alveolar bone, may reduce the risk of periodontal disease. A statistically significant ($P < 0.001$) inverse association exists between the serum concentrations of 25-hydroxycholecalciferol and periodontal disease levels in individuals aged 50 years or older (Dietrich *et al*., 2005; Bischoff-Ferrari *et al*., 2006). With respect to vitamin K, which is believed to be needed for bone mineralization (Cashman, 2007), its supplementation may pose some concerns for periodontal health. Microorganisms residing in the gingival crevices may have very limited access to nutrients in saliva (Shah and Gharbia, 1995). Among the microorganisms present are *Prevotella intermedia* and *Porphyromonas gingivalis* which are dependent on vitamin K in the gingival crevicular fluid (GCF), a serum transudate or inflammatory exudate. These microorganisms are involved in the pathogenesis of periodontitis. Supplementation with vitamin K may promote increased availability of nutrients to these organisms. There is also the possibility of enhanced growth of other bacteria, for example *Staphylococcus aureus* which requires this nutrient (Acar *et al*., 1978; Baronets, 2003).

15.7.2 Vitamin supplements in HIV-infection/AIDS

There is lack of consensus on the impact of HIV-infection on vitamin status. Beisel (2002) has suggested that many of the B-complex vitamins are unaffected, but reductions may be noted in riboflavin, pyridoxine, folate and cobalamin, with cobalamin being the only one adversely affected and influencing the course of the infection. In contrast, several reports indicate that HIV-infected individuals, despite a history of high dietary and supplemented intakes of micronutrients, often present with low serum levels of vitamins A, B6, B12, C, E, folate and the carotenoids (Enwonwu and Warren, 2001; Friis and Michaelson, 1998). A relatively recent cross-sectional study of 241

HIV-positive and 115 HIV-negative subjects showed significantly low plasma vitamin C in the former group, but no group differences in tocopherol (Stephensen *et al*., 2006). Some studies have reported abnormally low carotenoids, vitamin C and 25-hydroxycholecalciferol in HIV-infected subjects (Semba and Tang, 1999). The metabolic responses to infections may account for the discrepancies between various studies. Additionally, the effects of HIV infection on vitamin status are likely to be more pronounced among the less priviledged with habitually low micronutrient intakes.

15.7.3 Vitamin supplementation and oral cancer

Oral squamous-cell carcinoma (SCC) is the main type of oral cancer, which is preceded in most cases by pre-malignant lesions such as leukoplakia, submucous fibrosis and lichen planus (Varma *et al*., 2007). The vitamins preferentially used by patients for prevention and treatment of cancers include retinol, ascorbic acid, tocopherols and β-carotene, although the efficacy of some of these is in doubt (Werneke, 2007). The main risk factors for SCC are alcohol and tobacco (smoking and smokeless) usage. In several developing countries, chewing of betel squid is a major risk factor.

High intake of fruits and vegetables reduces the risk of several cancers including SCC. It must be emphasized that fruits and vegetables contain important, non-essential nutrients besides vitamins and minerals. Using p53 and bcl2 oncoproteins as prognostic markers, vitamin A therapy in high dose has been shown to be effective against some pre-malignant oral lesions except in chronic heavy smokers (Varma *et al*., 2007). Similarly, other workers have reported an elevated risk of oral premalignant lesions (OPLs) in current smokers receiving high supplements of vitamin E and/or β-carotene (Maserejian *et al*., 2007). While case-control studies suggest that vitamins C, E and the carotenoids may reduce the risk of OPLs, clinical trials do not appear to support these claims for vitamin E and β-carotene (Maserejian *et al*., 2007).

This is a subject area riddled with controversies (Brown and Kane, 2006). Some workers attribute the increased risk of oral cancer in chronic heavy smokers to diminished folate status, altered folate form distribution and increased genetic damage in the buccal mucosa (Gabriel *et al*., 2006), with the buccal cell micronuclei index serving as a putative biomarker. Although the number of micronuclei in buccal mucosal cells of heavy smokers is higher than in non-smokers, no significant association has been demonstrated between systemic or oral mucosal folate status and the number of micronuclei. Localized folate and vitamin B12 deficiencies may be promoted by hydrocarbons in tobacco smoke which inactivate these vitamins. Alcohol, another major risk factor for oral cancer, promotes malabsorption, increased excretion and abnormal folate metabolism. Use of anti-folate drugs has featured prominently in cancer chemotherapy because of the biological roles of this vitamin in nucleic acid synthesis and DNA repair

processes. A recent report (Smith *et al*., 2008) suggests that folic acid may play a dual role in cancer. Folate supplementation prior to establishment of neoplastic foci may retard the development of the tumor, but if the foci are already well established, the supplementation will promote growth of the preneoplastic cells (Kim, 2004; Song *et al*., 2000).

15.7.4 Orofacial clefting and vitamins

Orofacial clefts (QFCs), including cleft lip with or without cleft palate (CLP), and cleft palate alone (CP) affect approximately 1 in 1000 and 1 in 2500 infants, respectively (Itikala *et al*., 2001). In QFCs, molecular biological processes are modified by nutrients, including vitamins, which influence the expression and silencing of genes during embryogenesis. Vitamins associated with OFCs in humans, either in excessive or deficient amounts, include thiamine, riboflavin, niacin, folate, biotin, pyridoxine, ascorbate, vitamin A and vitamin B12. Biotin deficiency affects the proliferation of human embryonic palatal mesenchymal cells in culture (Takechi *et al*., 2008). Histones are involved in the replication as well as the repair of DNA and evidence has been provided that biotinylation of histones increases in response to cell proliferation and differentiation (Stanley *et al*., 2001). Vitamin A is of interest in palatogenesis. The retinoid receptors, namely retinoic acid receptor (RAR), retinoic X receptor (RXR) and their sub-types play important roles in cell differentiation and proliferation during embryonic development. An excess of retinoic acid can inactivate its receptors, resulting in cellular damage and impaired retinoic acid-mediated signaling. In contrast, maternal vitamin A deficiency during pregnancy is believed to promote the occurrence of oro-facial clefts in the offspring (Hozyasz and Chelchowaska, 2004).

Folate sufficiency and metabolism play an important role in embryonic development and there is evidence that periconceptual folate supplementation reduces the risk of neural tube defects (NTDs) and very likely, OFCs (Boyles *et al*., 2008). This has been confirmed in several other studies (Krapels *et al*., 2006; VanRoij *et al*., 2004).

Following mandatory folic acid fortification of foods in the USA in 1998, a study of prevalence ratios of OFCs in 45 states and the District of Columbia, relative to pre-fortification showed a small decline in non-Hispanic whites only. This was observed exclusively in women who did not smoke during pregnancy and who also had prenatal care in the first trimester (Yazdy *et al*., 2007). In a hospital-based case–control study in the Boston, Philadelphia and Toronto areas, no association was observed between OFCs and maternal use of multivitamins containing folic acid (Hayes *et al*., 1996). In contrast, an epidemiological study in California revealed that maternal use of multivitamins containing folic acid significantly reduced the occurrence of CLP and CP by 50% and 27%, respectively (Shaw *et al*., 1995), an observation consistent with a subsequent report showing that

periconceptual supplementation with folic acid alone reduced the risk of CLP by 50% (VanRoij *et al*., 2004). The latter and the reported increased risk of OFCs in a large case-control study in which mothers received dihydrofolate reductase, a folic acid antagonist, provided additional evidence in support of the probable role of adequate folate status in the prevention of oro-facial clefts (Hernandez-Diaz *et al*., 2000). Polymorphisms in the 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene increase the risk of OFCs. In a Norwegian population-based study which included 362 families with CPL and 191 families with CP, a 39% reduction in the risk of CPL with folic acid supplementation was observed (Boyles *et al*., 2008).

15.8 Conclusions

Motivations for the widespread, growing use of vitamin supplements are still poorly understood. Even more enigmatic is the special appeal of these micronutrients to the well-educated, high socioeconomic, apparently well-nourished groups who really have no physiological need for them. Vitamin supplements compensate for relevant deficiencies in special groups in the rich developed world, but are more useful for the widespread, often severe dietary deficiencies encountered in poor developing countries. In both instances, the best strategy is good judgment in the choice of a wide variety of foods, including appropriately fortified foods.

Every vitamin has a safe range of intake. The toxicity or risk of harm from a vitamin supplement is determined not only by the level of intake, but also by many other poorly understood factors (Mulholland and Benford, 2007). These variables include supplement formulation, water solubility and numerous host factors such as genetic profile, age, infections/disease state, concurrent use of prescribed and over-the-counter medications and lifestyle behaviors such as abuse of alcohol and tobacco (Eberhardie, 2007; Webb, 2007; Werneke, 2007; Prentice *et al*., 2007). In some individuals, the margin of safety between the therapeutic and toxic doses of a vitamin may be very small. For example, supplementation with a high dose of vitamin C in subjects heterozygous for hemochromatosis and thalassaemia or those predisposed to formation of renal stones, heightens the chances of developing kidney damage particularly if the subjects are on methotrexate chemotherapy at the same time (Sketris *et al*., 1984).

There is still a severe dearth of reliable, evidence-based data for accurate clinical assessment of use the of dietary vitamin supplements, their efficacy, toxicity and interactions with medications and other lifestyle factors. Herbert (1996) questions the benefits, if any, of the widespread use of vitamin supplements by most Americans who are generally well nourished. About one and half decades ago, Kim and colleagues (1993) reported no difference in mortality rates between Americans who took dietary supplements regularly and those who did not. More disturbing are relatively recent observations

of the increased cancer risk and mortality associated with some vitamin supplementations. Supplementation with β-carotene increased the incidence of, and mortality from, lung cancer in current and ex-smokers (Werneke, 2007). Similarly, in a prospective cohort studies of 77 721 men and women from Washington State, USA, vitamin E supplementation increased the risk of lung cancer in current smokers (Slatore *et al*., 2008). In the most recently published SENECA (Survey in Europe on Nutrition and the Elderly; a concerted action) study, which examined individuals residing in fifteen European towns, dietary supplements increased all-cause mortality risk among smokers (Brzozowska *et al*., 2008).

Another issue of increasing concern is the potential antagonism or reversal of efficacy of prescribed chemotherapies by dietary vitamin supplementations. Sulfadoxine pyrimethamine (SP), an anti-folate, is a popularly prescribed anti-malarial drug in tropical countries where malaria is holoendemic. Folic acid supplement may increase the failure rate of this drug (Metz, 2007).

15.9 Assessment of vitamin status

Ideally, clinicians should have a good knowledge of the dietary supplements habitually consumed by their patients before they can offer meaningful advice to the latter. It is also necessary to assess the nutritional status of the subjects to determine those who really need additional dietary support. Assessment of vitamin status is not very easy (Tomkins, 2003). Plasma vitamin concentrations are often used, but their diagnostic values are questionable since they are influenced by many factors such as infections, inflammation and exercise, among others, and may not reflect true deficiencies (Filteau, 1999; Stephensen, 2000; Tomkins, 2003; Prentice *et al*., 2007). For example, infection-induced alterations in the synthesis or release of the hepatic acute phase proteins (APPs), produce marked reduction in circulating levels of several vitamins including retinol, pyridoxine, folate, riboflavin, ascorbic acid and tocopherol (Prentice *et al*., 2007). If the subject is marginal in retinol status, the acute phase response (APR) can bring plasma retinol concentration transiently down to a deficiency level (Filteau, 1999). The plasma retinol level rebounds as the compartmentalized vitamin is released from the liver bound to retinol binding protein, after resolution of the APR (Stephensen, 2000). Some investigators have cautioned that immediate vitamin supplementation to correct the temporary, adaptive changes elicited by infection-induced APR, may precipitate unintended adverse complications (Prentice *et al*., 2007). Assessment of vitamin status can also be carried out using some metabolic markers. These include accumulation of homocysteine in circulation resulting from its impaired remethylation to methionine as a consequence of folate and/or vitamin B12 deficiency, and methylmalonic acid accumulation caused by vitamin B12 deficit.

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16

Trace elements and oral health

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Abstract: This chapter first gives a short overview of the importance of good nutrition for healthy teeth, the classification of trace elements and the trace element content of teeth. It then gives an overview of the role of fluoride, iron, copper, zinc and selenium on oral health. For each nutrient, the effects of both inadequate and excessive intakes are discussed, and food sources and dietary requirements are given. Interactions between nutrients and the effect thereof on oral health are discussed where applicable.

Key words: copper, fluoride, iron, oral health, selenium, zinc.

16.1 Introduction

Oral health and dental diseases have an impact on eating ability, nutrition and health, both in childhood and later in life. This includes people living with a disease (e.g. AIDS), condition (e.g. congenital abnormalities such as cleft palate) or treatment (e.g. radiation therapy) that affect the mouth (DePaola *et al.*, 2006). Dental status can have an impact on food choice and intake of key nutrients and is closely associated with nutritional status, particularly the elderly (Marcenes *et al.*, 2003; Sahyoun *et al.*, 2003; Sheiham *et al.*, 2001; Sun Lee *et al.*, 2004). This chapter will, however, not focus on the effect of oral health on the nutritional status, but on the effects of nutritional status on oral health.

Childhood malnutrition has a number of effects on the dentition. A cross-sectional survey of children aged one to 13 years showed that malnutrition delayed tooth development, affected the age distribution of dental caries and resulted in a higher number of caries in primary teeth (Alvarez *et al.*, 1990). A longitudinal study of dental caries in the primary teeth of children showed that malnutrition during the first year of life may result in an increased risk of dental caries three to four years later, possibly because

of a deleterious effect on the formation of tooth enamel early in life (Alvarez *et al*., 1993). Children with severe early childhood caries were found to have compromised nutritional status, particularly iron status (Clarke *et al*., 2006). Adequate overall nutrition from a very young age is therefore vital for healthy teeth.

This chapter concentrates on the effect of trace elements in the diet on oral health. Curzon and Cutress (1983, p2), for the purpose of their book *Trace Elements in Dental Disease* defined trace elements as all the naturally occurring elements in the periodic table excluding the major components of hydroxyapatite – calcium, phosphorus, oxygen and hydrogen. In the WHO/FAO report *Trace Elements in Human Nutrition and Health* (WHO/FAO, 1996, p3) the definitive feature of a 'nutritionally significant trace element is either its essential intervention in physiological processes or its potential toxicity when present in low concentrations in tissues, food or drinking water'. The term 'trace' was used by this Expert Consultation when the concentration of an element does not exceed 250 $\mu g\ g^{-1}$ of matrix. One can argue that resistance to dental caries is a physiologically important function (WHO/FAO, 1996, p4) and thereby declare that the element fluoride is essential. The concentration of trace elements varies in the different anatomical parts of the mouth and during different stages of the lifecycle. Consequently, specifying a cut-off value to define trace elements poses problems. On the basis of the current literature available on oral health, the following elements were considered to be the most important and were selected for further discussion in this review: fluoride, iron, copper, zinc and selenium. Whilst iodine might be related to tooth eruption and malocclusion, inadequate intakes of boron could affect the periodontium by impairing wound healing and bone calcification (DePaola *et al*., 2006) and molybdenum may have a beneficial effect on the incidence and severity of dental caries (Expert group on Vitamins and Minerals, 2002), these trace elements are not further elaborated upon.

16.2 Trace element content of teeth

It has been suggested that teeth are suitable indicators of trace element exposure because of their physical stability which causes the element to be retained. The trace element content of mineralized dental tissue is related to those elements incorporated into the apatite crystals during the mineralizing period and to those which diffuse into the tissue after completion of mineralization. The trace element content of teeth therefore reflects not only the biological environment during the time of tooth development, but also the oral and vascular environments associated with the erupted tooth (cited in Brown *et al*., 2002).

A comparison of trace element concentrations in caries-affected versus healthy extracted primary and secondary teeth led a group of Polish

researchers to postulate that lower concentrations of zinc, iron, copper, nickel, selenium and strontium together with higher concentrations of chromium, cobalt, lead and cadmium can be a risk factor for dental caries (Gierat-Kucharzewska and Karasiński, 2006). Equally, the saliva of children with and without dental caries may have a different trace element composition (Zahir and Sarkar, 2006).

A study comparing the trace element content of primary teeth of children from Uganda and the United Kingdom (UK) suggested that the country of origin and the physical environment may play a role in tooth composition (Brown *et al*., 2002, 2004). When compared to teeth from Uganda, UK teeth had significantly more zinc and the authors argued that this could be explained by the absence of malnutrition and the use of zinc in insecticides, drugs and paint (Brown *et al*., 2004). On the other hand, Tvinnereim *et al*. (1999) did not find a correlation between tooth zinc concentration and available data on zinc in drinking water and the discharge of zinc from industrial sources.

16.3 Fluoride

16.3.1 Fluoride and dental caries

Fluoride is important for the integrity of bones and teeth and is an important protective factor against dental caries. Fluoride enhances tooth mineralization and remineralization, reduces tooth demineralization, thus contributing to the stability of enamel crystal, and inhibits the metabolism of the acid-producing bacteria responsible for dental caries (ADA Reports, 2005; DePaola *et al*., 2006; Moynihan and Petersen, 2004). Fluoride affects dental caries both pre-eruptively and post-eruptively.

Pre-eruptively, during tooth development, fluoride is incorporated into the developing tooth's mineralizing structure and helps increase resistance to acid demineralization (ADA Reports, 2005). The assimilation of ingested fluoride in calcified, pre-eruptive teeth is most dramatic during infancy, decreasing with age (DePaola *et al*., 2006).

The main protective action of fluoride is topical, after the teeth have erupted (Sheiham, 2001). People of all ages benefit from the topical effects of fluoride whether or not they had pre-eruptive systemic fluoride as children (ADA Reports, 2005). The findings of more than 800 controlled trials showed that fluoride, either in water or in toothpaste, is the most prophylactic agent against dental caries (cited in Sheiham, 2001).

16.3.2 Sugar intake, fluoride and dental caries

Sugar is a well-known risk factor for the development of dental caries. Exposure to fluoride alters the sugar–caries relationship. A systematic review carried out by Burt and Pai (2001) concluded that, where there is

good exposure to fluoride, sugar consumption is a moderate-to-mild risk factor for caries in most people. On the other hand, in persons who do not have regular exposure to fluoride, sugar consumption is likely to be a more powerful indicator of risk of caries. With widespread use of fluoride, sugar consumption has a role to play in the prevention of caries but this role is not as strong as it is without exposure to fluoride. Sheiham (1991) argued that, where fluoride in drinking water is 0.7–1 ppm, or where over 90% of toothpastes available are fluoridated, the safe level of sugars consumption would be up to 15 kg/person/year, compared to 10 kg/person/year in the absence of fluoride.

16.3.3 Fluorosis

The exposure range between too little and too much fluoride is not wide. Excessive fluoride exposure causes dental fluorosis, a permanent hypomineralization, mottling and discolouration of tooth enamel. This occurs when too much fluoride is ingested prior to tooth eruption (i.e. during enamel formation – between birth and approximately 14 years of age) (ADA Reports, 2005; DePaola *et al*., 2006). Examples would be if young children ingest excess fluoride through dietary fluoride supplements or fluoridated toothpaste.

Four main risk factors for fluorosis have been documented, namely the use of fluoridated drinking water, misuse of fluoride supplements, fluoridated toothpaste and food processing, specifically foods and infant formula reconstituted with fluoridated water (ADA Reports, 2005; dePaola *et al*., 2006; Levy, 2003; Mascarenhas, 2000). Having multiple sources of ingested fluoride is an additional risk factor. The use of fluoridated toothpaste by very young children increases the risk of dental fluorosis (Tavener *et al*., 2006; Wang *et al*., 1997), since it is often swallowed rather than expectorated (DePaola *et al*., 2006). Fluoridated salt programmes may increase the risk of dental fluorosis, as was shown in Mexico (Vallejos-Sánchez *et al*., 2006).

Dental fluorosis is more common in countries that have higher levels of fluoride in their water supplies. In a review paper, Sheiham (2001) stated that the prevalence of dental fluorosis ranges from 3–42% in low fluoride areas and 45–81% in areas with optimal water fluoridation. Beverages consumed during infancy influence the risk of primary tooth fluorosis and the risk is mostly due to the fluoride concentration of water used in reconstituting beverages. A study by Marshall *et al*. (2004) showed that infant beverages, particularly infant formulas prepared with fluoridated water, can increase the risk of fluorosis in primary teeth.

A general increase in the prevalence of dental fluorosis has been observed. In the United States (USA), for example, 22.8% of school children presented with dental fluorosis in a national survey conducted by the National Institute of Dental Research in 1986–87, versus 32% in the NHANES

survey that was done in 1999–2002 (Beltrán-Aguilar *et al*., 2005). This represents a 9% increase in the prevalence in a period of less than 15 years.

16.3.4 Sources of fluoride

Fluoride is a universally present element found in varying concentrations in all natural waters. Water fluoridation is the process of adjusting the amount of fluoride that is present naturally in water to an optimal level for protection against tooth decay. Many countries have fluoride added to their drinking water, at the controlled level of 1 mgl^{-1} (1 ppm). This recommended level of 1 ppm fluoride is considered optimal and safe for the prevention of caries. There is little benefit from water with fluoride above 1 ppm, while fluorosis only becomes significant above 1 ppm. This concentration, therefore, offers maximal protection against dental caries with minimal risk of dental fluorosis (ADA Reports, 2005).

Water fluoridation benefits all residents served by community water supplies, regardless of their social or economic status, provided that they have access to the water supplies. Water fluoridation is particularly beneficial for individuals who have less access to oral health care and alternative fluoride resources (ADA Reports, 2005). The approximate 20% of the world's population who do not have access to safe drinking water (WHO, 2003) will not benefit from fluoridation of drinking water.

Dietary fluoride is obtained mostly from fluoridated drinking water and fluoride in foods and beverages made with fluoridated water. The fluoride contribution of beverages depends on the fluoride concentration of the water supply. Drinking water and water-based (non-dairy) beverages provide the bulk of fluoride intake for most people, accounting for 66–80% of fluoride intake in adults in the USA (Lennon *et al*., 2005). It appears that bottled waters have lower concentrations of fluoride than fluoridated tap water (Zohouri *et al*., 2003). In societies where bottled waters are preferred to tap water, fluoride ingestion may be less than optimal and reduced caries protection could occur (cited in Moynihan, 2005). There are limited data available on the bioavailability and absorption of fluoride from artificially versus naturally fluoridated water. A study in humans showed that any differences in fluoride bioavailability between naturally and artificially fluoridated drinking waters were small compared with the naturally occurring variability in fluoride absorption (Maguire *et al*., 2005).

Most toothpastes contain added fluoride. Other fluoride-containing oral health care products include, for example, mouth rinses and gels, dental sealants and fluoride tablets. However, populations in many developing countries do not have access to these fluoride-containing oral health care products because of practical or economic reasons. One of the World Health Organization's (WHO) policies, therefore, is to support the widespread use of affordable fluoridated toothpaste in developing countries (WHO, 2003). The WHO Global Oral Health Programme also looks at the possible use

of milk and salt fluoridation (WHO, 2003). A recent review concluded that fluoridation of milk is effective in preventing dental caries in areas where the fluoride concentration of drinking water is suboptimal and where there is an existing school milk programme. The authors recommended that milk fluoridation programmes should aim to provide fluoridated milk for at least 200 days per year and should commence before the children are four years of age (Bánóczy and Rugg-Gunn, 2007). In rural communities in Chile, fluoridated milk and milk derivatives are provided for school children through the School Feeding Programme. The fluoridated milk provided for the school children was shown to be effective for caries prevention (Weitz *et al*., 2007).

The fluoride content of breast milk is relatively low (DePaola *et al*., 2006). In the lactating mother, low dosage supplements of fluoride, such as 1.5 mg/day, do not affect the fluoride content of breast milk (Mann and Truswell, 2002, p183). The American Dental Association does not endorse oral fluoride supplements for infants younger than six months of age (Fitzsimons *et al*., 1998). Infants older than six months and with exposure to less than 0.3 ppm fluoride in their drinking water need dietary fluoride supplements of 0.25 mg fluoride per day (Nainar and Mohummed, 2004; ADA Reports, 2005).

In certain regions, additional sources of fluoride may need to be considered, such as tobacco and pan masala in India (Yadav *et al*., 2007). Tea is also a source of fluoride. In the case of green tea, the content ranges between 1 and 2 ppm, whereas the levels in black tea are reported to be five times higher. Contrary to previous beliefs, the more recent evidence suggests that tea polyphenols, rather than the fluoride, contribute to its anticariogenic potential (Wu and Wei, 2002).

Overall, the preventive benefits of fluoride from all sources are determined by the concentration of fluoride used, the route of use (ingestion or topical application) and the type of agent used (whether it is in water, a tablet, rinse or gel) (DePaola *et al*., 2006).

16.3.5 Fluoride requirements

The dietary reference intakes (DRI) of the US Institute of Medicine includes adequate intakes (AI) for fluoride. The AI is a goal for the nutrient intake of individuals. In the case of fluoride it refers to the amount consumed to reduce the occurrence of dental caries maximally in a group of individuals without causing unwanted side effects. Adequate intakes of fluoride are set at between 0.5 and 1.0 mg/day for young children between six months and eight years of age, 2 mg/day for children 9–13 years of age, 3 mg/day for children 14–18 years of age and in adults 4 mg/day in men and 3 mg/day in women. Adequate intakes for fluoride are not affected by pregnancy and lactation. To determine more accurately the DRIs for fluoride,

further research is needed on the exposure to fluoride from all sources together with its ability to prevent dental caries and the risk of inducing fluorosis (Institute of Medicine, 1997).

Fluoride can be toxic when consumed in excessive amounts (ADA Reports, 2005). The tolerable upper intake level (UL) defined in the DRIs is the maximum level of daily nutrient intake that is likely to pose no risk of adverse effects to members of the healthy general population. From the age of nine years and through adulthood, the UL for fluoride is 10 mg/day (this includes intake from food, water and supplements). For children younger than nine years, the UL is much lower: 0.7 mg/day for infants from six months and younger, 0.9 mg/day for infants 7–12 months, 1.3 mg/day for 1–3-year-old children and 2.2 mg/day for 4–8 year-old-children (Institute of Medicine, 1997).

16.4 Iron

16.4.1 Iron and oral health

Iron in the body serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells and as an integral part of important enzyme systems in various tissues (FAO/WHO, 2001, p195). Iron deficiency may affect tooth and caries development, but as yet human data are lacking (DePaola *et al*., 2006). Excessive iron in drinking water has been shown to be associated with dental extrinsic stains (Pushpanjali *et al*., 2004).

Oral signs of iron deficiency include glossitis, angular cheilitis, mucosal atrophy (which increases susceptibility to carcinoma) and candidosis (Touger-Decker *et al*., 2005, p114). Geriatric patients with mucosal pathology were shown to have significantly lower serum iron concentrations than those without this oral disease (Sweeney *et al*., 1994). Cancers of the mouth, pharynx and larynx are the seventh most common cause of death from cancer (World Cancer Research Fund/American Institute for Cancer Research, 2007, p245). There are some indications that iron deficiency, *per se* or in combination with copper and selenium, may be related to oral cancer (Bhattathiri, 2001; Khanna and Karjodkar, 2006). According to a systematic review *Food, Nutrition, Physical Activity, and the Prevention of Cancer*, no conclusion on the role of iron in oral cancer can be made because of limited evidence (World Cancer Research Fund/American Institute for Cancer Research, 2007, p245).

Saliva plays a major role in oral health and changes in the quantity produced may alter the oral health status. In a randomized, placebo-controlled trial, oral supplementation with 60 mg iron twice a day for three months had no effect on salivary flow rate among individuals with hyposalivation and low serum ferritin levels (Flink *et al*., 2006).

16.4.2 Sources of iron

Dietary iron exists in two forms, haem and non-haem. Haem iron is found in foods from animal sources, especially meat, fish and poultry. Non-haem iron is found mainly in plant foods. Absorption of non-haem iron is affected by enhancers and inhibitors present in the meal. Ascorbic acid and/or haem iron in the meal enhances the absorption of non-haem iron, whereas phytate (found mostly in cereals), polyphenols (found mostly in vegetables), tannins (in tea), oxalates and calcium (particularly from milk and milk products) inhibit its absorption (Mann and Truswell, 2002; Samman, 2007a; FAO/WHO, 2001). Because the bioavailability of dietary iron varies worldwide, the WHO set standards for high-, moderate- and low-bioavailability diets, in which respectively more than 15%, approximately 10% and less than 5% of dietary iron is absorbable. Diets with high iron bioavailability contain generous amounts of foods rich in enhancers and haem iron, and the inhibitor content is low as cereals are often refined. Diets with moderate iron bioavailability include limited amounts of enhancers. Diets with low iron bioavailability have a high inhibitor content, negligible amounts of enhancers and little haem iron, and are based largely on cereals and legumes (Mann and Truswell, 2002). Good dietary sources of iron are organ meats, fish, meat and poultry.

16.4.3 Iron requirements

In the DRIs of the Institute of Medicine, the fractional absorption for iron is taken as 18% for adults. Thus the RDAs from the Institute of Medicine are comparable to the high bioavailability estimates of the WHO. Iron requirements for adult females are higher than for adult males (18 mg/day versus 8 mg/day). Iron requirements increase with pregnancy (27 mg/day). The UL for adults is set at 45 mg/day (Institute of Medicine, 2001).

16.5 Copper

16.5.1 Copper and oral health

Copper is part of many proteins of the human body, including copper-containing enzymes (e.g. amine oxidases, ferroxidases, cytochrome, superoxide dismutase), copper-binding proteins (e.g. metalloprotein) and low molecular weight ligands. Its general physiological functions relate to connective tissue formation, iron metabolism, the nervous system, melanin pigment formation, cardiac function and cholesterol metabolism, and immune function (Turnlund, 2006) Copper has been shown to inhibit – SH-containing enzymes and may prevent acid production in dental plaque and caries in rodents (cited in Bowen, 1994).

Studies on desalivated rats showed that copper incorporated in sugar by crystallization during the manufacturing process is an effective cariostatic agent and reduces the cariogenic potential of sugar (Rosalen *et al*., 1996a). Combinations of iron, copper and fluoride co-crystallized with sugar appear to have an additive cariostatic effect, probably by affecting lactic acid formation and reducing bacterial colonization (Rosalen *et al*., 1996b). A double-blind randomized *in vitro* study showed that significantly less enamel demineralization occurred following a cariogenic challenge when copper and fluoride were present in combination, compared with fluoride treatment alone, yet copper alone had no significant protective effect (Abdullah *et al*., 2006).

In a review of oral submucous fibrosis, Tilakaratne *et al*. (2006) highlighted the aetiological role of frequent chewing of areca nut for long periods. Since areca nut is high in copper, the possible role thereof as a mediator of fibrosis was put forward by Rooban *et al*. (2004).

16.5.2 Sources of copper

Copper is found in low concentrations in a variety of foods. Information on the copper content of foods is, however, incomplete and databases often contain missing values. Reported values can vary because of the analytical method, sampling procedure, recipe, cooking method and the part of the country from which samples are collected (Turnlund, 2006).

Nevertheless, copper is widely distributed in nature, both in plants and animals (WHO/FAO, 1996, p127). It appears that the copper content of plants is not influenced significantly by the soil in which the plants have grown, yet in the case of leafy vegetables the copper content can increase markedly if the leaves are polluted by substances high in copper. A potentially significant source is the contribution that copper pipes can make to copper intake from drinking water. This may vary from less than 0.1 mg/day in hard water areas, to ten times that level in areas with very soft water (WHO/FAO, 1996, p128).

A crude grouping of food sources of copper into rich, intermediate and poor sources is given in Table 16.1 (from text provided by Turnlund (2006), based on data from the Nutrition Data System for Research version 5.0_35, 2004).

16.5.3 Copper requirements

The RDA for copper is 340 μg/day for 1–3-year-old children and increases progressively up to 900 μg/day for adult men and women. Copper requirements increase with pregnancy (1000 μg/day) and lactation (1300 μg/day). The UL for adults is set at 10 000 μg/day (Institute of Medicine, 2001).

Table 16.1 Food sources of copper (from Turnlund, 2006)

Richest sources[a]	Intermediate sources[b]	Poor sources[c]
• Shellfish • Nuts and seeds (including cocoa powder) • Legumes • Bran and germ portion of grains • Liver and organ meats	• Most grain products • Most products containing chocolate • Fruits and vegetables such as dried fruits, mushrooms, tomatoes, bananas, grapes, potatoes	• Fruits and vegetables not listed under intermediate sources • Chicken • Fish • Dairy, particularly cow's milk

[a] 0.3 to more than 2 mg/100 g, i.e. 50–300 nmol g^{-1}.
[b] 0.1 to 0.3 mg/100 g, i.e. 20–50 nmol g^{-1}.
[c] <0.1 mg/100 g, i.e. 20 nmol g^{-1}.

16.6 Zinc

16.6.1 Zinc and oral health

Zinc is an essential trace element and is present in all body tissues and fluids. Zinc functions in the catalysis of various enzymes, in the maintenance of the structural integrity of proteins and in the regulation of gene expression (Institute if Medicine, 2001). Clinical manifestations of zinc deficiency have been described, including delayed healing of wounds, burns and decubitus ulcers (King and Cousins, 2006). This has been attributed to a reduced rate of epithelialization, decreased wound strength and diminished collagen synthesis (Touger-Decker *et al*., 2005, p277).

The general wound healing properties of zinc have also been documented specifically in conditions affecting the mouth. Zinc supplementation postponed the development and reduced the severity of mucositis and dermatitis in head and neck cancer patients undergoing radiotherapy (Lin *et al*., 2006), possibly by preventing infection by *Candida* spp. and staphylococci (Ertekin *et al*., 2003).

Zinc concentrations in human primary teeth were shown to vary significantly with caries status, as well as tooth type and root length (Tvinnereim *et al*., 1999). Zinc deficiency has been shown to be related to several oral health concerns. A study with rats showed that zinc deficiency can be a potential risk factor for oral and periodontal diseases (Orbak *et al*., 2007). Oral candidiasis appears to be related to nutritional deficiencies, including low serum levels of zinc in elderly, hospitalized patients (Paillaud *et al*., 2004). Higher maternal plasma zinc concentrations may be associated with a lower risk for oral clefts in their offspring (Tamura *et al*., 2005; Krapels *et al*., 2004).

Zinc is involved in the physiology of taste function and plays an important role in taste perception. Taste disturbances are an oral sign of zinc

deficiency (Touger-Decker *et al*., 2005, p114). Goto *et al*. (2001) suggested that taste abnormality caused by zinc deficiency may be the result of a combination of a decrease in the sensitivity of the peripheral taste nerve (long-term effect) together with possible changes in the sensitivity of the central nervous system (short- and long-term effects).

Age and sex may play a role in the relation between zinc and taste disturbances. First, zinc seems to be more important for taste acuity in males than females (McDaid *et al*., 2007). Second, the decline in taste acuity with age may be dependent on the zinc status. This was illustrated in the ZENITH study on older Europeans which showed that the age-related decline in sensitivity for salt taste was associated with zinc deficiency (Stewart-Knox *et al*., 2005) and that zinc supplementation has the potential to enhance salt acuity in those over the age of 70 years (Stewart-Knox *et al*., 2008). Although taste acuity declines with age, the curative effect of zinc supplementation on taste acuity is not affected by age (Ikeda *et al*., 2008).

A decrease in taste acuity can be reversed by the administration of zinc. Oral zinc administration normalized taste perception and taste bud anatomy in patients with taste sensitivity (cited in Ripamonti *et al*., 1998) and improved gustatory function in patients with idiopathic dysgeusia (Heckmann *et al*., 2005). Not all studies with zinc supplementation showed a positive effect on taste acuity. In head and neck cancer patients undergoing radiotherapy, for example, Ripamonti *et al*. (1998) showed that zinc administration slowed down the worsening and accelerated the improvement of taste acuity, whereas Halyard *et al*. (2007) showed that zinc administration did not prevent taste alterations commonly found in these patients. Zinc supplementation also did not correct disturbances in taste perception, particularly for the sour modality, in haemodialysis patients (Matson *et al*., 2003). In terms of zinc toxicity, ingestion of high doses of zinc (more than one gram) results in a metallic taste in the mouth (Mann and Truswell, 2002, p163).

16.6.2 Sources of zinc

Zinc is found in a variety of foods, but its bioavailability from different foods is highly variable. Zinc in animal products is more readily absorbed than from plant foods (Samman, 2007b). Cereal grains, legumes and nuts are rich in phytate, which binds zinc in the intestine and reduces its absorption (Samman, 2007b). The phytate:zinc molar ratio in the diet has been used to estimate the absorption of zinc. A phytate:zinc molar ratio below 5 is associated with relatively high absorption of zinc, a phytate:zinc molar ratio between 5 and 15 with moderate absorption and a phytate:zinc molar ratio above 15 with low absorption (King and Cousins, 2006).

Because the bioavailability of zinc varies widely worldwide, the WHO set standards for high-, moderate- and low-availability diets, in which, respectively, 50%, 30% and 15% of dietary zinc is absorbable (cited in King

and Cousins, 2006). Diets with high zinc availability are generally refined diets low in cereal fibre, low in phytate content, with a phytate:zinc molar ratio below 5, and adequate protein content primarily from animal sources such as meat and fish. Diets with moderate zinc availability are generally mixed diets containing animal or fish protein and with a phytate:zinc molar ratio between 5 and 15. Diets with low zinc availability are generally high in unrefined, unfermented and ungerminated cereal grain, where the phytate:zinc molar ratio exceeds 15 (FAO/WHO, 2001, p263). Rich sources of zinc are red meat, whole-grain cereals, pulses and legumes (FAO/WHO, 2001, p258). Table 16.2 summarizes the zinc and phytate content of selected foods, as well as giving an estimation of the amount of absorbable zinc they contain. Zinc concentration and its variation appear to be characteristic of specific foods and food groups, respectively, and seem not to depend on the geographical origin of the food. Oysters have been found to be the exception, with extremely high variations in the zinc content, which is strongly influenced by habitat contamination (Scherz and Kirchhoff, 2006).

16.6.3 Zinc requirements

In the DRIs of the Institute of Medicine, the fractional absorption for zinc is taken as 0.41 and 0.48 for men and women, respectively. Thus the RDAs from the Institute of Medicine data are comparable to the high- and moderate-availability estimates of the WHO (King and Cousins, 2006). The RDA for zinc is set at 3 mg/day for children from seven months to three years old, and 11 mg/day for men and 8 mg/day for women. Zinc requirements

Table 16.2 Zinc and phytate content of different foods and estimated amount of absorbable zinc (Brown *et al*., 2001)

Food	Zinc content (mg/100 g)	Phytate content (mg/100 g)	Estimated absorbable zinc (mg/100 g)
Liver, kidney	4.2–6.1	0	2.1–3.1
Meat (beef, pork)	2.9–4.7	0	1.4–2.4
Poultry (chicken, duck)	1.8–3.0	0	0.9–1.5
Seafood (without oysters)	0.5–5.2	0	0.2–2.6
Eggs	1.1–1.4	0	0.6–0.7
Dairy products	0.4–3.1	0	0.2–1.6
Seeds and nuts	2.9–7.8	1760–4710	0.3–0.8
Bread (from white flour and yeast)	0.9	30	0.4
Whole grain cereal	0.5–3.2	211–618	0.1–0.3
Beans, lentils	1.0–2.0	110–617	0.1–0.2
Refined cereals (e.g. white flour/rice)	0.4–0.8	30–439	0.1
Vegetables	0.1–0.8	0–116	<0.1–0.4
Fruits	0–0.2	0–63	<0.1–0.2
Tubers	0.3–0.5	93–131	<0.1–0.2

increase with pregnancy (11 mg/day) and lactation (12 mg/day). The UL for zinc is 40 mg/day, which includes zinc intake from food and drinks, fortified foods and supplements (Institute of Medicine, 2001).

16.7 Selenium

16.7.1 Selenium and oral health

Selenium is an antioxidant and essential trace element in living organisms. Because of its anti-oxidant properties, selenium may offer potential benefits in oral tissue wound healing (Touger-Decker, 2005, p278). In a randomized controlled trial, 400 μg/day of supplemental selenium given for six months to oral cancer patients treated with radiotherapy resulted in significantly better antioxidant status compared to patients who did not receive the supplement (Elango *et al*., 2006). Before the intervention, all the cancer patients had significantly lower antioxidant status than non-cancer controls.

Epidemiological data and results from several animal studies show that ingestion of selenium is associated with a higher prevalence of caries. Excessive selenium disrupts the formation of the enamel matrix and subsequent mineralization of the tooth (cited in Bowen, 1994). A study on Wister rats suggested positive effects of selenium on diabetes-induced structural alterations in the mandible (Delilbasie *et al*., 2002).

Although there are some indications that selenium may be protective against oral cancer (Touger-Decker, 2005, p114), a recent report on *Food, Nutrition, Physical Activity, and the Prevention of Cancer* states that no conclusion on the role of selenium in oral cancer can be made because of limited evidence (World Cancer Research Fund/American Institute for Cancer Research, 2007, p245).

The margin between an adequate and toxic intake of selenium is quite narrow. Overexposure or selenosis may occur from consuming high selenium foods grown in seleniferous areas in, for example, Venezuela and some areas of China. The most common sign of poisoning is loss of hair and nails, but the skin, nervous system and teeth may also be involved. Garlic odour on the breath is an indication of excessive selenium exposure from breathing out of dimethylselenide (Burk and Levander, 2006; Mann and Truswell, 2002, p180–181).

16.7.2 Sources of selenium

Rich sources of selenium are fish and organ meats (0.4–1.5 $\mu g\ g^{-1}$ fresh weight), followed by muscle meats, cereals and grains and dairy products. Fruits and vegetables are generally poor sources, containing less than 0.1 $\mu g\ g^{-1}$. In contrast to animal sources, the concentration of selenium in plant foods reflects the selenium content of the soils, and this may vary

considerably by geographic area (Burk and Levander, 2006; Mann and Truswell, 2002, p179). Average total daily intakes of selenium are thus subject to significant variability.

16.7.3 Selenium requirements

The RDA for selenium relates to the intake needed to maximize the activity of the plasma selenoprotein glutathione peroxidase, an oxidant defence enzyme. From the age of nine years through to adulthood, the RDA for selenium is set at 55 μg/day, with no difference between males and females. Selenium requirements are slightly increased during pregnancy (60 μg/day) and lactation (70 μg/day). The UL for selenium is set at 400 μg/day and includes selenium intake from food and supplements (Institute of Medicine, 2000). Bioavailability and nutrient–nutrient interrelationships may be important considerations in establishing requirement and dietary adequacy (Burk and Levander, 2006).

16.8 Lead

Martin *et al*. (2007) stated that associations between childhood lead exposures and dental caries in children have been reported for over 30 years, but the findings and conclusions varied. Moss *et al*. (1999) hypothesized that lead exposure can affect dental caries by three possible mechanisms. First, exposure to lead during salivary gland development may adversely affect the ability of the gland to produce adequate amounts of saliva. Second, lead incorporated into the tooth structure before the tooth erupts into the mouth may result in defective enamel that is more susceptible to caries. Third, lead may interfere with the bioavailability of fluoride by binding to fluoride ions in saliva and plaque. According to Martin *et al*. (2007), each of these potential mechanisms remains unproven, with published studies for each which both support and do not support the mechanism. The lead content of foods is very low. Lead, which accumulates in teeth, is of interest because of its wide distribution in the environment. There are indications that environmental lead exposure is a risk factor for dental caries (Moss *et al*., 1999). As lead exposure relates to environmental pollution and not food intake, the role of lead in oral health is not covered in this chapter.

16.9 Conclusions

Data published during the last decade on the effect of dietary trace elements on oral health is limited. The role of fluoride in the prevention of dental caries is well established and, with the increasing use of fluoride, the prevalence of dental fluorosis is increasing. Fluoride reduces the cariogenic

potential of sugar, as does copper when crystallized with sugar. An additive effect is obtained when copper is combined with either fluoride or iron. The role of zinc seems to be mostly in terms of taste acuity. For most of the other trace elements, strong scientific evidence showing an effect of trace elements in food on oral health is limited or lacking.

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17

Alcohol and oral health

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Abstract: Heavy alcohol consumption, generally defined as daily intake of ≥50 g of pure ethanol and/or frequent binge drinking occasions, is related to more than 60 medical conditions, both acute (e.g. suicide, homicide, motor vehicle crashes) and chronic (e.g. liver cirrhosis, chronic pancreatitis, haemorrhagic stroke). Head and neck cancers are not the only oral conditions associated with such behaviour, as the list also includes dental erosion (due to the acidic components of alcoholic drinks and to gastrio-oesophageal reflux), maxillofacial trauma and dental injuries (due to accidents, assaults, falls, etc. and consequent to acute intoxication), growth deficiency, such as abnormal maxillary/mandibular growth and disturbed odontogenesis (symptoms of the foetal alcohol spectrum disorders (FASD) due to alcoholism in pregnancy), sialosis and, possibly, dry mouth (due to autonomic dysfunction and fatty infiltration in salivary glands which can precede and accompany liver cirrhosis during chronic alcohol abuse), dental caries and periodontal disease (among chronic alcoholics who neglect themselves with poor oral hygiene and diet). Although community-based anti-alcohol campaigns have been developed in many countries, they are unlikely to be effective in the long term, unless restrictive measures (price rise, reduced availability and public consumption) are applied. The long-term effectiveness of the various forms of treatment at the individual level is not related to the type of treatment but to the timeliness of the intervention and cooperation of the patient.

Key words: alcohol, alcoholism, oral disease, public health.

17.1 Introduction

According to the World Health Organization, there are almost two billion people worldwide who consume alcohol, which is the most common drug of abuse, and almost 80 million with diagnosable alcohol abuse disorders. The alcohol-related global burden of disease, estimated to be 4%, is as high as the burdens of disease of tobacco and high blood pressure. Indeed, excessive alcohol consumption is causally related to more than 60 different

medical conditions, some of them, such as suicide, homicide and different forms of accidents, are acute, while other conditions, such as liver cirrhosis, chronic pancreatitis, haemorrhagic stroke and various forms of cancer, are chronic consequences of alcohol use.

High alcohol consumption has a deep impact on oral health. Some systemic alcohol-related conditions may indirectly affect oral health. These are the cases of missing teeth caused by caries or periodontal disease among chronic alcoholics, who generally neglect themselves and have poor oral hygiene; of maxillofacial and dental traumas and osteomyelitis, mostly owing to vehicle crashes among binge drinkers; of tooth erosion arising from gastric reflux; of various forms of abnormal prenatal craniofacial maldevelopment consequent to fetal alcohol syndrome; of stomatitis caused by several micronutrient deficiencies; of sialosis and dry mouth with impact on caries development.

In addition, high alcohol consumption has a direct impact on oral health. The principal effect of heavy drinking is the high risk of cancer of the oral mucosa, which could also be increased by the use of alcohol-containing mouthwashes. Dental erosion is also associated with frequent alcohol consumption, because of precipitation of salivary proline-rich proteins caused by polyphenols present in most alcoholic drinks, the high concentration of organic and inorganic acids and the habit of keeping the alcoholic drink in the mouth. Occasionally, flambé drinks may inflict superficial to mid-second-degree burns of the oral mucosa.

17.2 Overall effect of alcohol on general health

An alcohol is an organic compound in which a hydroxyl group is bound to a carbon atom of an alkyl group. Common types of alcohol include isopropyl, ethylene glycol, glycerol, phenol, methanol and ethanol. The term "alcohol" as used in medicine and by the lay public typically applies to ethanol, rather than other alcohols. Ethanol (CH_3CH_2OH) is therefore the active ingredient in alcoholic beverages. For consumption purposes it is produced by fermentation of carbohydrates, such as simple carbohydrates in fruit and starch (which is previously hydrolysed during a process called malting) in grains, by yeasts. Spirits, such as whisky, brandy and vodka are produced by distillation of fermented products. Carbohydrate fermentation is incomplete in beer and complete in wine, with resulting alcohol contents between 3–8% and 7–18% by volume, respectively. Distilled products, such as liqueurs and spirits, are 30% or greater alcohol by volume (Wikipedia *Alcoholic beverage*. Available at http://en.wikipedia.org, last access, February 15, 2008). Other than these primary alcoholic beverages, there are the secondary alcoholic drinks, such as alcoholic cocktails, which are made with primary beverages. A cocktail is a mixed drink usually containing one or more alcoholic beverages, flavourings and one or more fruit juices, sauces,

honey, milk, cream or spices, and so on. A sub-group of cocktails are premixed and bottled cocktails, generally known as alcopops, RTDs (ready to drink), or FABs (flavoured alcoholic beverages). Alcopops are generally made from vodka, beer, whisky, rum to reach an alcohol concentration of 4–7% by volume, are sweet and fruit flavoured because they are mixed with cola, lemonade, ginger ale, and so on (Wikipedia *Alcopop*. Available at http://en.wikipedia.org, last access, February 15, 2008).

Alcohol in drinks is generally measured in units. One alcohol unit (AU) equates to 10–12 ml of pure ethanol (approximately $^1/_3$ American fluid ounce), contained in a standard bottle or a can of regular beer, a glass of dinner (125 ml) wine, a small glass (30 ml) of spirits and a measure (60 ml) of aperitif.

Alcoholic beverages are a heterogeneous group of beverages, with variable number, type and concentration of components. The only common components are ethanol and water. In addition, beers contain, on average, carbon dioxide, minerals, proteins, acids, polyphenols (total phenol content 500–1000 mg l^{-1}, partly attributable to polyphenols) and are rich in carbohydrates (30–75 g l^{-1}). Wines contain carbon dioxide, alcohols, vitamins, acids, minerals, carbohydrates, polyphenols (red wines, 1000–4000 mg l^{-1}; white wines, 200–300 mg l^{-1}), fatty alcohols, fatty acids, esters, aldehydes, terpenes, ethereal oils, volatile bases are among the few common components of spirits and liqueurs. The pH values are round 4.0 for beers and most spirits and 3.5 for wines (Mosedale and Puech, 1998; Klampfl *et al*., 2000; Scalbert and Williamson, 2000; Dreosti, 2000).

All alcohols are toxic, but ethanol is less toxic because the body can metabolize it rapidly. The various alcoholic drinks also contain substances that interact synergistically or antagonistically with ethanol. A group of metabolically active carcinogenic components of alcoholic drinks are nitrosamines. Ethanol blocks nitrosamine metabolism in the liver, thus allowing it to circulate to other organs, such as kidneys and oesophagus, where it can be activated into carcinogens. Beer usually contains a high nitrosamine level, although concentrations have declined recently; spirits also contain nitrosamines, while wine does not (Berger, 1998).

Another important group of substances contained in alcoholic beverages that have an effect on human health are polyphenols – plant secondary metabolites, which play an important role in human health in relation to their potent antioxidant activity (Schijlen *et al*., 2004). Polyphenols also appear to inhibit the metabolic activation of carcinogenic free radicals (Visioli and Galli, 2002) and can induce apoptosis (Hsu *et al*., 2002; Hakimuddin *et al*., 2004). All plant derivative foods, including alcoholic drinks, contain different types of polyphenols. Beer contains high levels of anthocyanins and their polymers. Wine, particularly red wine, has a high content of different polyphenols, such as quercetin, resveratrol, anthocyanins and tannins. Spirits aged in wooden barrels, such as brandy and whisky, contain tannins extracted from the barrel walls by the ethanol–

water solution during ageing (Scalbert and Williamson, 2000). The highest antioxidant potential of alcoholic drinks has been found for red wine, which has a reducing power 10- to-20-fold higher than the normal reducing power of human plasma (Lugasi and Hovari, 2003; Katalinić *et al*., 2004) and the ingestion of 300 ml of red wine causes an increase of the total antioxidant capacity of serum by 18% after one hour and 11% after two hours (Whitehead *et al*., 1995).

There is wide variability among countries in which alcohol beverages are consumed, in terms of per capita average alcohol (ethanol) consumption, drinking pattern and preferred type of alcoholic beverage. Alcohol effects are affected not only by the volume of alcohol consumption, but also by other variables, such as the pattern of drinking, especially heavy binge drinking. For example, in Europe, three groups of countries are traditionally identified according to the prevalent drinking culture (wine drinking in the South, beer drinking in the Centre and spirit drinking in the North). There is considerable variability within such groups and within countries and new patterns are evolving rapidly (e.g. increasing consumption of wine in northern European countries and increasing prevalence of binge drinking, in particular among women). In fact, with similar annual per capita alcohol consuming levels of about 11 l and similar adult drinking prevalence of 75%, the alcohol-attributable fraction of overall mortality is almost double in eastern than in western Europe (12.0% versus 6.8%, respectively). The explanation of such a difference presumably lies in the different drinking habits between drinkers from eastern and western (particularly Mediterranean) countries, the first group heavily consuming beverages with high ethanol concentration during weekends, the second daily but moderately consuming beverages with moderate to low ethanol concentration during meals (Simpura and Karlsson, 2001; Britton and McKee, 2000).

Overall, the alcohol-related global burden of disease has been estimated to be 4%. Thus, alcohol accounts for about as much of the burden of disease as tobacco (4.1%) and high blood pressure (4.4%) and is preceded only by the burdens caused by underweight (9.5%) and unsafe sex (6.3%) (WHO, 2002; Ezzati *et al*., 2002; Room *et al*., 2005).

17.2.1 Alcohol drinking guidelines

All the above points should be considered in order to give sensible advice regarding individual recommended limits of alcohol consumption. Moderate alcohol use is defined as daily one AU for women, two for men. A safe *maximum* daily alcohol consumption is generally regarded as three units for a man, two for a woman.

The limit discriminating between moderate and heavy alcohol drinking has been generally set at 40 g/day and is defined as tolerable upper alcohol intake level (TUAL) (Burger *et al*., 2004). Nevertheless, national drinking guidelines vary from country to country. For example, in the UK the recom-

mended alcohol consumption level is of ≤2–3 AU/day for women (the number of AU in various alcoholic drinks is displayed in Table 17.1), ≤3–4 AU/day for men and ≤1–2 AU/day for pregnant women (Department of Health, 1995). In the USA, the recommended level is ≤1 AU/day for women and ≤2 AU/day for men to be consumed together with the main meals (US Department of Agriculture, US Department of Health and Human Services, 1995). In Scandinavia, the recommended level is ≤15 g of alcohol daily for women and ≤20 g for men, not exceeding 4–5% of total energy intake (Nordic Council of Ministers, 1996). In Italy, adults are recommended to drink moderately (≤4 AU/day) beverages containing low ethanol concentration, such as beer and wine, during the mean meals, whereas no alcohol intake is recommended during pregnancy (National Institute for Research on Food and Nutrition, 2003).

17.2.2 Alcohol metabolism

Most ethanol, rapidly absorbed through the gastric and duodenal mucosa, is metabolized by the liver, with a small fraction also metabolized by oral and other mucosae of the upper digestive tract (Dong *et al*., 1996). The enzyme acetaldehyde dehydrogenase (ADH) catalyses ethanol oxidation to acetaldehyde, which is oxidized into non-toxic acetate by the enzyme acetaldehyde dehydrogenase (ALDH). In turn, acetate is oxidized into fatty acids, carbon dioxide and water. It has been demonstrated that the highest rate of ethanol that the human organism is able to metabolize does not vary and is not affected by ethanol concentration, by ethanol tolerance or by persistently heavy drinking. Therefore, a high alcohol intake in a single occasion, as in the case of binge spirit drinking, leads to acetaldehyde accumulation in the oral mucosa, which promotes the formation of highly reactive free radicals and singlet oxygen. Free radicals and singlet oxygen cause irreversible damage to important macromolecules, such as DNA and DNA-repairing enzymes, with consequent carcinogenic activity. This mechanism

Table 17.1 Number of units of alcohol in various alcoholic drinks

Beverage	Amount	Number of units of alcohol
Spirits	70 cl bottle	32
Spirits	One measure	1
Fortified wine e.g sherry	70 cl bottle	16
Wine (12% alcohol)	75 cl bottle	9
Wine (10% alcohol)	75 cl bottle	7.5
Wine (10% alcohol)	One glass	1
Beer, cider (8% alcohol)	Pint	4.5
Beer, cider (5% alcohol)	Pint	3
Beer, cider (3.5% alcohol)	Pint	2
Beer, cider (3.5% alcohol)	Half pint	1

also helps to explain the multiplicative carcinogenic effect of heavy smoking and drinking, since acetaldehyde is also produced by tobacco combustion, with a consequent over-accumulation of this substance in the oral mucosa. Moreover, ethanol increases membrane permeability of the oral mucosa epithelial cells, thus promoting the penetration of other tobacco carcinogens such as nitrosonornicotine (Wight and Ogden, 1998; Ogden and Wight, 1998; Llewellyn *et al*., 2001).

Alcohol drinking is also strongly associated with the risk of development of liver cirrhosis, which may result in impaired metabolism of carcinogens, and in impaired immunity. A diet poor in fruits and vegetables is also typical of heavy drinkers.

17.2.3 Alcohol and public health

A systematic review of the management of alcohol use disorders from the public health point of view has reached the following conclusions (Room *et al*., 2005):

- individuals who obtain help for a drinking problem, especially in a timely manner, have better outcomes than those who do not receive help. Nevertheless, the type of help has little effect on long-term outcomes;
- treatment intensity and duration are not associated with more pronounced improvements in outcome;
- medically based inpatient treatment is more costly but generally not more effective than non-medical outpatient treatment;
- there is little evidence that the type of treatment affects the long-term outcome.

National and community-based approaches have been developed in many countries, with different levels of success. The first recourse in case of public concern about alcohol-related problems is to implement public information campaigns. Nevertheless, despite the widespread use of these measures, they are not likely to be effective in the long term (Foxcroft *et al*., 2003). A more effective community-based preventive approach is controlling alcoholic drink price and availability. Tax increases have been shown positively to affect some acute and chronic drinking-related causes of death, such as cirrhosis, driving deaths and violent crime (Chaloupka *et al*., 2002). Another, not less important and effective public health measure, is the reduction of alcohol-related vehicle casualties, by means of countermeasures, such as forbidding driving above a given blood alcohol concentration level (Shults *et al*., 2001).

The potential benefits, in terms of oral cancer alcohol-attributable mortality prevented fraction of an alcohol drinking preventive campaign implemented on only heavy drinking individuals (the so-called high-risk approach) can be estimated using the national figures of heavy drinking prevalence

(WHO, 1999), oral cancer mortality (Ferlay *et al*., 2001) and the alcohol attributable fractions of deaths for any cause and for oral cancer (Room *et al*., 2005). We have given results for four European countries, two from eastern (Czech Republic, Poland) two from western (France, UK) Europe, with different heavy drinking prevalence in Table 17.2. The prevented fraction was higher than 7% among males and ranged between 2% and 3% among females, according to different heavy drinking prevalence values. In terms of number of prevented deaths, the highest number was found for French males ($n = 123$), because of the high heavy drinking prevalence, the numerous population and the high cancer mortality rate.

17.2.4 Alcohol misuse

Alcohol misuse, defined as a daily intake in excess of five AU, is the most common form of drug misuse. The World Health Organization estimates that there are almost two billion people worldwide who consume alcohol with almost 80 million people with diagnosable alcohol use disorders caused by misuse (WHO, 2004). Alcohol should only be drunk in moderation, with meals, and when consumption cannot put others at risk. Alcohol should not be consumed by children, women trying to conceive or already pregnant, anyone about to drive a motor vehicle, fly a plane or be involved in any other skilled activities, healthcare workers about to treat patients, and anyone taking prescription or over-the-counter medicines.

The consumption of alcohol is rising throughout the world and as many as 15% of all visits to doctors are alcohol-related, and even up to 25% of dental patients may have abused or continue to misuse alcohol. The probability of developing alcoholism is greater in several groups, especially those for whom alcohol is freely available (Table 17.3).

Table 17.2 Potential impact on alcohol-related oral cancer mortality of a high-risk preventive campaign with a 33% effectiveness in reducing prevalence of heavy alcohol drinking in four European countries

Country	Heavy drinking prevalence (%)		Crude oral cancer mortality (per 100000)		Estimated prevented oral cancer deaths		Estimated oral cancer mortality prevented fraction (%)	
	Males	Females	Males	Females	Males	Females	Males	Females
Czech Republic	28.0	8.0	5.44	1.16	20	2	7.4	3.3
Poland	23.7	3.6	4.12	1.40	56	13	7.2	3.1
France	15.9	1.0	5.91	1.12	123	7	7.2	1.8
UK	6.0	2.0	2.38	1.49	54	13	7.2	2.9

Table 17.3 Occupational risk factors for alcoholism

Publicans and other workers in the alcoholic drinks industry
Entertainers
Commercial travellers
Bored housewives
Bachelors over 40
Armed forces
Doctors
Dentists

The difficulties of recognizing whether a patient has been taking alcohol are shown by a survey in a British teaching hospital, where it was found that over 30% of patients attending the casualty department had detectable blood alcohol levels. Medical staff, who at the same time were attempting to detect inebriation clinically, underestimated the true extent of the problem by 19%. Recognition of the alcoholic is notoriously difficult and, even if the disorder is suspected, the history is often a hopelessly unreliable guide to the amount of alcohol consumed. The CAGE questionnaire may be helpful: a positive response to any of the following questions suggests a diagnosis of alcoholism:

- Have you ever felt the need to Cut down on drink?
- Have you ever felt Annoyed by criticism of your drinking?
- Have you ever felt Guilty about drinking?
- Do you drink a morning Eye opener?

Two or more affirmative answers indicate probable alcoholism (Mayfield *et al*., 1974; Ewing, 1984; Allen *et al*., 1988). Laboratory investigations that may be diagnostically helpful include raised blood levels of alcohol, carbohydrate-deficient transferrin isoforms (CDT) gamma-glutamyl transpeptidase and other hepatic enzymes, macrocytosis and folate deficiency with no obvious cause. Macrocytosis alone is one of the earliest signs of alcoholism; later there may be macrocytic anaemia.

17.2.5 Treatment of alcoholism

Treatment for alcoholism includes:

- harm reduction, by drinking in a less damaging way and accepting help to deal with the crises and the physical, behavioural and social damage that result from drinking;
- to learn to abstain from alcohol: best achieved with the support of others such as Alcoholics Anonymous;

- admission to hospital to manage rehabilitation and ensure abstinence;
- nutrition replacement for missing thiamine and folate;
- use of drugs to reduce dependence (naltrexone, acamprosate or chlormethiazole) or to cause unpleasant side effects if alcohol is taken (disulfiram). Reactions can be severe and can be seen when any alcohol is used – even the small amounts in medicines or toiletries or mouthwashes – for up to 1 week after disulfiram is taken.

17.2.6 Abstinence or withdrawal syndrome

If the supply of alcohol is reduced or cut off, or if blood alcohol levels fall sharply, the abstinence or withdrawal syndrome (AWS) may appear. Common alcohol withdrawal symptoms are morning 'shakes', typically trembling of the hands, but possibly involving the whole body. Morning nausea and vomiting are sometimes called 'toothbrush heaves'. Alcoholics frequently resort to a drink first thing in the morning to relieve these withdrawal symptoms ('the morning eye-opener' or 'morning livener'). Other alcohol withdrawal symptoms are:

- diarrhoea
- sweating
- rapid pulse and raised blood pressure
- confusion – disorientation in time and place
- agitation – from mild anxiety to terror
- pseudo-epileptic fits
- illusions
- misperceptions – e.g. a spot on the wall seen as a cockroach
- hallucinations – usually of sight and hearing
- delusions – often but not always paranoid.

When the above symptoms are associated, the full-blown syndrome is called 'delirium tremens' or 'DTs'. The symptoms of alcohol intoxication and withdrawal can mimic many other psychiatric disorders so patients are often misdiagnosed. The whole withdrawal syndrome lasts about a week and requires medical supervision and the use of benzodiazepines or chlormethiazole (Petti and Scully, 2005).

17.2.7 Health effects of alcohol consumption

Alcohol use may be associated with a range of risks, especially if the alcohol is taken to an extent that interferes with central nervous system (CNS) function. The mortality rate in alcoholism is significantly raised: over 15 years it may be about 300% above the norm. Alcohol has been shown to be causally related to more than 60 different medical conditions (Fig. 17.1) and, for 27 of them, the evidence of the relationship to alcohol consumption is judged to be sufficient. Some of these conditions, such as suicide,

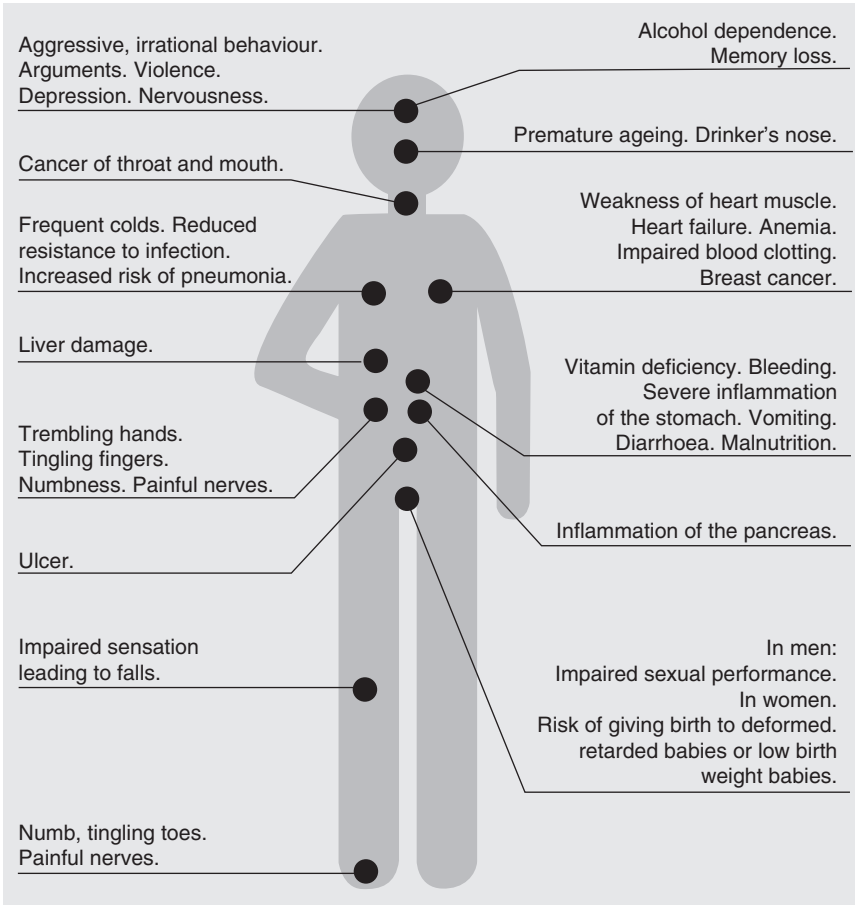

Fig. 17.1 Health effects of heavy drinking (from Babor *et al.*, 2001).

homicide, different forms of accidents (e.g. falls, poisoning, accidents caused by flames or by motor vehicles) are acute consequences of alcohol use. Other conditions, such as liver cirrhosis, chronic pancreatitis, haemorrhagic stroke and various forms of cancer are chronic consequences of alcohol use.

Alcohol as a CNS depressant, initially releases inhibitions, but impairs the capacity to reason. It eventually interferes with cerebellar function causing ataxia and motor incoordination and unconsciousness. The acute effects of alcohol are mainly on judgement, concentration and coordination, and are dose related (Tables 17.4 and 17.5). Alcohol at blood levels above 35 mg dl^{-1} (the legal maximum for driving in some countries) impairs judgement. Signs of intoxication (slurred speech, loss of restraint and ataxia) are clinically obvious at blood alcohol levels above 100 mg dl^{-1}. At a blood

Table 17.4 Increased risks for alcohol drinkers

Trauma	Accidents, suicides, assaults
Cancer	Mouth, larynx, pharynx and oesophagus, liver, stomach, colon and rectum and possibly breast
Behavioural	Dependence, social problems, encephalopathy, neuropathy, clinical depression and suicides

Table 17.5 Effects of chronic alcohol use on body systems

Body system	Possible effects	Significant biochemical changes
Cardiovascular	Cardiomyopathy Hypertension Ischaemic heart disease (IHD) Dysrhythmias	
CNS	Intoxication Dementia Wernicke–Korsakoff syndrome Neuropathies	Raised blood alcohol Decreased thiamine levels
Oesophageal	Gastro-oesophageal reflux Mallory–Weiss syndrome	
Gastric	Gastritis Ulcers	
Intestinal	Malabsorption of glucose and vitamins	Reduced folate, thiamine and vitamins B12, A, D, E and K
Pancreatic	Pancreatitis	Raised serum amylase
Hepatic	Hepatitis Fatty liver (steatosis) Cirrhosis	Raised gamma glutamyl transpeptidase Raised liver enzymes Raised bilirubin Reduced albumin
Haematological	Pancytopenia Immune defect	Reduced haemoglobin Reduced platelet count Leukopenia Reduced blood clotting factors II, VII, IX, X Macrocytosis
Reproductive	In men, temporary impotence and longer-term loss of potency, shrinking testes and penis and reduced sperm count. In women, menstrual cycle disrupted, increased miscarriages, low birth weight babies, birth defects and foetal alcohol syndrome	

alcohol level above 200 mg dl^{-1}, some drinkers become aggressive and combative and can find themselves in conflict with others or the law. After a large alcoholic binge, suppression of protective reflexes, such as the cough reflex, can result in inhalation of vomit and death. A small person, particularly female, is affected at a lower alcohol intake than a larger person or one used to regular alcohol intake (Rehm *et al*., 2003; Britton *et al*., 2003; WHO, 1999).

Conditions that can be caused or aggravated by alcohol include particularly:

- injuries, including maxillofacial, from accidents or assaults. Alcohol is an important, if not the main, causal factor in over 25% of road traffic accidents and also in many other accidents or assaults;
- liver disease. Acute alcoholic hepatitis may follow binge drinking. Cirrhosis commonly results from chronic alcoholism;
- immune defects, impaired wound healing and predisposition to infections, especially pneumonia and tuberculosis. Alcohol also contributes to the spread of sexually transmitted infections, including HIV;
- inadequate diet – which can cause nutritional defects leading to immune defects and peripheral polyneuropathies (burning hands and feet), pellagra, amblyopia (visual defects) and organic brain disorders (Wernicke's encephalopathy and Korsakoff's psychosis);
- cancers.

Moreover, during pregnancy, alcohol drinking has a detrimental effect on foetal development (Table 17.5).

Whereas health risks of heavy alcohol consumption and alcohol abuse are well known, there are also studies indicating beneficial effects of lower intake levels, for conditions such as diabetes mellitus and many cardiovascular diseases, thus generating the evidence for a J-shaped risk curve between alcohol consumption and these diseases (Corrao *et al*., 2000), while the risk curve of alcohol consumption and total mortality would be U-shaped, with a decreased risk in light drinkers compared to non-drinkers and then an increasing risk as alcohol consumption increases (Stanley and Mazier, 1999). A summary of the alcohol risk functions for some important alcohol-related conditions is displayed in Table 17.6.

17.3 Systemic effects of alcohol, influencing orofacial health

17.3.1 Oral neglect: caries/periodontal disease

Chronic alcoholics may neglect themselves as they are preoccupied with their addiction and they may have very poor dental hygiene. Alcoholics have a high incidence of decayed missing filled teeth and also more missing teeth compared to non-alcoholics. Alcoholics have an increased rate of chronic, advanced generalized periodontitis with inflamed gingivae, loss of

Table 17.6 Alcohol consumption and relative risks of mortality for important alcohol-related conditions[a]

Condition	Alcohol consumption (g/day)					
	0 ≤ 1	1–10	10–20	20–30	30–40	40–50
CVD[b] men	1.00	0.83	0.78	0.77	0.77	0.79
CVD[b] women	1.00	0.86	0.85	0.90	0.96	1.05
Colon cancer	1.00	1.07	1.21	1.38	1.57	1.79
Breast cancer	1.00	1.04	1.12	1.21	1.31	1.41
Haemorrhagic stroke	1.00	1.08	1.25	1.46	1.69	1.96

[a] from Britton *et al.*, 2003.
[b] CVD = cardiovascular diseases.

stippling, blunting of the interdental papillae and deep pocketing with associated bone loss.

Alcoholics had in one study, statistically significant more absent, traumatized, major cavitated and extracted teeth. Addicts had a worse severity index and dental index (based on both the number and severity of decayed, missing and filled teeth; 13.13 versus 4.74). Furthermore, dental pathology developed in addicts at younger ages than in non-addicts with 56.8% versus 5.4% of patients younger than 38 years having a semi-quantitative dental index score higher than 10, respectively (OR for semi-quantitative dental index score higher than 10 = 22.98, 95% CI = 5.57–200.65, $P < 0.000\,000\,1$) (Reece, 2007). Severe alcoholics have a high risk of periodontal breakdown and tooth loss. To what extent these findings are caused by general/oral neglect alone (in combination with nicotine abuse) is at present unknown (Hornecker *et al.*, 2003). Significantly fewer teeth and more caries are found in alcoholics and there is a tendency for alcoholics to have more endodontically treated teeth than controls, but no difference in the number of periapical lesions in endodontically treated teeth. Horizontal bone loss and the presence of calculus is more frequent in alcoholic men than in alcoholic women. Significantly more horizontal bone loss was observed in alcoholic non-smokers than in non-alcoholic non-smokers. In the non-smoking groups, alcoholics had significantly more periodontal destruction than did non-smoking controls (Enberg *et al.*, 2001).

In patients undergoing treatment for alcohol use disorders, significant levels of dental caries, gingival inflammation, soft tissue abnormalities and tooth erosion were seen (Araujo *et al.*, 2004). Measures of dental pathology (including tooth loss, carious teeth and periodontal disease) correlated significantly with alcohol-related indicators. The associations were more evident for males than for females, which is consistent with some studies of alcohol-related medical consequences (Kranzler *et al.*, 1990). Age, low income, low education level, smoking and alcohol abuse seemed to be risk markers for edentulism (Mack *et al.*, 2003).

However, unlike several reports from the United States, dental health in a UK sample of alcoholics was not compromised, but mucosal health was a cause for concern. Neither plaque index scores nor mean subject pocket depths were correlated with alcohol consumption. Overall mean decayed, missing and filled teeth (DMFT) was 15.4; age-specific mean DMFT and tooth loss of the sample were closely similar to the 1988 United Kingdom adult dental health survey data. The prevalence and severity of tooth wear and attrition were greater in the sample than levels described in the literature. Trauma to teeth and oral mucosae was noted in 25% of the sample. Seven oral mucosal lesions (including one treated carcinoma) were detected. Furthermore, 21% of the alcoholics were malnourished (body mass index, BMI < 20) (Harris *et al*., 1996). No major differences were found in Danish alcoholics with respect to number of teeth and dental caries (number of decayed, missing, filled surfaces – DMFS), when compared to reference figures for the general population. Considering untreated decay, however, 3–5 times more actual decayed surfaces were found among the alcoholics.

In the multivariate analyses, neither DMFS, nor untreated decay were found to be associated with alcohol-related indicators. These variables were, however, related to variables of social background and dental health behaviour. An association was found with duration of alcoholism and social situation, but when the analysis was controlled with respect to number of teeth present this relationship was eliminated. In contrast, dental erosion was related to duration of alcoholism irrespective of confounding control of dental health behaviour and social situation. Hence, the study indicates that oral health in alcoholics can be explained mainly by social situation and dental health behaviour and not by variables associated directly with alcohol consumption. An exception was the presence of dental erosion, which was associated with the exposure to alcohol (Hede, 1996). In another study, measures of oral hygiene, dental care and periodontal parameters were significantly worse and the number of teeth requiring treatment was higher in alcoholics with or without cirrhosis than in healthy subjects and in non-alcoholic patients with cirrhosis. Alcoholics had fewer teeth and more caries than patients without alcohol abuse and healthy controls. The dental and periodontal status of patients with non-alcoholic cirrhosis did not differ from the control group. The severity and duration of liver disease had no influence on dental and peridontal disease (Novacek *et al*., 1995). There was a tendency for the alcoholics <45 years of age to have more endodontically treated teeth. Horizontal bone loss and the presence of calculus were more frequent in alcoholic men than in alcoholic women. Significantly more horizontal bone loss was observed in the group of alcoholic non-smokers than in non-alcoholic non-smokers. In the non-smoking groups, alcoholics had significantly more periodontal destruction than the non-smoking controls (Enberg *et al*., 2001).

Among patients aged 20–75 years, the mean DMF score was significantly higher among alcoholic patients than among non-alcoholic patients (26

versus 23, respectively). This difference was greater among patients aged 20–39 years (20 versus 14, respectively) than among those aged 60–75 years (29 versus 27, respectively). The positive association between alcoholism and dental disease (OR = 2.24) remained after sequential stratification for several confounding factors (Niquille *et al*., 1993).

There are no direct studies investigating whether an association exists between dental caries and dietary polyphenol (PP) intake. However, there are *in vitro* and human studies concerning the effect of PP on cariogenic and non-cariogenic microbiotas. In a clinical trial, adult subjects who drank coffee, tea, barley coffee or wine, had 10-fold lower levels of mutans streptococci and lactobacilli in saliva and dental plaque than control subjects, while total streptococci and viable microbiota levels were similar (Signoretto *et al*., 2006).

In the absence of salivary proteins, extracts from tea, cocoa, wine and other plant material have bacteriocidal or bacteriostatic activity against mutans streptococci (Yamamoto and Ogawa, 2002; Smullen *et al*., 2007; Sasaki *et al*., 2004; Daglia *et al*., 2007). However, such antibacterial activity may be reduced or completely annulled in the presence of salivary proteins, such as proline-rich proteins or by their surrogates, because of the well-known capacity of PP to form complexes with proteins and enzymes (Cushnie and Lamb, 2005). This observation suggests that the antibacterial activity of PP would be insignificant *in vivo*. In addition, the antibacterial activity of dealcoholized wines against *Streptococcus mutans*, *S. oralis*, *S. intermedius* and *S. constellatus* seems attributable to organic acids rather than to PP (Daglia *et al*., 2007). Adherence inhibition also has been reported for tea, barley coffee, cocoa, wine, apple extracts on *S. mutans* and *S. sobrinus* and *Actinomyces viscosus* (Smullen *et al*., 2007; Yanagida *et al*., 2000; Xiao *et al*., 2000; Papetti *et al*., 2007). Interestingly, this activity seems to persist in the presence of salivary proteins (Xiao *et al*., 2000). In summary, frequent PP intake causes a decrease in oral levels of cariogenic bacteria, with eventual positive effects on dental caries prevention and treatment. This activity seems to be due to the ability of these compounds to inhibit bacterial adhesion to enamel, while an effect on bacterial growth and viability seems implausible *in vivo*.

17.3.2 Orofacial trauma

One of the most important dental complications of excessive alcohol intake is maxillofacial trauma and head injuries, and unconsciousness in such patients may be due at least in part to the alcohol itself. In a New Zealand study, alcohol was most frequently involved in mild and moderate head injuries and these patients were more likely to have been assaulted than those admitted with severe head injuries (Goodisson *et al*., 2004). In the UK, 11% of all falls were associated with alcohol consumption (Hutchison *et al*., 1998). Assault caused 24% of the facial injuries. The commonest sites for

assault were in the street, followed by public drinking establishments. More women than men were assaulted at home. Alcohol consumption was related to 55% of assaults and 8% assaults were with bottles or glasses. Road traffic accidents (RTAs) accounted for 5% of the facial injuries. Alcohol had been consumed by 15% of RTA victims. The 15–25 age group suffered the greatest number of facial injuries caused by assault and RTAs and had the highest number associated with alcohol consumption. At least 22% of all the facial injuries in all age groups were related to alcohol consumption within four hours of the injury. In the over-15 age groups, alcohol consumption was associated with 90% of facial injuries occurring in bars, 45% on the street and 25% in the home. Assault, RTA and alcohol consumption conveyed an increased risk of serious facial injury (Hutchison *et al*., 1998).

Alcohol clearly plays a major role in facial trauma in UK (Shepherd *et al*., 2006; Warburton and Shepherd, 2002, 2006; Smith *et al*., 1998, 2003; Stephens *et al*., 1996; Brickley and Shepherd, 1990, 1995; Telfer *et al*., 1991). In Greenland, violence was aggravated and involvement of alcohol was seen in four out of five cases. Regulation and deregulation of alcohol in Greenland however, had only temporary effects on the occurrence of jaw fractures (Thorn *et al*., 1986).

17.3.3 Tooth wear

Tooth erosion may arise from gastritis and reflux (Simmons and Thompson, 1987; Young, 2005) and oral malodour may be exacerbated (see Chapter 6). Tooth wear patterns attributed to the physical wear of teeth by clenching and grinding (attrition) in alcoholic patients are more consistent with chemical damage (erosion) than attrition (Smith and Robb, 1989). The teeth in alcoholic patients have significantly more wear than age- and sex-matched controls. Tooth wear is most marked in males and in those whose alcohol consumption was continuous rather than in the form of episodic binges. Wear appeared to be erosive in nature and in 40% of the sample it affected the palatal surfaces of the upper anterior teeth. (Robb and Smith, 1990).

17.3.4 Foetal alcohol syndrome

Alcohol is now the most common teratogen apart from smoking. Foetal alcohol syndrome (FAS) is one cause of abnormal prenatal craniofacial maldevelopment and affects growth, CNS and orofacial features (Johnston and Bronsky, 1995). Alcoholism in pregnancy can lead to FAS, the prevalence of which is similar to that of Down's syndrome or to spontaneous abortion. Most affected children have short stature, microcephaly is common and associated with a low IQ, difficulties in eating and speech, muscular incoordination, deformities in the small joints of the hand, altered palmar creases and malformation of the ears. The FAS patient is irritable as an infant, hyperactive as a child and highly unsociable as an adult.

The first descriptions in the modern medical literature of a distinctly recognizable pattern of malformations associated with maternal alcohol abuse were reported in 1968 and 1973 and, since that time, substantial progress has been made in developing specific criteria for defining and diagnosing the condition. The adverse effects of alcohol on the developing human comprise a spectrum of structural anomalies and behavioural and neurocognitive disabilities, most accurately termed foetal alcohol spectrum disorders (FASD). FASD is characterized mainly by a distinct pattern of craniofacial malformations, physical and mental retardation. The FASD continuum includes FAS, partial foetal alcohol syndrome, alcohol-related birth defects and alcohol-related neurodevelopmental disorder.Two sets of diagnostic criteria are now used most widely for evaluation of children with potential diagnoses in the FASD continuum, that is, the 1996 Institute of Medicine criteria and the Washington criteria. (Hoyme *et al*., 2005). The revised Institute of Medicine Diagnostic Classification System and the diagnostic criteria for FAS and FASD are outlined elsewhere (Calhoun *et al*., 2006).

In one large study from Finland, 11% of FAS children were born prematurely, 70% demonstrated prenatal growth deficiency and 45% were microcephalic. Other than growth deficits and the cardinal facial features outlined below, the most common major and minor anomalies noted were camptodactyly (55%), 'hockey stick' or other altered palmar creases (51%), refractive errors (40%), strabismus (38%), dental crowding (43%), nail hypoplasia (38%), genitourinary anomalies (22%) and congenital heart defects (18%) (Autti-Rämö *et al*., 2006).

Experimental studies have shown that alcohol has a direct toxic effect on the ectodermal and mesodermal cells of the developing embryo, particularly in the cells destined to give rise to dentofacial structures (i.e. cranial neural crest cells) and other effects such as abnormal cranial and mandibular growth and altered odontogenesis (Sant'Anna and Tosello, 2006; Mutsvangwa and Douglas, 2007). Orofacial features of FASD include hypoplastic maxillae, low nasal bridge, indistinct philtrum with a hypoplastic upper lip and other features, including small teeth with dysplastic enamel (Naidoo *et al*., 2005, 2006a, 2006b; Jimenez-Farfan *et al*., 2005; Johnston and Bronsky, 1995; Rosenlicht *et al*., 1979). Comparisons of FAS with age-, sex- and race-matched controls discloses a triad of facial profile differences:

- frontal bossing,
- palatal plane tipped up in the front with proclined upper incisors and a sharp nasolabial angle acquired from digit habits,
- above-average length of the mandibular body.

Collectively, these generate the perception of midface hypoplasia, although the midface actually is unremarkable in size and position. A high prevalence of chronic digit habits is a secondary consideration in FAS,

leading to localized skeletodental problems (Gir *et al*., 1989). FAS children have vertically and horizontally underdeveloped maxillae, together with features of long face syndrome with large gonial angles and a short ramus in relation to total face height. There is also a tendency for the development of an anterior open bite, which appears to be compensated for by an increase in the vertical dimension of the anterior alveolar process to bring the incisor teeth into occlusion. The latter adaptation occurred mainly in the mandible (Naidoo *et al*., 2006b).

Maternal alcoholism also interferes with epidermal growth factor (EGF) expression during initial dentinogenesis and amelogenesis and in the secretion and maturation of the dentine and enamel, therefore, which may cause a reduction of dentine and enamel formation (Sant'Anna *et al*., 2005). Small teeth, enamel alterations, and delayed eruption have been observed after ethanol exposure. Epidermal growth factor receptors (EGF-Rs) participate in dental proliferation and differentiation.

Lower first molar morphogenesis when investigated in mouse foetuses exposed to ethanol during gestation showed delayed differentiation, degenerative changes in dental epithelial tissues and reduced dental size, and also enhanced immunoreactivity to EGF-R type 1 and EGFR type 2 (erbB-2) indicating a delay in dental morphogenesis (Jiménez-Farfán *et al*., 2005). However, studies of the dental age and skeletal age of children with FAS compared with matched controls are inconclusive since growth of individuals is often irregular and a more complete appraisal of the entire skeleton and an evaluation of the entire dentition, rather than just the mandibular teeth, might improve the correlation between the variables (Naidoo *et al*., 2006a). FAS patients have statistically significantly more dentofacial anomalies than controls. The mean DMFT score for the FAS sample was slightly higher, although not significantly different from that of the controls and the decayed component made up the largest part of the index in both groups. The prevalence of enamel opacities between FAS and controls is not significantly different and averaged around 15% for both groups. More than three-quarters of both the cases and the controls demonstrated the presence of plaque and almost two-thirds demonstrated gingival bleeding on probing (Naidoo *et al*., 2005).

17.3.5 Oral mucosa

There may be folate deficiency or other anaemia, causing glossitis and sometimes angular stomatitis or recurrent aphthae. Carcinoma is another possible outcome (see Chapters 3 and 14).

17.3.6 Sialosis

A rare manifestation of alcohol abuse is bilateral painless swelling of the parotids or other major salivary glands (sialosis). Enlarged parotid glands

may precede liver disease and is due to fatty infiltration or acinar hypertrophy. Many reports have indicated that between 30% and 80% of patients with alcoholic cirrhosis have sialosis (Borsanyi and Blanchard, 1960, 1961; Brick, 1958; Mandel and Baurmash, 1971; Kastin and Mandel, 2000; Mandel and Hamele-Bena, 1997; Borsanyi, 1963; Bonnin *et al*., 1954) but, if that were universally true, one would expect sialosis to be far more commonly seen than it is. Only 26% of the patients in a recent study had a history of alcohol abuse and only six had liver damage as confirmed by serum biochemistry (Scully *et al*., 2008).

The causes of sialosis are varied and the very diversity of associated conditions is intriguing. However, it is now recognized that many of these aetiological factors may act to produce sialosis through the common feature of autonomic nerve dysfunction and it is possible that there are functional changes in salivary aquaporin water channels (Mandic *et al*., 2005). Samples from parotid, submaxillary and von Ebner salivary glands of chronic alcoholic individuals show enlargement of major ducts, heterogeneous expression of cytokeratins and athrophy in epithelial cells, desquamated cells and stasis of content, and ductal hyperplasia in von Ebner glands. The lymphoplasmocytic infiltration does not represent the typical lymphocytic focus seen in Sjögren's syndrome or other connective tissue pathologies (Ferraris *et al*., 1999; Carda *et al*., 2004; Ferraris *et al*., 2000; Carranza *et al*., 2005). There appear to be qualitative histopathologic variations between sialosis with different origins (Carda *et al*., 2005).

17.3.7 Dry mouth

The effects of alcohol on salivation have not been clearly defined. The parotid flow rate over the initial post-stimulatory five-minute period was raised by 44% in ethanol-dosed rats and the salivary sodium concentration was also raised, in line with higher salivary flow rates. There were no histopathological changes related to ethanol or sucrose dosing, but stereological analysis showed a 64% increase in the proportional volume of intralobular vascular tissue in ethanol-dosed rats. These quantified histological findings suggested that parotid intralobular haemodynamics may be altered after chronic ethanol-dosing and this may contribute to the hypersecretory response exhibited by the ethanol-dosed rats (Berry and Scott, 1990).

In cirrhotic subjects the parotid contained proportionally more adipose but less acinar tissues than in controls. The submandibular gland showed a proportional increase in adiposity and reduction in fibrovascular tissues but no noticeable reduction in its acinar proportional volume (Scott *et al*., 1988a). Resting salivary flow was raised threefold in the alcohol group and the protein and electrolyte concentrations were also altered in this saliva. However, there were no intergroup differences in parotid flow or composition following gustatory stimulation by 6% citric acid.

The results contrast with the wide parotid functional changes known to occur in stimulated parotid saliva in alcoholic cirrhosis and suggest, therefore, that the direct salivary effects of chronic alcohol abuse may be less important than the damaged liver function in contributing to the salivary disturbance reportedly occurring in alcoholic cirrhosis (Scott *et al*., 1988b). The salivary findings of increased flow rate, protein and amylase levels indicate that hypertrophy and increased acinar function may be a component of the parotid enlargement and that, furthermore, a fatty replacement of functional gland tissue is probably not involved. In addition, the salivary electrolyte changes that were found, increased potassium with effectively decreased sodium excretion, suggest that the elevated aldosterone level commonly found in cirrhotic patients affects salivary secretions in these patients in much the same way as it does in patients with hypertension (Abelson *et al*., 1976).

In contrast, mean simulated parotid saliva flow rates were significantly lower in patients with alcoholic cirrhosis compared with alcoholic and non-alcoholic control subjects in another study (Dutta *et al*., 1989). A similar reduction was observed in mean basal parotid saliva flow rate in patients with alcoholic cirrhosis that reached statistical significance ($p < 0.05$) in comparison with non-alcoholic control subjects. In addition, the concentration of sodium, bicarbonate and total proteins in stimulated parotid saliva was significantly ($p < 0.005$) lower in patients with alcoholic cirrhosis compared with the two groups of control subjects (Dutta *et al*., 1989). Mean stimulated parotid saliva flow rate was significantly ($p < 0.01$) lower in alcoholic subjects than in matched control subjects. Reduction in parotid saliva flow rate was associated with a statistically significant decrease in total protein and amylase secretion in this group of patients. In addition, secretion of immunoreactive EGF, a specific salivary protein, was also markedly reduced in alcoholic patients. None of the parotid saliva samples from the alcoholic subjects had detectable bioactivity of EGF in saliva. These data suggest that chronic alcohol ingestion is associated with significant changes in parotid saliva secretion and its composition, which may perpetuate and compound ethanol-induced injury to the upper gastrointestinal tract (Dutta *et al*., 1992).

Concentrations of Na^+ in saliva of patients with idiopathic chronic pancreatitis and of beta2-microglobulin in saliva of patients with idiopathic and autoimmune chronic pancreatitis were significantly elevated compared to those of the control group. In submandibular and parotid gland scintigraphy, the peak count density ratio of patients with all chronic pancreatitis and washout ratio of patients with alcoholic and idiopathic chronic pancreatitis were significantly lower than those of the control group. Salivary gland function was frequently impaired in the course of chronic pancreatitis of various etiologies. Salivary gland dysfunction might be the result of a common pathophysiological effect of alcohol in patients with alcoholic chronic pancreatitis and of the aggressive immune mechanism against the

pancreatic and the salivary ducts in patients with autoimmune and idiopathic chronic pancreatitis (Kamisawa *et al*., 2003).

17.3.8 Osteomyelitis

Trauma and odontogenic infections are the most prevalent causes of osteomyelitis of the jaws, which in the vast majority of cases affected the mandible, although alcohol and/or tobacco use was reported in at least one-half of the cases surveyed (Koorbusch *et al*., 1992). The development of infection, non-union and related complications after the repair of mandibular fractures correlates with a history of tobacco and alcohol use and open reduction internal fixation of multiple fractures (Furr *et al*., 2006). Wound healing may also be impaired in the severe chronic alcoholic, and alcoholism may underlie osteomyelitis following mandibular fractures (Ferguson *et al*., 1991). Uncooperative alcoholics with psychosocial handicaps, and general as well as local periodontitis, are found to be especially liable to consolidate their fractures at a slower rate than the average patient (Adell *et al*., 1987).

One of the most prevalent negative factors associated with osteoradionecrosis patients was the continued heavy use of alcohol and tobacco by 86% of them (Kluth *et al*., 1988).

Alcohol-addicted patients have a high risk of intercurrent complications during the postoperative period. In addition to the predisposition to infection, alcohol withdrawal syndrome is potentially life threatening in these patients (Heil *et al*., 1992). No difference in the rate of overall complications was seen between the patients who experienced withdrawal and those who did not, although patients who experienced withdrawal did have a statistically significant ratio of non-flap-related to flap-related complications (Weinfeld *et al*., 2000). Acute alcohol withdrawal in the first 72 hours after maxillofacial surgery is associated with a high incidence of flap loss. Therefore, patients at significant risk during alcohol withdrawal should undergo detoxification preoperatively (Gallivan and Reiter, 2001).

17.4 Direct effects of alcohol influencing orofacial health

17.4.1 Enamel erosion

Several intrinsic factors are involved in the development of dental erosion, such as salivary flow rate, buffering capacity and composition, pellicle formation, tooth composition, and extrinsic factors, such as chemical (pH, titratable acidity, phosphate and calcium concentration, fluoride content of the material in contact with the tooth) and behavioural (eating and drinking habits, lifestyle, excessive consumption of acids) factors. Although low pH plays a crucial role in determining tooth wear and the evidence that acidic

foodstuffs and beverages play a crucial role in the development of erosion is convincing, the density of acids in the food/beverage, that is, the titratable acidity, is more important than the pH itself, as well as other behavioural factors, such as the contact time between the acidic food/beverage and the dental surfaces (Lussi *et al*., 2004; Zero and Lussi, 2005).

The pH of most alcoholic beverages is acidic, with values around 4, and the concentration of organic and inorganic acids is high. Indeed, it is important that the pH of incompletely fermented drinks, such as beer and wine, is acidic because it helps prevent contamination by other microorganisms, while the concentration of carbonic acid in alcopops (and in cocktails) is high because of the presence of soft drinks or fruit juices in the beverage (Klampfl *et al*., 2000).

Most alcoholic beverages are involved in determining dental erosion, but the major role is for alcopops (O'Sullivan and Curzon, 1998; Rees *et al*., 1998; Zero and Lussi, 2006) and cocktails (Chuajedong *et al*., 2002; Rafeek *et al*., 2006), which combine the erosive potentials of primary alcoholic beverages and of soft drinks and/or fruit juices, at the same time. Among primary drinks, white wines are more erosive than red wines, because of higher concentration of titratable acids (Rees *et al*., 2002), while beers (Lissera *et al*., 1998; Noguiera *et al*., 2000) and ciders (Rees and Griffiths, 2002) have a moderate erosive potential. The astringency of alcoholic beverages is likely to be another factor promoting tooth wear. Indeed, this taste which is typical of some alcoholic beverages is due to presence of high levels of polyphenols, mostly tannins, which bind salivary proteins, such as proline-rich proteins and mucopolysaccharides, causing their precipitation, with consequent sensation of astringency owing to loss of lubrication of the oral mucosa and teeth and simultaneous decreased protection of teeth from acids (Dreosti, 2000).

Discomfort of the teeth is acknowledged to be a problem for those people making or tasting wines regularly which, because of wine's acidity, may have a deleterious erosive effect on teeth (Mandel, 2005; Meurman and Vesterinen, 2000; Mok *et al*., 2001; Gray *et al*., 1998; Ferguson *et al*., 1996; Chikte *et al*., 2005; Wiktorsson *et al*., 1997; Chaudhry *et al*., 1997). In fact, dental erosions are so frequent among wine merchants (Chaudhry *et al*., 1997), winetasters (Wiktorsson *et al*., 1997; Gray *et al*., 1998; Chikte *et al*., 2005) and winemakers in general (Chikte *et al*., 2005), who keep wine in their mouth for long time, as to be considered an occupational hazard.

The erosive effects vary between wines (Chikte *et al*., 2003). Riesling style wine is more erosive than champagne style and both are more than claret (Mok *et al*., 2001). Most white wines tested were at least as erosive as orange juice, while some wines, notably the cava, were significantly more erosive than orange juice (Rees *et al*., 2002). Red wines may also be erosive (Lupi-Pegurier *et al*., 2003) and all ciders tested are acidic and had

considerable erosive potential *in vitro* which was broadly similar to that of orange juice (Rees and Griffiths, 2002). Many commercially available designer drinks also have considerable erosive potential (Rees and Davis, 2000) as do alcoholic soft drinks (O'Sullivan and Curzon, 1998) such as Hooch alcoholic lemonade (Rees *et al*., 1998). Fluoride gels or varnishes may significantly reduce such enamel erosion (Jones *et al*., 2002; Sorvari *et al*., 1994).

Dental erosion is strongly associated with drinking behaviour. In fact, it is more frequent and severe among those whose alcohol consumption is continuous rather than in the form of episodic binges (Smith and Robb, 1989; Robb and Smith, 1990; Chuajedong *et al*., 2002; Rafeek *et al*., 2006) and it is affected by the time the drink is kept into mouth before swallowing. Several epidemiologic studies report a time-dependent association between chronic alcoholism and dental erosion, independently of socioeconomic status, with prevalence values as high as 50% (Smith and Robb, 1989; Robb and Smith, 1990; Hede, 1996; Araujo *et al*., 2004). The typical dental erosions of heavy alcohol drinkers generally affect the palatal surfaces of the upper anterior teeth (Smith, 1990).

Summarizing, the evidence of the association between alcoholic beverage intake and dental erosion is convincing, with secondary drinks showing greater erosive potential than primary drinks and with an important role of drinking behaviour, particularly those habits characterized by frequent intake and long retention of the drink in the oral cavity.

17.4.2 Facial burns

Facial burns are occasionally caused by flambé drinks, usually while drinking and spilling the whisky on the flame during a social gathering and festivity. Alcohol flames inflict mainly superficial (56%) to mid-second-degree burns in a relatively small area of body, usually the head, followed by the upper extremity and trunk (Jang *et al*., 2006).

17.4.3 Mouthwashes

The alcohol content of mouthwashes can be as high as 27% which is equivalent to that of 54-proof liquor (Table 17.7). Mouthwashes increase the time the mucosa is in contact with alcohol and those with a high content of alcohol can cause hyperkeratotic lesions both in laboratory animals and humans. Nevertheless, systematic reviews provide varying conclusions, at the moment, the evidence of a causal relationship between the use of alcohol-containing mouthwashes and the development of oral cancer is not sufficient (Carretero Pelaez *et al*., 2004; Cole *et al*., 2003; McCullough and Farrah, 2008; La Vecchia, 2008). In the absence of a clear evidence-based positive or negative response, prescription of alcohol-containing mouthwashes should, therefore, be made with prudence, particularly among children and high-risk subjects.

Table 17.7 Alcohol concentration in mouthwashes

Mouthwashes	%v/v Alcohol
Listermint	13.0
Listerine Mouthwash	26.9
Scope Mouthwash	19.0
Search	15.3
Mentadent Mouthwash	12.0
Oral B Dental Rinse	9.4
Colgate Fluorigard Daily	5.0
Macleans Active Mouthguard	17.5
Safeway ExtraStrength antiseptic	26.9
Safeway Antiplaque Mouthwash	7.7
Corsodyl	7
Pearl drops Smokers 1+1	14.2
Perio Aid	6.5
Colgate Total (Plax) red	8.0–8.3
Colgate Total (Plax) green	5.0–5.2
Plax (Pfizer – USA only)	8.7
Cepacol	14.5
Listerine Coolmint (other flavours also)	21.6
Colgate Fluoriguard	5.0
Oral B Anti-Plaque Dental Rinse Alcohol Free	0.0
RetarDEX oral spray and oral rinse	0.0
Colgate rinse alcohol-free	0.0
TheraBreath	0.0
Dentyl pH	0.0
Macleans Alcohol Free Smoothmint Mouthwash	0.0
Yotuel Whitening Mouthwash	0.0
Frador coolmint mouthwash	0.0
Cariax Gingival	0.0
Elmex	0.0
Meridol	0.0
Colgate Fluoriguard	0.0
Perio Aid New Formulation	0.0

Note: *Medicina Oral* 2004, 9 (2) 116–23 Table 1 shows different alcohol concentrations for Spanish versions of some of the above mouthwashes, e.g. Perio Aid 11.6% rather than 6.5%.

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18

Malnutrition as an etiological factor in dental caries disparity

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Abstract: A diet including frequent consumption of foods high in carbohydrates is a known risk factor for dental caries. Nevertheless, economic and environmental barriers encourage low-income parents and children to consume foods high in sugar and low in nutrients. Such foods are generally more readily available to low-income populations owing to their relatively low cost compared to foods low in energy density and high in nutrients (such as fruits and vegetables). The objective of this review is to inform dental providers of barriers that families face in following dietary guidelines and to suggest methods that have been shown to be successful in encouraging children to eat healthy foods. Also reviewed are suggestions for how societies might encourage healthy eating in children, through stricter oversight and better funding of nutrition programs in child care settings and schools. High-sugar foods are both more readily available in low-income neighborhoods and cheaper to purchase than are healthier options. Thus there are both environmental and economic barriers to healthy eating in low-income populations. Furthermore, there are cultural and socioeconomic differences in attitudes and parenting practices that may contribute to dietary-induced dental caries, such as pressure to eat. Dental providers should encourage parents to expose their children regularly to healthy foods such as fresh vegetables to increase preferences for these items. However, providers also need to recognize the financial constraints on obtaining a healthy diet. More attention to the types of foods provided in government-subsidized meal programs could also serve to ameliorate the problem.

Key words: cariogenic agents, dental care for children, dental caries, diet, nutrition policy.

18.1 Introduction

Diet plays a role in all major oral diseases – particularly dental caries, periodontal diseases, and oral cancer – but no source of inequity is greater than

the relationship between sugar and tooth decay. A major etiologic factor among the poor is the substitution of high energy, low nutrient-density foods for lower energy density, high-nutrient foods. We should not view this caries disparity as caused by the willful consumption of too much soda or candy by children whose diet is poorly controlled by their parents – a common view of dental professionals. Parents themselves consume low-cost-per-calorie drinks and foods (Drewnowski and Bellisle, 2007). Rather, this is part of a larger problem of malnutrition (Clarke *et al.*, 2006).

Soda, candy, chips and cookies are a source of cheap calories. They substitute for fruits and vegetables and are responsible, in part, for a constellation of developmental problems and delays. Hypocalcification defects and tooth decay are often visible signs of more serious health and social problems in these children. A low-cost diet may result in the child being overweight and then obese, with subsequent problems of diabetes and cardiovascular disease. A dental practitioner, hygienist, therapist, assistant, nurse, or medical doctor who fails to understand these linkages and connections is likely to offer ineffective advice and guidance. Indeed, there are few studies that demonstrate any impact of counseling on diet and dental caries (Kay and Locker, 1998). One aspect of this problem that should not be underestimated is the gulf between a healthy diet as seen by relatively well off dental and medical practitioners – for whom the cost of high nutrient foods is not a barrier – and the world inhabited by those who face oral health disparities.

Culture and socioeconomic status influence attitudes toward weight and efforts to eat a healthy diet. Wardle and colleagues (2004) surveyed 1248 female adolescents and found that socioeconomic status (SES) was related to attitudes about their own weight and the weights of others. High SES adolescents were more aware of social ideals regarding slimness and had more family and friends trying to lose weight. They also defined a lower body mass index as 'fat' than did adolescents with low SES. Similarly, Barker and colleagues (2008) found that educational attainment is associated with consumption of fruits and vegetables, with less consumption occurring in those with fewer educational qualifications.

Cultural and socioeconomic differences in attitudes toward weight and healthy eating have also been observed in parents. Focus group studies of low-income mothers have revealed that many view an infant who is large for his/her age as being in good health, whereas health care professionals would find that same infant to be overweight (Baughcum *et al.*, 1998; Bentley *et al.*, 1999). A desire to have a large infant results in feeding practices aimed at increasing the infant's weight, such as adding cereal to formula and feeding frequently. Signs of distress in infants and children also are interpreted as signs of hunger, thus access to food is given on a regular and continuous basis, increasing the risk of both caries and obesity.

Carnell and colleagues (2005) found that, across income levels, parents of 3- to 5-year-old children generally show poor awareness of their child's

current weight status and often fail to identify a child as overweight. This lack of awareness is likely to contribute to unhealthy feeding practices. High intake of unhealthy snack foods and low intake of fruits and vegetables have both been linked to high parental pressure to eat. The parenting style that includes high pressure to eat is more commonly observed among male, non-white parents and parents of younger children (Brown *et al*., 2008). In contrast, covert control of children's diets (keeping unhealthy foods out of the home and avoiding fast food restaurants) is associated with healthy child nutrition practices. Such covert control of a child's diet is positively associated with parental level of education (Brown *et al*., 2008).

Some parental control methods, such as using foods as a reward or being highly restrictive of sweets, have been found to create the unintended outcome of children eating more unhealthy sweets (Birch, 1999). Parents can increase children's acceptance of fruits and vegetables by providing frequent exposure to them in the home (Cooke, 2007).

When asking a parent to change child feeding practices, a counselor or health professional should recognize and address the complex nature of parenting styles. It may also be helpful to explain to the parent that frequent taste experience (eight or more occasions) with a new healthy food (such as sweet red pepper or tofu) is required to increase children's acceptance of these items (Cooke, 2007).

18.2 Stress and depression

Stress, including pressures of work and limited time to parent, as well as parental depression itself, may further lead to substitution of baby bottles for breast-feeding and to other maladaptive feeding behaviors (Weinstein *et al*., 1996). The sweetened drinks placed in baby bottles appear to satisfy babies and provide quiet and order in crowded, stressed, low-income households.

Depression has many adverse effects on the behavior and health of low-income women and on the health of their children. These women often have low energy and fatigue, reduced problem-solving abilities and concentration and low self-esteem. Therefore, they are not likely to readily respond to health care providers' requests that they see as inconvenient, labor-intensive, or problematic.

The prevalence of depressed low-income women in the USA and the United Kingdom is shocking, with 25% of low income young women and mothers with young children meeting the criteria for depression (Miranda *et al*., 2003; Siefert *et al*., 2000). Epidemiological studies show a peak of initial onset of depression in childbearing and childrearing years (Kessler *et al*., 1994). Episodes are recurrent in 50–70% of new cases and the severity of subsequent episodes tends to increase over time (Kupfer,

1991). According to a survey conducted by the Royal College of Midwives, 20% of young mothers in the UK also suffer from depression (Dent, 2007).

What do the depressed look like to dental personnel? Women within a depressive episode seem sad and tired and report feeling 'empty'. They may be irritable or angry, blaming others when frustrated. They may appear disinterested, rude, or argumentative during a discussion about altering diet. They may seem indecisive about taking action. Recent reports of depressed women with a history of abuse point to distrust of health providers and a strong sense of self-reliance (Grote *et al*., 2007).

As a clinician, what should you do if you believe the person who brought the child to the practice is depressed? First, inquire if there is anyone else who can take responsibility for the child's future visits, diet, tooth cleaning, and so on. Frequently, there is a competent person in the extended family who may be willing to take on additional child care activities. Second, limit dietary or other recommendations; they should be simple and very 'doable'. Finally, explore resources in your community and learn how to make a referral to a mental health professional. If a rapport develops, the patient may talk about her problems, presenting you with the opportunity to refer her for treatment.

18.3 Counseling caregiver about diet

Generally, parents and children are aware that high-energy, low-nutrient-density foods are not good for them. Few parents need to be told that vegetables and fruits are good for kids or that candy is bad for their teeth, but advice along these lines is largely ineffective. There is little evidence that providing dietary information and advice itself to parents has a meaningful influence on the diets of children.

The discussion about depression sets the stage for our comments concerning dietary counseling. There is little evidence that providing dietary information and advice itself to parents has resulted in meaningful improvements in preventing chronic disease, modifying dietary fat and increasing fruit and vegetable intake (Ammerman *et al*., 2002). Recently, however, a brief counseling technique was found to be effective in treating addictive behaviors. This technique, motivational interviewing (MI), was successfully used to alter nutrition (Kolasa, 2005; Richards *et al*., 2006). This technique also was successfully used with the parents of 6- to 18-month-olds at high risk for early childhood caries (Weinstein *et al*., 2004). A clinician using the technique asks the parent questions to trigger verbal expressions concerning risk and desired outcomes (for example, 'Tell me about your family's teeth', or 'What do you want for your child?'). The clinician listens carefully and summarizes what the parent has said, thereby showing empathy and identifying internal motivation.

Next, parents who perceive a problem with the *status quo* are asked if they would like to look at a menu of choices. The choices on the menu are then discussed. Obstacles are identified, choices are made and a plan of action is created. The clinician follows up by phone call or by appointment. Remember, unhealthy teeth are not the only problem that malnutrition presents. Counseling need not focus only on the dentition. The MI approach is presented in more detail in Weinstein's workbook (Weinstein, 2002).

18.4 Impacts of poor diet

Left untreated, cavities turn into abcesses and create great discomfort for children. In a study conducted at the Children's Hospital and Medical Center in Seattle, 40% of emergency room visits were for dental caries and pain (Zeng *et al*., 1994), and other studies have found that as many as 20% of all children's dental visits are scheduled because a child has been in pain (Edelstein, 2002). Tooth decay in the primary dentition is a strong predictor of problems later in life as well.

Tooth decay may be a marker for other pediatric conditions (Ayhan *et al*., 1996). Chronic dental problems contribute to distractibility, other behavior problems, poor sleep, acute pain and difficulties with speech and eating. Dental problems may exacerbate inequality. In addition, it is important to understand and to communicate to the parents that infections (dental caries is an infection) in the primary dentition are related to infections in the permanent dentition. There are therefore short- and long-term reasons why the primary dentition should be infection-free.

Guided by an adaptation of Patrick and Erickson's model of health-related quality of life to oral health-related quality of life (Gift and Atchison, 1995; Gift *et al*., 1997), researchers have identified five domains in which oral health can affect daily functioning: (1) impairment (e.g. pain, discomfort), (2) physical functioning (e.g. eating, sleeping), (3) social functioning (e.g. interactions with others, school performance), (4) psychological functioning (e.g. self-esteem, appearance) and (5) oral health perceptions (i.e. self-assessment of overall oral health and experience with dentists). Objective measures of oral health are correlated with impacts. The better the oral health, the fewer the negative impacts. There is increasing evidence that early tooth decay results in a child's poorer growth and feeding. Thus the problem comes full circle in that poor diet leads to tooth decay and one of tooth decay's impacts is poor eating.

18.5 Model of caries disparity

Patrick and others (2006) have provided a conceptual framework that permits grasping the larger influences of society, community and family on

individual behavior and ultimately on oral health status and quality of life (Fig. 18.1). This model permits an understanding of how various aspects of diet and eating affect health status and lead to disparities. Thus, diet is an important aspect of the problem but surely must be seen in context to have value in determining policies or counseling individual patients and their families.

The macroenvironment includes advertising and government policy such as price supports for sugar. While some countries ban television advertising of certain products during periods when children are most likely to watch, they permit advertisements for foods that are most associated with the disparities being discussed here. To the extent that governments control the broadcast spectrum, it can be argued that they give a subsidy to these advertisers, which exacerbates the oral health disparity problem. On the positive side, some governments, including the United States may have dietary guidelines (Table 18.1). The USA government also provides a toolkit for health professionals containing valuable information on diet and counseling (see website http://www.Health.gov/dietaryguidelines).

Community, especially the physical environment, can promote or help defeat oral health equity. For example, schools may provide facilities for toothbrushing or they may allow vending machines that provide soda.

The interpersonal environment is also a factor in oral health disparities. To the extent that schools offer curricula rich in nutrition science, children have the opportunity to learn why healthy diets are important. Parents may not have had the opportunity to learn about nutrition. In many countries, access to personal dental services is very limited, further reducing the opportunity for families to learn what constitutes a tooth friendly dietary pattern.

18.6 Ecological model of what people eat: tooth decay is part of a larger problem

To the extent that what we eat is the source of tooth decay and part of a larger problem of food choices and offerings, it is helpful to look at an ecological model of what we eat (Fig. 18.2) (Story *et al.*, 2007).

18.7 Family influences on taste preferences

Parents strongly influence what kids eat and like. Parental style in which dairy products and vegetables are presented to young children results in children eating more of these healthy foods. On the other hand, pressuring children or using foods as rewards may backfire (Birch, 1999).

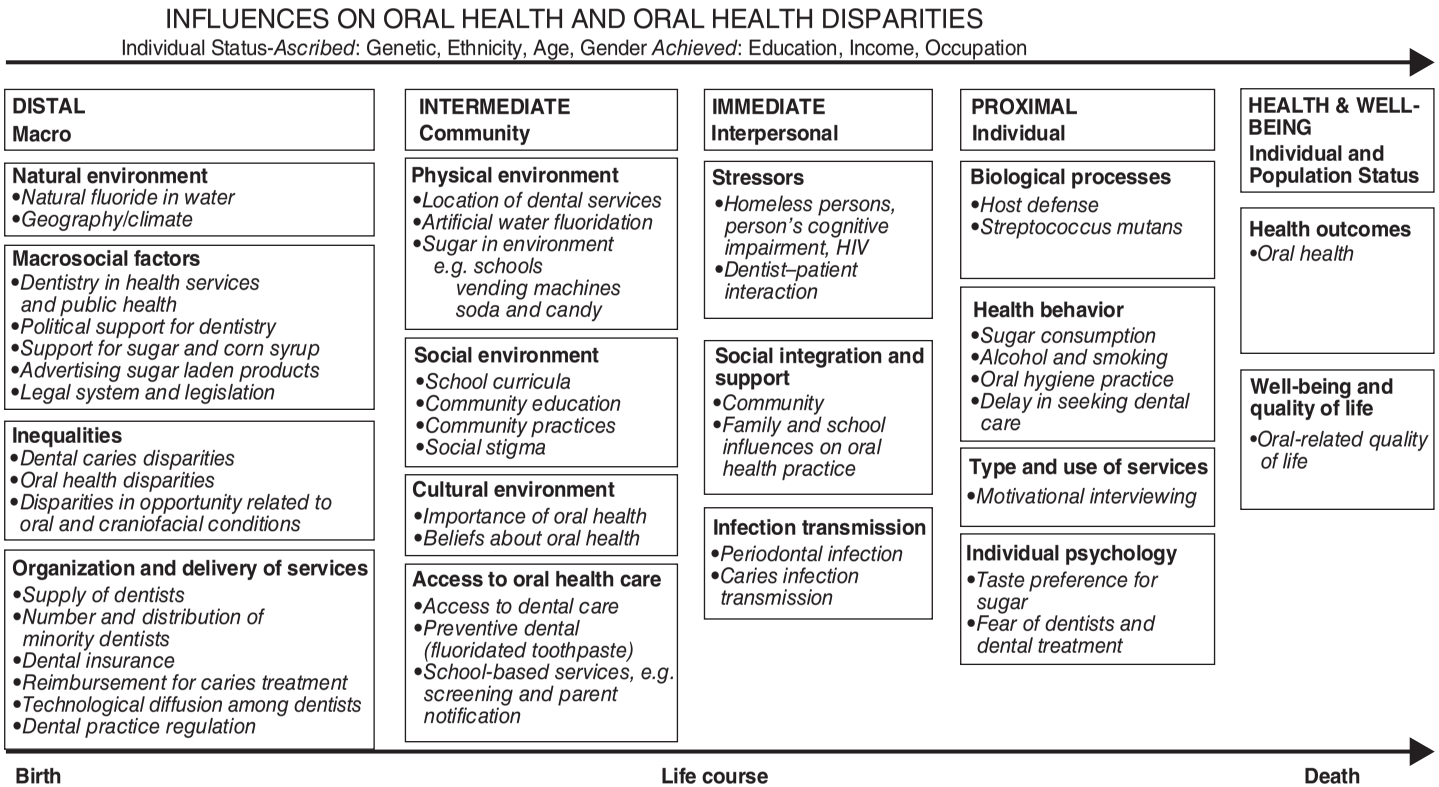

Fig. 18.1 Influences on oral health and oral health disparities. Based on Patrick and Erickson, 1993 and Schulz and Northridge, 2004. Boxes contain only selected examples of influences in italics; readers may be able to suggest additional examples.

Table 18.1 Recommendations from *Dietary Guidelines for Americans,* 2005

	Key recommendations	Specific recommendations for children and adolescents
Food groups to encourage	• Consume a sufficient amount of fruits and vegetables while staying within energy needs. Two cups of fruit and 21/2 cups of vegetables per day are recommended for a reference 2000-calorie intake, with higher or lower amounts depending on the calorie level. • Choose a variety of fruits and vegetables each day. In particular, select from all five vegetable subgroups (dark green, orange, legumes, starchy vegetables and other vegetables) several times a week. • Consume three or more ounce-equivalents of whole-grain products per day, with the rest of the recommended grains coming from enriched or whole-grain products. In general, at least half the grains should come from whole grains. • Consume three cups per day of fat-free or low-fat milk or equivalent milk products.	• Consume whole-grain products often; at least half the grains should be whole grains. Children 2 to 8 years should consume two cups per day of fat-free or low-fat milk or equivalent milk products. Children of 9 years of age and older should consume three cups per day of fat-free or low-fat milk or equivalent milk products.
Fats	• Consume less than 10% calories from saturated fatty acids and less than 300 mg/day of cholesterol and keep trans fatty acid consumption as low as possible. • Keep total fat intake between 20–35% of calories, with most fats coming from sources of polyunsaturated and monounsaturated fatty acids, such as fish, nuts and vegetable oils. • When selecting and preparing meat, poultry, dry beans, and milk or milk products, make choices that are lean, low-fat, or fat-free. • Limit intake of fats and oils high in saturated and/or trans fatty acids and choose products low in such fats and oils.	• Keep total fat intake between 30–35% of calories for children 2–3 years of age and between 25–35% of calories for children and adolescents 4 to 18 years of age, with most fats coming from sources of polyunsaturated and monounsaturated fatty acids, such as fish, nuts and vegetable oils.
Carbohydrates	• Choose fiber-rich fruits, vegetables and whole grains often. • Choose and prepare foods and beverages with little added sugars or caloric sweeteners, such as amounts suggested by the USDA Food Guide and the DASH Eating Plan. • Reduce the incidence of dental caries by practicing good oral hygiene and consuming sugar- and starch-containing foods and beverages less frequently.	

Adapted from *Dietary Guidelines for Americans*, 2005.
http://www.health.gov/dietaryguidelines/dga2005/document/html/executivesummary.htm) accessed March 5, 2008.

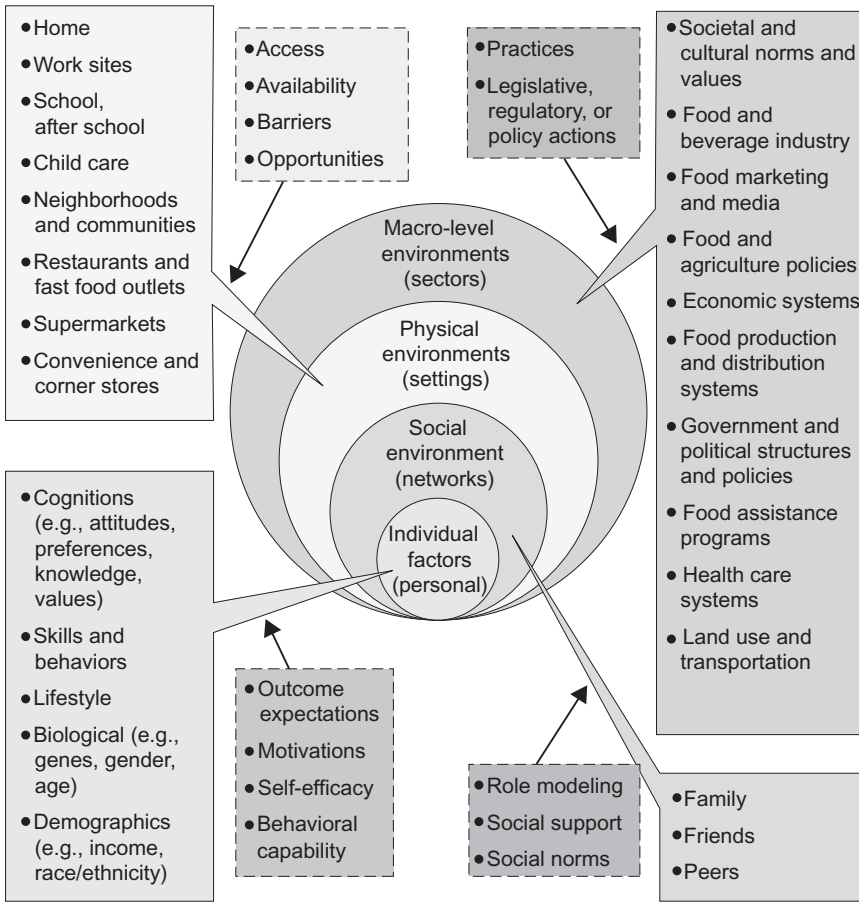

Fig. 18.2 An ecological framework depicting the multiple influences on what people eat.

18.7.1 Home

Americans consume more than two-thirds of their total calories from foods prepared at home. The strongest factors associated with healthy eating behavior at home are availability and accessibility (Cullen *et al*., 2003). Availability means that the foods – fruits and vegetables – are available in the home. Accessibility means that the healthy foods are in a location that makes them easy to get (such as a fruit bowl on the table). Parental social support for healthy eating, such as regular meal times and family meals, also are strong influences. Parents who consume fruits and vegetables generally have children who do the same. On the other hand, the presence of soft drinks is strongly correlated with soft drink consumption in children (Grimm *et al*., 2004) and experiments in which high caloric drinks were replaced

within the home with non-caloric beverages resulted in reduced body mass in adolescents who had been heavy consumers (Ebbeling *et al.*, 2006). Relatively simple changes in the home, where most calories are consumed, can readily remediate the ill effects of poor aspects of diet. Counseling should focus on such simple changes.

18.7.2 Child care

Many American children under five years old spend time in child care settings every week and more than four in 100 spend nearly the entire workday week in child care. These settings have largely been ignored as opportunities to improve nutrition and reduce the disparities associated with poor diets. The United States Department of Agriculture (USDA) is a major supplier of meals and snacks for three million of these children. USDA operates under nutrient-based dietary standards, but these standards offer no specific guidance regarding high-calorie foods containing large amounts of fats and sugars (Rosso and Weill, 2006). The Sure Start program for childcare in the UK mentions a good diet and encourages toothbrushing to reduce tooth decay in its practice guidance, but the guidance does not appear to contain specific policies about dietary advice (Sure Start, 2008: Practice Guidance chapter 10, Family Health). In the United States, local dental professionals often serve as advisers to Head Start and other childcare centers and can positively influence policies within these centers. The American Dietetic Association provides advice to Head Start regarding nutrition standards for childcare programs (American Dietetic Association, 1999) and Head Start has specific standards (Head Start Bureau, 1998).

18.7.3 Schools

Considerable efforts are going into improving meals at schools in several countries. In the United States, food at school can come from the National School Lunch Program and the National School Breakfast Program and is governed by US federal dietary guidelines. More than half the states have adopted policies that govern school meals outside the federal programs – including vending machines – but the standards are generally weak. Recent federal policies require school districts to adopt local school nutrition policies, but this effort is just beginning (Institute of Medicine, 2007).

The UK has voluntary Target Nutritional Specifications (TNS) for manufactured products used in school meals, adopted in 2005. They are already in use in Scotland as part of the Hungry for Success policy and in Wales as part of the Appetite for Life program. The policies set maximum levels of total fat, saturated fat, total sugars and salt for food products. There has also been work providing free school meals or encouraging changes in school meals, but overall nutrient levels are still lower than encouraged and sugar is still a problem. Table 18.2 gives some of the food standards

Table 18.2 United Kingdom standards for school meals

Food/food groups	Interim food-based standards for school lunches from 2006 (revised 2007)	Food-based standards for school food other than lunches from 2007	Final food-based standards for school lunches from 2008 (primary) and 2009 (secondary)
Salt and condiments – restricted	● No salt shall be available to add to food after the cooking process is complete. Salt shall not be provided at tables or service counters. Condiments, such as ketchup and mayonnaise, may only be available in sachets or in individual portions of not more than 10 g or 1 teaspoonful		
Snacks – restricted	● Snacks such as crisps must not be provided. Nuts, seeds, vegetables and fruit with no added salt, sugar or fat are allowed. Dried fruit may contain up to 0.5% vegetable oil as a glazing agent.		
	Savoury crackers and breadsticks can only be served with fruit, vegetables or dairy food as part of school lunch.	Savoury crackers and breadsticks must not be provided.	Savoury crackers and breadsticks can only be served with fruit, vegetables or dairy food as part of school lunch.
No confectionery	● Confectionery such as chocolate bars, chocolate coated or flavoured biscuits, sweets or cereal bars must not be provided		
Cakes and biscuits – restricted	● Cakes and biscuits are allowed at lunchtime but must not contain any confectionery.	Cakes and biscuits must not be provided.	Cakes and biscuits are allowed at lunchtime but must not contain any confectionery.
Drinking water	● Free, fresh drinking water should be provided at all times.		
Healthier drinks	● The only drink permitted during the school day are plain water (still or sparkling); skimmed, semi-skimmed or lactose-reduced milk; fruit juice; vegetable juice; plain soya, rice, or oat drink enriched with calcium; plain fermented milk (e.g. yoghurt) drink; combination drinks; flavoured milk. Tea, coffee and low-calorie hot chocolate also permitted.		

for UK schools (School Food Trust, 2008). These standards are clearly in line with efforts to reduce oral health disparities through improvements in diet.

The UK and the EU are clearly struggling with policies about vending machines. Policies still allow some flavored milks and juices must be at least 50% fruit juice (School Food Trust, 2008). Consumption of food from vending machines has been shown to be related to tooth decay (Maliderou *et al*., 2006). Further efforts along these lines will help reduce inequities related to diet.

18.7.4 After school

Many after-school programs receive food aid because they serve disadvantaged and minority youth. The US government supports these programs through the After School Snack Program and the Summer Food Service. Little is known about the quality of these programs and what impact they may have on oral health disparities that stem from diet. Nonetheless, these programs – serving millions of children – are another avenue by which government and care providers can reduce oral health disparities.

18.8 Food access

Food insecurity is a growing problem throughout the world and this contributes to lack of a good diet. A great deal of evidence indicates that lack of access to cheap, healthy foods contributes to the disparities in diet-related diseases such as tooth decay (Baker *et al*., 2006). Prices are higher and availability poorer for vegetables and fruits in low-income neighborhoods in the USA and Europe. Poor neighborhoods have more mini-marts and fewer full service supermarkets than more affluent areas (Glanz and Yaroch, 2004).

18.9 Food marketing

Aggressive food marketing both on television and radio and now on the internet contribute to the oral health/caries disparity. Child- and youth-directed media are loaded with advertising that aims to influence purchasing behavior. Attempts to foster brand loyalty begin when children are toddlers. Virtually all of these advertisements are for candy, snacks, sugared cereals and fast food. Experts believe that the advertisements will soon extend to voice messages on cell phones, instant messaging and video games. Parents may be largely unaware of the extent of this advertising or its impact.

18.10 Conclusions

Diet is important in all oral diseases. This chapter has addressed the role of diet in the inequality of burden of dental caries. It has viewed dental caries as more than the ignorant or stupid consumption of too much sugar-dense food but as a form of malnutrition. Soda, candy, chips and cookies are a source of cheap calories and in the poverty diet they substitute for fruits and vegetables. This high-energy, low nutrient-density food problem is ubiquitous in the world and responsible for many chronic oral health problems.

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19

Tea as a functional food for oral health

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Abstract: Tea, an infusion prepared from leaves of *Camellia sinensis*, has long been considered a beverage with functional properties, owing to its medicinal, health promoting and disease prevention properties in humans. Since oral diseases exert a significant impact on a person's overall health, investigation into how tea may benefit oral health is important. This chapter represents an updated review of the available literature on the *in vitro*, *in vivo*, animal and human studies demonstrating the effects of tea and tea polyphenols on oral pathogens associated with oral/dental diseases and the implications of tea and tea polyphenols in the prevention of these diseases.

Key words: *Camellia sinensis*, functional foods, natural oral antimicrobials, oral pathogens, tea and oral health, tea polyphenols.

19.1 Introduction

The tea beverage is an infusion of leaves of the evergreen shrub *Camellia sinensis*, a member of the Theaceae family. Second only to water, tea is the most popular and widely consumed beverage in the world today. Teas are classified into three major types depending on the manufacturing process (Fig. 19.1): the non-fermented green tea, the semi-fermented oolong tea and the fully fermented black tea (Cabrera *et al.*, 2006). The term 'fermentation' refers to the enzyme-catalyzed oxidation that takes place during the tea manufacturing process. The oxidation is catalyzed by the polyphenol oxidase present in the tea leaves (Mukhtar and Ahmad, 2000). Green tea is produced from steaming fresh leaves at high temperatures, thereby inactivating the oxidizing enzymes and maintaining its original composition of polyphenols (Mukhtar and Ahmad, 2000). When tea leaves are ground and incubated at about 40°C for 60 minutes, the polyphenol oxidase converts the original polyphenols to a number of other products such as theaflavins, theaflagallins and thearubigens, which are typical of the black tea. A lesser

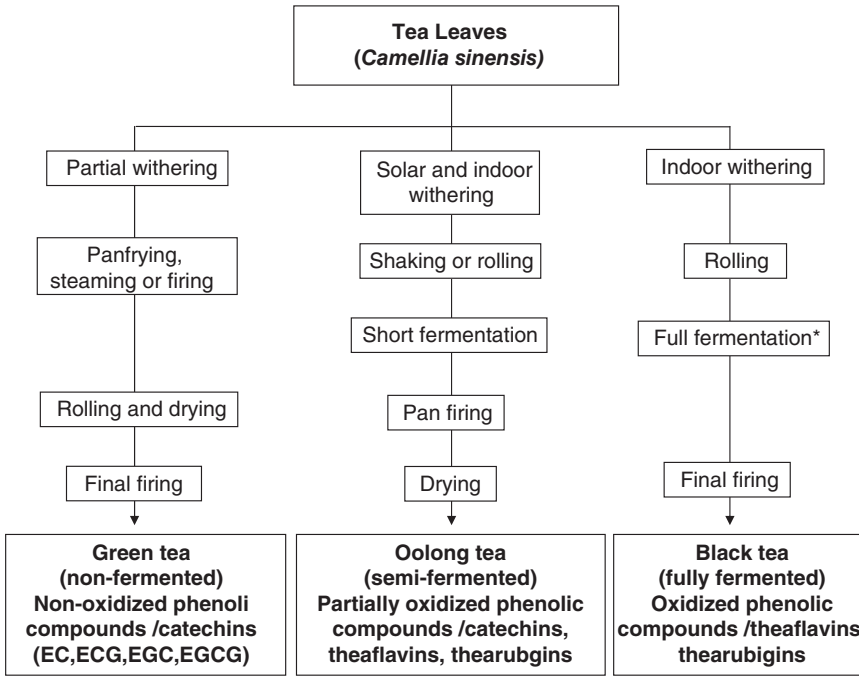

Fig. 19.1 Processing scheme of tea and polyphenols.

incubation time, for example 25–35 minutes, gives rise to an intermediate product, the oolong tea. Tea is on the US Food and Drug Administration's (FDA) list of compounds generally recognized as safe (GRAS) (FDA, 2006).

The chemical composition of tea is quite complex, consisting of polyphenols, catechins, caffeine, amino acids, carbohydrates, protein, chlorophyll, volatile compounds, fluoride, minerals and other undefined compounds (Graham, 1992). Among these, the polyphenols, polyphenolic compounds based on the isoflavan structure, constitute the most interesting group of components making up approximately 36% of the dry weight of the tea leaf. In a cup of tea, the polyphenols concentration is approximately 1 mg ml^{-1} (Sakanaka *et al.*, 1989). The simplest polyphenolic compounds in tea are the catechins, which are colorless, astringent and water-soluble. Six of these occur in considerable quantities in the tea beverage: (+)-catechins, (–)-epicatechin (EC), (+)-gallocatechin (GC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC) and (–)-epigallocatechin gallate (EGCG) (Fig. 19.2).

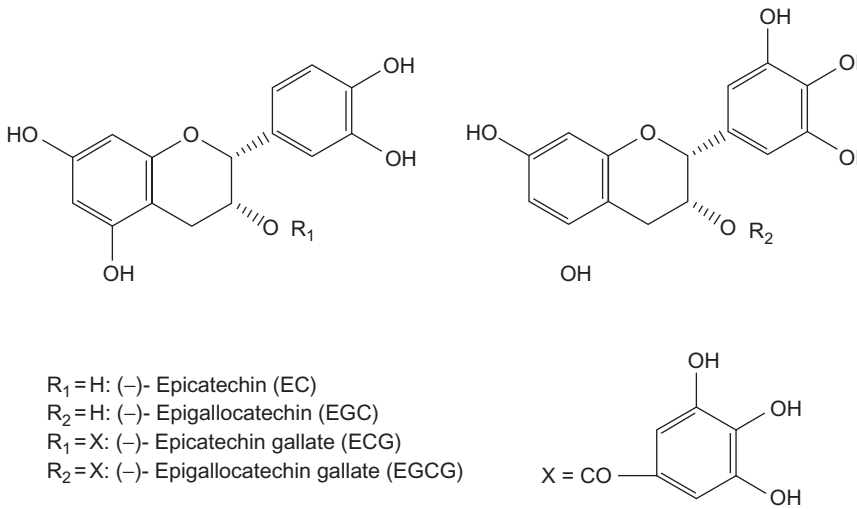

Fig. 19.2 The basic structural formulas of tea catechins.

The EGCG and EGC are most important to many biological functions, such as antioxidants, anti-rancidity and antimicrobial activities. These compounds act primarily on bacterial membranes, producing damage that leads to cell death. EGCG also binds to a wide variety of proteins, especially the non-globular extended proteins with high proline content (Fang *et al.*, 2003; Sah *et al.*, 2004; Tachibana *et al.*, 2004; Ermakova *et al.*, 2005; Palermo *et al.*, 2005; Jobstl *et al.*, 2006). It is important to note that tea polyphenols are chemically different from plant tannins (plant polyphenols) and are not harmful (Hamilton-Miller, 1995). Although green tea contains more catechins than black tea or oolong tea, black tea is also a rich source of polyphenols and/or antioxidants, with an estimated 75–82% being thearubigins. The latter can be cleaved into theaflavins in the environment of the gastric lumen (Rechner *et al.*, 2002). In most incidences, polyphenols from green or black tea possess similar health-promoting properties which have been demonstrated in numerous human, animal and *in vitro* studies (Wiseman *et al.*, 1997; Cabrera *et al.*, 2006).

In recent years, the importance of oral health and its impact on a person's overall health have been emphasized by dental researchers, clinicians and professionals alike (Beck and Offenbacher, 1998; Offenbacher *et al.*, 1998; Beck *et al.*, 2000; Xiong *et al.*, 2006). The general public has become more aware of measures and lifestyles that promote oral health, thus preventing oral diseases such as dental caries, periodontitis, oral cancer and others. In recent years, the potential of tea in promoting oral health and preventing oral diseases has generated much interest among dental researchers. Literature regarding this topic has been presented in several review articles

(Hamilton-Miller, 1995, 2001; Wu and Wei, 2002; Cabrera *et al.*, 2006; Wu, 2006; Friedman, 2007; Yang *et al.*, 2007). The following presents an updated summary of available *in vitro* and *in vivo* investigations on tea and its potential effect on oral health and disease.

19.2 Anticariogenic potential of tea

Although the introduction of fluoride has resulted in the reduction of dental caries, the latter is still the most common infectious disease of mankind and is especially prevalent in children and people with xerostomia (dry mouth) (Chiappelli *et al*., 2002; Tinanoff *et al*., 2002). In adults, the incidence of root caries was found to increase dramatically with age (Winston and Bhaskar, 1998). Thus, control of caries is of major importance in dentistry and will continue to be so for the foreseeable future. Studies in the 1940s and 1950s provided the first clue to the cariostatic effects of tea and fluoride was thought to be the active component (Gershon-Cohen and Mc, 1954). Subsequent reports suggested that tea consumption led to a reduction in dental caries in humans and experimental animals and that tannins contributed to the inhibitory effect (Elvin-Lewis and Steelman, 1968; Rosen *et al.*, 1984). Despite the animal data supporting the positive relation between tea and dental caries prevention, it was not until the past decade that researchers paid attention to the benefits of tea and its relevance to oral health. Several recent reviews are available on the anticariogenic properties and oral health benefits of tea (Hamilton-Miller, 1995, 2001; Wu, 2006).

19.2.1 *In vitro* antibacterial studies

Tea and its polyphenols have been demonstrated to inhibit growth, acid production, metabolism and glucosyl transferase (GTF) enzyme activity of mutans streptococci and dental plaque bacteria (Hamilton-Miller, 2001; Cabrera *et al.*, 2006). Green tea extract or polyphenols have been reported to inhibit *in vitro* growth or viability of *Streptococcus mutans* (Bhalla *et al.*, 1997; Hirasawa *et al.*, 2006; Smullen *et al.*, 2007). Similar findings have been reported in black and oolong teas showing inhibition of *in vitro* growth of selected cariogenic and periodontal pathogens (Wei *et al.*, 1999; Sarkar *et al.*, 2000). The monomeric polyphenols extracted from oolong tea extract (OTE) were found to bind proteins and exerted synergistic antibacterial activity against oral streptococci, including *S. mutans* and *Streptococcus sobrinus* (Sasaki *et al.*, 2004). OTE was also effective in reducing the rate of acid production by mutans streptococci (Matsumoto *et al.*, 1999). This activity may be attributed to the inhibition of EGCG on lactate dehydrogenase activity (Hirasawa *et al.*, 2006).

It is well documented that the GTFs of mutans streptococci play an essential role in the synthesis of adherent glucans from sucrose, thus

promoting plaque formation and adherence (Hamada and Slade, 1980; Banas and Vickerman, 2003). Suppression of GTFs activity, and thereby plaque adherence, has been an important target in reducing plaque-related diseases. Many *in vitro* studies demonstrated that tea polyphenols inhibited *S. mutans* GTFs and suppressed the sucrose-dependent cell adherence (Kashket *et al.*, 1985; Hattori *et al.*, 1990; Otake *et al.*, 1991; Nakahara *et al.*, 1993; Xiao *et al.*, 2000). Most of these findings have been reported in studies using OTE (Nakahara *et al.*, 1993; Ooshima *et al.*, 1993). OTE and the purified polymeric polyphenols from OTE inhibited GTFs of mutans streptococci. The inhibition was enhanced as the degree of polymerization of the catechins increased (Hamada *et al.*, 1996). Matsumoto *et al.* (2003) examined the effect of OTF6, a specific oolong tea polymeric polyphenol, on the functional domains of *S. mutans* GTFs. They reported that OTF6 reduced glucan synthesis by inhibiting the glucan-binding domain of *S. mutans* GTF-B. These workers previously reported that OTE inhibited the adherence of mutans streptococci to saliva-coated hydroxyapatite by reducing cell surface hydrophobicity (Matsumoto *et al.*, 1999).

Besides GTFs, amylase is one of the factors that may contribute to the cariogenicity of starch-containing foods. Kashket and Paolino (1988) found that tea beverage was inhibitory to salivary amylase activity. In agreement with this study, Zhang and Kashket (1998) also demonstrated that tea inhibited amylase activity and that black tea exhibited higher inhibition than green tea. They attributed the inhibition to the theaflavins, the high molecular weight polyphenols predominantly found in black tea. Since starch-containing foods such as crackers and cakes may serve as slow-release sources of fermentable carbohydrate in the oral cavity, tea consumption may reduce the cariogenic potential of foods (Zhang and Kashket, 1998). Based on the above reports, it is apparent that tea polyphenols exert an anti-caries effect, at least in part, via an antimicrobial mode of action rather than an action on de/remineralization of enamel.

19.2.2 Animal studies

Animal studies have shown that specific pathogen-free (SPF) rats infected with *S. mutans* and then fed a cariogenic diet containing green tea polyphenols demonstrated significantly lowered caries scores. Supplementing the drinking water of rats with 0.05% crude tea polyphenolic compounds along with a cariogenic diet also significantly reduced total fissure caries lesions (Otake *et al.*, 1991). Similar animal studies using oolong tea also demonstrated reductions in plaque accumulation and caries development (Ooshima *et al.*, 1993; Ooshima *et al.*, 1998). It was suggested that OTE contained active substances that affected virulence factors other than the GTF enzymes (Ooshima *et al.*, 1998).

Linke and LeGeros (2003) investigated the effect of a standardized black tea extract (BTE) on caries formation in inbred hamsters fed a regular and

a cariogenic diet. It was found that BTE, compared with water, significantly decreased caries formation by 56.6% in hamsters on a regular diet and by 63.7% in hamsters on a cariogenic diet ($p < 0.05$). In the cariogenic diet group, BTE reduced the mandibular caries score of the hamsters slightly more than the maxillary caries score. These workers suggested that frequent intake of black tea may significantly decrease caries formation, even in a diet containing sugars.

19.2.3 Human studies

Tea drinking has been associated with lower caries levels in humans. Caries were found to be significantly lower among children who drank a cup of tea immediately after lunch and that the tea polyphenols, rather than fluoride, were responsible for the anticariogenic effects (Sakanaka *et al.*, 1989). In Tunisia, tea drinking has also been attributed as one of the factors in the declining prevalence of caries (Abid, 2004). Rinsing with 0.2% Chinese green tea was found to decrease plaque and gingival index significantly (You, 1993), while rinsing with oolong tea extract significantly inhibited plaque deposition in volunteers. The numbers of mutans streptococci in unstimulated whole saliva were not significantly reduced (Ooshima *et al.*, 1994).

Worldwide, 80% of the tea consumed is black tea which is also a popular drink in Europe and North America. Wu *et al.* (2001) reported on *in vivo* rinsing with tea extracts on human dental plaque regrowth/metabolism, composition of cariogenic microflora and pH/fluoride of saliva and plaque. Short-term frequent rinses with tea inhibited subsequent regrowth and glycolysis of human supragingival plaque bacteria compared to the water rinse group. A controlled clinical study in adult humans found that rinsing with black tea 10 times a day for 7 days resulted in a significantly less pronounced pH-fall, lower plaque index ($p < 0.05$) and lower numbers of mutans streptococci and total oral streptococci in plaque but not in saliva. It was suggested that black tea and its polyphenols may benefit human oral health by inhibition of dental plaque, its acidity and its cariogenic microbiota (Wu *et al.*, 2001; Wu *et al.*, 2004). Another study reported that gargling with black tea for 60 seconds reduced the bacterial count from the expectorate, 5 and 60 minutes post-gargling. This activity was potentiated by sodium lauryl sulfate. Although a mouth rinse containing 0.047% thymol (Minty Brett) demonstrated significantly higher antimicrobial activity, the black tea extract group showed a longer duration of activity (Esimone *et al.*, 2001).

At present, natural antimicrobial agents have been incorporated into oral hygiene products as adjuncts to mechanical plaque control. Although there are limited controlled clinical trials available to substantiate their efficacy claims, oral products containing tea extracts are popular on the market. Tea polyphenol varnish was used to treat 107 children on their

deciduous molars and incisors. New caries incidence in these teeth decreased 66% one year after treatment compared to the non-treated control group. The caries prevention effect of tea polyphenol varnish was significantly better on occlusal surfaces than on the mesial and distal surfaces of teeth (Feng *et al.*, 1997). Reports on other products such as polyphenol tablets and tea chewing gums are also available, but their efficacies in plaque or caries reduction have not been demonstrated (Liu and Chi, 2000; Shu *et al.*, 2007).

19.2.4 Tea fluoride

The introduction of fluoride has resulted in a reduction of the incidence and severity of dental caries (Strohmenger and Brambilla, 2001; Marinho *et al.*, 2003; de Oliveira, 2006). The ability of fluoride to prevent demineralization of dental hard tissues and enhance remineralization is recognized as one of its major mechanisms of action (Hicks *et al.*, 2004; Lynch *et al.*, 2004). Although fluoride levels in plaque and resting saliva are low and may be insufficient to exert effective antimicrobial activity (Lynch *et al.*, 2004), it has been known to interfere with the growth and metabolism of oral bacteria *in vitro* (Shani *et al.*, 1998; Wiegand *et al.*, 2007). Fluoride is currently thought to exert itself on plaque, gingivitis and initial caries lesion development owing to topical effects rather than systemic ones. Professionally applied fluoride varnishes and fluoridated toothpastes/mouth rinses provide high levels of fluoride and are effective topical applications for fluoride delivery and for the prevention of dental caries (Ogaard, 1999; Topping and Assaf, 2005; Ogaard *et al.*, 2006; Weitz *et al.*, 2007; Azarpazhooh and Main, 2008).

Besides providing many dietary trace elements, teas are also a natural source of fluoride (Kaczmarek, 2004; Lu *et al.*, 2004). Fluoride is taken up from the soil by the tea plant through passive diffusion and is concentrated in the tea leaves by transpiration (Wei *et al.*, 1989). Owing to differences in soils, types of tea leaves, infusion times and methods of analysis, a great deal of variation in fluoride content has been noted (Wei *et al.*, 1989; Jenkins, 1991; Chan and Koh, 1996; Hayacibara *et al.*, 2004). Wei *et al.* (1989) found that a 15-minute infusion resulted in a mean fluoride concentration of 1.75 ppm for 15 Chinese teas, 1.24 ppm for 11 Ceylon/Indian teas and a negligible amount for six herbal teas. As the brewing time increased, so did the fluoride content in the infusions. In general, one cup of tea (100 ml) may contain up to 0.6 mg of fluoride and black tea has been reported to contain the highest fluoride content among all teas (Kavanagh and Renehan, 1998; Kaczmarek, 2004; Cao *et al.*, 2006; Lung *et al.*, 2008; Malinowska *et al.*, 2008).

The caries preventive effect of teas was first thought to be due to its fluoride content, although studies have indicated that tea polyphenols may affect dental plaque formation and metabolism as well (Yu *et al.*, 1995; Wei

et al., 1999). A previous animal study showed that rats consuming black tea (prepared from fluoride-free water) over a two-week period had a significantly lower rate of caries than those consuming non-fluoridated water. These authors suggested that black tea consumption attenuates the development of caries in young, caries-prone rats (Touyz and Amsel, 2001).

In humans, regular tea drinking may result in low levels of fluoride retained in the oral cavity. Wu *et al.* (2001) showed that fluoride accumulated in plaque and saliva in subjects after a 7-day rinsing regimen with black tea extract 5 to 10 times a day. While examining the bioavailability of fluoride from black tea, Simpson *et al.* (2001) reported that, after rinsing with tea, 34% of the fluoride was retained in the oral cavity with strong binding to enamel particles. These studies suggested that tea may be an effective vehicle for fluoride delivery to the oral cavity where it may interact with the oral tissues and their surface integuments.

The amount of fluoride that may be ingested after drinking tea has been a topic of interest to many researchers. Wei *et al.* (1989) estimated a daily fluoride intake from tea infusion made with fluoridated water (0.7 ppm) of 1.05 mg. These authors also reported that 'tea contributes up to one-fifth of the optimal daily fluoride intake (0.33 mg) if an 8-year old child (27 kg) consumes one cup of tea . . .' In another study, Jenkins (1991) reported that a total of 9 mg fluoride could be consumed by subjects who drank large quantities of tea. When using the assumption of 2.5 cups/day, 150 ml per cup and 2.2 ppm fluoride diluted in half with milk for children, the amount of fluoride ingested may range from 0.1 mg to 1.08 mg. The optimal concentration of fluoride through ingestion has been reported to be 0.05–0.07 mg F/kg body weight, while an acute lethal dose has been calculated as 35–70 mg fluoride/kg body weight. Therefore, it is very unlikely that enough tea could be consumed at one time to cause a lethal overdose (Sofuoglu and Kavcar, 2008). Even so, most studies on fluoride consumption from tea concluded that the total fluoride intake needed to be assessed prior to supplementation (Kiritsy *et al.*, 1996).

Dental fluorosis may occur when fluoride levels in water exceed twice the optimal concentration (Yeung, 2008). In most instances, dental fluorosis only occurs during tooth development and the over ingestion of fluoride is reflected in those teeth undergoing active mineralization. A high prevalence of dental fluorosis has been reported in children who have had a heavy intake of tea starting at a very young age, in countries or areas where ground water fluoride levels are high (Shomar *et al.*, 2004; Chandrajith *et al.*, 2007). At present, human studies are relatively limited regarding the effect of tea consumption on dental fluorosis. While teas are unlikely to cause fluorosis by themselves, they may be significant contributors to the total fluoride intake of children when high fluoride is found in the drinking water (Simpson *et al.*, 2001). In addition, the early use of high fluoride containing oral products in children undergoing tooth formation needs to be done in moderation. As for inhibition of dental caries, the amount of

fluoride retained in the oral cavity after rinsing may be the most important parameter. If the concentration is sufficient, fluoride may bind to the enamel and salivary pellicle components leading to topical effects and perhaps caries prevention.

19.3 Tea and periodontal diseases

Tea catechins have been reported to exert antimicrobial activity against the suspected periodontal pathogen *Porphyromonas gingivalis* (Sakanaka *et al.*, 1996; Sakanaka and Okada, 2004). BTE, theaflavins and epicatechins with gallate moiety suppressed *in vitro* growth and viability of *P. gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum*. Inhibition of coaggregation between *F. nucleatum* and *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), *Streptococcus sanguis, S. mutans, S. sobrinus* or *Actinomyces viscosus* was also noted (Wei *et al.*, 1999; Wei, 2001).

Proteinases such as gingipain, collagenases and matix metaloproteinases are known to play important roles in the development of periodontal diseases (Reynolds *et al.*, 1994; Potempa *et al.*, 2000; Sorsa *et al.*, 2006). Tea catechins such as ECG and EGCG inhibited both eukaryotic and prokaryotic cell-derived collagenases, which are involved in the disruption of the collagen component of the gingival tissues of patients with periodontal disease (Osawa *et al.*, 1991; Makimura *et al.*, 1993; Demeule *et al.*, 2000; Maeda-Yamamoto *et al.*, 2003). Inhibition of certain proteases of *P. gingivalis* (Wei *et al.*, 1999; Wei, 2001; Okamoto *et al.*, 2004), protein tyrosine phosphatase of *P. intermedia* (Okamoto *et al.*, 2003) and production of toxic metabolites by *P. gingivalis* (Sakanaka and Okada, 2004) have also been reported.

Alveolar bone resorption is one of the characteristic features of periodontitis and involves the removal of both the mineral and organic constituents of the bone matrix, mediated by multinucleated osteoclasts and matrix metalloproteinases (MMPs). Various *in vitro* studies have shown that tea catechins EGCG were capable of inhibiting the formation of osteoclasts, the expression of MMP-9 in osteoblasts (Yun *et al.*, 2004) and the survival of osteoclasts through caspase-mediated apoptosis (Nakagawa *et al.*, 2002; Yun *et al.*, 2007).

Thus far, *in vivo* randomized controlled trials that effectively assess the benefits of tea on periodontal health are lacking. A human study investigated the effect of tea polyphenols formulated in chew candies on gingival inflammation over a four-week period (Krahwinkel and Willershausen, 2000). Although the approximal plaque index (API) and sulcus bleeding index (SBI) determined at day 7 and day 28 suggested that tea polyphenols might exert a positive influence on gingival inflammation, no statisti-

cally significant differences were noted between the test group and the placebo group. Although a high amount of tea catechins may be consumed by a tea drinker on a daily basis, it is not known whether an effective concentration of tea catechins may be present in the gingival crevicular fluid or the circulating blood to exert oral health benefits. A combined use of mechanical treatment and the application of green tea catechins using a slow release local delivery system were found to be effective in improving periodontal status including the reduction in pocket depth and the suppression of peptidase activities in the gingival crevicular fluid (Hirasawa *et al.*, 2002).

19.4 Tea and halitosis

Oral malodor (halitosis) is a common complaint that affects a large proportion of the population (Eli *et al.*, 1996; Scully and Rosenberg, 2003). Halitosis is predominantly caused by the overgrowth of proteolytic, anaerobic bacteria on surfaces within the mouth, including periodontal pockets and the posterior dorsum of the tongue. These bacteria degrade sulfur-containing amino acids and produce volatile sulfur compounds (VSCs) such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide (Loesche and Kazor, 2002). The enzyme L-cysteine desulfhydrase (CD) catalyzes the degradation of L-cysteine to produce pyruvate, ammonia and H_2S. The L-methionine-α-deamino-γ-mercaptomethane-lyase (METase) catalyzes L-methionine to α-ketobutyrate, ammonia and CH_3SH (Pianotti *et al.*, 1986; Esaki and Soda, 1987). Other compounds, such as volatile fatty acids and diamines can also contribute to oral malodor (Iwakura *et al.*, 1994).

Previous reports have shown that tea extracts and polyphenols inhibited *in vitro* growth and VSC production by oral anaerobes associated with halitosis, that is *F. nucleatum*, *P. gingivalis* and *P. intermedia* (Wu *et al.*, 1999; Zhu *et al.*, 1999). Tea polyphenols such as EGCG precipitated CH_3SH which resulted in their odor-reducing activity *in vitro* (Yasu da and Arakawa, 1995; Lodhia *et al.*, 2008). At sub-MIC minimal inhibitory concentration levels, EGCG suppressed the expression of L-cysteine desulfhydrase of *F. nucleatum* and resulted in reduced H_2S production. Proteomic analysis of cytosolic proteins of EGCG grown *F. nucleatum* identified nine proteins whose expressions were affected. Among these, both 3-hydroxybutyryl-CoA dehydrogenase and pyruvate oxidoreductase were downregulated leading to reduction of butyric acid and propionic acid syntheses (Coulburn *et al.*, 2008). Both of these volatile fatty acids contribute to oral malodor (Kapatral *et al.*, 2003).

Based on *in vitro* studies, tea may have the potential to reduce halitosis but this has not been fully explored. A recent human study reported that

green tea powder exerted a greater deodorizing effect against VSCs in exhaled mouth air compared to chewing gums and mints. However, the effect was found to be transient (Lodhia *et al.*, 2008)

19.5 Tea and oral cancer

Cancer is a product of DNA-damaging attacks from the environment, through infection by viruses and the persistence of habits that expose individuals to DNA-damaging agents such as tobacco products and/or the ingestion of alcohol.

The potential of tea for oral cancer prevention has been suggested by researchers based on a wide range of biological activities of tea and its polyphenols demonstrated in *in vitro* and animal studies (Hsu *et al.*, 2002; Gonzalez de Mejia *et al.*, 2005; Schwartz *et al.*, 2005; Ho *et al.*, 2007). Tea polyphenols have been shown to reduce DNA damaging effects introduced by the environment, infection or behavior. It is suggested in literature reviews and studies that tea polyphenols such as EGCG may suppress the formation of cancer by two mechanisms: first, a reduction in oxygen free radicals because of the electrophilic capacity of polyphenols and second, EGCG and other polyphenols act as chemopreventive agents through a suppressor regulation of pathways that are linked to NF-kB and transcription expression of c-jun, c-fos, and iNOS. The regulation of these pathways results in an increase in apoptosis, differentiation and a reduction in cell cycle associated growth (Yamamoto *et al.*, 2004).

In vitro studies have shown that green tea and its constituents selectively induced apoptosis in oral carcinoma cells and EGCG inhibited the *in vitro* growth and invasion of oral carcinoma cells (Hsu *et al.*, 2002). It was proposed that the chemopreventive effects of green tea polyphenols may involve a p57-mediated survival pathway in normal epithelial cells, while oral carcinoma cells undergo an apoptotic pathway. A study by Yamamoto *et al.* (2004) found that green tea polyphenols protected normal epithelial and salivary gland cells from reactive oxygen species, chemical or irradiation-induced damage. The observed selective bioactivity may be the result of different responses to tea extracts and to EGCG between malignant and normal cells of the human oral cavity. Since cancerous cells have been shown to be more sensitive to oxidative stress than normal cells (Weisburg *et al.*, 2004), EGCG may create different oxidative environments favoring either normal cell survival or tumor cell destruction (Maeda-Yamamoto *et al.*, 2003). Using an *in vitro* multi-stage carcinogenesis model for oral cancer, Khafif *et al.* (1998) have previously demonstrated that cancerous oral epithelium was less responsive than normal or dysplastic tissues after treatment with EGCG. Research from these findings may lead to applications of naturally occurring polyphenols to enhance the effective-

ness of chemo/radiation therapy by promoting cancer cell death while protecting normal cells.

The antimutagenic and anticarcinogenic potentials of tea extracts and polyphenols have also been investigated using animal models including those for oral cancer (Cabrera *et al.*, 2006; Yang *et al.*, 2007, 2008). In an induced rat oral cancer model, Srinivasan *et al.* (2008) examined the effect of green tea polyphenols on the activities of tumor-associated factors, including phase II enzymes of the tongue and oral cavity. They reported a significant decline in the number of tumors, tumor volume and oral squamous cell carcinoma in both simultaneous and post-green tea polyphenols treated animals. They also found that, upon the supplementation of green tea polyphenols, there was a significant increase in the activity of phase II enzymes and a significant decrease in the activity of phase I enzymes. It was proposed that green tea polyphenols, through their modulating role, inhibited the formation of tumors. Vidjaya Letchoumy *et al.* (2008) examined the efficacy of the black tea polyphenols for the pre-initiation of induced hamster buccal pouch carcinogenesis. They found that tea polyphenols inhibited phase I enzymes and enhanced phase II enzymes. Significant reduction in the tumor incidence, oxidative DNA damage and expression of CYP1A1 and CYP1B1 isoforms were also observed. In another study by Ho *et al.* (2007), EGCG was found to inhibit matrix metalloproteinases and urokinase-plasminogen activator, resulting in the suppression of the invasion and migration of human oral cancer cells.

Despite numerous *in vitro* and *in vivo* studies, the relationship between tea consumption and cancer risk has not been conclusively demonstrated in humans (Ide *et al.*, 2007; Yang *et al.*, 2008). Moreover, limited human studies are available on the chemoprevention of tea against oral cancer. Li *et al.* (1999) treated patients with oral mucosa leukoplakia topically with a mixed tea for 6 months and found reductions in the size of oral lesions, cell proliferation and the incidence of micronucleated exfoliated oral mucosa cells. Black tea has also been demonstrated to benefit these patients by decreasing the micronuclei frequencies and chromosomal aberrations of the oral mucosal epithelium (Halder *et al.*, 2005). Consumption of green or black tea has been found to reduce tobacco-associated DNA damage by inducing cell growth arrest and apoptosis (Pal *et al.*, 2007). Schwartz *et al.* (2005) studied the molecular and cellular effects of green tea on oral keratinocytes of smokers. During the course of green tea administration, smoking-induced DNA damage was decreased, cell growth was inhibited and the percentage of cells in S phase was reduced, although accumulation of cells in G1 phase was observed. The DNA content also became more diploid and the presence of biochemical markers for apoptosis increased. These workers proposed that drinking green tea reduced the amount of DNA damage by inducing cell growth arrest and apoptosis. At present, further *in vitro* and human studies are needed to provide a mechanistic basis in substantiating the chemoprevention potential of tea against oral cancers.

19.6 Antimicrobial activity against fungi associated with oral infections

Fungal infections including oral candidiasis have increased significantly and the trend is expected to continue in the foreseeable future owing to the expanding population of immuno-suppressed and non-immunocompromised critically ill patients (Ostrosky-Zeichner *et al.*, 2002; Pfaller and Diekema, 2007). Approximately 30–50% of the human population harbors *Candida* species as part of their normal oral microbiota (Epstein *et al.*, 2002). These fungi can become pathogenic under specific conditions and produce opportunistic infections in the oral cavity of individuals, especially those with immunodeficiencies (Ellepola and Samaranayake, 2000; Coogan *et al.*, 2006).

The antifungal activities of tea extract or EGCG have been studied against fungal species including *Candida glabrata*, *Clavispora lusitatiae*, *Cryptococcus laurentii*, *Filobasidiella neoformans*, *Issatchenkia orientalis*, *Saccharomyces cerevisiae*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* (Turchetti *et al.*, 2005). At present, reports on tea and tea catechins on growth or viability of fungal species frequently associated with oral infections have been limited. *In vitro* studies have demonstrated that neither green tea extract nor EGCG inhibited growth of *Candida albicans* (Okubo *et al.*, 1991; Simonetti *et al.*, 2004). However, when combined with butylated hydroxyanisole (BHA), hypha formation of *C. albicans* was inhibited (Simonetti *et al.*, 2004). This synergy was thought to be related to an impairment of the barrier function in microorganisms and a depletion of thiol groups. Synergy between EGCG and azole antifungals such as amphotericin B or fluconazole have also been reported (Hirasawa and Takada, 2004). It was suggested that EGCG disturbed the folic acid metabolism which in turn inhibited ergosterol production of *C. albicans*.

19.7 Antimicrobial activity against viruses associated with oral infections

Viral infections are common in the oral cavity of humans, including localized or systemic infections. Among these, members of the human herpesvirus (HHV) and human papillomavirus (HPV) families are the most common causes of primary viral infections of the oral cavity (Cheng *et al.*, 2002). Herpes simplex virus (HSV) encodes 11 or 12 glycoproteins and four of them: gD, gB, gH and gL, are required for viral entry into host cells (Campadelli-Fiume *et al.*, 2007). Interactions leading to alteration of these glycoproteins may inhibit their function in the virus–cell fusion process, thus inactivating the HSV. In an effort to test EGCG and other purified green tea catechins as topical agents against HSV, Issacs *et al.* (2008) found that EGCG was capable of inactivating multiple clinical isolates of herpes

simplex virus type 1 (HSV-1) and HSV-2. It was suggested that the inactivation was caused by the binding of EGCG to viral envelope glycoproteins such as gB and gD which are essential for HSV infectivity. Since EGCG was found to be stable in the vaginal pH range (pH 3.8–4.5), efforts are underway to determine the feasibility of EGCG formulations as efficient topical microbicides for HSV inactivation. However, its utility as a microbicide for oral HSV infections may be more challenging owing to the higher pH environment of the oral cavity buffered by saliva.

19.8 Conclusion

In recent years, the general public in America and other western countries have embraced tea drinking as a healthy habit owing to their awareness of tea's protective effects against many diseases. Numerous laboratory, human and epidemiological studies have provided evidence demonstrating the multiple mechanistic action of tea polyphenols and supporting the various health benefits of tea consumption. The literature reviewed in this chapter clearly points to the effectiveness of tea and its constituents against oral pathogens and their virulence factors. The potential of tea consumption to prevent oral infections and diseases has, therefore, been emphasized.

Considering the importance of oral health and its contribution to a person's overall well being, further investigations of how tea consumption has an impact on oral health are warranted. More in-depth studies into the mechanistic actions of tea polyphenols and the functionality of tea in preventing oral diseases are needed. This can be accomplished through a multidisciplinary research approach to experimental designs and strategies. Randomized controlled clinical and epidemiological studies will provide evidence about the relationship between tea consumption and the lowered risk of oral infections or diseases. Studies defining the extent of oral health benefits and the establishment of the safe range of tea consumption associated with these benefits are also important. To achieve these goals, the distribution and metabolism of tea polyphenols and their bioavailablity in the oral cavity need to be researched. At present, one of the major concerns in tea research is the lack of standardization among tea preparations employed in the numerous *in vitro* and *in vivo* experiments. Tea preparations often differed in the types and sources of tea leaves used, manufacturing procedures and extraction methods. Analytical data for tea preparations usually were not provided. The use of standardized preparations of tea with known bioavailability and polyphenolic content will avoid the conflicting results in laboratory and cohort studies reported by researchers in different laboratories and countries. Because of the beneficial effects it exerts within the oral cavity as well as its long recognized bioregulatory properties, tea may be considered as a functional food for oral health.

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20

Probiotics and oral health

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Abstract: Probiotic bacteria are regarded as good for health in contrast to pathogenic bacteria. This chapter introduces probiotics into the oral health perspective. Bacteria suitable for ameliorating gastrointestinal disorders cannot be directly used in the mouth owing to their potential acidogenicity and detrimental effects on teeth. However, the first randomized controlled trials with properly selected probiotics have shown their potential in controlling dental caries and oral yeast infections. This chapter discusses the results from both experimental and clinical studies with probiotics and also outlines future possibilities for probiotic therapy for oral and dental diseases.

Key words: dental caries, oral disease, periodontal disease, probiotic, yeast infection.

20.1 Probiotics: the concept

In 1908 Ukrainian-born bacteriologist Ilya Mechnikov, working at the Pasteur Institute in Paris, received the Nobel Prize in medicine together with Paul Ehrlich for studies on immunity. Mechnikov had studied the microbiota of the human intestine and developed a theory that senility is due to poisoning of the body by the products of gut bacteria. To prevent the multiplication of certain organisms, he proposed a diet containing milk fermented by lactobacilli, which produce large amounts of lactic acid. He also laid the foundation for the present-day dairy industry. The concept of probiotics was thus born. Probiotics are by definition 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (Guarner *et al*., 2005). In other words, these bacteria are regarded as good for health in contrast to pathogenic bacteria. Today, a variety of commercial products have been developed that contain probiotics, or claimed probiotic strains, in food or juice preparations, or in tablets, capsules or lozenges. However, only a few probiotic preparations have been

tested in a randomized controlled trial setting. Ailments and diseases of the gastrointestinal tract have been the indicators of where probiotic therapy has been most successfully employed. This chapter introduces probiotics from the oral and dental health perspective.

20.2 Probiotic species

Probiotic strains mainly belong to the genera *Lactobacillus* and *Bifidobacterium*, but also some streptococci and even strains of *Escherichia coli* have been investigated for their putative probiotic effect. Probiotics have mainly been used as support therapy for gastrointestinal diseases but they have also been applied, for example, to patients with atopic disorders and allergy, and in urinary tract and gynaecological infections (Gill and Prasad, 2008). Nevertheless, scientific evidence of the extraintestinal probiotic approaches is still weak and more studies are called for in this area (Lee *et al*., 2008). In general, the delicate balance between and within bacterial species in gut and other members of the commensal microbiota and host tissue may partly depend on probiotic characteristics of individual species. Only a small proportion of species in the gut microbiota has been identified (Cani and Delzenne, 2007).

20.3 Putative mechanisms of probiotic action

In order to affect host tissue beneficially or to inhibit the commensal microbiota, a probiotic strain needs to be established in the mouth. Thus, attachment to oral surfaces, to mucosal membranes and teeth is regarded as an important first step for an eventual probiotic action in the mouth. After attachment, colonization, growth and multiplication should take place for a more permanent effect. However, thus far no probiotic strain has been shown to be capable of permanent colonization in the mouth. Consequently, this finding has led to the recommendation for the continuous administration of a probiotic product. On the other hand, experimental studies on the binding patterns of probiotics to oral surfaces have shown great variation among strains and surfaces tested (Haukioja *et al*., 2006). This finding emphasizes the need to identify the best probiotic strains for each particular purpose. At the same time, safety aspects need to be taken into account. For example, adhesion of *Lactobacillus casei* Shirota was found to be inferior compared with *Lactobacillus acidophilus* in a test model system using bovine teeth, but the latter strain is claimed to be cariogenic and thus might not be advisable for oral health purposes (Lima *et al*., 2005).

In addition to their adhesion characteristics, the coaggregation ability of putative probiotic species is considered to be important (Kolenbrander, 1988; Reid *et al*., 1990; Boris *et al*., 1998). These characteristics, however,

have not been systematically tested from the oral health perspective. Recently, a study from our laboratory evaluating coaggregation of known probiotic species and putative probiotic candidates revealed that this phenomenon is strain-dependent, with *L. acidophilus* NCFM and *L. casei* 921 showing about 90% capacity to coaggregate with *Fusobacterium nucleatum*, an important bacterium in dental biofilm formation (Stamatora *et al*., 2007a). An *in vitro* study by Kang *et al*. (2005) has shown the ability of *Weissella cibaria* to coaggregate with *F. nucleatum*, thus contributing to better adhesion to oral epithelial cells. *W. cibaria* has been introduced as an effective probiotic species in the oral cavity providing stable reduction of the production of volatile sulphur compound by various oral pathogens (Kang *et al*., 2006). These sulphur compounds are thought to be responsible for bad breath and, subsequently, if probiotics reduce their occurrence in the mouth, a new indication for probiotic therapy might be established.

Another possible mechanism of probiotic action may be mediated by bacteriocins. *Streptococcus mutans* has been shown to be susceptible to the bacteriocin lacticin (Galvin *et al*., 1999). When studying the effect of lactobacilli on mutans streptococci, the inhibitory substance isolated from lactobacilli appeared to be pH dependent in an *in vitro* study where zones of growth inhibition on agar plates were apparent only at pH values below 5. In the same study, growth curve experiments showed a statistically significant inhibition between series with and without the isolated substance (Meurman *et al*., 1995). However, the nature of the inhibitory substance(s) still remains to be determined. This is a particularly challenging area of research in the complex microenvironment of oral biofilms. pH as such regulates the microbial composition and one suggested mechanism by which lactobacilli affect other species is their efficient production of lactic acid and survival in an acidic environment.

20.4 Probiotics as support therapy for systemic diseases

Probiotic organisms are thought to act through a variety of mechanisms including: (i) competition with potential pathogens for nutrients or adhesion sites, (ii) detoxication, (iii) production of antimicrobial substances and (iv) local and systemic immunomodulation (Silva *et al*., 1987; Lewis and Freedman, 1998; Isolauri *et al*., 2001).

The suggested modulation of host immune responses by probiotics is interesting and may explain the observed effects beyond the gastrointestinal tract. Immune inductive sites in the oral cavity are located in the lymphoid tissue of the lingual and pharyngeal tonsils and the adenoids. The role of these anatomic structures as inductive sites of mucosal immunity has been shown by intranasally delivered vaccines (Wu *et al*., 1997). Dendritic cells scattered in mucosal surfaces are essential in the antigen presentation and activation of T cells. Depending on the signals from dendritic cells,

either immune tolerance or an active immune response towards a specific antigen may occur (Banchereau and Steinman, 1998). A marked production of interleukin-10 by dendritic cells in the gut mucosa has been registered after administration of a probiotic mixture (Hart *et al*., 2004). Taken together, probiotic administration has been shown to have an effect on a variety of disease states such as asthma, cancers, diabetes and arthritis. However, the exact mechanisms of action, optimum dose, frequency and duration of treatment required still remain open. Further general discussion about these aspects is beyond the scope of the present article (for review, see Gill and Prasad, 2008).

20.5 Probiotic strains and oral microbiology

Knowing the diversity and number of microbial species in the oral cavity, the permanent establishment of external probiotic species in oral biofilms is difficult. Concequently, no permanent colonization has been observed after probiotic administration except in one reported case where the subject had received *Lactobacillus rhamnosus* GG containing milk products from early childhood as support therapy for an allergy. This subject remained positive for the bacterium at the age of 19 years (Yli-Knuuttila *et al*., 2006). Hence, the case indicates that permanent colonization is possible but probably requires early colonization and repeated exposure.

Dental plaque, with its complex structure, presents a great challenge for the insertion of new microbial species which subsequently affect on the microbial balance in the biofilm. But there is every reason to expect that the same mechanisms as observed in other parts of the gastrointestinal tract also hold true in the mouth. Thus, in addition to local effects on the commensal microbiota, externally administered probiotics might also affect immune functions and other defensive systems in the oral cavity. However, there are no studies of these aspects. Neither is it known if 'natural' probiotic strains exist in the mouth, although one study has shown that naturally occurring lactobacilli inhibited cariogenic mutans streptococci and that the effect was more pronounced in caries-free subjects (Simark-Mattson *et al*., 2007). Table 20.1 outlines the anticipated action of probiotics in the mouth (Meurman and Stamatova, 2007a).

20.6 Experimental and clinical studies of the effect of probiotics on oral and dental diseases

Table 20.2 gives examples of studies where probiotic strains have been investigated for their effect on oral microorganisms. *In vitro* studies have shown zones of inhibition of *S. mutans* growth produced by lactobacilli, but there is no evidence as to whether inhibition was due to changes in pH or

Table 20.1 Anticipated mechanisms of probiotic action in the mouth

Putative oral probiotic mechanism	Possible effect
Competitive attachment to oral surfaces	Reduction in attachment of pathogenic microorganisms
Colonization	Competition for colonization sites with pathogenic species
Inhibition of bacterial metabolism	Reduction in pathogenic species
Interference with bacterial metabolism	Reduction in harmful bacterial end products such as volatile sulphur compounds
Antimicrobial effect	Reduction in pathogenic species

was related to the secretion of specific substances such as bacteriocins (Kõll-Klais *et al*., 2005; O'Connor *et al*., 2006; Stamatova *et al*., 2007a).

Displacing *S. mutans* or other cariogenic microorganisms from their binding sites on dental surfaces or in oral biofilms is another means by which probiotics might exert a beneficial effect. However, attachment of probiotics to oral surfaces has shown great variation among the strains and surfaces tested and more studies are needed to identify the best putative strains in this regard (Lima *et al*., 2005; Haukioja *et al*., 2006). Here, selecting non-cariogenic putative probiotic strains is a necessity from the dental health point of view.

Even though the mechanisms of probioic action are not known, there is increasing evidence from clinical intervention studies showing that probiotics may indeed exert beneficial effects in the oral cavity. Table 20.2 gives examples of such studies. The emphasis in clinical trials has mainly been on dental caries and promising results have been obtained. However, probiotic effects on oral yeast infections have also been of interest. Randomized controlled trials on the effect of probiotic intervention on periodontal disease have not been published. The latter area of research is difficult, taking into account the fact that periodontal disease is caused by the subgingival microbiota. By adopting the idea that the oral cavity itself might be a habitat for probiotic species, one study has shown that the composition of oral lactobacilli varies with respect to periodontal health (Kõll-Klais *et al*., 2005). The same authors found that lactobacilli from periodontally healthy patients showed a lower antimicrobial activity against *S. mutans* than the strains from chronic periodontitis patients. In order to inhibit bacteria in deep periodontal pockets, it would be necessary to develop different application modes from the probiotic preparations currently available. However, in general, the first randomized placebo controlled trials with probiotics have shown that oral and dental diseases might be interesting targets for this therapy but more studies are needed for further evidence.

Table 20.2 Laboratory and clinical investigations of probiotics and oral health. The asterisk denotes a study related to both caries and periodontitis

Type of study	Probiotic species/strains	Effect	Means of administration	Reference
Dental caries				
Clinical trial	*Lactobacillus reuteri* ATCC 55730 *L. reuteri* ATCC PTA 5289	Reduction in salivary *Streptococcus mutans* levels	Lozenge	Çaglar *et al.*, 2008
In vivo	*L. reuteri* ATCC 55730	Reduction of *S. mutans*	Straw and tablet	Çaglar *et al.*, 2006
Clinical trial	*Lactobacillus rhamnosus* GG, ATCC 53103	Reduction of the risk of the highest level of *S. mutans*	Cheese	Ahola *et al.*, 2002
Clinical trial	*L. rhamnosus* GG, ATCC 53103	Reduction of the risk of the highest level of *S. mutans*	Milk	Näse *et al.*, 2001
In vitro	*Lactobacillus plantarum, L. rhamnosus, L. paracasei* and *L. salivarius*	Inhibition of *S. mutans* growth	–	Kõll-Klais *et al.*, 2005
Clinical trial	*Bifidobacterium* DN-173 010	Reduction in salivary mutans streptococci	Yoghurt	Çaglar *et al.*, 2005
In vitro and clinical study*	*Weisella cibaria* CMS1	Inhibition of *S. mutans* biofilm	–	Kang *et al.*, 2006
In vitro	*Lactococcus lactis* DPC3147	Effective inhibition of oral *S. mutans* due to lactacin 3147	–	O'Connor *et al.*, 2006
In vitro	*Lactoc. lactis* NCC2211	Modulation of the growth and colonization of *Streptococcus oralis* OMZ607, *Veillonella dispar* OMZ493, *Actinomyces naeslundii* OMZ745 and *Streptococcus sobrinus* OMZ176	–	Comelli *et al.*, 2002

Periodontal disease				
In vivo	*Streptococcus salivarius* K12	Suppressed growth of black-pigmented bacteria in saliva samples and also of bacteria implicated in halitosis	Mouthwash	Burton *et al.*, 2006
In vitro and clinical study*	*W. cibaria*	Ability to inhibit biofilm formation	Mouthwash	Kang *et al.*, 2006
In vivo	*L. reuteri*	Reduction of gingivitis and plaque	Probiotic formulation	Krasse *et al.*, 2006
In vivo	*L. casei* 37	Extended remission after treatment of chronic generalized periodontitis	Periodontal dressing	Volozhin *et al.*, 2004
In vitro	*L. casei* ATCC 4646	Compared to *Porphyromonas gingivalis* and *Fusobacterium nucleatum, L. casei* exhibited low IL-8 production by human dental pulp cells	–	Thaweboon *et al.*, 2006
In vitro	*Lactobacillus bulgaricus*	Inhibition of oral streptococci and *Aggregatibacter actinomycetemcomitans*	–	Stamatova *et al.*, 2007a,b
Oral *Candida* infections				
Clinical trial	*L. rhamnosus* GG (ATCC 53103), *L. rhamnosus* LC705, *Propionibacterium freudenreichii* ssp *shermanii* JS	Reduction in growth of *Candida* and positive effect on hyposalivation	Cheese	Hatakka *et al.*, 2007
In vitro	*Lactobacillus fermentum* Ess-1	Inhibition of *Candida albicans* and *Candida glabrata*	–	Rönnquist *et al.*, 2007
In vivo	*Lactobacillus acidophilus* LAFTI L10	Enhanced clearance of *C. albicans* in mice	–	Elahi *et al.*, 2005

20.7 Safety aspects

It is understandable that safety is of major concern with any bacteriotherapy. The probiotic strains identified are microorganisms originally isolated from the normal human microbiota or are dairy strains used for centuries in milk fermentation (Vanderhoof and Young, 2008). Safety aspects of probiotics have mainly been focussed on possible infections caused by the strains and, secondly, on antibiotic resistance problems. In Finland, where probiotic milk products have been widely used for more than 10 years, the incidence of *Lactobacillus* bacteraemia has been keenly followed. There is evidence to show that probiotic strains may cause infections in immunosuppressed and long-term hospitalized patients (Salminen *et al.*, 2004). However, the average incidence in Finland was 0.3 cases/100 000 inhabitants/year in 1995–2000 and thus increased probiotic use has not led to an increase in *Lactobacillus* bacteraemia (Salminen *et al.*, 2002).

Antibiotic resistance genes may also be carried by probiotic species, which is of concern if species with such characteristics are widely used, as is the case for example with functional food products (Klare *et al.*, 2007). However, there is no evidence that the use of such products would have affected the antibiotic resistance situation in populations.

From the oral health perspective, additional safety aspects of probiotics would require their safe-for-teeth characteristics to be verified. Here, selecting non-cariogenic and non-virulent strains has been particularly emphasized (Burton *et al.*, 2006). Furthermore, the proteolytic activity of the different probiotic strains utilized has been investigated and it was found that *Lactobacillus* strains are not likely to degrade host tissue components (Stamatova *et al.*, 2007b). This observation is important for maintaining mucosal and gingival health.

20.8 Future trends

The first placebo-controlled clinical intervention studies have shown promising results regarding the use of probiotics for dental caries in children and for controlling oral yeast counts in the elderly. However, more trials are needed and several questions remain to be answered before further recommendations can be made (Meurman and Stamatova, 2007b). In future, probiotic products will inevitably be developed specifically for oral health purposes. These products are supposed to be useful as support therapy in controlling dental infections and yeast infections. If the mechanisms of probiotic action are shown to be immunomodulatory, then new indications such as ameliorating oral discomfort caused by mucosal diseases such as lichen planus might be possible. Furthermore, these ‘extra-oral’ effects, similar to the extraintestinal effects of probiotics, may include helping patients with reduced salivary flow as was observed in the trial on elderly

Table 20.3 Possible uses of probiotics in oral medicine and dentistry

Target	Anticipated mechanism
Prevention of dental caries	Interfering with attachment and colonization of cariogenic bacteria Inhibition of sugar fermentation by cariogenic bacteria
Prevention of periodontal disease	Interfering with attachment and colonization of periodontal pathogens Interfering with oral biofilm development
Prevention of oral yeast infections	Inhibiting oral *Candida* strains
Ameliorating oral discomfort	Affecting local immunity and non-specific defence mechanisms of the mouth Stimulating salivary secretion
Support therapy for oral mucosal diseases	Affecting local immunity and non-specific defence mechanisms of the mouth Stimulating salivary secretion

patients with high oral yeast counts (Hatakka *et al.*, 2007). In general, however, we are still in the vanguard of research with regard to probiotics and oral health. Table 20.3 lists some indications that might prove interesting in the future in this respect. As discussed above, apart from studies of dental caries and oral yeast infections, scientific evidence is still weak or practically non-existent concerning the other indications mentioned.

20.9 Conclusion

Research data on probiotics from an oral health perspective are still sparse. Neither the general mechanisms of probiotic action, or those operating in the oral cavity, are known even though controlled clinical trials have shown some positive effects of probiotic intervention. So far, studies of dental caries dominate the literature in this area and oral health-related research with probiotics is still in its infancy. However, there is every reason to believe that new indications for therapy will be introduced in oral medicine and dentistry following research and development into functional foods and other commercial products containing probiotics.

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21

Oral care gum products

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Abstract: Various oral care gum products (chewing gums) have been designed to promote oral health through their saliva-stimulating action or throught the direct pharmacological effect of gum constituent on oral tissues. Because of the generally pleasurable experience associated with gum chewing, various gum products can be used to dispense pharmacological agents by means of the 'slow-release' mechanism rendered possible by gum chewing. Gum products containing sugar substitutes have been used extensively to limit dental caries and periodontal infections, and to facilitate salivation in hyposalivation patients, but they have also gained use in various medical and physiological tests.

Key words: chewing-gum, dental caries, medical use of chewing-gums, saliva stimulation, sugar substitutes.

21.1 Introduction: notes on early history; rationale for the use of oral care gums

Modern oral care gum products can be traced back to early times, when people enjoyed chewing various gummy substances. The early Greeks reportedly chewed the gummy material obtained from the mastic tree (*Pistacia lentiscus*), while the Maya Indians chewed chicle (the natural, latex-like exudation obtained from the sapodilla tree (*Achras zapota*) more than a thousand years ago. Chewing gum made with chicle and other resinous materials gradually developed during the late 1800s and gained widespread popularity. One of the earliest possible uses of a chewing gum-like product was recently revealed by an archaeology student in Finland, who discovered a 5000-year-old piece of chewing gum – a lump of birch bark tar (which may have also been used for other purposes). It has anyway been postulated that Neolithic people used gummy substances of plant origin as an antiseptic to treat gum infections; birch bark contains phenolic substances that exert antiseptic effects.

Oral care gums are designed to promote oral health via their saliva-stimulating action or through the direct effect of gum ingredients on oral tissues. Gum-chewing stimulates salivation mechanically, but also chemically, owing to the sweeteners normally present in gums. Mechanical and chemical stimuli increase the oral levels of innate salivary defence factors. Owing to the generally pleasurable experience associated with gum-chewing, gums can be used to dispense pharmacological agents, whose administration would otherwise be difficult. Administration of such substances often presumes their slow release into saliva; gum-chewing renders this possible. Slow-release may also be advantageous for caries-preventing substances such as fluorides and those sugar substitutes that actively affect the caries process. Oral care gums can also be designed to dampen periodontal and gingival infections, to prevent halitosis and to facilitate salivation in hyposalivation patients; the alleviating effect of gum-chewing can be of value in trying to maintain a normal quality of life for patients. Various gummy products have also been used to study occlusion, jaw muscle fatigue and other oral conditions. Consequently, oral care gums can play a significant role in the administration of health-promoting agents and procedures.

21.2 Oral care gums in relation to other oral care adjuvants; advantages and disadvantages

Consumer products recommended for oral health care include chewing gums, chewable tablets, toothpastes, mouthwashes, sprays, gels, salivary substitutes ('artificial saliva'), pacifiers (experimental products for infants), and so on. Scientific research and oral physiological reasoning suggest especially that products that render active chewing and sucking functions possible are ideal oral care products. Gum-chewing normally occasions a type of pumping effect that can effectively squeeze biologically active salivary constituents and pharmacological agents into pits and fissures of the teeth.

An objective examination of oral care gums shows that their use can be accompanied by various negative effects (Table 21.1). Although some of the detrimental aspects listed in Table 21.1 can be considered rare or exaggerated, attention must be paid to common drawbacks of gum-chewing. Subjects with occlusal problems may need to avoid gum-chewing. A particular environmental problem arises from the disposal of used gum. Although engineers have developed non-adhesive gums, a gum base that disintegrates after use would be equally welcome.

Ignoring the 'pop culture' impression occasionally associated with gum-chewing, one can examine the situation purely from a physiological point of view: gum-chewing stimulates salivary glands. This can be considered a beneficial oral biological reaction and constitutes the basis for most oral care applications. Current and potential future utilisation of gum products is described in Table 21.2, which offers a broader view of the diversified

Table 21.1 Possible negative effects of gum-chewing

1. Frequent use of sugar gum can increase the oral levels of caries-inducive microorganisms and the incidence of caries.
2. Gum ingredients may irritate the oral mucosa. Examples: cinnamon may cause stomatitis; patients with oral lichen planus may show contact allergy to spearmint oil; occupational asthma may result from coating chewing gum with raspberry powder; monomers (e.g. butadiene) liberated from the gum base latex material may be toxic or allergic; aspartame may provoke headache in some susceptible individuals; organic acids may chelate calcium.
3. Intensive gum-chewing may contribute to temporomandibular disorders.
4. Gum-chewing may in some cases be responsible for irritable bowel syndrome.
5. Gum-chewing may have consequences for anaesthesia (resulting from obstruction of the trachea and oesophagus, and from stimulated production of gastric juices which increase the risk of regurgitation and aspiration; refuted by some authors).
6. Dental bacteraemia, possibly responsible for endocarditis, may result from simple daily manoeuvres such as tooth brushing and chewing gum.
7. Gum-chewing may be associated with risk of oesophageal and cardiac adenocarcinoma (refuted by some authors).
8. Forensic medicine reports suggest that sudden deaths may be linked to aspiration of chewing-gum.
9. Children can have the habit of inserting foreign bodies (chewing-gum) into nose, ear, etc.
10. Children with immature oral motorics may not like chewing gum.
11. The used gum may constitute an unwelcome form of refuse.*

* The Singapore government promulgated in 1992 a ban on chewing gum. The government later relented. Considering the pharmacological uses of medical gum products (Table 21.2), a categorical ban on chewing gum may be considered excessive self-defence. However, consumers should be blamed for improper disposal of used gum. A Japanese company has included a pad of small paper sheets in the gum packages to wrap used gums.

medical applications in this field. The final target tissues and functions of several of the listed applications are located beyond the oral cavity. They must be mentioned, however, since the masticatory organ is involved in all cases; all gum products are, *de facto*, chewed. All chewable products affect saliva, the oral mucosa, dental plaque and the teeth.

21.3 '*In sputo veritas*' – gum-chewing stimulates salivation and the chemical defence of saliva

Saliva can be regarded as an effective natural 'mouthwash'. Gum-chewing increases the sheer volume of saliva and, consequently, the salivary levels of host-defence factors. Gum-chewing facilitates the chemical host-defence associated, for example, with the following natural factors: the buffering system (largely based on bicarbonate and phosphate ions), the innate salivary F^-, immunoglobulins (such as secretory IgA and other agglutinins),

Table 21.2 Consumer groups who may benefit from the use of sugar-free oral care gums (1) and use of gums as experimental tools in medical tests (2)

1. Consumer groups

1. Expectant mothers and mothers during children's first years of life. Other family members and baby minders with intimate contact with infants.
2. Children attending day-care centres and kindergartens (emphasis on early caries prevention).
3. Elderly subjects (gum products may be substituted with pastilles, lozenges, etc.).
4. Orthodontic patients: gums are available that do not damage orthodontic appliances.
5. Certain occupational workers (at bakeries and confectionery and car battery factories).
6. Athletes (sports beverages may contain Ca-chelating fruit acids and fermentable sugars). Prolonged (30 min) salivary stimulation by gum-chewing after an erosive attack can reduce dental wear.
7. Dry mouth patients (Sjögren's syndrome and other hyposalivation patients). Chewing gum can be used to identify patients with a slow salivary flow rate and buffering capacity.
8. Handicapped subjects. Handicap may include mental problems, lack of dexterity and other disabilities.
9. Hospital patients: long-term hospital care of severely ill patients should avoid causing oral health problems by restricting the administration of sugary medication.
10. Patients with impaired glucose intolerance.
11. Diabetic subjects.
12. Patients with allergies toward certain cereal products (some starch syrups may contain traces of wheat).
13. Regurgitation patients (frequent regurgitation may cause dental erosion).
14. 'Extreme culinarians' (individual who habitually consume large quantities of acidic foods, such as salad dressing, citrus fruits and certain national foods containing abundant citric acid, tartaric acid and related compounds; dental erosion may result).
15. Subjects suffering from halitosis (which results mostly from Gram-negative bacteria thriving in low oxidation–reduction environments, frequently on the dorsal area of the tongue).
16. Patients with laryngopharyngeal reflux (chewing gum after a meal may be a useful adjunctive antireflux therapy and can thus help reduce the postprandial oesophageal acid exposure). Patients with hypersecretion of HCl may benefit from regular gum chewing.
17. Bulimic patients may benefit from the use antacids, cheese and xylitol chewing-gum.

2. Use of gums in medical investigations

18. Stimulation of peripheral circulation by muscle contraction (i.e. gum-chewing, causing moderate muscle activity, may positively influence the cerebral blood flow). The jaw muscles may recover quickly from prolonged chewing in subjects without the temporomandibular disorder. Gum-chewing equalises the blood flow supply of mastication muscles on both working and non-working sides.
19. Chewing gum can be used to study the chewing cycle kinematics of subjects with deepbite malocclusion, the masticatory efficiency, the functional relationship between masticatory muscles, the mandibular and head movements, the masticatory laterality, the cleft palate speech, etc.

Table 21.2 *Continued*

20.	Gum-chewing may speed colon surgery recovery. Gum-chewing may ameliorate postoperative ileus (still a controversial topic).
21.	Gum-chewing may positively affect memorial facilitation and context-dependent memory effects, such as initial learning and subsequent recall of a word list (a debated research finding).
22.	Chewing gum ('inter-meal oral stimulation') may suppress appetite, especially the desire for sweets, may reduce snack intake and may moderate the rising obesity rates.
23.	Gum-chewing may positively influence intra-alveolar bone optical density (in patients with clinically different periodontal tissue conditions).
24.	Maintaining alertness and performance in sleep-deprived individuals (gum containing caffeine).
25.	Gum-chewing may increase neuronal, age-dependent activity in the brain.
26.	Smoking cessation (use of anti-smoking gums).
27.	Alleviation of certain otologic conditions (such as equalisation of air pressure when travelling by plane). Prevention of otitis media in infants (using xylitol gum).
28.	Drug delivery in general: a special example is anti-fungal therapy. Generally, in the treatment or prevention of local diseases in the mouth.

salivary enzymes (such as peroxidase, lysozyme and amylase), the iron-binding glycoprotein lactoferrin, pH-rise- and glycolysis-promoting factors, various anionic antimicrobial proteins, tyrosine-rich peptides (such as statherin which stabilises the calcium and phosphate salts of saliva, binding to hydroxyapatite, thus governing the mineralisation process), proline-rich proteins (that also bind to hydroxyapatite in addition to exerting antimicrobial effects), antimicrobial histidine-rich proteins ('histatins'), and so on. Even the polymorphonuclear leucocytes of saliva can be regarded as a defensive factor. The normal transudate of the gingival crevices in turn contributes to the chemical defence of the periodontal tissues: the massaging action of gum-chewing on the gingivae may exert positive effects. Gum-chewing obviously stimulates all salivary glands.

21.4 Target groups for the administration of oral care gums

Several manufacturers market chewing gums designed to limit the incidence of dental caries. Several other patient groups can also benefit from gum-chewing (Table 21.2). Occupational workers such as those working in confectionery factories can experience cariogenic challenges owing to the sugar dust to which they are frequently exposed. It has been shown that it is possible to lower the caries risk associated with airborne sugar and flour dust by providing xylitol–sorbitol chewing gum and xylitol tablets in the workplace for free use over a three-month period (Masalin, 1992). This assessment was based on a decrease in the number of workers with high

salivary counts of mutans streptococci (MS), when the workers used these products.

Patients with mental retardation and other handicaps can pose serious challenges for health-care workers, family members and the individuals themselves. The oral health of residents in mental hospitals, psychiatric institutions and homes for the elderly are normally the responsibility of the nursing staff in these institutions. Most studies of elderly and handicapped subjects have employed dentifrices, mouthwashes and chewable lozenge-type products. The few studies carried out with gum products have provided promising results. The authors of the Hungarian WHO trials concluded that their studies 'unconditionally show the practical value of partial substitution of sucrose by xylitol in solid sweets [several xylitol gum brands were included in the regimen] as a caries-preventive measure' in institutionalised children, predominantly orphans, but also hearing- and sight-impaired or blind subjects aged between 6 and 12 years (at baseline examinations) (Scheinin *et al*., 1985).

The Veterans Administration Medical Center trial in Dayton, Ohio, indicated that the use of xylitol- or sorbitol-containing gums and hard candy reduced the incidence of supragingival root-surface caries, the most effective products being those containing xylitol (Mäkinen *et al*., 1996a). In another study among 'frail, elderly people', gum-chewing significantly improved oral health. The acceptance of the gums (a chlorhexidine acetate/xylitol gum and a xylitol gum) was reported to be high (Simons *et al*., 2002).

21.5 Examples of special oral care gum products

21.5.1 Sugar-free gums

The rationale behind replacing sucrose with other bulking agents in chewing gums stems from the proven cariogenicity of sucrose. Sugar alcohols (polyols) currently replace sucrose in most oral care gums aimed at caries reduction. Historically, sorbitol was one of the first bulking agents of the polyol type that was used in chewing gums. The sweetening power of sorbitol is, however, insufficient, and intense sweeteners and xylitol have therefore been added to sorbitol gums to improve their organoleptic qualities. Sorbitol also supports the growth of certain strains of mutans streptococci and displays a nugatory effect on dental plaque growth. Dental plaque can also become adapted to sorbitol (Kalfas *et al*., 1990). Several gum manufacturers have switched to gums that contain xylitol as the only sweetener, or to gums containing xylitol (as the first ingredient listed on packages) combined with sorbitol, maltose syrup, maltitol, palatinit or related polyols.

Sugar-containing gums continue to be marketed worldwide, although their sales have been declining in some countries. For example, in Finland, the sale of sugar gum currently constitutes only 1% of total chewing-gum

sales, the majority of gums containing polyols. Polyol-containing chewing gums have become popular research tools and consumer items. Caries trials and laboratory experiments with sugar-free polyol gums are described below and in Table 21.3.

21.5.2 Fluoride-containing gums

Fluoride-containing chewing-gums can be regarded as slow-release vehicles of active fluoride compounds, which normally dissolve rapidly in saliva. Use of fluoride gums provides repetitive topical application of low levels of fluorides, prolongs the elevation of salivary fluoride levels and simultaneously stimulates the salivary flow rate, which in turn promotes remineralisation, clearance of cariogenic substrates and increase of the salivary and plaque pH value (Itthagarun and Wei, 1997). The compound most frequently used in gum products has been NaF, although Na_2FPO_3, SnF_2, and amine fluorides have also been effective. Use of fluoride-containing gums requires caution, since some individuals may be in the habit of using larger quantities of chewing gum.

21.5.3 Stain-removing gums

Studies of gum products formulated to inhibit the formation of extrinsic stain on enamel surfaces have suggested that sodium hexametaphosphate (NaHMP; sodium polymetaphosphate, $(NaPO_3)_x$, a mixture of polymeric metaphosphates; actually not a hexamer), a long-chain condensed phosphate (at 4–7% level in the gum), significantly reduced stain formation compared to a no-gum treatment (Bartizek *et al*., 2003; Biesbrock *et al*., 2004; Porciani *et al*., 2006). This salt was studied earlier because of its anti-tartar and anti-caries effects and has lately been promoted as an enamel-whitening agent (Baig *et al*., 2005). A physiochemical consequence of the NaHMP application is an increase in the hydrophilicity of the enamel surface (van der Mei *et al*., 2003). Users of NaHMP should note that this salt depolymerises in aqueous solutions to form sodium trimetaphosphate and sodium orthophosphates. It has also been suggested that baking soda ($NaHCO_3$) has a whitening effect when used in a gum formulation (Mankodi *et al*., 2001).

A well-known negative consequence of chlorhexidine (CHX) application is tooth staining. Gum-chewing can be of use in the discoloration of CHX-affected teeth (Yankell and Emling, 1997).

21.5.4 Calculus-removing gums

Gum products showing anti-calculus effects include those containing sodium tripolyphosphate and tetrasodium pyrophosphate (both at a 1% concentration; Porciani *et al*., 2003) or ascorbic acid (with or without carbamide;

Table 21.3 Human caries studies on polyol-containing gum products. The reduction percentages of caries are given compared with a control group that received a normal diet, fluoride treatment or sucrose products [older references in Mäkinen (1989, 2000); later references shown below]

Study location	Product(s) tested; (chewing time, if reported)	Duration (years)	Polyol dose (g/day)	Caries reduction (%)
Denmark 1973	Sorbitol chewing gum	2	3.6	10
Finland 1975	Xylitol chewing gum	1	6.7	>82
USA 1983	Sorbitol chewing gum	2	Not given (2 sticks)	Not significantnt
Hungary 1985	Xylitol chewing gum, candies, dentifrice	2–3	14–20	37–45
French Polynesia 1988	Xylitol chewing gum	3	About 20	58–62
Canada 1990	Xylitol chewing gum	1–2	1.0–3.9	52
Finland 1988	Xylitol chewing gum; all subjects[a]	2	7–10	30–57
	high-risk subjects[a] (5 min)	3	7–10	59–84
Belize 1995, 1996	Xylitol chewing gum; permanent teeth[b]	3.3	<10.7	up to 73
	deciduous teeth[c] (5 min)	2	<10.7	up to 63
USA 1996	Xylitol chewing gum, pastilles; root surface caries (<3 min for pastilles; 15 min for gum)	1.8	8.5	80
Estonia 2000	Xylitol chewing gum, pastilles[d] (10 min)	2–3	5	50–60 (Alanen *et al*., 2000)
Finland 2000	Xylitol chewing gum; (used by mothers)	ca. 1.75	6	70 (in children; Isokangas *et al*., 2000)
Hungary 2001	Sorbitol-mannitol chewing gum (>20 min)	2	Not given[e]	38.7 (Szöke *et al*., 2001)
Lithuania 2001	Xylitol chewing gum ('at least 10 min')	3	2.95	21–36[f] (Machiulskiene *et al*., 2001)
Sweden 2006	Xylitol chewing gum; (used by mothers)	1	2	40 (in children; Thorild *et al*., 2006)

[a] Long-term effects (after up to 5-year use) have been reported (Isokangas *et al*., 1993).
[b] 16-month use of xylitol gum following the 3.3-year use of sucrose gum reduced caries significantly (Mäkinen *et al*., 1998a). '<10.7' indicates the maximum, supervised usage (at schools) per day and subject.
[c] The 2-year use of xylitol gum protected the erupting permanent teeth against caries (Hujoel *et al*., 1999), i.e. long-term effects were involved.
[d] Saliva stimulants were given only on school days (about 200 per year). Gums were as effective as pastilles (hard candies of the 'Läkerol-type'; Alanen *et al*., 2000).
[e] Three sticks of a sorbitol-mannitol gum were used per day. Assuming that a stick weighs 2.7 g, the daily polyol level was 5.26 g.
[f] The authors failed to recognise that, in their study, xylitol gum was the only gum that lowered the decayed, missing and filled tooth surfaces (DMFS) increment compared with the no-gum group after 3 years. The original data were rectified by Hayes (2002). 'To still observe a significant caries-lowering effect of xylitol with such a small dosage is quite remarkable' (Hayes, 2002).

Lingström *et al*., 2005). It is possible that the positive observations made in dentifrice studies could be applied in gum formulation; a preparation consisting of various phosphates and NaF may be considered worth experimenting with (Sowinski *et al*., 2000), as well as those containing pyrophosphate supplemented with certain zinc salts, triclosan or diphosphonate (Volpe *et al*., 1993).

21.5.5 Gum products targeted at periodontal and gingival inflammation

The experience obtained with CHX rinses has been applied to gum products (review: Imfeld, 2006). CHX, a bisbiguanide with strong bacteriostatic activity, has normally been used as a diacetate or as a digluconate. Various gum formulas containing CHX have expectedly reduced plaque growth and improved gingival health (e.g. Ainamo and Etemadzadeh, 1987; Ainamo *et al*., 1990; Simons *et al*., 1999), and reduced gingivitis (Smith at al., 1996). Chewing a CHX gum (about 5 mg CHX per piece) twice a day and using CHX rinses (approx. 0.2%) have frequently yielded similar results. It is also normal for CHX to increase the plaque-reducing effect of xylitol (Simons *et al*., 1999). Smith *et al*. (1996) and Imfeld (2006) rightly emphasised the shortcomings of CHX applications: tooth staining, bitter taste and toxicity, which limit the application of CHX to short-term use.

Xylitol gum prolonged the effect of CHX therapy on oral MS levels, maintaining long-term caries pathogen suppression (Hildebrand and Sparks, 2000). It has been claimed that, although chewing a regular sugar-free gum reduces plaque accumulation at sites of predilection for caries, there is only little or no effect at sites of predilection for gingivitis (Hanham and Addy, 2001). However, such studies have often used a too-small number of gum pieces (or at a too-low daily frequency) and ignored the active effects of bulk sweeteners (xylitol and sorbitol).

The numerous antibacterial phytochemicals isolated from plants have found their way to gum products as well. This is an ever-escalating field of research and application: a large number of efficacious herbal oils, antioxidants and other substances is available. For example, a pycnogenol-containing gum reduced gingival bleeding and plaque accumulation in a two-week study (Kimbrough *et al*., 2002). Pycnogenol, an extract from French maritime pine bark, exhibits almost panacea-like properties, acting as an antioxidant, as an inhibitor of viral replication, as a suppressor of the expression of pro-inflammatory cytokines, and so on. It is a complex mixture of pine bark substances, including polyphenolic monomers, dimers and higher oligomers. The higher procyanidin oligomers are especially active and ideal oral care adjuvants for use in gum products.

Manuka honey (used as a chewable 'honey leather') exerts antimicrobial properties in reducing dental plaque, gingivitis and periodontal disease (English *et al*., 2004). Honey in general has been used in wound dressing for thousands of years. The manuka honey (from the nectar collected from

Leptospermum scoparium which grows in New Zealand and Australia) has antioxidant potential, quenches free radicals (Henriques *et al*., 2006) and contains a 5.8-kDa component that stimulates immune cells (Tonks *et al*., 2007). Honeys in general can make suitable sweeteners in gums provided that the cariogenicity of the sugar components can be minimised.

Other substances of plant origin include xanthorrizol (1,3,5,10,bisabolatetraen-3-ol) from the dried rhizomes of *Curcuma xanthorriza*, which has exhibited antibacterial activity especially against oral pathogens (among those that are associated with dental caries), as well as against *Actinomyces viscosus* and *Porphyromonas gingivalis* which are associated with periodontal disease (Hwang *et al*., 2000). These compounds have a tendency to penetrate the dental plaque biofilm and to reduce plaque growth. Such compounds may be suitable chewing gum ingredients.

21.5.6 Chewing gum and orthodontic treatment

Patients with malocclusions have retention sites that are difficult to clean. When these subjects wear orthodontic appliances, they are under a great risk of developing dental caries. One study investigated the effect of 28-day use of polyol-containing chewing gums on dental plaque and mutans streptococci. Gum that contained xylitol as the predominant sweetener reduced the caries risk in young orthodontic patients (Isotupa *et al*., 1995). The subjects did not report any harmful sticking of the gum to appliances and no damage was observed in orthodontic arches, brackets or bands. Mastic gum reduced the salivary levels of *Streptococcus mutans* and lactobacilli in orthodontic patients (Aksoy *et al*., 2007). This author is aware of dental schools in the United States, Japan and Finland, where the training of orthodontists includes an encouragement to recommend chewing xylitol-gum as a means of reducing caries risk.

21.5.7 Trimetaphosphate gum

A three-year study of school-age children using sodium trimetaphosphate (TMP) as a chewing-gum additive produced a significant 23.3% caries reduction for the TMP-sucrose gum group and a 47.6% reduction for the TMP-non-sugar group, compared to the no-gum group (Finn *et al*., 1978). Each gum stick (used thrice daily) contained 45 mg of TMP. Earlier studies on animals had shown that a TMP-supplemented diet had reduced caries by up to 79%.

21.5.8 Gum products in clinical and biological tests

The number of applications of chewable gum products (Table 21.2) is too great to be discussed in this context. The importance of these applications should once again be underlined: whatever the rationale behind the diversi-

fied applications, all gums employ the masticatory organ and can have remarkable effects on the oral cavity.

21.6 Clinical caries trials: school programmes

Of the polyol gum trials shown in Table 21.3, a total of eight studies can be regarded as school programmes, that is the gum use took place predominantly at school and was supervised by teachers. In a trial, where one of the three daily chewing episodes took place at school (on school days), a sorbitol gum reduced the caries increment by 10% over two years (Möller and Poulsen, 1973). In the WHO Hungary trial, polyol gum constituted an important part of the preventive regimen (Scheinin *et al.*, 1985). Most polyol-gum school programmes have provided encouraging results; caries rates have significantly dropped in subjects receiving xylitol gum. Similar conclusions were reached by Peng *et al.* (2004). In the Estonian school programme xylitol gum was as effective in caries reduction as hard xylitol candy (Alanen *et al.*, 2000). This result suggests that it may not be necessary to employ strong mastication to achieve satisfactory caries prevention and that xylitol probably displays active pharmacological effects on dental caries.

21.6.1 Clinical caries trials: mother–child studies

A series of long-term clinical studies has dealt with caries in the mother–child relationship. In the Finnish programme, mothers used xylitol chewing-gum during a 21-month period, when their infants were 3 to 24 months old. Mothers in the comparison groups received customary fluoride or CHX treatments. The children did not receive any of these treatments. Already by the age of three years, the children of xylitol-consuming mothers exhibited significantly reduced oral counts of MS compared with children whose mothers had received conventional fluoride and CHX treatments. The reduction in the intrafamiliar transmission of MS caused a remarkable caries-limiting effect in the deciduous teeth of the infants examined two years later (Isokangas *et al.*, 2000). Results of microbiological measurements, when the children were six years old, were in congruence with the three-year data (Söderling *et al.*, 2001, and references therein). Preliminary results of caries registrations of the children at the age of ten years showed that approximately the same caries-preventive effect had continued (to be published). It is likely that, in addition to mothers, other family members in intimate contact with infants can also transmit the caries infection. High MS counts in mothers can thus be transmitted to children in normal kissing situations, or when mothers test the temperature of the contents of the baby bottle in their own mouth.

These results were verified in a Swedish trial (Thorild *et al.*, 2006, and references therein). Consequently, a relatively simple, cost-effective, home

application of a preventive strategy based on habitual use of xylitol-containing chewing-gum by mothers has resulted in a significant improvement in the oral health of their children.

21.6.2 Results of ancillary studies of long-term caries trials

Saliva and plaque samples of subjects participating in several long-term trials on oral care gums have been analysed for various organic compounds and enzymes, for the salivary and plaque levels of mutans streptococci and lactobacilli, and for the amount and adhesiveness of dental plaque. Long-term use of a 100% xylitol gum decreased the whole saliva invertase–sucrase activity and the salivary levels of free sialic acid (Mäkinen *et al*., 1996b). Lowered invertase–sucrase levels speak for overall reduced sugar-exploiting capacity, while the diminished sialic levels suggest reduced enzymatic breakdown of salivary mucins. These findings are in congruence with suggestions of a general decrease in carbohydrate metabolism as affected by habitual use of xylitol, which generally increases the overall nitrogen metabolism of dental plaque (Mäkinen and Scheinin, 1982; *vide infra*).

The plaque mass and plaque index scores, as well as the oral levels of MS and lactobacilli, have generally decreased significantly during habitual use of xylitol gum. Historically, the first observations on plaque quantity were made during the 1970s (Mäkinen and Scheinin, 1975, and references therein) and in numerous studies over the past 30 years on oral bacterial levels. Some of the latest investigations elucidated the relationship between the dose and frequency of xylitol use with the reduction of MS levels in saliva and plaque. The bacterial levels were reduced with increased dosage of xylitol, with the effect levelling off between 6.88 g/day and 10.32 g/day (Milgrom *et al*., 2006). A two-year trial involving the use of 6.6 g xylitol per day (in four chewing episodes) resulted in significantly reduced salivary and plaque levels of mutans streptococci and salivary levels of lactobacilli (Mäkinen *et al*., 2008). The latter study suggested that such effects can be long term, since significantly reduced bacterial scores were measured 15 months following the termination of the gum programme. Similar long-term effects were earlier observed in the Finnish Ylivieska study (Söderling *et al*., 1991; Isokangas *et al*., 1993, and references therein).

21.7 Effect after 'sugar challenge'

Xylitol-containing chewing gums have been tested for their possible dampening effect on the acid attack caused by sucrose rinsing. Accordingly, chewing a xylitol-containing gum immediately following a 10% sucrose rinse resisted the sugar-associated acid attack significantly more effectively than a sorbitol-containing gum (Söderling *et al*., 1989). This suggests that xylitol, when present in a chewing gum, can exert a short-term pH-

increasing chemical effect on dental plaque that produces acids as a result of previous exposure to sucrose. The result also further confirmed the selectivity of the polyol effects on oral biological processes; pentitols and hexitols do exert specific effects on dental plaque. This observation was verified in an independent study, which concluded that 'regular use of xylitol-sweetened gum may serve to reduce the acidogenic potential of dental plaque' (Aquirre-Zero *et al*., 1993).

The acidogenic potential of plaque after sucrose challenge has been studied with carbamide-containing gums (Imfeld *et al*., 1995; Gopinath *et al*., 1997). Based on biochemical reasoning, carbamide, which is present in small concentrations in normal saliva, produces ammonia after hydrolysis by plaque enzymes. The ammonia neutralises acids formed in plaque following a sucrose challenge. Similar sugar challenge tests have been carried out with fluoride-containing gums; the salivary fluoride levels increased during and after chewing, and prolonged chewing (20 to 45 minutes) almost always returned the plaque's pH value to neutral following a sucrose rinse (Oztaş *et al*., 2004). Chewing gums containing intense sweeteners (aspartame, saccharin or acesulfame K) can also increase the pH value of plaque following a sugar challenge (Park *et al*., 1995).

21.8 Biochemical effects in short-term polyol gum use

In addition to serving as softeners in oral care gums, polyols also have a remarkable effect on dental plaque. These substances were initially believed to have only a bulking role. The scientific literature is replete with studies showing that xylitol, a sugar alcohol of the pentitol type, reduces the counts of oral MS and lactobacilli, reduces the mass and the adhesiveness of plaque and exerts selective effects on plaque metabolism. Sorbitol, owing to its six-carbon nature (and its close structural relationship to glucose), either stimulates the growth of MS or has no particular effect. The xylitol-specific effects result from the pentitol nature (the 'non-glucose structure') of the xylitol molecule. The following biochemical reactions characterise dental plaque and whole saliva affected by xylitol:

- Plaque deprived of its required hexose-based dietary sugars, in the presence of xylitol, converts its normally carbohydrate-dominated metabolism into one where various reactions of nitrogen metabolism predominate. Consequently, 'xylitol plaque' and 'xylitol saliva' display elevated transaminase activity and higher levels of ammonia, basic amino acids, protein and nitrogen (Mäkinen and Scheinin, 1975, 1982). The xylitol-associated increase in plaque protein levels has also been observed by other authors (Rølla *et al*., 1980; Kertész *et al*., 1985). Ammonia (which can result from deamination of amino acids) neutralises plaque acids.

- Plaque grown during xylitol consumption contains smaller amounts of insoluble, sticky polysaccharides than plaque grown during sucrose consumption.
- Dental plaque and whole saliva obtained from xylitol-using subjects display lower 'sucrase- and invertase-like' activities than those obtained from subjects receiving a normal diet. These enzymes attack sucrose and maltose molecules and are, therefore, involved in acid production.
- 'Xylitol plaque' produces less lactic acid than 'sucrose plaque'. In the presence of xylitol, the cells of MS also produce less lactic acid than the hexitols (sorbitol and mannitol) do. Most MS strains produce acids almost exclusively by glycolysis (Embden–Meyerhof pathway). The carbon skeleton of xylitol does not directly enter the glycolytic pathway.
- In the presence of xylitol, the cells of MS undergo distinct alterations in ultrastucture, exhibiting degrading cells, autolysis, intracellular vacuoles and lamellated formations in the cytoplasmic membrane, independent of the concentrations of xylitol. Sorbitol, mannitol, fructose, glucose, lactose and sucrose did not cause such effects (Tuompo *et al*., 1983).
- Cells of MS grown in the presence of xylitol contain smaller amounts of lipoteichoic acid (LTA) compared with cells grown in the presence of sucrose (Rølla *et al*., 1980; Tuompo *et al*., 1983). LTA plays a key role in the adhesiveness of sucrose-grown MS.
- The adhesiveness of the cells of MS onto glass surfaces decreases significantly in the presence of xylitol, compared with sorbitol. Xylitol also increases the ratio of soluble polysaccharides to insoluble ones, shows a lower degree of cell–cell aggregation, compared with sorbitol and increases the calcium content of plaque.

21.9 Gum products and remineralisation

The saliva excreted from the acini is normally supersaturated with calcium phosphate. Saliva contains peptides that stabilise calcium and phosphate ions, governing their precipitation as calcium phosphates. The supersaturated state reflects evolutionary expediency: minor calcium deficiencies in the tooth enamel can normally be corrected by the mineral ions present in the surrounding saliva. This remineralisation is a normal repair mechanism of the teeth. In simple chemical terms, the conditions for remineralisation are sufficiently high salivary calcium and phosphate levels and sufficiently high salivary pH ('acid attacks' reduce the remineralisation rate). By chewing polyol gums, which stimulate salivation and do not cause acid attack, the above chemical conditions can be met. Clinical studies suggest that habitual use of xylitol-containing gums is associated with significant remineralisation of caries lesions (Scheinin and Mäkinen, 1975; Mäkinen *et al*., 1995) and that rehardening is also encountered in dentine lesions

(Mäkinen *et al*., 1995, 1998b). Laboratory experiments (Vissink *et al*., 1985; Steinberg *et al*., 1992; Miake and Yanagisawa, 2000) and animal tests (Havenaar *et al*., 1984) support this finding.

The sulphate-rich oligosaccharide funoran (from the seaweed *Gloipeltis furcata*) has generated interest owing to its remineralisation-enhancing chemical effect, especially when combined with NaF, calcium hydrogenphosphate and xylitol (Saeki *et al*., 2000; Miake and Yanagisawa, 2000). Oral care gums containing this mixture are currently available in some Asian markets. Combinations of xylitol with other organic and inorganic compounds in a gum can significantly increase the rate of remineralisation. Such compounds include calcium lactate (Suda *et al*., 2006) and casein phosphopeptide-amorphous calcium phosphate nanocomplexes (Shen *et al*., 2001; Reynolds *et al*., 2003, Iijima *et al*., 2004). The latter ('Recaldent' formula) are chemically interesting, owing to the presence in the casein phosphopeptide of several phosphoseryl residues that contribute to the stabilisation of nanoclusters of amorphous calcium phosphate in metastable solutions (Reynolds 1998). Some of the natural calcium-stabilising salivary factors (e.g. the statherins) contain similar phosphoseryl groupings.

A regular bulk sweetener can also stabilise salivary calcium: the xylitol molecules stabilise calcium phosphate solutions, maintaining the state of supersaturation, compared with sucrose, which normally allows almost instantaneous precipitation of calcium phosphate(s) (Mäkinen and Söderling, 1984). In the presence of polyols, apatite crystals can form (Mäkinen *et al*., 1989). Such bioinorganic effects are common to dietary polyols, as shown in studies with sorbitol gum (Kashket *et al*., 1989). However, the nugatory effect of sorbitol (a 'glucose polyol') on dental plaque and mutans streptococci places it apart from the pentitols ('non-glucose polyols'). Among other physiochemical properties of xylitol is its ability to increase plaque calcium levels. This calcium (partly in soluble form) has been assumed to be available in the remineralisation processes (Mäkinen, 1989).

21.10 Public endorsement of oral care gum products

It is imperative that public health policy concerning oral health allows the promotion of food and food components that can assist in reducing oral diseases. Clinical studies have demonstrated that the use of the xylitol sweetener in oral care gum products can significantly reduce the incidence of dental caries and may also alleviate gingival and periodontal infections. Public health evaluation of xylitol-containing gum products has been carried out during the past 30 years. This evaluation has resulted in recommendations in the use of xylitol in publicly supported caries prevention programmes. Endorsing organisations have included professional dental associations, non-profit organisations and government departments. Notably,

Table 21.4 Public institutions, regulatory bodies and professional organisations who have endorsed or recommended the use of xylitol chewing gum in caries prevention

Organisation	Countries
National Dental Association	Canada, Finland, Estonia, France, Hungary, Iceland, Ireland, Malta, Norway, Peru, South Africa, South Korea, Switzerland, Sweden, Taiwan, The Netherlands, Turkey, United Kingdom, USA
Ministry of Health	Finland, Italy, Japan
Other dental associations	China (National Committee for Oral Health), Japan (School Dentists' Association), United Kingdom (British Dental Health Foundation), Switzerland ('Toothfriendly' Sweets International)
Armed forces	Finland, USA
Other organisations	Germany (German Consumers' Foundation), The Netherlands (Ivory Cross), Scotland (The Scottish Intercollegiate Guidelines Network), World Health Organisation

Finnish public health centres have participated in the dissemination of xylitol-related information to patients. Table 21.4 gives examples of institutions that have recommended the use of xylitol chewing gum in caries prevention in various countries. Emphasis is placed on xylitol, since it is the only bulk sweetener present in oral care gums that has received worldwide public recommendations as a caries-limiting agent.

21.11 Practical instructions for studies on oral care gums; incompatibilities among gum ingredients

The daily consumption level of oral care gums, frequency of use per day, chewing time per episode and length of the overall treatment depend on the purpose of the application of the product. Gum products aimed at limiting dental caries may normally be used by regular consumers without specific restrictions; gum use is most often self-limiting by nature. Although frequent and long-term chewing of sugar-free gums normally assists in caries prevention, it is necessary to recall the following precautionary physiologic aspects:

1. Patients with severe occlusal problems should chew only for short periods at a time or even avoid gum use completely. Such subjects may benefit from salivary stimulation from suckable hard-candy-type products. Xylitol candies can be as effective in caries reduction as xylitol gums (Alanen *et al.*, 2000).

2. The minimum plaque pH value is often attained during the first 1–3 minutes following the sugar challenge. Consequently, clinical trials on sugar substitutes should not involve chewing times longer than about 5 minutes. Prolonged chewing may mask the desired pharmacological effect of the tested sweetener, when the salivary effect becomes dominant.
3. Xylitol gum can exert long-term effects on dental plaque. Such effects can be achieved even after a single exposure to xylitol. The chemical mechanism of the xylitol effect suggests that it may be possible to influence the sugar-exploiting capacity of dental plaque by also chewing xylitol gum before a sugary meal (standard instructions call for the use of xylitol gum after sugar intake). The impact of the long-term effect may invalidate studies that employ too-short washout periods.
4. The acid attack caused by sugar gum will weaken after 20–30 minutes of continuous chewing (owing to the neutralising effect of saliva). This finding has wrongly been exploited to claim that the use of sugar gum is safe; the acid attack of sucrose may have already had its detrimental effects during the first minutes of chewing.
5. Prolonged chewing (after 20 minutes) may significantly affect the results of salivary tests. Even extended chewing of paraffin may positively influence certain parameters of parotid saliva (such as the flow rate, pH value and protein concentration; Jensen *et al*., 1998).
6. The levels of certain salivary ingredients (such as F^-) may be higher on the chewing side of the oral cavity, compared with the non-chewing side. This possibility should be considered in oral physiological investigations.
7. There are no physiologically inert bulking agents currently used in gum products. The strong and specific effects of bulk sweeteners such as erythritol, xylitol, sorbitol and maltitol, serve as examples.
8. Differences in preferences for chewing gums may influence subject compliance in long-term trials. Decisive factors include gum shape, texture and taste. All chewing gums normally increase salivation during the first few minutes. However, after about 10 minutes of continuous chewing, the salivary flow rate may be equal to or even higher than that measured with paraffin stimulation.
9. Studies on infants should perhaps employ fully soluble, small-size pastille- or lozenge-type products instead of gums. Use of strong flavours should be avoided.

Most physiologically active substances customarily used in dental care gum products are compatible with the bulking agents present in gums. For example, bicarbonate, urea, several zinc salts and fluoride compounds seem to be compatible with polyols. Xylitol and F^- act synergistically on the metabolism of caries-associated bacteria (Maehara *et al*., 2005). However, the presence of sodium lauryl sulphate and larger amounts of fructose may

lower or even nullify the advantageous effect of xylitol on MS. In spite of this, a 1:1 mixture of xylitol and fructose has been shown to reduce plaque mass compared with sucrose.

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22

Novel drug delivery systems for therapeutic intervention in the oral environment

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Abstract: The research of the late Professor Sid Kalachandra toward the development of novel drug delivery systems for oral infections is described in the context of related work in the field over the past quarter of a century. In the case of persistent oral infections, the dosages of conventionally delivered antiviral, antibacterial and antifungal medications can lead to toxic side effects. The development of a biocompatible copolymer (ethylene vinyl acetate, EVA) into a drug-loaded mouth guard may allow medication to be delivered locally to the infection site at a lower concentration over an extended period of time. Studies with different antimicrobial medications have shown sustained, steady release from EVA and it is hoped that such a device will prove to be safe and effective in patients with oral opportunistic infections like those associated with HIV/AIDS. It is necessary that the design of the system allow for medication to be released near the gums, preserve the drug's integrity and allow for adjustment, all while still being biocompatible. The amount of medication released from the system can be adjusted by the amount of drug initially loaded or by the use of surfactants. While studies are currently underway to characterize the clinical efficacy of this drug delivery system, the ultimate success of such oral drug delivery systems may depend on their ability to release multiple medications simultaneously to suppress the combined viral, bacterial and fungal infections that can opportunistically attack severely immunocompromised individuals.

Key words: copolymer, dental materials, drug delivery, oral environment, oral therapy, polymer device.

22.1 Polymeric systems appropriate for oral drug delivery: history and background

Polymeric systems have been a backbone for the development of new drug delivery systems over the past few decades. The use of such devices in

dentistry is a relatively new area of research with the exception of the release of ions from polyalkenoate cements and their predecessors, silicate cements, inhibiting secondary caries and promoting bone growth (Patel *et al.*, 1998; Riggs *et al.*, 2000). The use of polymers as matrices to release antimicrobial, antiviral and other agents orally is a relatively novel area of research in dentistry. Materials such as 'compomers' (*comp*osite plus ion*omer*) (Ahrends and Ruben, 1998; Behrend and Guertsen, 2001), orthodontic adhesive resins (Rothwell *et al.*, 1998) and methacrylate-based copolymers (Patel *et al.*, 1998) have all been reported as matrices for the release of fluoride ions.

Current areas of research in which drug-loading and release are being investigated include release of chlorhexidine digluconate from polymeric materials (Addy and Handley, 1981), delivery of antimicrobials to treat periodontal disease from implantable copolymers (Wilson and Wilson, 1993) and the control of *Candida albicans* through the use of drug-loaded polymeric materials (Lee *et al.*, 2005; Yue *et al.*, 2004). Methacrylate-based systems as drug carriers have been less studied; Patel *et al.* (1994) reported that a tetrahydrofurfuryl methacrylate/poly(ethyl methacrylate) (THFMA/PEM) cold cure polymer system was used as a delivery system for chlorhexidine diacetate (CDA) and other drugs for the treatment of *Candida* infections in immune suppressed or palliative care patients (Parker *et al.*, 1997; Patel *et al.*, 2001).

Ethylene vinyl acetate is a copolymer containing the semi-crystalline polyethylene and amorphous poly(vinyl acetate) (VA) and this combination is commonly used in drug delivery devices (Baker, 1987, p. 161). The permeability properties of these copolymer films change substantially with the VA content and thus it is possible to tailor the release rate to a desired value by small changes in the membrane composition. It is known that an increase in crystallinity reduces the diffusivity within the polymer (Donbrow and Friedman, 1975). Analysis of study results showed that release of acyclovir (ACY) and CDA increased as the vinyl acetate comonomer was increased from 28 to 40%. The release rate of a drug from a device can be influenced by coating the device with a different polymer (Rhodes and Porter, 1998). This approach reduces the release rate of the drug owing to an increase in the diffusive path length and the release rate can be adjusted by the thickness of the coating (Huang *et al.*, 2000).

22.2 Oral diseases that are potential candidates for local drug delivery

The oral cavity is a unique environment, which has been utilized as a minimally invasive access point for administration of systemic medications (see Rathbone *et al.*, 1994 for an excellent review). However, the disease states encountered in the oral cavity often pose new and difficult challenges. The

keratinized epithelium in the mouth (masticatory mucosa) is the least permeable region in the mouth (resembling the epidermis of the skin, which is permeable to certain creams and ointments), accounting for 25% of the oral mucosa. The lining mucosa is stratified squamous non-keratinized epithelium similar to the tissues of the esophagus and the uterine cervix and accounts for 60% of the oral mucosa (Squier, 1991). Nonetheless, the healthy epithelial mucosa of the mouth are surprisingly non-absorbent compared to, for example, the nasal mucosa; and the rapid, though complex (Weatherell *et al.*, 1994) clearance of many potential drugs by saliva and the actions of the tongue limit the effectiveness of approaches such as mouthwashes and dentifrices. Higher concentrations of medications, sufficient to have a therapeutic effect when administered conventionally, are often accompanied by systemic toxicity (for example, excess ganciclovir can lead to decreased levels of blood cells – anemia; excess acyclovir can produce easy bruising or bleeding; excess metronidazole may cause seizures, or may affect liver function; and excess amoxicillin can cause gastrointestinal disturbances, may affect kidney or liver function, or lead to easy bruising or bleeding), particularly if they are absorbed in the gastrointestinal tract and metabolized by the liver. Therefore, interest has increased in therapeutic applications with extended exposure to the oral cavity and targeting the local sites of infection.

Oral opportunistic infections in immunocompromised patients are often persistent and difficult to heal, resulting in significant morbidity. Although available systemic therapies are in many cases less than ideal, the advantages of local drug delivery have yet to be realized owing to the inadequacies of current delivery systems. Oral diseases are common in immunosuppressed (HIV/AIDS) patients and are often the first signs of HIV/AIDS infection. Patients are treated with different systemic medications depending on the particular oral manifestations they present. The standard treatment of chronic HIV-associated periodontitis is thorough scaling and root planing, local delivery antibiotics, oral hygiene instructions, three-month recall and an at-home antimicrobial mouth rinse. For more aggressive forms of HIV-associated periodontitis such as necrotizing ulcerative periodontitis (NUP) and linear gingival erythema, systemic antibiotics are typically prescribed. About 10% of cases of HIV-associated periodontitis do not respond to conventional local therapy and systemic antibiotics (Murray, 1994). With the substantial morbidity attributed to HIV-associated oral diseases, for example NUP, it is increasingly important that these lesions be treated by the most effective, least invasive and least painful means. NUP is a painful and debilitating condition and frequently the patient is unable to achieve adequate nutrition or oral hygiene. This can lead to increased bleeding and an increased risk of HIV transmission (Murray, 1994). To a lesser degree, the same can be said of HIV-associated periodontitis, and given the increasing evidence of the strong association between periodontal disease and systemic complications, including cardio-

vascular disorders, diabetes and kidney disease, there is growing emphasis on maintenance of oral health in all individuals as an essential component of preventive medicine (Garcia *et al.*, 2001).

Commonly in HIV/AIDS patients there can also be oral manifestations caused by any of a number of viral infections which can include:

- Epstein–Barr virus (EBV);
- VZV (varicella virus, zoster virus, human herpes virus 3 (HHV-3) and varicella zoster virus (VZV) – all referring to the same viral pathogen which is responsible for 'Shingles');
- Herpes simplex virus (HSV) and cytomegalovirus (CMV) or human herpes virus-6 (HHV-6);
- Kaposi's sarcoma-associated herpes virus (KSHV) or HHV-8 (Cavert, 2005).

Finally, another oral condition we have recently considered that might prove to be a suitable target for oral drug delivery is the erosive form of oral lichen planus (Curran *et al.*, 2008). Unlike the other syndromes described in this section that are caused by a bacterial, fungal or viral agent; lichen planus is a chronic disease of unknown etiology that affects 0.5–1% of the population. While the cause of oral lichen planus has not been definitively established, it is thought to be an autoimmune disorder, but the altered self-peptide is unknown. The role of autoimmunity in the pathogenesis is supported by many clinical and cytological features, including:

- chronicity (onset in adults)
- predilection for females
- association with other autoimmune diseases
- depressed immune suppressor activity
- the presence of autocytotoxic T-cell clones.

Most familiar to clinicians is the asymptomatic reticular form of lichen planus that appears most commonly as a lace-like plaque on the buccal mucosa and requires no treatment. Less familiar is erosive lichen planus (ELP) which is characterized by diffuse erythema with desquamation and ulceration of the oral tissues. ELP accounts for up to 46% of cases of lichen planus. ELP can cause significant morbidity. It can have a major impact on quality of life because of the severe pain and burning that often accompanies this disease. Patients often cannot maintain normal nutritional or oral hygiene habits. As mentioned for the other disorders enumerated in this section, this in turn can compromise dental, periodontal and general health.

The most common treatment for erosive lichen planus is topical application of anti-inflammatory agents such as corticosteroids of various potencies in a tasteless gel or ointment form, sometimes in conjunction with systemic corticosteroid therapy. Topical therapy involves applying a small

amount of the agent to the affected area 3–4 times per day (Lozada-Nur and Miranda, 1997). Repeated application is necessary because saliva dilutes the ointment or gel, thus reducing the effectiveness of the therapy. With topical corticosteroids, applications are tapered to the amount of medication that is necessary to maintain oral comfort. Problems with this plan include difficulty in calibrating individual dosing, ingestion of excess medication, compliance with multiple daily dosing and a complicated taper routine. Patients often apply too little medication and skip doses; this inconsistent therapy can lead to exacerbation of lesions; it then becomes difficult to regain control of the condition. Repeated administration of systemic corticosteroids is not without significant side effects (high blood glucose levels, glaucoma and cataract formation, osteoporosis, occurrence of acne, sleeplessness and weight gain).

22.3 Design goals for modern oral drug delivery systems

The delivery of antiviral and antimicrobial drugs within safe and effective exposures in the oral cavity is an important goal in controlled release formulations. We have sought to develop a simple method involving a biocompatible copolymer (ethylene vinyl acetate, EVA) to deliver drugs in lower, locally effective concentrations and at near constant rates over an extended period of time (Kalachandra *et al.*, 2002; Lin *et al.*, 2003). This EVA copolymer system is able to release many of the drugs we have tested at an essentially constant rate over several weeks and therefore we hypothesized it would be useful for the treatment of oral infections.

Oral maladies, associated with HIV/AIDS are commonly the first manifestations of the disease and remain problematic in patients not treated adequately with the latest drug regimens (Murray, 1994). In the worst cases, HIV-associated oral lesions cause unbearable discomfort and may increase the rate of acquisition of additional opportunistic infections owing to a breakdown in the oral epithelium, leading to further morbidity.

Conventional treatment of these oral infections consists of systemic administration of antibacterial, antifungal or antiviral drugs at such high concentrations – 3–4 g per day – that significant toxicity from the drugs may ensue. To avoid potential side effects, and deliver therapeutic agents directly to the site of these oral infections, we sought to develop a novel, oral drug-delivery device to address these HIV/AIDS complications, with the possibility of beneficial application in the general population as well (Kalachandra *et al.*, 2002; Kalachandra *et al.*, 2006).

Delivery of a drug molecule to a target tissue that needs medication is known to be a complex process. Many factors will affect the successful delivery. In oral drug delivery systems, these factors can be biological factors, drug factors and delivery system factors (texture, taste, appearance, release characteristics, size, shape, allergenicity, etc.).

The specific requirements of the drug delivery system include:

- a delivery device design that would encourage compliance
- a design that would reduce variability in drug delivery
- biocompatibility of all components
- a design that would primarily release medication in the region of oral infection, but could provide benefit throughout the mouth for drugs that could be transported by salivary flow
- a design that preserves drug integrity during the manufacturing process
- a system that would allow some adjustment of drug delivery rate, since it was not possible to predict with complete accuracy the necessary delivery rate in the context of continuous local delivery.

22.4 Actual devices based on biocompatible materials

CDA has both antifungal and, to a limited extent, antiviral activity, as well as being an effective antibacterial, making it a multifaceted antimicrobial. Therefore, it has been the initial drug that we have chosen to characterize in the majority of our *in vitro* testing and the only drug used so far in our human clinical safety testing (Arnold *et al.*, 2008).

We have demonstrated sustained drug release properties with an EVA copolymer system. All release studies exhibited an initial 'burst' effect (higher amounts of drug released in the first 1 to 2 days) followed by a slow and sustained drug release. In order to avoid free radicals generated during polymerization, which is the case in some monomer/polymer systems (possibly degrading the drugs), copolymer systems were chosen rather than unpolymerized monomer systems. Studies of drug delivery systems based on other polymers/copolymers have also been performed. The systems include polyethyl methacrylate (PEMA), copolymer of ethyl methacrylate and *n*-hexyl methacrylate (poly(EMA-co-HMA)) and polymer blends of these polymers. Based on the drug delivery and mechanical properties of all these systems, EVA copolymer system was chosen for use in clinical investigation. Therefore, the majority of our developmental work on oral drug delivery devices has been focused on the EVA copolymer system, primarily using the 40 wt% vinyl acetate form. The coating of drug-loaded films with drug-free layers (either with more 40 wt% vinyl acetate, or even with other forms such as 32 wt% vinyl acetate EVA) has been used to reduce the release rate of certain drugs. But for the most part, and especially in our clinical trials, we have exclusively utilized the very simply prepared drug-loaded EVA films and mouth guards described below.

Polymer casting solutions were prepared by dissolving first the drug and then 18 g of EVA (Elvax®; clinical grade containing 40 weight % vinyl

acetate, obtained from DuPont Chemicals, Wilmington, DE) copolymer beads in the appropriate ratio (for example, 40:1 for 2.5 wt% CDA) in 65 ml of dichloromethane in a stoppered conical flask. The solution was stirred with a magnetic stir rod at room temperature overnight and the entire volume was then poured into a 14 cm diameter glass PYREX® Petri dish. Each solution was allowed to dry into a film overnight in a fume hood by evaporation of residual solvent at room temperature (see Fig. 22.1).

For drug release testing, we typically cut three 3 cm × 3 cm × 0.08 cm drug loaded polymer thin square films from the dry films. The square films (see Fig. 22.2) were placed in a volume of 10 ml distilled water at 37°C to collect the drug released daily. Alternatively, for clinical studies, the films were further processed into custom-made mouth guards. Models of the participants' teeth and gums were made using dental impression material and stone (see Fig. 22.3). The mouth guards were fabricated by using heat to soften the drug-loaded film and vacuum to adhere the film to the model (see Fig. 22.4). After the film re-solidified and cooled, the mouth guard was trimmed to cover the teeth and gum tissue (see Fig. 22.5).

To remove surface (non-entrapped) drug excess, which we have described as responsible for the 'burst effect' in our descriptions of *in vitro* drug release profiles, the mouth guards were then soaked in 100 ml of distilled water for 24 hours. Following the 24-hour soak they were rinsed twice for approximately 20 seconds in 70% ethyl alcohol to assure removal of contaminants during processing and handling.

Fig. 22.1 Drug-loaded EVA copolymer film of approximately 0.8 cm thickness formed after overnight drying at room temperature to remove dichloromethane cosolvent.

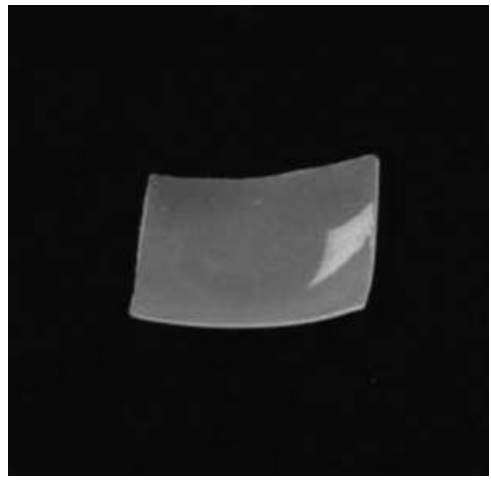

Fig. 22.2 3.0 cm square film sample cut from drug-loaded EVA copolymer film. These squares were employed to measure quantitatively drug release rates by immersion in distilled water or other test media changed daily, with spectrophotometric measurements taken of drug concentrations in the daily media samples. Triplicate samples taken from different regions of the films typically showed excellent reproducibility, suggesting good lateral homogeneity of the drugs contained in the films.

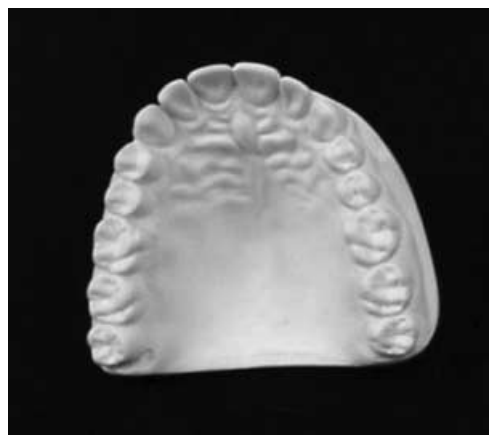

Fig. 22.3 Stone impression positive model created from alginate impression taken of the maxillary (upper) arch. This simple method enables proper fitting of the mouth guard devices and also allows preparation of multiple identical mouth guards.

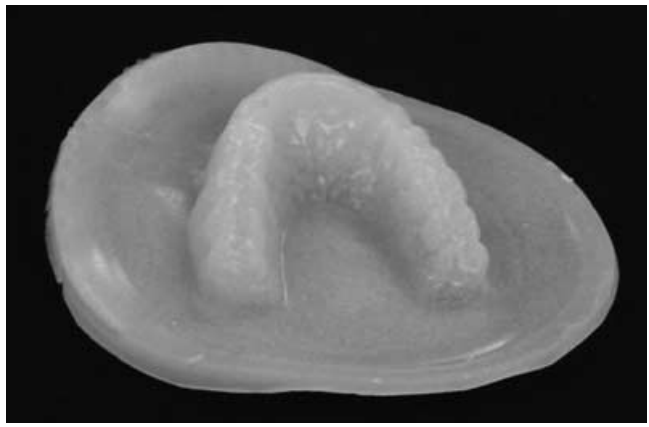

Fig. 22.4 Product of vacuforming device using drug-loaded EVA film heated and then lowered onto the stone model. As shown, a significant amount of film material is left that is generally saved as retention samples.

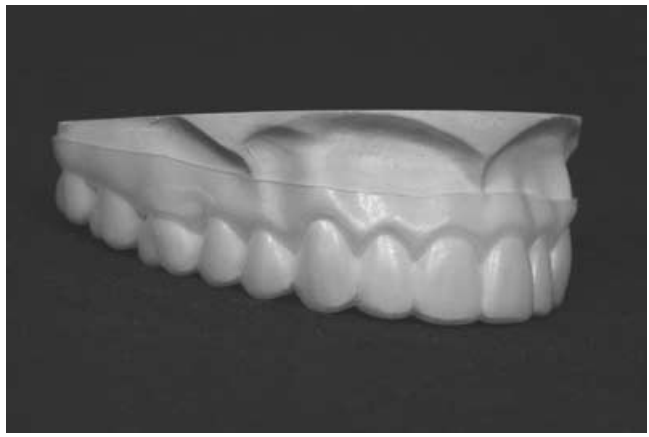

Fig. 22.5 Demonstration of the final mouth guard drug delivery device formed from EVA copolymer fitted onto the stone model of the maxillary arch. To achieve contact of the device with the peridontium, approximately 5 mm of material was left above the gum line. The bottom side of the film, which we observed routinely to have a visually rough surface and was later confirmed by scanning electron microscopy and microbiostatic testing to be enriched in drug concentration relative to the smooth top side of the film, was oriented to be in direct contact with the teeth and gums in an effort to direct drug delivery to the immediate contact sites.

22.5 Use of additives to accelerate release and solubility of problematic (water-insoluble) drugs

The release rate of the antifungal drug nystatin from EVA was studied with the addition of non-ionic surfactants Tween 60 and Cremophor RH 40. Studies have shown that the solubility of sparingly water-soluble drugs can be increased through the addition of surfactants. The addition of surfactants promoted a much higher release of Nystatin from a chewing gum formulation used as a drug delivery device. There are other reports of enhanced drug release owing to the addition of surfactants. Nystatin release was quite low in distilled water or in phosphate buffered saline, consistent with its known poor aqueous solubility. The rate of release was substantially enhanced by addition of surfactants and, as we have found for many other drugs, by increasing the initial amount of drug loaded into the EVA copolymer as well (Kalachandra *et al.*, 2002). When surfactant molecules are dissolved in water above the critical micelle concentration (CMC), they form aggregates known as micelles. The formation of micelles can increase the solubility of sparingly soluble substances in water (Patel *et al.*, 2001; Kalachandra *et al.*, 2002). The release rate of Nystatin increased with addition of surfactants probably due to incorporation into the surfactant micelles which allow the poorly soluble Nystatin to partition better into aqueous environments. Also, it is possible that the surfactant lowers the interfacial tension between the polymer matrix and the dissolution medium; either one or both mechanisms in combination would be consistent with our observations of increased release rates.

22.6 Maintaining and assuring stability of drugs during device manufacturing

One of the beneficial aspects of the mouth guard manufacturing procedure we have employed is that it avoids exposure of the drugs either to significantly elevated temperature (as needed in polymer extrusion or molding processes) or to free radicals (as in polymerization of monomeric molecules). Nonetheless, to assure that the therapeutic drugs we wish to dispense using the oral drug delivery device remain unaltered throughout the entire process of solvent evaporation and even device storage and release into the mouth, we had to employ a variety of approaches. For the antimicrobial CDA, we developed (Arnold *et al.*, 2008) microbiological activity assays based on suppression of colony growth of periodontal pathogens such as *Porphyromonas gingivalis* and several oral yeast species including *Candida albicans*.

Drug stability during film casting and release has also been demonstrated by characterizing the drug-loaded EVA films using ^{1}H nuclear magnetic resonance (NMR) and cross-polarization/magic angle spinning (CP/MAS)

^{13}C solid state NMR. ^{1}H NMR spectra were used to investigate the drug stability during the film casting and after release into aqueous media (Kalachandra *et al.*, 2005, 2006). The NMR spectral measurements showed that chemical shifts for ganciclovir (GCY) and GCY released from EVA were identical, demonstrating that GCY remained unchanged during the film casting and release processes.

22.7 Future trends: multi-drug devices

The development of a novel delivery system will permit the use of specific topically active agents that are not absorbed by the gastrointestinal tract and that cannot be easily administered intraorally over a prolonged period of time, owing to salivary clearance. Such a new delivery system could provide optimal pharmacodynamic drug release, whether that is slow, constant release or perhaps using some other pattern, assuming the device could be appropriately adjusted. Initially, *in vitro* rate of drug release measurements helped provide a basis for establishing a novel approach (treatment modality) for sustained intra-oral drug delivery over extended time periods. Then, clinical testing with devices releasing a single drug agent helped establish pharmacodynamic ranges of local (salivary) levels within acceptably low systemic (serum) levels of drug release. At the same time, measurement of a variety of oral health parameters (periodontal pocket depths, oral bacterial and fungal levels, along with bleeding on probing and gingival index, to name a few) has allowed quantitative and early assessment of possible efficacy in limited gingival disease conditions. In forthcoming studies, we plan to examine further both the oral health parameters mentioned above and the resolution of HIV-associated oral diseases.

In ideal cases, for diseases using a single organism causality, or using drugs with a sufficiently broad spectrum, we hope that a return to, and maintenance of, oral health can be achieved using a single drug oral delivery system. Many diseases of the oral cavity may be complex enough to require a multi-faceted approach. Indeed, suppression of significant numbers of bacteria may result in opportunistic infection by yeasts (*Candida*) and fungi, as has been described in the intestinal tract and the vagina (Mardh *et al.*, 2003), as well as in the oral cavity (Cannon *et al.*, 1995).

Thus, oral drug delivery systems may achieve their optimal effectiveness when they release not one, but several agents. Such 'therapeutic cocktails' could presumably be optimized, given enough time for research, for each possible oral disease condition that might exist in the general population. Or, combining another emerging advance in the medicine of tomorrow, *ex vivo* testing of patient samples against an array of possible drug cocktails could point to the most effective combination for a specific patient. Such an 'individualized medicine' approach is already being described (Evans and Relling, 2004) as a possible future treatment for other systemic

infectious conditions, but could perhaps be most safely evaluated (and most easily observed for effectiveness) in either oral or dermatological applications.

22.8 Dedication

Dr. Siddugari (Sid) Kalachandra, Research Professor in the University of North Carolina's School of Dentistry and Principal Investigator of the research projects described in this chapter, unexpectedly passed away on March 14, 2008. His wife Renuka Devi predeceased him and he is survived by his son and daughter-in-law, Drs. Krishna and Sowmya Kishor. This chapter is dedicated to them as the legacy that 'Kala' intended.

Dr. Kalachandra was born in Venkatagiri, Andhra Pradesh, India in 1939. He received his early education in India, culminating in a PhD in Physical Organic Chemistry from the University of Madras in 1970. He then taught for eight years at Madras Christian College before bringing his family to the United States. After appointments at the University of West Virginia, the University of Texas and the University of Florida, he came to the Uni-

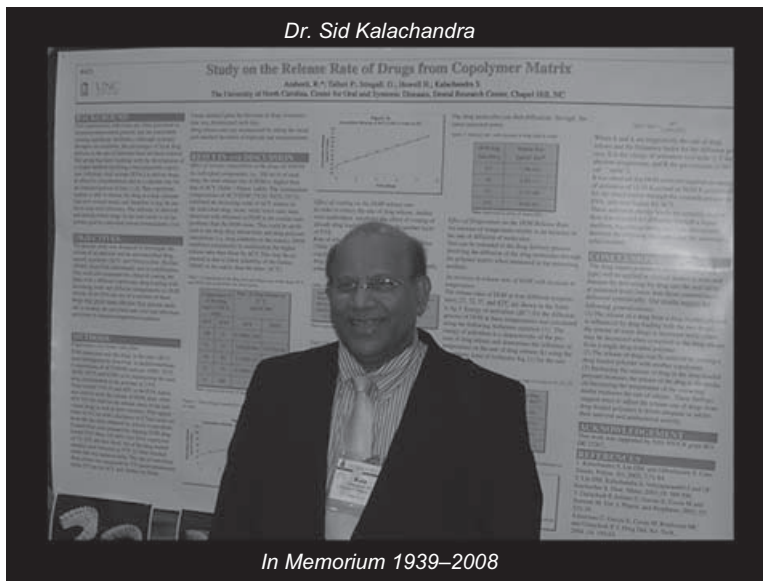

Fig. 22.6 Professor Kalachandra, shown here, as he proudly presented some of his group's preliminary research findings at the 2007 New Orleans Meeting of the International Association for Dental Research and the American Association of Dental Research. Affectionately known as Kala to his many friends and colleagues around the globe, his jovial good nature, boundless curiosity and passion for his research will doubtless leave a positive impact on the fields of dental research, materials research and polymer chemistry.

versity of North Carolina at Chapel Hill, where he worked in the Dental Research Center and the Department of Periodontology on the synthesis and characterization of materials for use in dentistry.

Dr. Kalachandra traveled widely; he was Visiting Professor at Queen Mary and Westfield College of the University of London from 1996 to 2002 and Research Professor at Virginia Tech from 1992 to 2000. He was a kind, gentle, loving man who will be missed greatly by his family, friends and collaborators from around the world. A memorial service at UNC on March 30, 2008 was attended by hundreds of friends, family and colleagues.

22.9 Acknowledgements

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23

Formulating tooth-friendly beverages, confectionery and oral care products

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Abstract: Beverages, confectionery and oral care products may cause dental caries and/or erosion. In the first part of this chapter, the properties of such products which may be detrimental to the dental hard tissues are discussed. In the latter part of the chapter, methods for modifying products to render them 'tooth friendly' are reviewed and explained, and examples from the literature are discussed.

Key words: beverage, caries, dental erosion, food, tooth friendly.

23.1 Introduction

When considering the effects of beverages, confectionery and oral care products on dental health, there are four main factors which must be taken into account:

- acids
- sugars
- calcium and phosphorus concentrations and
- fluoride

Sugars contribute to dental caries: the metabolism of dietary sugars by plaque bacteria which results in acid production and consequent dissolution of the enamel and dentine. Dietary acids contribute to dental erosion: the dissolution of the dental hard tissues by acids not of bacterial origin – in this case, extrinsic acids which form a constituent of the beverage, confectionery or oral care product. Calcium and phosphorus, in the form of phosphate, inhibit acid-mediated dissolution of the dental hard tissues owing to the common ion effect. Fluoride has a complex mechanism of action but is

generally accepted to protect teeth against acidic dissolution by depositing a layer, and/or reservoirs, of fluoride-rich mineral on the hydroxyapatite crystal surface.

The diet-related pathogenic conditions of dental caries and erosion have been discussed elsewhere, in Chapters 1 and 4, respectively. This chapter comprises a summary of the properties of beverages, confectionery and oral care products that contribute to dental health problems, followed by discussion of how such products may be modified to render them 'tooth friendly'.

23.2 Properties of beverages which may compromise dental health

Beverages may cause, or contribute to, dental caries (Kidd and Joyston-Bechal, 1997); the link has been repeatedly emphasised in *in situ* studies (Duggal and Curzon, 1989; Tahmassebi and Duggal, 1996; Jensen *et al.*, 2000) and epidemiological investigations (Ismail *et al.*, 1984; Marshall *et al.*, 2003, 2007; Sohn *et al.*, 2006). The reason for this is, of course, that many drinks contain fermentable sugars. Carbonated drinks and fruit drinks and juices often contain sucrose: Coca Cola™, for example, contains 10.6% w/w sugars, as does Sprite™. Fruit drinks often contain sucrose and, if they contain a significant proportion of fruit juice, they will also contain fructose: Orange and apple juices contain around 10–11% total sugars. Tea and coffee are often sweetened with sucrose. Sports drinks, which are designed to provide energy and electrolytes during and after periods of strenuous exercise, often contain glucose; Lucozade™ contains 17% and Powerade™ contains 26.5% carbohydrate (all data obtained February 2008). Sucrose is considered to be the most cariogenic of the sugars but glucose, fructose and other sugars are also metabolised by plaque bacteria and contribute to the caries process (Kidd and Joyston-Bechal, 1997).

Many popular beverages are also acidic and are undersaturated with respect to hydroxyapatite; $S_{HA} < 1$ (Larsen, 1975) and could thus be expected to cause dental erosion. Beverages which have been shown to be erosive in laboratory studies include carbonated soft drinks (Rytomaa *et al.*, 1988; Grobler *et al.*, 1990; Lussi *et al.*, 1993), fruit-based and fruit-flavoured juices, drinks and waters (Rytomaa *et al.*, 1988; Grobler *et al.*, 1990; Lussi *et al.*, 1993; Brown *et al.*, 2007), herbal teas (Brunton and Hussain, 2001; Phelan and Rees, 2003), wines (Meurman and Vesterinen, 2000), sports drinks (Rytomaa *et al.*, 1988; Meurman and Frank, 1991) and fruit-flavoured alcoholic drinks (Rees and Davis, 2000). Tea, coffee, still mineral waters and milk- or yogurt-based drinks are usually regarded as unlikely to cause dental erosion (Rytomaa *et al.*, 1988) and, although carbonated mineral waters may be erosive *in vitro*, they are considerably less so than many other soft drinks (Parry *et al.*, 2001).

The erosivity of drinks is related to their pH, buffer capacity and the type of acid(s) in the drink. The pH of common non-alcoholic beverages (excluding mineral waters) is typically in the range 2.5–4.0, with an average value of 3.2 or 3.3 quite widely accepted (Feldman and Barnett, 1995; Lussi and Jaeggi, 2006). Wine has a pH of 3–4 (Lussi and Jaeggi, 2006; Waite and Daeschel, 2007); beer is typically higher, at around 3.8–5 (Nogueira *et al.*, 2000; Lussi and Jaeggi, 2006). The most common acids to be found in these drinks are weak organic acids including citric (citrus fruit-based drinks), malic (apple-based drinks) and phosphoric (colas) acids; other acids include tartaric acid (grape juices and wine), lactic acid (dairy-based drinks), ascorbic acid (vitamin C, found in many fruits and added to some drinks) and quinic and shikimic acids (cranberry drinks). Buffer capacity is an interesting and perhaps controversial topic and is discussed in Section 23.5.

23.3 Properties of confectionery which may compromise dental health

Confectionery is often a significant dietary source of fermentable carbohydrate, particularly in younger individuals, and has been considered to be a causal factor of dental caries for decades, since the pioneering work of Bibby in the 1950s (Bibby *et al.*, 1951). The sugars in confectionery are readily metabolised by plaque bacteria resulting in a decrease in plaque pH and demineralisation of the underlying enamel or dentine. Plain chocolate, for example, contains around 60% sugars, while biscuits and cakes may contain between 10 and 50% sugar. The sugar content of confectionery such as boiled sweets may be considerably higher.

Confectionery may also be acidic. Fruit-flavoured or 'tangy' sweets, such as those flavoured with lemon, lime, rhubarb and other sour-tasting flavours, may contain organic acids such as citric and tartaric acid, either from the fruits themselves or as acidity and taste regulators. It has been demonstrated that acidic sweets can cause dental erosion *in situ* (Lussi *et al.*, 1997), which can presumably be attributed to a reduction in salivary pH in the vicinity of the tooth surface similar to that observed by Jensdottir (Jensdottir *et al.*, 2005b).

23.4 Properties of oral care products which may compromise dental health

Here it is necessary to draw a distinction and make clear the scope of this chapter. In discussing tooth-friendly oral healthcare products, the products themselves, any detrimental affects they may have on tooth tissues and how to modify the products to prevent this, will all be discussed. Products which are designed to *protect* the teeth from *subsequent* erosive or carious epi-

sodes will not be included, however, this is a much broader interpretation of the adjective 'tooth friendly' and implies a longer-term, protective effect rather than a straightforward 'is product X harmful or not?' approach.

Few oral care products could genuinely be described as detrimental to oral health, if they are used as directed by the manufacturer, as distinct from instances of tooth abrasion from excessive or abusive toothbrushing, for example. The question of tooth abrasion by toothpastes, particularly in combination with erosion, is an interesting one, but beyond the scope of this chapter and the reader is referred to review articles on the subject (Barbour and Rees, 2006; Addy and Shellis, 2006). There are, however, some other concerns regarding oral healthcare products which could detrimentally affect oral health and it is pertinent to consider these here.

Sugars are typically not found in oral care products, but acids may be, and there have been reports of erosive mouth rinses and related products. The pH of mouth rinses varies between 2 and 7, although most fall between 6 and 7. A case report of moderate to excessive use of diluted TCP™ as a mouth rinse over a 2-year period identified this agent as causing extensive erosion in an otherwise healthy patient. TCP™ is a general purpose antiseptic solution available in the UK and elsewhere and may be used as a mouth rinse if diluted 1:5 with tap water. Its erosivity is, on reflection, not surprising given that Poyser and colleagues' investigation revealed that the TCP™ preparation had a pH of 2.4 (Poyser *et al.*, 2004). In a laboratory study, Pretty and co-workers investigated a number of popular mouth rinses available in the UK and found that one (Listerine™, Pfizer), caused substantial enamel erosion and this happened only after 14 hours over exposure to the mouth rinse (Pretty *et al.*, 2003). Pontefract has also identified acidified sodium chlorite as a potentially erosive mouth rinse and demonstrated that it caused comparable erosion to orange juice in an *in situ* study (Pontefract *et al.*, 2001).

There has also been one interesting report describing dental erosion by oral care products sold in Finland, but not caused by acids. Rytomaa and coworkers observed that one mouth rinse (Calcusan™) caused four times more erosion than the positive control, a sports drink, and attributed this to chelation by ethylene diamine tetraacetic acid (EDTA) in the formulation, which was, arguably bizarrely, designed to prevent accumulation of heavy metals on the oral mucosa. (Rytomaa *et al.*, 1989). It appears, from internet research, that this mouth rinse may have been withdrawn from sale.

23.5 Acids and dental erosion: approaches to, and mechanisms of, reducing acid-mediated tooth dissolution

The simplest approach to modification of products to reduce dental erosion is to address directly the properties of the product which lead to the erosion

in the first place. Applications of these approaches are described below; it is the intention in this section to outline the mechanism of action of such approaches.

Hydroxyapatite, the main constituent of enamel and dentine, is sparingly soluble in acids and solubility increases with decreasing pH. Thus the simplest means of reducing hydroxyapatite dissolution rate and thus enamel or dentine erosion, is by raising the pH of the dissolving solution. This may be achieved by reducing the concentration of acid in the product, but this may not be possible or practical if, for example, the acids originate from fruits in a fruit juice or drink. An alternative approach is partially to neutralise the acid by adding buffering chemicals.

An important and related issue is the *type* of acid and the extent to which it buffers changes in pH. Strong acids such as hydrochloric acid dissociate fully in aqueous solution, resulting in equal concentrations of H^+ and Cl^- and negligible concentrations of the HCl molecule. Weak acids, by contrast, do not dissociate fully and an equilibrium is established between the dissociated H^+ and X^- (where X^- is the acid anion) and undissociated HX molecules. The significance of this for dental erosion can be comprehended when one considers that, when hydroxyapatite dissolves, it releases OH^- ions which neutralise some of the H^+ ions from the acid. In a strong acid, this will result in an increase in pH and a consequent reduction in dissolution rate. In a weak acid, however, a reduction in concentration of H^+ stimulates more of the HX molecules to dissociate, releasing further H^+ (and X^-) and maintaining the low pH. Thus dissolution will proceed at a more sustained, higher rate in a weak acid than a strong acid if the buffer capacity is appreciable at the pH of the solution. Although the relationship between acid characteristics such as pK_a, concentration and dissolution rate is apparently complex (Margolis and Moreno, 1992; Margolis *et al.*, 1999; Barbour and Shellis, 2007), it is conceivable that judicious choice of acid in acidic products may be used in future to aid formulation of low-erosive products.

Buffering is often measured by 'titratable acidity': the molar equivalent of OH^- required to adjust the pH of a drink to a predetermined value – often 7. This is of dubious relevance since it is difficult to imagine a situation in which a drink would be in contact with a tooth for sufficient time for the pH to be raised from, say, 3.3 to 7. Titratable acidity is widely quoted in the literature, perhaps because it is easy to measure in the laboratory, although it can be argued that if a drink has pH of 3.3, buffering at pH 4, or 5, or 6, is barely relevant to the *in vivo* situation. Of greater applicability is a measure of buffer capacity which applies at the original pH of the drink. A recently published study describes the use of differential buffer capacity in this regard (Barbour and Shellis, 2007) and other authors have discussed related parameters (Jensdottir *et al.*, 2005a).

Another means of reducing the rate of hydroxyapatite dissolution in acidic solution is to increase the concentration(s) of the component ions of hydroxyapatite in the dissolving solution. Thus, by adding calcium or phos-

phate to an acidic solution, one raises the degree of saturation with respect to hydroxyapatite (S_{HA}) of that solution and reduces the thermodynamic driving force for dissolution. Both calcium and phosphate are effective, but phosphorus is more complicated as it will take different chemical states according to the pH of the solution. One may obtain PO_4^{3-}, HPO_4^{2-} or $H_2PO_4^-$, depending on pH, and only the former affects S_{HA} and thus the hydroxyapatite dissolution rate. Even when this is taken into account, it has been shown that calcium is more effective than phosphate at reducing erosion. In one study, enamel softening was compared in solutions with the same S_{HA} but different calcium and phosphate concentrations, and those with higher calcium caused less softening than those with higher phosphate (Barbour *et al.*, 2005a).

Adjustment of pH, acid type and S_{HA} are thus perhaps the simplest chemical approaches to formulating acidic products which could be described as 'tooth friendly'. Supplementation with fluoride is another potentially useful route of investigation. The mechanism of action of fluoride with respect to caries prevention is complex and not entirely characterised, but its effectiveness with respect to erosion is even less clear. Fluoride supplementation of erosive foodstuffs is discussed in Section 23.7. Of course, the acceptable concentration of fluoride in an oral healthcare product may be much higher than that in a food or beverage product and thus fluoride in oral mouth rinses may help to render potentially erosive products tooth friendly. This is discussed in Section 23.9.

Another approach is to incorporate constituents into products which deposit a protective layer on the tooth surface, much as the saliva deposits a layer of proteins known as the pellicle on the tooth surface. The pellicle is known to have a protective, albeit short-lived, function in that it delays the onset of erosion. If a drink, item of confectionery or oral healthcare product could be designed that deposits a protective, pellicle-like layer on the tooth, this may prove a beneficial alternative or adjunct to the more conventional methods described above.

Applications of these technologies to beverages, confectionery and oral healthcare products are discussed in turn in Sections 23.7–23.9.

23.6 Sugars and dental caries: approaches to, and mechanisms of, reducing carious tooth loss

There are three main approaches to formulating beverages and confectionery which are non-cariogenic: elimination of the substrate for bacterial metabolism, interference with the plaque biofilm, or a means of protecting or 'strengthening' the tooth mineral against dissolution. The first is primarily achieved via the substitution of sugars with non-cariogenic sweeteners; the second may be achieved by incorporating substances into the product which interfere with bacterial properties or processes such as attachment,

membrane integrity, metabolism and biofilm formation. These two approaches are intimately linked; several sugar substitutes also interfere with the plaque biofilm in some way, and thus these will be discussed together. The third, the protection of the tooth tissues against carious dissolution, primarily concerns fluoride supplementation of foods and, predominantly, drinks. Oral care products are unlikely to pose a cariogenic risk to the consumer.

The most common means of formulating beverages and confectionery with low cariogenic potential is to utilise non-cariogenic sweeteners in place of sugar. By far the most common are the sugar alcohols, also known as polyols, such as xylitol, sorbitol and mannitol (Van Loveren, 2004). All of the sugar alcohols have proven to be hypo- or non-acidogenic when tested with *in vitro* biofilms of oral bacteria and in clinical plaque pH tests, although there are some concerns that bacteria such as *Streptococcus mutans* may adapt to ferment sorbitol under conditions of frequent exposure.

It is widely accepted that xylitol is the most effective sugar alcohol in terms of preventing caries. It has been claimed that xylitol is not only non-cariogenic but actively *anti*-cariogenic, but this has been disputed in a review of the subject (Scheie and Fejerskov, 1998). Although there is evidence that supports the anticariogenic claim with chewing gum, it seems that xylitol reduces the proportion of mutans streptococci in dental plaque (Soderling *et al.*, 2001) and can reduce binding of plaque, and mutans streptococci, to tooth surfaces (Soderling *et al.*, 1991) – similar outcomes have not been observed with the use of xylitol-containing lozenges (Birkhed *et al.*, 1979; Tenovuo *et al.*, 1997).

Sorbitol is also an effective non-cariogenic sweetener, and although some studies suggest that it is less effective than xylitol, there is also evidence to contradict this (Van Loveren, 2004). Most sorbitol studies focus on its use in chewing gums, in which it is associated with reduced caries compared to sucrose-containing gums or no gum at all. Sorbitol, xylitol and other sugar alcohols in gums are discussed further in Chapter 21.

Other, 'intense' sweeteners may also be used as sugar substitutes in confectionery products. Such compounds contain no nutritive value and cannot be fermented by plaque bacteria; thus they do not cause caries. These include common synthetic compounds such as aspartame, acesulfame–potassium, saccharin and sucralose (Kidd and Joyston-Bechal, 1997) and some plant-derived products such as stevioside (Kinghorn *et al.*, 1998). Isomaltulose (palatinose) is a structural isomer of sucrose and is another alternative sweetener which may result in a reduction in caries, although it has been shown to be fermented by a significant proportion of oral bacteria. This may explain the observation that isomaltulose causes less caries than sucrose, but is still cariogenic to a limited degree (Matsuyama *et al.*, 2003).

The identification of substances which interfere with the caries process is fraught with difficulty. Biofilms are structurally, chemically and biologically complex structures, and vary from person to person and site to site. A

chemical which reduces, for example, *Streptococcus mutans* adherence in a single-species *in vitro* study may have no impact whatsoever on *in vivo*, multi-species plaque biofilms. Simple studies such as these abound in the literature and they are undoubtedly important as a means of identifying potential routes to controlling caries, but they must be taken as just that; preliminary studies which, if the results are promising, should be taken to indicate that further, more sophisticated, studies are required.

Fluoride supplementation of water and other beverages is controversial among certain societal groups, particularly those favouring so-called alternative medicine. 'Fluoride phobia' is largely unfounded and there is no doubt that fluoridation of mains water supplies has significantly improved oral health in a number of countries. The one criticism levelled at water fluoridation which merits serious consideration is the increased risk of fluorosis; there is, in fact, a trend which indicates greater occurrences of fluorosis in areas with fluoridated water (Whelton *et al.*, 2004), although it is necessary to weigh up the disadvantages of a certain incidence of fluorosis against the benefits of caries reduction. Fluoridation of drinks other than water is discussed in Section 23.7.

It is perhaps surprising that, five decades after it was suggested that fluoride could reduce caries, the mechanism of action of fluoride is still a matter of some debate. The accepted wisdom and the view held by many, is that the fluoride ion substitutes for the hydroxyl ion in the hydroxyapatite structure, thus forming fluorapatite or a mixed fluor-hydroxyapatite (ten Cate, 1999). These fluoride-containing apatites are less soluble than hydroxyapatite and require a lower pH in order to dissolve. Since only the outer layer of the crystallites is exposed to the oral environment, topical fluorides which cause the deposition of a very thin layer of fluoride-containing apatite on the surface of the hydroxyapatite effectively render the interface with the oral environment a fluoridated one.

Although this mechanism does have a role to play in caries prevention, it is not thought to be the only way in which fluoride operates. It is known that fluoride concentrations in oral fluids following topical application remain elevated for much longer than would be expected from oral clearance rates (Duckworth and Morgan, 1991). This has been attributed to the formation of a reservoir of soluble fluoride-containing material which gradually releases fluoride; this is most commonly thought to be poorly crystalline calcium fluoride or a closely related material (ten Cate, 1997). The calcium fluoride-like mineral provides a reservoir of fluoride which may be released into the oral environment for an extended period after initial exposure and this is gradually converted to fluorapatite (ten Cate, 1997). Why this should provide greater protection than pure fluorapatite is not clear. What is apparent, however, is that the formation of calcium fluoride deposits occurs during topical application of fluoride-containing oral healthcare products but not from the relatively low concentrations found in fluoridated water.

It has been demonstrated that fluoride interferes with the metabolism and growth of pathogenic oral bacteria (Baehni and Takeuchi, 2003). Most of these effects, however, occur at fluoride concentrations considerably higher than those which are typically found in oral fluids and it is generally accepted that the most important role of fluoride in caries prevention is the chemical alteration of the tooth mineral and not the effects on plaque bacteria *per se* (ten Cate, 1999). Applications of these technologies to beverages, confectionery and oral healthcare products are discussed in turn in Sections 23.7–23.9.

23.7 Formulating tooth-friendly beverages

Many drinks, particularly carbonated drinks, are available in 'diet' or 'low-calorie' versions, in which the sucrose is replaced by one or more of the non-cariogenic sweeteners discussed in Section 23.6 in order to afford the desired sweetness. Such drinks are generally considered to cause minimal caries, since there is little or no fermentable substrate for the plaque bacteria (Kidd and Joyston-Bechal, 1997).

Fluoride supplementation of drinks has also shown promise with respect to caries prevention. Fluoridation of milk was first suggested in the 1950s as a means of reducing or preventing caries on a community-wide basis (Yeung *et al.*, 2008). Fluoridated milk is believed to be effective in reducing caries in children (Banoczy *et al.*, 1983; Gyurkovics *et al.*, 1992; Bian *et al.*, 2003; Weitz *et al.*, 2007) and may be a particularly useful approach in rural communities where water fluoridation is not practical (Weitz *et al.*, 2007). Of course, this does not technically fall into the definition of tooth-friendly beverages. Milk itself is neither cariogenic nor erosive, owing to its near-neutral pH and high concentration of calcium. The same can be said of the high levels of fluoride which occur naturally in some teas; the fluoride may offer dental benefits but the original drink cannot be said to be genuinely tooth-*unfriendly*. Fluoride has, however, been added to an experimental sports drink and this was found to cause a lower incidence of caries in rats than an unfluoridated drink (Sorvari *et al.*, 1986). Nevertheless, elimination of fermentable sugars remains the main means of generating non-cariogenic beverages.

'Diet' drinks only address half of the problem; non-cariogenic beverages are usually of comparable pH and acid content to the sugared versions and may cause dental erosion (Jensdottir *et al.*, 2006). Perhaps as a result of the success of fluoridated water and milk in reducing caries, some investigators have considered fluoridation of drinks to reduce erosion. Sorvari and co-workers tested the drink discussed above with respect to erosion and found that the addition of 15 ppm fluoride significantly reduced erosion on lingual surfaces (Sorvari *et al.*, 1988). Other investigators have found that fluoride supplementation of otherwise erosive beverages reduced the extent of

erosion (Fuks *et al.*, 1973; Shabat *et al.*, 1975). Larsen, on the other hand, observed that supplementation of drinks with 4–6 ppm fluoride had little effect on erosion and in one case prevented an erosive lesion developing only to promote the formation of a carious lesion instead (Larsen, 2001). In a subsequent study, Larsen concluded (despite the misleading article title) that supplementation of drinks with 3–8 ppm fluoride could reduce erosion if the initial drink pH was above 3, but not if it was below this value (Larsen and Richards, 2002).

Fluoride supplementation of drinks, whether for caries or erosion prevention, does not appear to have become a mainstream topic in the literature. This is perhaps because, in order to be effective, the drink would presumably have to be consumed regularly, and to the exclusion of other beverages, and there are ethical and practical problems with advising a patient to drink a particular commercially available drink on a regular basis. Furthermore, individual drink manufacturers may be reluctant to add fluoride to drinks in the face of vociferous anti-fluoride campaigners, at least without the support of a reputable association such as Toothfriendly International (TI, see below) or a national dental association.

In the mid-1970s, important progress in the field of non-erosive beverages was made by adding calcium to soft drinks (Reussner *et al.*, 1975). Grenby discussed this and a number of subsequent applications of calcium and phosphate supplementation, predominantly *in vitro* and animal studies, in a thorough review in 1996 (Grenby, 1996). A series of *in vitro* and the first human *in situ* reports from the Addy group in 1999 described the development of a fruit-based beverage with a reduced erosive potential (West *et al.*, 1999; Hughes *et al.*, 1999a, 1999b). The experimental drink had an elevated pH, compared to many soft drinks, of 3.8, and a calcium concentration of 10 mM. Although this only resulted in a degree of saturation of $S_{HA} \sim 0.10$ it was, perhaps surprisingly, very effective in reducing erosion both *in vitro* and *in situ*, with the drink causing approximately one-quarter of the erosion of an orange juice with a similar pH but a lower calcium concentration. More recently, Attin and co-workers have demonstrated that solutions with concentrations as low as 1 mM calcium or 0.5 mM calcium, 0.5 mM phosphate and 0.03 mM fluoride, significantly reduced erosion in citric acid and a number of commercially available soft drinks (Attin *et al.*, 2003, 2005). Hara and Zero have also demonstrated that the calcium concentration of a range of drinks correlates well with their ability to cause erosion *in vitro* (Hara and Zero, 2008).

There have been other developments in the field of low-erosive drinks which work by different mechanisms than the addition of calcium. In one study of a series of food gums, it was demonstrated that addition of 0.02%w/v xanthan gum or carboxymethylcellulose to citric acid solutions (pH 3.2) significantly reduced the hydroxyapatite dissolution rate *in vitro* (Barbour *et al.*, 2005b). Xanthan was also incorporated into an experimental soft drink in an *in situ* trial, but did not offer any benefit in terms of enamel

erosion in comparison with the same drink without xanthan (West *et al.*, 2004). The *in vitro* study described above also demonstrated that addition of 0.02%w/v pyrophosphate, tripolyphosphate and polyphosphate ($n = 25$) reduced the hydroxyapatite dissolution rate by up to 64% (Barbour *et al.*, 2005b).

Recently, selected food proteins have been suggested as potential anti-erosive ingredients in beverages (Barbour *et al.*, 2008; Hemingway *et al.*, 2008). In a series of *in vitro* studies, the milk protein casein and the hen egg protein ovalbumin were shown to reduce the hydroxyapatite dissolution rate substantially in citric acid solutions representative of soft drinks. Dissolution was reduced by 50% at pH 2.8–3.6 by the addition of 0.02% whole casein. Addition of casein phosphopeptide–amorphous calcium phosphate to a sports drink also reduced dental erosion, although this was attributed in part to the elevation in pH, rather than or in addition to the effect of the peptide itself (Ramalingam *et al.*, 2005). Although this work is in a preliminary phase of development, such food proteins may, in future, find application in beverages with reduced erosion potential.

23.8 Formulating tooth-friendly confectionery

Since a World Health Organisation recommendation in 1983, there have been concerted efforts to promote the oral health benefits of tooth-friendly sweets in several countries. The first instance of this was in Switzerland in 1985, when a campaign was mounted to promote sweets which were non- or hypo-acidogenic as determined using intra-oral plaque pH measurements (Guggenheim, 1995). Sweets which fulfilled the requirements were labelled with a 'Happy Tooth' symbol for easy identification by the consumer and an advertising campaign was mounted to raise public awareness of the benefits of choosing these over other, cariogenic sweets. The initiative was very successful and similar campaigns have since been developed in countries including Germany, Japan, Korea and Turkey (Guggenheim, 1995). Toothfriendly International (TI) is a not-for-profit organisation, based in Switzerland, which arranges product testing to determine cariogenicity and erosive potential and assess whether they might be awarded tooth-friendly status. Example of intra-oral plaque pH curves for two confectionery products, one which contained sugar and one classed as tooth friendly, are shown in Fig. 23.1. It can be seen that the sugar-containing confectionery caused plaque pH to fall by approximately 2.5 pH units (Fig. 23.1a), whereas the tooth-friendly confectionery caused a much smaller reduction in plaque pH by around 0.3 pH units (Fig. 23.1b). The subsequent sucrose challenge is included to demonstrate that the model is robust.

Many items of confectionery classified as tooth friendly by TI are chewing gums and these are discussed in Chapter 21. Other tooth-friendly sweets typically contain no, or virtually no, sucrose or other fermentable carbohy-

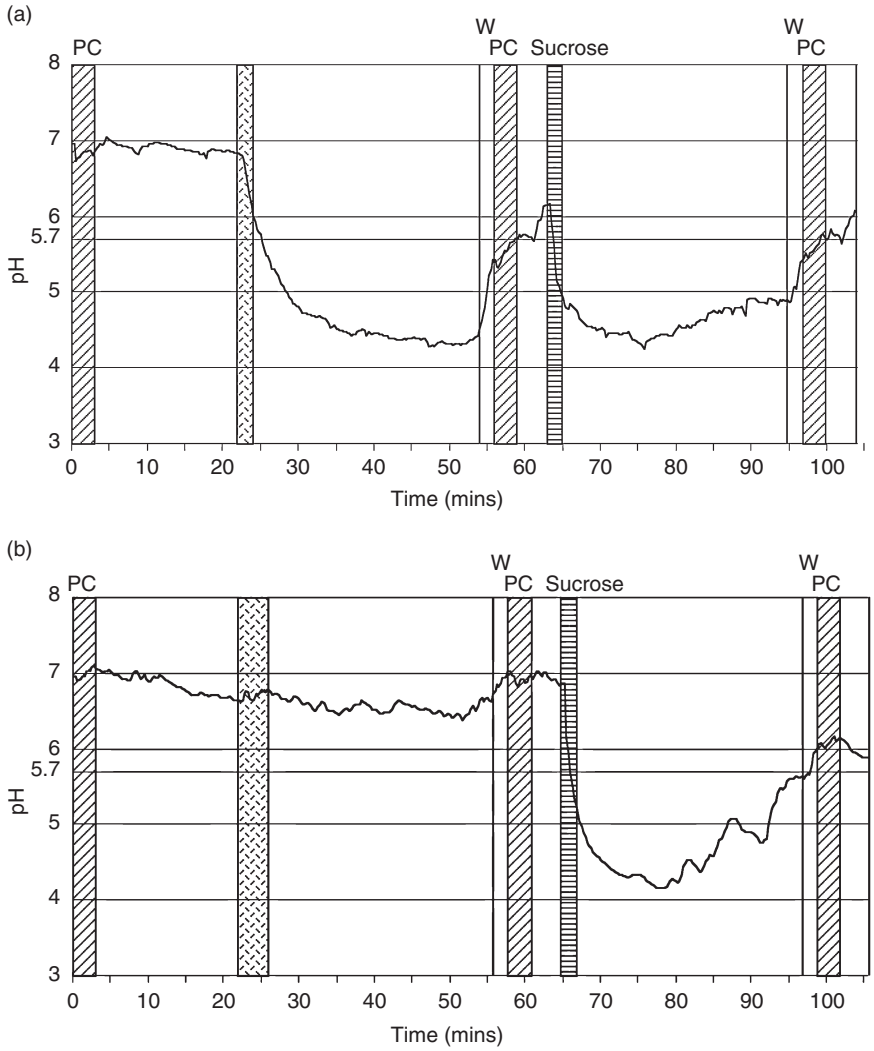

Fig. 23.1 Intra-oral plaque pH curves for a sugar-containing confectionery product (a) and a tooth-friendly confectionery product (b). The sugar-containing product caused a substantial fall in plaque pH, whereas the tooth-friendly product caused only a minimal change in plaque pH. The author is indebted to Prof T Imfeld, University of Zurich, for providing these figures.

drates, relying instead on artificial sweeteners to impart sweetness (Section 23.6). This is a fairly straightforward and established means of rendering sweet products non-cariogenic. More challenging is the formulation of tooth-friendly sweets that have a tart or sour taste and chocolate products.

Examples of tart or sour sweets include lozenge-style 'boiled' sweets which are designed to be sucked rather than chewed and which are often flavoured with citrus, rhubarb or other sour flavourings. These sweets usually contain acids which are released into the oral environment during consumption, as described in Section 23.3. Jensdottir and co-workers have developed a modified acidic sweet with an elevated concentration of calcium (Jensdottir *et al.*, 2007). Addition of 82.5 mg calcium lactate to a 5 g sweet had no effect on salivary flow rate but dramatically increased salivary calcium concentration, thus reducing the likelihood that such sweets would cause dental erosion.

This seems an entirely reasonable method to reduce the erosive potential of hard sweets designed for sucking; the slow release of calcium that occurs as the sweet slowly dissolves will continually replenish the calcium in the saliva and elevate S_{HA}. Whether it would work with a chewy sweet, which may be consumed much more quickly, is unclear and appears not to have been tested yet. Doubtless this will prove to be an interesting field of research in the future.

Chocolate is generally believed to be cariogenic, although there are some conflicting reports. Bibby reported that chocolates had a high decalcification potential in his seminal 1951 study (Bibby *et al.*, 1951). It appears that different chocolates – milk/dark/white, difference cocoa content – have different cariogenicities (Grenby and Mistry, 1995; Verakaki and Duggal, 2003). While artificial sweeteners have proven acceptable in many other forms of confectionery, it is not so straightforward in the case of chocolate and chocolate products. Low-sugar chocolates have been formulated and marketed for diabetics, but these are generally considered inferior in terms of taste and may be associated with flatulence (Zumbé and Brinkworth, 1992). Any attempt to formulate non-cariogenic chocolate bars, therefore, is likely to involve addition of caries-reducing compounds rather than reduction in the sucrose substrate. There is evidence for anti-cariogenic action of certain extracts from cocoa bean husk (Ooshima *et al.*, 2000, 2001) and supplementation of chocolate with sodium caseinate may reduce its cariogenic potential (Reynolds and Black, 1987). As yet, few tooth-friendly chocolate products appear to be available; only one is listed on the TI database (13/2/08). It seems that there is scope for further work on this topic.

23.9 Formulating tooth-friendly oral care products

Of the mouth rinses which have been shown to cause dental erosion, TCP™ and acidified sodium chlorite are rarely used and, it seems, are not suitable for use over any prolonged period owing to the very low pH values (Pontefract *et al.*, 2001; Poyser *et al.*, 2004). It appears that the calcium-chelating Calcusan (Rytomaa *et al.*, 1989) is no longer on the market, at least as far

as the author could ascertain from internet research. It may be possible to supplement these mouth rinses with fluoride to prevent erosion, but it seems superfluous to suggest changes to the formulations of these rather obscure mouth rinses when there are numerous, more popular, alternatives available.

There has been some debate, however, regarding the erosive potential of Listerine™, which is a popular and widely available brand. Pretty's study demonstrated that it caused significant erosion *in vitro*, albeit after 14 hours of continuous exposure (Pretty *et al.*, 2003). Although the author is not aware of any published rebuttal to this research, it ought to be noted that 14 hours of continuous exposure to a mouth rinse could certainly be considered excessive. Interestingly, since Pretty's paper was released, a fluoride-containing Listerine™ product has been launched, but there do not appear to be any reports of its erosive potential in the literature.

23.10 Future trends

A great deal has been achieved in the formulation of tooth-friendly products over recent decades. The work of Toothfriendly International and its associated national bodies in identifying and labelling tooth-friendly confectionery has surely had a beneficial impact on the oral health of many individuals. Tooth-friendly beverages are a more recent development, particularly with regard to drinks with low erosive potential, but it seems now that there are a number of technologies available for creating such products and it remains for individual manufacturers to work out the details of the formulations. The few oral care products which might be described as tooth-unfriendly are comparatively obscure, with the exception of Listerine™, and this author considers that its erosive potential is likely to be clinically insignificant.

There are some interesting areas for development, for example, in products which use some of the more novel means of inhibiting mineral dissolution, such as the deposition of organic films on the tooth surface which replace or supplement the salivary pellicle.

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24

Functional foods and oral health

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Abstract: 'Functional foods' are foods or dietary components that have a beneficial effect on one or more target functions in the body beyond basic nutrition. Food is classified as any substance or product, whether processed or not, that is ingested by humans; thus it includes solids, including chewing-gum, and liquids. Common oral conditions could benefit substantially from the development of functional foods. The findings of research on foods containing minerals, animal products and plant products are considered in terms of their beneficial effects on caries, periodontal disease, tooth wear and oral mucosal disease. International regulation of functional food is also considered.

Key words: antioxidants, cheese peptides, fluoride, food, milk, oral health, polyphenols, probiotic, regulation.

24.1 Introduction

The development of research into adding therapeutic benefit to food is welcome. In dentistry there is a well-established practice of fluoridating water for the prevention of dental caries. The use of other foods to promote oral health is another step in the same direction. As the mouth is at the beginning of the gastrointestinal tract, the potential to capture oral health benefits from the emerging developments in functional foods is considerable. This chapter will consider common oral conditions which could benefit substantially from the development of functional foods. These are caries, periodontal disease, tooth wear and oral mucosal disease including candidiasis.

'Functional foods' are foods or dietary components that have a beneficial effect on one or more target functions in the body beyond basic nutrition. The concept was developed in Japan in the 1980s where the Ministry of Health and Welfare saw the potential to use food as a vehicle to address the nation's spiralling health care costs. They introduced a regulatory system

to approve certain foods with documented health benefits in the hope of improving the health of the nation's ageing population (Arai, 1996).

Food is classified according to Article 2 of Regulation (EC) No. 178/2002 as any substance or product, whether processed or not, that is ingested by humans. This definition encompasses solids, liquids, beverages, water incorporated into food and products such as chewing gum. Although the term 'functional food' is widely used, there is no internationally agreed system for classifying foods as functional. In their regulations, the Japanese Ministry of Health refers to 'foods for specified health use' as opposed to 'functional food'. A vast number of foods and food products could be labelled functional owing to their natural antioxidant, mineral and vitamin content. For example, limes used as a source of vitamin C on board ships in previous centuries to prevent scurvy, could be classified as functional. Some foods which are modified by the addition of vitamins and minerals to promote general health and prevent disease or disorders could also be classified as functional, for example, cereals with added calcium and vitamins. Other modified foods address specific health concerns such as flour fortified with folic acid to prevent neural tube defects and maxillofacial clefts, or salt with added iodine to prevent iodine deficiency-related thyroid disorders. Probiotics are also added to food to promote alimentary health. The term probiotic is a relatively new word meaning 'for life' and it is currently used to label bacteria associated with beneficial effects for humans and animals. In addition, functional foods can have therapeutic action as with spreads and yoghurt drinks with added plant stanol ester which lowers blood cholesterol.

In addition to ingredients, the physical attributes of certain foods may also confer oral health benefits. Consumption of high fibre foods, for example, peanuts, increases salivary stimulation which has a beneficial effect on oral health. Chewing gum also increases the production of saliva; mechanical stimulation of saliva by chewing is further increased by the addition of flavouring to gum. Thus high fibre foods and chewing gum may be considered functional as they can exert positive effects on oral health which are mediated by the cleansing and protective properties of saliva. Saliva has a wide range of protective properties including: both specific and non-specific antimicrobial properties; it has a capacity to buffer acids owing largely to its bicarbonate content which increases at higher flow rates; it acts as a reservoir of minerals for enamel remineralization; it acts as a lubricant (owing to its high molecular weight glycoprotein content) for speech, swallowing and air flow; and it forms a protective pellicle on the teeth which acts as a diffusion barrier for plaque acids.

Foods which have a positive impact on processes or functions affecting oral health are explored here, thus an inclusive interpretation of functional food is adopted. Unmodified and modified foods with both general and specific oral health benefits are considered. Trace elements and vitamins required for normal physiological processes are beyond the scope of this

chapter, albeit they too could be considered functional. The impact of food on the development of oral disease and disorders has been well documented and this relationship is outlined for the major oral diseases as a background against which the positive impact of foods on oral health is explored.

24.2 Foods associated with oral diseases and disorders

The two most common diseases in the mouth are dental caries, which affects an estimated 95% of the world's population (Aguilera Galaviz *et al*., 2002), and periodontal disease which affects an estimated 15% of the population and is most common in older age groups. Another condition which affects a large proportion of the population is tooth surface loss, the prevalence of this condition internationally is not well defined but in two national surveys tooth wear into dentine was found to affect 38% of Irish (Whelton *et al*., 2007) and 36% of UK 16–24-year-olds (Kelly *et al*., 2000), and 93% of Irish and 89% of UK dentate 65+ year-olds. Although not considered to be a disease, progressive tooth surface loss is increasingly recognized as a potential challenge to the maintenance of a functioning dentition for life. The fourth condition which has been linked to dietary habits and will be explored is oral cancer. Among men, it is the eighth most prevalent cancer worldwide (Petersen, 2005); it can be fatal if not diagnosed and treated early. Tobacco use and excessive alcohol consumption have been estimated to account for about 90% of cancers in the oral cavity; the oral cancer risk increases when tobacco is used in combination with alcohol or areca nut (Reibel, 2003). Tobacco chewing also causes oral cancer (Cogliano, 2004). Of these four conditions, dental caries, tooth wear and oral cancer are linked to dietary content and patterns of eating. In the case of periodontal disease, progression is purportedly faster in undernourished populations (Enwonwu, 1995). This is more likely to be due to the compromised immune status of undernourished people rather than any specific effect of diet on periodontal disease. The development of scurvy as a result of vitamin C deficiency is well recognized. Vitamin C is essential for collagen synthesis in connective tissue and the periodontal ligament has a very high turnover of collagen. The detrimental effects of vitamin C deficiency on the collagen fibres of the periodontal ligament is seen as increased levels of periodontal disease in those with scurvy. However, periodontal disease occurs in well-nourished individuals and vitamin C deficiency is not a factor in the majority of periodontal disease seen in developed countries, where the main causes of the disease are bacterial infection and genetic predisposition.

The role of sugar and other fermentable carbohydrates in dental caries is undisputed. Dental caries develops when acid demineralization of enamel increases either due to increased acid attack or to reduced host resistance to disease. Dental enamel is a highly mineralized tissue made up of 96%

organic material and 4% water. In its healthy state it is in a constant see–saw between demineralization and remineralization.

The inorganic content of enamel consists of a crystalline calcium phosphate known as hydyoxyapatite $Ca_{10}(PO_4)_6(OH)_2$. The dissolution of these crystals by acid produced by plaque bacteria on the enamel surface is the start of the caries process. However, when the enamel is in equilibrium, caries does not progress as the enamel is quickly remineralized by the supersaturated reservoir of minerals present in plaque and saliva. Irreversible demineralization occurs when demineralization exceeds remineralization and the equilibrium is upset, as can happen with an increase in the frequency of ingestion of foods or drinks containing sugar.

The multifactorial nature of dental caries and the interplay between the different factors is illustrated in Fig. 24.1. Caries is represented by the intersection of the three circles denoting the host, substrate and bacteria. The quantity and quality of saliva present, as well as the tooth morphology and any plaque retentive factors, have an impact on the host's susceptibility, which is depicted by the host circle. The greater the host's susceptibility to disease, the greater chance that caries will occur, although it will not develop without the presence of both bacteria and substrate. The bacteria circle indicates the need for the presence of acidogenic species for caries to develop. Caries will not develop in the absence of bacteria. *Streptococcus mutans*, a Gram-positive acidophilic bacterium is usually associated with the initiation of caries. In addition to producing lactic acid when it ferments dietary polysaccharides to disaccharides, *S. mutans* produces extracellular glutans which adhere to enamel, allowing the bacterium to colonize the smooth enamel surface. Other acid-producing microorganisms such as *Lactobacillus* species contribute to the caries process once the initial demineralization has taken place and a nidus for non-adhesive bacteria is created.

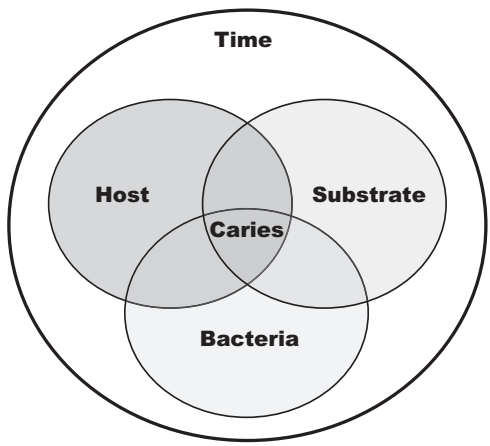

Fig. 24.1 The multiple factors involved in the caries process.

The key to the third circle is that microorganisms need substrate to produce acid. Both monosaccharides and disaccharides can be used for bacterial plaque metabolism. The fourth circle encompassing the other three signifies the importance of time, as caries takes time to develop. The length of time the bacteria have access to substrate at the plaque enamel interface plays a major role in the progress of caries.

As well as demineralization by plaque acids, enamel is also prone to demineralization by ingested acids or by regurgitated gastric acids. Such demineralization has a different pattern to dental caries and results in a more generalized loss of tooth substance. Early signs of tooth surface loss are the loss of surface characteristics of the teeth, increase in shine and thinning of the incisal edges or cupping on the cusps of the molars. As the enamel surface is lost, the dentine becomes visible and eventually the tooth surface loss can progress to the substantial loss of the vertical height of the teeth.

Acids such as citric, phosphoric, malic, tartaric and carbonic acids present in fruits and drinks such as fruit juices, squashes, carbonated soft drinks and energy drinks pose a threat to the integrity of the dentition. Although fruit and fruit juices are components of a healthy diet, their pH is usually well below that which dissolves enamel. They have the potential, if consumed excessively (Milward *et al*., 1994; Järvinen *et al*., 1991), to cause a slow and insidious loss of tooth substance, which will eventually compromise the function of the dentition. Tooth surface loss associated with daily fruit juice consumption has been reported among children as young as 5 years old (Harding *et al*., 2003). As improvements in oral health have increased the likelihood of retention of teeth for life (Whelton *et al*., 2006; Kelly *et al*., 2000; Slade *et al*., 2007), there is a greater awareness of the need to avoid the gradual wearing away of teeth over time, which can accompany an acidic diet.

Other foods associated with oral disease are alcohol and betel nut. High levels of alcohol consumption are associated with oral cancer. Betel nut chewing is a common practice in some developing countries and is also associated with a high incidence of oral cancer.

24.3 Foods which can be used to prevent and treat oral disease

A number of dietary constituents are available which have positive oral health benefits. Some of these are modified to benefit oral health; others have oral health benefits in their unmodified state. Identification of the active ingredients in foods which have positive oral health benefits can allow the further exploitation of the ingredient to benefit oral health. The identification of naturally occurring fluoride in water as the agent responsible for enamel mottling and prevention of dental caries led to its isolation

Table 24.1 Examples of food products which can be used to prevent and treat oral disease

Minerals
Fluoride
Calcium
Animal products
Milk
Cheese
Probiotics
Antimicrobials
Plant products
Polyphenols
Tea
Phosphates
Cocoa
Fibre
Sugar alcohols

and subsequent use in oral health products as well as in water, salt and milk. Examples of other foods and food products which have positive oral health benefits or which are currently under investigation for their potential contribution to oral health are listed in Table 24.1. For convenience they are classified as minerals, animal products and plant products.

As described, foods can function to prevent or protect against a variety of oral conditions including dental caries, periodontal disease, tooth surface loss and oral mucosal diseases including candidiasis. The role of minerals, animal food products and plant food products in protecting against each condition will be considered, although the vast majority of research has focused on the prevention of dental caries. Possible contributing factors to this narrow research focus are the high prevalence of dental caries and the relative ease with which dental caries can be measured. The efficacy of anticariogenic foods can be evaluated in contrast to periodontal disease activity and progression of tooth surface loss which are not as amenable to measurement.

24.4 Minerals and caries

24.4.1 Water fluoridation

Aside from calcium and phosphate, which are important in enamel mineralization, fluoride is the most effective dietary additive for the prevention of dental caries. Fluoride increases tooth resistance to caries and foods with fluoride could be classified as functional foods. The adjustment of fluoride in water was an early example of adjusting a food to benefit oral health. This method of preventing dental caries preceded the concept of functional foods

by 40 years. Fluorine is a naturally occurring element, found in freshwater, seawater, soil, rocks and in foods. Fluoride also occurs naturally in seawater, fish and tea, as well as in many ground waters and surface waters. The fluoride concentration in fresh surface water is generally low, ranging from 0.01 mg/L to 0.03 mg/L. In seawater, fluoride is found at approximately 1.5 ppm. Marine plants and animals are therefore constantly exposed to large amounts of fluoride. Fluoride in soils is derived primarily from minerals, for example, fluorspar or cryolite. In plants, the accumulation of soil fluoride is usually low, except for a few species such as tea which build up high levels. Dried tea leaves have a widely varying concentration of fluoride (approx. 4–400 ppm). When brewed, this is reduced to between 1 and 6 ppm depending on the amount of dry tea used, the fluoride concentration of the water and the brewing time. Levels of fluoride found in fish have been reported to be 0.05–0.17 ppm where bone fragments are excluded. Earlier analyses reporting between 0.6 to 2.7 ppm may have been carried out on samples which contained bones. With its high affinity for calcium, most absorbed fluoride is deposited in bones, and fish bones are a source of concentrated fluoride levels. Other dietary constituents which contain fluoride are fluoridated water and beverages reconstituted with fluoridated water. Most other foods have fluoride concentrations well below 0.05 ppm.

In the early days, scientists thought that the anticaries activity of fluoride was a pre-eruptive systemic effect as a result of its incorporation into the enamel crystal to form hydroxy fluorapatite during the time of enamel formation. This crystal is more stable and resistant to acid demineralization than hydroxyapatite. However, investigators have failed to show a consistent correlation between anticaries activity and the specific amounts of fluoride incorporated into the enamel. The theory of pre-eruptive fluoride incorporation as the sole or principal mechanism of caries prevention has been largely discounted.

The current theory is that fluoride acts topically on the teeth in a number of different ways. A low but slightly raised ambient level of fluoride in the oral cavity is effective in preventing dental caries in erupted teeth. The predominant effect is thought to be that fluoride increases the rate at which enamel remineralizes following dissociation of the hydroxyapatite crystal $Ca_{10}(PO_4)_6(OH)_2$ (ten Cate and Featherstone, 1991; ten Cate *et al.*, 2003). A low level of fluoride in the mouth, particularly at the plaque/saliva/enamel interface is of most benefit in inhibiting the demineralization of sound enamel (ten Cate, 1999; Featherstone, 1999). These low levels are effective in shifting the enamel balance from demineralization, which involves the loss of calcium, phosphate and carbonate, to remineralization by these minerals which are concentrated in plaque and saliva and are available for re-uptake by the enamel surface. As plaque pH is lowered owing to metabolism of carbohydrates by cariogenic bacteria, fluoride is released from dental plaque in response to lowered pH at the tooth–plaque

interface (Tatevossian, 1990). This fluoride is then incorporated with the calcium and phosphate into fluoridated hydroxyapatite which is more stable and less acid soluble than hydroxyapatite (Moreno *et al*., 1974).

Fluoride also has effects on bacterial metabolism. As fluoride concentrates in dental plaque, it inhibits acid production from the bacterial metabolism of carbohydrates and affects bacterial production of adhesive polysaccharides (Hamilton, 1990). In laboratory studies, when a low concentration of fluoride is constantly present, *S. mutans* (one of the cariogenic bacteria), produces less acid (Rosen *et al*., 1978; Bowden *et al*., 1982; Bowden, 1990; Marquis, 1990). Whether this decreased acid production reduces the cariogenicity of these bacteria in humans is unclear (Van Loveren, 1990). The inhibition of acid production is thought to be due to a combination of:

- reduction of intracellular pH of the bacteria
- inhibition of proton-translocating ATPases, which are involved in sugar uptake by the bacterial cell
- inhibition of key glycolytic enzymes such as enolase.

In addition to preventing caries, fluoride can disrupt the normal pattern of enamel formation or amelogenesis during the period of tooth formation. Both the central incisors and the first permanent molar teeth start to form in the perinatal period. By the age of 5, the crowns of these teeth and the enamel are fully mineralized; during the intervening period the enamel is susceptible to the effects of fluoride. The effect of fluoride on the appearance of enamel is dose-related. In temperate climates fluoridation of the domestic water supply at 1 ppm will have no effect on the appearance of the enamel for the vast majority. For some, paper white patches or fine white lines will be visible on close inspection or on drying of the teeth. In a few cases, up to 25% of the tooth surface will have small, opaque, paper white areas scattered irregularly over it. In his studies on the effects of fluoride on teeth, Dean (1934) developed an index to describe the appearance of teeth. He classified these appearances as normal, questionable and very mild. In areas where fluoride is naturally occurring at higher levels, for example 3–4 ppm, the appearance of fluorosis can be more obvious, ranging from what Dean described as mild to severe forms. Mild fluorosis is described as being more extensive than very mild, affecting up to 50% of the tooth surface. In moderate fluorosis, all enamel surfaces of the teeth are affected and surfaces subject to attrition show wear; brown stain is frequently a disfiguring feature. In cases of severe fluorosis, all surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting; brown stains are widespread and teeth often present a corroded-like appearance. The latter forms of fluorosis occur only in cases of excess ingestion of fluoride during the period of enamel formation. They do not usually

occur as a result of water fluoridation at optimal levels. Whilst the introduction of fluoridated toothpaste has been a resounding success in the prevention of dental caries in both fluoridated and non-fluoridated communities, the avoidance of fluorosis caused by swallowing fluoridated toothpaste during the period of amelogenesis of the permanent incisors is important. In Ireland where water is fluoridated to between 0.6–0.8 ppm with a target of 0.7 ppm, fluoridated toothpaste is recommended only for children age 2 and older as younger children tend to swallow most of the toothpaste used to brush their teeth (Department of Health and Children, 2002).

Throughout the world it is estimated that about 317 million people drink artificially fluoridated water and that a further 40 million drink water in which the natural fluoride level is sufficient to provide a high degree of protection against tooth decay (WHO, 1994). Countries with fluoridation schemes include the United States, Canada, Mexico, Argentina, Ireland, United Kingdom, Spain, Australia, New Zealand, Hong Kong and Singapore. The United States is the most extensively fluoridated country in the world, with some 135 million people, over half the population, currently receiving artificially fluoridated water and another 10 million receiving naturally fluoridated water.

Within the European Community, the UK, Ireland and Spain are currently operating fluoridation schemes. In Ireland, fluoridation of public water supplies is mandatory and 71% of the population receives fluoridated water supplies. On average, Irish 15-year-olds with fluoridated water have 34% fewer decayed missing or filled teeth than those with non-fluoridated water supplies (Whelton *et al*., 2004). From 1964, drinking water in Ireland was fluoridated to 0.8–1.0 ppm. In 2007, levels were lowered to 0.6–0.8 ppm with a target of 0.7 ppm. Fluoride levels were lowered in response to the reported increase in the prevalence of enamel fluorosis among Irish children using fluoridated water supplies (Whelton *et al*., 2006). An EC directive (Council Directive, 1998) relating to the quality of water intended for human consumption (98/83/EC) sets a maximum admissible concentration (MAC) for fluoride at 1.5 parts of fluoride per million parts of water, irrespective of climate.

Many other vehicles for the delivery of fluoride for the prevention of dental caries have been shown to be effective. In areas where water fluoridation is not feasible, salt supplies have been fluoridated. The incorporation of fluoride in commercial and domestic salt has well demonstrated caries preventive benefits. Under the appropriate circumstances, fluoridated salt (250 ppm) has been shown to be as effective as water fluoridation. Fluoridated domestic salt is available in Switzerland, France and Germany. Fluoridated milk and sugar have been tested on a pilot basis. Incorporation of fluoride into sucrose which is the most widely recognized cariogenic food, would appear to have a lot of potential for targeted caries prevention. Research in this area has not, however, been advanced in recent years.

24.4.2 Milk fluoridation

Milk fluoridation programs are currently operating in about ten countries of the world. Studies on the clinical effectiveness of fluoridated milk in caries prevention have been carried out in several countries using different research methods. A Cochrane review (Yeung, 2005) concluded that there are insufficient studies with good-quality evidence examining the effects of fluoridated milk in preventing dental caries. Of the two randomized controlled trials (RCTs) which satisfied the review's inclusion criteria (Stephen *et al*., 1984; Maslak, 2004), Stephen used 7 ppm in 200 ml milk per day and Maslak used 2.5 ppm in 180–200 ml milk per day. These studies also varied in the delivery of fluoridated milk, one used a drinking cup and the other a straw. The results of the RCTs suggested that fluoridated milk was beneficial to school children, especially their permanent dentition; however, more well-conducted research is necessary. Another review by Bánóczy and Rugg-Gunn (2007) suggested that fluoridation of milk can be recommended as a caries-preventive measure where the fluoride concentration in drinking water is suboptimal, caries experience in children is high and where there is an existing school milk programme. They recommended that the programme should aim to provide fluoridated milk for at least 200 days per year and should commence before the children are 4 years of age.

24.4.3 Salt fluoridation

Fluoridation of salt (200–250 ppm) to prevent dental caries began in Switzerland in 1955. The effectiveness of salt fluoridation is well researched and documented (Marthaler, 2005). It is far more common in Europe than water fluoridation, being common in Germany, France and Switzerland, where some 30–80% of the marketed salt for domestic use is fluoridated (Marthaler, 2005) and in a further 30 countries worldwide including Bolivia, Ecuador, Colombia, Peru, Jamaica, Costa Rica, Mexico, Uruguay and Venezuela. A salt (sodium chloride) intake of 4 g per day provides 1 mg of fluoride which is considered the desirable level. A technical report produced by WHO and the Food and Agriculture Organization of the United Nations (FAO) recommended the consumption of less than 5 g sodium chloride (or 2 g sodium) per day as a population nutrient intake goal, while ensuring that the salt is iodized (WHO, 2003). This expert consultation stressed that dietary intake of sodium from all sources influences blood pressure levels in the population and should be limited so as to reduce the risk of coronary heart disease and stroke. Fluoride in salt is compatible with iodization and is cheaper than water fluoridation (Gillespie and Marthaler, 2005). However, its use to promote oral health may be seen as conflicting with the recommendation for population-wide reductions in salt intake in order to lower population blood pressure and associated cardiovascular outcomes worldwide (WHO, 2002) and may cause confusion for some people. Those on low salt diets may not reap the benefits.

24.4.4 Sugar fluoridation

The use of fluoride in sugar has been studied as a means of preventing caries in animal and human experiments and has provided promising results (Luoma, 1985). Theoretically, addition of fluoride to the point of caries attack by incorporating it in sugar seems logical. As plaque bacteria ferment sugar to produce lactic acid which demineralizes enamel, the presence of fluoride inhibits acid production and promotes remineralization of enamel. However, there has been some debate about the merits of fluoridating sugar, for example, as with salt, control of dietary sugar intake is a common health promotion message; adding fluoride to it may seem to be promoting the use of an undesirable threat to oral and general health.

Other difficulties lie in dealing with variation in consumption patterns and amounts. The few clinical studies which have been carried out were under controlled conditions among institutionalized children. A field trial in Indonesia (Mulyani and McIntyre, 2002) compared the impact of using a fluoride cocrystallized sugar containing 10 ppm fluoride in place of normal sugar among 176 children aged between seven and 19 living in two orphanages and a boarding facility for rural children from poor families. After 18 months of sugar supply, the children using fluoridated sugar had significantly fewer carious lesions than those who used normal sugar. Another study (Luoma *et al.*, 1979) among children with an intellectual disability aged 5–15 years and living in institutions, provided fluoride supplement in several sugar products of their diet; in candies, marmalades, jams, fruit juices and in sweet desserts corresponding to 10 ppm fluoride in the sugar. To two of the four daily candies was also added a $NaHCO_3 + KH_2PO_4$ mixture as a substitute for 2.5% of the sugar of the candy. The control children received the respective products without the additives. The mean increment of decayed, missing or filled surfaces (DMFS) in the 43 control subjects was 4.5 and in the 41 test subjects 2.6 lesions/100 surfaces at risk, in other words 42% reduction. Caries arrestment had occurred in these test subjects after the first year, while in the respective controls it was continuously increasing. Both studies suggest that sugar might be considered as a further vehicle for supplementary dietary fluoride in communities where there is a high caries prevalence or high caries risk and little exposure to fluoride.

24.4.5 Calcium and phosphate

Enamel and dentine are composed mainly of calcium and phosphate and, as enamel is in a constant state of remineralization and demineralization, remineralization is contingent on the presence of these minerals at the enamel surface. Chewing gum has been used as a vehicle for remineralizing minerals and has been investigated in a number of studies, but reports of its effectiveness are conflicting. Significant reductions in dental caries were achieved in two clinical studies of sugared chewing gums containing dicalcium phosphate dehydrate when compared with a control group (Finn and

Jamison, 1967; Richardson *et al*., 1972) over two and a half and two years, respectively. More recently, in an *in situ* study involving 12 volunteers, chewing urea-containing gum with no added calcium was compared with the same gum with added dicalcium phosphate and another containing casein phosphopeptide–amorphous calcium phosphate complexes (CPP–ACP) (Itthagarun *et al*., 2005). The two gums containing calcium additives reduced lesion depth and promoted greater mineral gain when compared with the control. However, a systematic review by Lingström *et al*. (2003) concluded that there was insufficient evidence to support a caries-preventive effect from adding calcium phosphate or dicalcium phosphate dihydrate to chewing gums.

Chow *et al*. (1994) found that experimental sugar-free gums with added 5% monocalcium phosphate monohydrate (MCPM) or an equimolar mixture of tetracalcium phosphate with dicalcium phosphate anhydrous (TTCP-DCPA) had a greater remineralizing or anticarious potential than did a dicalcium phosphate dehydrate gum. These calcium phosphate compounds or mixtures of compounds are more soluble than dicalcium phosphate dehydrate.

New methods of delivering effective remineralizing products are being developed on an ongoing basis. Chewing gum as a delivery vehicle may displace cariogenic snacks where chewing gum is used as an alternative to snacking, in addition to providing saliva stimulation with the accompanying increase in buffering capacity and enhanced remineralization of enamel.

24.5 Minerals and periodontal disease

The importance of minerals (and vitamins) in the prevention of periodontal disease has been investigated both directly and indirectly. The direct approach has involved the study of dietary intakes amongst populations with and without periodontal disease. Whilst cross-sectional dietary analyses are not ideal in the study of this sporadic chronic, and usually slowly progressing disease, they can be useful for hypothesis formulation.

Following a review of the effects of vitamin B-complex, vitamin C and dietary calcium on general wound healing, periodontal disease status and response to periodontal therapy, Neiva *et al*. (2003) concluded that the efficacy of prophylactic nutrient supplementation remains to be determined. On the other hand, low serum 25-hydroxyvitamin D(3) was found to be associated with periodontal attachment loss amongst adults aged >50 from the National Health and Nutrition Examination Survey 2001/02 (Dietrich *et al*., 2004). Using the same database, Yu *et al*. (2007) found that a low serum folate level was independently associated with periodontal disease in older adults. These results suggest that fortification of foods with 25-hydroxyvitamin D (3) and folic acid (a B-complex vitamin) may be useful in the promotion of periodontal health.

The indirect approach looks at the relationship between bone mineral density elsewhere in the body and periodontal disease. As well as exercise, a diet rich in calcium, phosphate and vitamin D helps to protect against loss of bone mineral density (BMD) with advancing age. If periodontal disease is related to loss of BMD, then such foods could be considered to protect against osteoporosis as well as periodontal disease. Evidence for the association between BMD and alveolar bone loss has been reported (Tezal *et al*., 2000; Brennan-Calanan *et al*., 2008), although other studies (Famili *et al*., 2005) failed to find an association.

Whilst there may be scope to fortify foods with vitamins and minerals to promote periodontal health, there is as yet insufficient evidence to support this development. However, the recommendation of foods which function to improve general bone health and prevent osteopenia may be warranted to promote alveolar bone health.

24.6 Minerals and tooth surface loss

Tooth surface loss describes the non-carious loss of tooth tissue, one of the causes of which is the consumption of acidic diets including acidic soft drinks. Some recent developments in food technology have attempted to address this problem through the buffering of foods or through the addition of minerals which reduce the demineralizing effect on the tooth surface. For example, whereas regular orange juice erodes enamel, Larsen and Nyvad (1999) found that orange juice, pH 4.0, supplemented with 40 mmoL/L calcium and 30 mmoL/L phosphate did not erode enamel as the calcium and phosphate saturated the drink with respect to apatite. Similarly, Jensdottir *et al*. (2005) reported lower erosive potential of soft drinks modified by the addition of calcium and phosphate in Iceland. Success has also been achieved in reducing the erosive potential of acidic sweets as demonstrated by Jensdottir *et al*. (2007). Sweets with 16.5 g of calcium lactate per kg reduced their erosive potential compared with non-calcium-containing control.

These and other advances in the modification of foods and drinks have illustrated the potential to reduce the erosive effect of acidic foods and beverages. Wider availability of these modified products may help to prevent irreversible tooth surface loss in the population and avoid its long-term consequences.

24.7 Animal products and caries

Extensive scientific evidence has illustrated the potential health benefits of biologically active peptides from food products. Some of the health-promoting effects are attributed to physiologically active peptides encrypted in dietary protein molecules. Rich sources of such peptides include milk,

cheese, egg and plants. These peptides are inactive within the sequence of the parent protein and can be released during gastrointestinal digestion or food processing (Korhonen and Pihlanto, 2003). Bioactive peptides can also be produced from milk proteins through fermentation of milk, by starters employed in the manufacture of fermented milks or cheese. For general health, antihypertensive peptides have been identified in fermented milk, whey and ripened cheese.

24.7.1 Protective properties of dairy products

The cariostatic effects of milk and cheese have been reported in a variety of studies over the last 25 years. These studies included research on animals, *in vitro* studies and human observational and experimental studies. Kashket and DePaola (2002) reviewed these effects and found that postulated mechanisms involved buffering, salivary stimulation, reduction of bacterial adhesion, reduction of enamel demineralization and/or promotion of remineralization by casein and ionizable calcium and phosphorus. The cariostatic properties of cheese may result from a combination of the effects described in addition to the action of biologically active peptides in the protein fraction and/or naturally occurring probiotics. The oral health-promoting potential of peptides and probiotics will be discussed in more detail, as they represent areas of interest in the rapidly growing functional food industry. Identification and isolation of the components of dairy products which protect against dental caries could lead to an enhancement of the oral health benefits and functionality of dairy products or other products to which these components were added.

24.7.2 Dairy peptides

Cows' milk has two major groups of proteins, caseins (insoluble at pH 4.6) which represent ~80% of the total protein and whey proteins (soluble in their native forms independent of pH; ~20% of total protein) (Aimutis, 2004). Whey proteins include minor dairy proteins such as enzymes, enzyme inhibitors, metal-binding proteins, vitamin-binding proteins and growth factors. The bioactive properties of some of these minor proteins have been identified and they are used in nutraceutical products (Fox, 2001). Cheese in its natural form contains a huge reservoir of peptides (Upadhyay *et al*., 2004). The technology for isolating these cheese peptides has been developed and future research will determine their bioactivity in the mouth.

The caries preventive capacity of casein phosphopeptides and their use in oral health products and chewing gum are the subjects of ongoing research. Casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) nanocomplexes, derived from bovine milk protein, casein, calcium and phosphate have been proposed as non-toxic, anticariogenic agents that could supplement the effects of fluoride. The suggested anticariogenic

mechanism for CPP–ACP is the stabilization of calcium and phosphate as bioavailable ions and localization of calcium and phosphate ions into plaque producing a favourable concentration gradient, thus depressing enamel demineralization and enhancing remineralization (Reynolds, 1998; Morgan *et al*., 2008). An alkaline, stable and highly soluble CPP–ACP has been trademarked as Recaldent™ and has been commercialized in sugar-free gum and mints and in dental professional products (Tooth Mousse™).

A two-year randomized clinical trial of the anticariogenic effect of sugar-free gum containing CPP–ACP nanocomplexes on approximal caries among a fluoridated low caries risk population has been reported (Morgan *et al*., 2008). The double-blind trial was carried out on Australian school children aged 11–13 years who were asked to chew their assigned sugar-free gum (test and control gum with and without 54 mg CPP–ACP per serving, respectively) for 10 minutes, three times per day. Whilst it would be interesting to ascertain the impact of the programme on the overall caries experience of participants, the publication only describes the effect on caries progression on approximal surfaces, estimated using digital radiographs taken at baseline and at 24 months. Data for 892 children in the test group and 857 children in the control group who completed the study revealed that the difference in the frequency distributions of the caries transition scores along an 8-point ordinal scale was significant (the odds ratio, OR = 0.82, $p = 0.03$). Caries progressed on 5.4% of approximal surfaces in the CPP–ACP gum group compared with 6.5% of surfaces among the control group. However, data on the overall effect on caries among the two groups would be required for the efficacy of the product to be determined.

Much of the earlier research on the anti-cariogenic effect of CPP–ACP was carried out using animal or laboratory-based studies or '*in situ*' studies using human volunteers who wear removable appliances (*in situ*) containing blocks of artificially demineralized enamel. CPP–ACP has been reported to increase the level of calcium phosphate in plaque (Reynolds *et al*., 2003), to inhibit *in situ* demineralization of enamel (Reynolds, 1987) and to enhance *in situ* remineralization (Shen *et al*., 2001; Reynolds *et al*., 2003).

Although most of the human studies had a small sample size (none exceeding 30) and were conducted under controlled conditions, the results of the Australian studies consistently show that products containing CCP–ACP produce greater subsurface remineralization compared with products with no CCP–ACP and that there is a dose–response effect, with higher doses producing greater remineralization. An independent study by Schirrmeister *et al*. (2007) compared three calcium-containing chewing gums, two chewing gums (one with zinc citrate and one without) containing dicalcium phosphate, calcium gluconate and calcium lactate, and one chewing gum containing CPP–ACP nanocomplexes, against a non-calcium containing gum. Conversely, this study found no significant difference in average remineralization or lesion depth between various calcium-containing compounds and controls. The authors concluded that the use of chewing

gum offers no additional remineralizing benefit to buccal tooth surfaces, even if the chewing gum contains calcium compounds. However, Itthagarun *et al*. (2005) found that CCP–ACP was similar to dicalcium phosphate dehydrate in reducing lesion depth or in lesion mineral content and that both products were significantly different from the control.

Whilst the current evidence on the effectiveness of foods containing casein derivatives in remineralizing enamel under real-life conditions is encouraging, the evidence is not universally accepted (Azarpazhooh and Limeback, 2008) and further human trials are needed.

24.7.3 Probiotics and prebiotics

The Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) have adopted a definition of probiotics as 'live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host' (WHO, 2001). Probiotics differ greatly and their mechanism of action is not clearly understood. However, they are likely to exert their effects through a variety of mechanisms including (Stanton *et al*., 2005):

1 competitive exclusion, prevention of colonization, cellular adhesion and invasion by pathogenic organisms
2 competition for essential nutrients
3 direct antimicrobial activity, e.g. production of bacteriocins
4 stimulation of the local and systemic host immune response.

The FAO and WHO (2001) suggest that the health benefits for which probiotics can be applied include conditions such as gastrointestinal infections, certain bowel disorders, allergy and urogenital infections. They also point to emerging evidence indicating that probiotics can be taken by otherwise healthy people as a means of preventing certain diseases and to modulate host immunity.

A prebiotic is defined (Gibson and Roberfroid, 1995) as 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon'. Inulin and oligofructose are commercially used prebiotics. They are commonly occurring plant storage carbohydrates comprising fructose polymers. Chicory and Jerusalem artichokes are sources of these fructans which stimulate the growth of intestinal bifidobacteria. These products are used in low-calorie foods and reported general health benefits are extensive, ranging from better calcium absorption to protection from bowel cancer. Oligofructose is used as a low-calorie sweetener in combination with other intense sweetners and is less cariogenic than sucrose (Kaur and Gupta, 2002). Other than their reduced cariogenicity, prebiotics as functional foods are less immediately relevant to oral health than probiotics. Probiotic organisms occur naturally in many products including dairy products. Research

efforts have focused on the identification of organisms with significant health benefits and the modification of foods by the addition of these organisms in the quantities required to confer their health benefits. In many cases, the naturally occurring concentration of these potentially beneficial organisms is insufficient to exert positive health effects, thus developments in food technology have aimed to develop stable cultures of probiotics at effective levels in foods. The dairy food industry has been particularly active in this field and probiotics are increasingly common in marketed dairy foods.

The most commonly used probiotic agents are bacteria from the *Lactobacillus* and *Bifidobacterium* genera, which form part of the normal healthy intestinal microbiota (Pham *et al*., 2008). Although the greatest body of probiotic research has focused on the colon, some progress has been made in the application of probiotics to promote oral health. A search using PubMed revealed that the earliest paper on the use of probiotics for oral health was published in Russian in 1996 (Morozova *et al*., 1996). The limited amount of research in this area has been reviewed by Meurman (2005), Caglar *et al*. (2005), Meurman and Stamatova (2007) and Twetman and Stecksén-Blicks (2008) and is covered in some detail in Chapter 20.

Lactobacillus rhamnosus GG (LGG) is one of the probiotics investigated for the prevention of dental caries. LGG is a strain of *L. rhamnosus* isolated from the intestinal tract of a healthy human being. GG represents the last names of two of the researchers who identified the probiotic bacterium, Sherwood Gorbach and Barry Goldin. It is particularly effective for the treatment of rotaviral diarrhoea (Allen *et al*., 2003) and it has been shown to inhibit cariogenic mutans streptococci. It has also been studied for its effect on dental caries. Näse *et al*. (2001) carried out a randomized double blind placebo controlled study (n = 594) to measure the effect of seven months' consumption of the LGG in milk on dental caries and caries risk in 1–6-year-old Finnish children. The results showed less dental caries in the LGG group and lower mutans streptococcus counts at the end of the study, especially in 3–4-year-old children. LGG reduced the risk of caries significantly, OR = 0.51, p = 0.004, when controlled for age and gender. The authors concluded that milk containing the probiotic LGG may have beneficial effects on children's dental health. They also pointed out that it does not ferment sucrose or lactose, increasing its safety for oral use.

LGG was combined with *Lactobacillus rhamnosus* LC 705 in cheese in another human intervention study in Finland (Ahola *et al*., 2002). The study investigated the effect of daily consumption of 5 × 15 g of the probiotic cheese for three weeks on caries-associated salivary microbial counts in young adults. Altogether, 74 18–35 year-old subjects completed this double-blinded, randomized, placebo-controlled study. Stimulated salivary secretion rates, buffer capacity and counts of salivary *S. mutans*, yeast and lactobacilli were evaluated before and after the intervention and after a 3-week post-treatment period. Although there was no statistically significant

difference between the groups in *S. mutans* counts after the intervention, at the 3-week post-treatment examination, these counts were significantly lower in the intervention group compared to the control group ($P = 0.05$). However, *S. mutans* counts decreased in 20% ($P = 0.01$) and yeast counts in 27% ($P = 0.005$) of all the subjects, regardless of the intervention group.

Wei *et al*. (2002) produced high titres of IgG antibodies against human cariogenic bacteria, *S. mutans* and *Streptococcus sobrinus* in bovine colostrum through immunization of pregnant cows with a multivalent vaccine. The purified immune product (IP) of this preparation has a number of anticariogenic properties, such as inhibition of streptococcal adherence to saliva-coated hydroxyapatite and inhibition of glucosyltransferase enzymes. Wei *et al*. (2002) demonstrated that IP added to LGG-fermented milk maintained its antibody activity against cariogenic streptococci during the expected shelf-life of the product. From the anticariogenic point of view they concluded that it may be beneficial to add bovine-specific antibodies against mutans streptococci to probiotic LGG-containing milk products.

Lactobacillus casei has also been evaluated for its caries protective effects. In an early animal study, Michalek *et al*. (1981) illustrated a potential caries effect of *L. casei* when present in sufficient levels in rats. Rats monoinfected with *S. mutans* developed higher levels of caries than rats with greater than 1% *L. casei* in their plaque. Busscher *et al*. (1999) also demonstrated the potential of *L. casei* from a bioyoghurt to inhibit oral streptococci. Petti *et al*. (2001) illustrated the activity of yoghurt containing *Lactobacillus bulgaricus* against *S. mutans* following 8 weeks of twice daily consumption. At 8 weeks, the mean count for mutans streptococci was lower in the test than in the control group who were consuming casein-free soyabean ice cream (3.6 versus 4.0 log colony-forming units per ml (cfu ml); $P = 0.02$).

Lactobacillus reuteri has shown activity against *S. mutans*, when given in yogurt (Nikawa *et al*., 2004) water or lozenge form (Caglar *et al*., 2006, 2008) illustrating the potential for further development of foods containing this probiotic to control dental caries.

Current research on probiotics includes characterization of human oral lactobacilli and the selection of potential probiotic strains for oral health. Kõll *et al*. (2008) identified 22 strains, most of which had antimicrobial properties against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis, Prevotella intermedia* and *S. mutans*. Thus research continues to progress in this area which may have a major impact on the future prevention and treatment of oral disease.

Other antimicrobial bacterial products have also been identified and purified from dairy products. An example with potential anticaries activity is lacticin 3147, which is a broad-spectrum lantibiotic produced by the food-grade strain *Lactococcus lactis* DPC3147. O'Connor *et al*. (2006) demonstrated that a lacticin-powdered fermentate was effective in inhibiting a

range of natural cariogenic *S. mutans* strains and that a lacticin-powdered ingredient is effective in reducing this pathogen in human saliva. Further research to determine its effect on biofilms is required to determine the potential of this powder as a nutritional additive for dairy products with the added benefits of preventing dental caries. A small number of probiotics with potential for reducing caries risk by decreasing the level of *S. mutans* in the mouth have been identified and it is likely that this number will increase in the future as our knowledge and understanding of this area continues to improve. Probiotics may not replace conventional preventive approaches but the incorporation of strains with even slight oral health benefits in commonly consumed foods such as dairy products may provide a useful improvement in oral health at the population level. Further well-conducted randomized controlled human trials will be needed to test the efficacy of existing and new probiotics in controlling dental caries. The use of probiotics to prevent or control oral disease looks like a promising area for future development.

24.7.4 Other animal products for the control of dental caries

Chitin is a polysaccharide, *N*-acetyl-D-glucosamine, found in the outer skeleton of insects, crabs, shrimps and lobsters and in the internal structures of other invertebrates. Chitin is the main source of production of chitosan which has demonstrated activity against *S. mutans* (Virga *et al*., 2002; Sano *et al*., 2003; Bae *et al*., 2006), in particular against its adhesive properties (Tarsi *et al*., 1997; Sano *et al*., 2003). Its use in a chewing gum with anticaries effects has been demonstrated (Shibasaki *et al*., 1994; Hayashi *et al*., 2007 a and b). The results suggest that chitosan gum has potential as a new functional food to help control dental caries.

24.8 Animal products and other oral diseases

Increased intake of dairy products has been associated with a lower prevalence of periodontal disease. Using data from 12 764 adults in the third US National Health and Nutrition Examination Survey, individuals in the highest quintile of intake of dairy products were 20% less likely to have periodontitis than those in the lowest quintile ($P = 0.024$ for trend) (Al-Zahrani, 2006). In Japan, a study involving 942 adults showed that those eating ≥55 g lactic acid foods per day had a significantly lower prevalence of deep probing depths (PD) and severe clinical attachment loss (CAL) compared to those not eating these foods, after adjusting for confounding variables; the odds ratios for generalized deep PD and severe CAL were 0.40 (95% confidence interval (CI): 0.23–0.70) and 0.50 (95% CI: 0.29–0.87), respectively. (Shimazaki *et al*., 2008). These studies suggest a protective benefit of dairy products against periodontal disease but further research is required to clarify this relationship and identify the specific protective factors.

Compared with research on foods with caries protective properties, there has been little progress in the development of foods with specific periodontal benefits. Development of probiotics in this area has focused on application of these products as therapeutic agents rather than as food ingredients (Krasse *et al*., 2006; Teughels *et al*., 2007).

There is some preliminary evidence that probiotic microorganisms can prevent or delay the onset of certain cancers outside the mouth (WHO, 2001). However, it is too early to make definitive clinical conclusions regarding the efficacy of probiotics in cancer prevention and further research is required.

The use of probiotics for the control of *Candida albicans* has also been studied. *Candida* is the most frequently isolated yeast species in the mouth, of which *C. albicans* is the most common (Odds, 1988). In a review of published studies, Odds (1988) found that the reported prevalence of *C. albicans* in healthy individuals was 17.7% (range, 1.9–62.3%), whereas mean prevalence in hospitalized individuals (without clinical candidiasis) was 40.6% (range, 6.0–69.6%). Only a proportion of the population colonized by *C. albicans*, develop candidiasis (Cannon and Chaffin, 1999).

Hatakka *et al*. (2007) demonstrated that a mixture of probiotics (*L. rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freudenreichii* ss. *shermanii* JS) delivered in 50 g cheese divided in two portions per day reduced the prevalence of oral *Candida* spp. and diminished the risk of hyposalivation and the feeling of dry mouth in the elderly, over a 16-week period. Over 16 weeks, the prevalence of a high salivary yeast count ($\geq 10^4$ cfu/mL) decreased in the probiotic group from 30% to 21% (32% reduction). In contrast the prevalence of a high yeast count increased from 28% to 34% in the control group, who chewed cheese containing only *Lactococcus lactis* as a starter culture with no added probiotic strains. Probiotic intervention reduced the risk of high yeast counts by 75% (OR = 0.25, 95% CI: 0.10–0.65, $p = 0.004$). Another interesting aspect of this study was that those who had regularly used lactic acid bacteria-containing products before the study had high yeast counts ($\geq 10^4$ cfu/mL) less often than those who did not (25% vs. 38%; $p = 0.03$). The findings with regard to salivary flow rates were encouraging, as the median unstimulated salivary flow increased in the probiotic group, from 0.18 mL/min at the beginning to 0.22 mL/min at the end, and decreased in the control group, from 0.22 mL/min to 0.18 mL/min. Probiotics decreased the risk of hyposalivation by 56% (OR = 0.44, 95% CI: 0.19–1.01, $p = 0.05$); this outcome warrants further investigation.

24.9 Plant products and caries

As sugar is the food most commonly associated with caries, sugar substitutes, many of which are derived from plants, are commonly associated with caries prevention. The replacement of fermentable non-milk extrinsic sugars by sugar alcohols or artificial sweeteners is an example of food modification

for oral health; such modifications make confectionery 'safe for teeth' as they carry a reduced risk of dental caries.

24.9.1 Sugar-free chewing gum

Sugar-free chewing gum is a food marketed on the basis of its positive effect on oral health. Sugar-free gum stimulates salivary flow through mechanical and gustatory stimulation of the glands. This not only increases the clearance of sugars and other fermentable carbohydrates from the teeth and the oral cavity but faster flowing saliva retains more bicarbonate ion and has greater buffering capacity. The ability of saliva to neutralize plaque acids increases as its bicarbonate ion concentration increases with faster flow rates. Thus chewing sugar-free gum after meals helps to increase the rate at which plaque pH returns to normal after consumption of sugars. Sugar substitutes are advised as an alternative to sugar as they do not provide a substrate for fermentation by bacteria.

24.9.2 Sugar alcohols

The oral health benefits of the substitution of sugar by various sweeteners have been studied. Sugar alcohols are commonly used as sweeteners and are non-cariogenic, they include sorbitol, xylitol, mannitol, erythritol and isomalt. The potential of the 5-carbon polyol, xylitol, to improve oral health has been extensively examined. Whilst other sugar alcohols and artificial sweeteners are not fermentable and so are tooth friendly, xylitol has the added benefit of having a direct effect on plaque bacteria and enhanced remineralization (Trahan, 1995; Hildebrandt and Sparks, 2000). Sorbitol-sweetened gums simulate saliva without causing a drop in the critical pH and have been shown to be similar to xylitol gum in terms of caries control (Machiulskiene *et al*., 2001).

24.9.3 Polyphenol-containing compounds

Polyphenols are phytochemicals that are found in food substances produced from plants such as fruits, vegetables, cereals, olive, dry legumes and chocolate and beverages, such as tea, coffee and wine; they are active against a wide spectrum of microbes. Although a polyphenol deficiency state has not been identified; these chemicals are believed to play a biologically active role and have been shown to be potentially immunomodulating (Shapiro *et al*., 2007). Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another. The main groups of polyphenols are flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans. Flavonoids are themselves divided into six subclasses, depending on the oxidation state of the central pyran ring: flavonols, flavones, flavanones, isoflavones,

anthocyanidins and flavanols (catechins and proanthocyanidins) (D'Archivio *et al*., 2007). Studies on animals or cultured human cell lines indicate a role for polyphenols in the prevention of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes and osteoporosis. However, it is very difficult to extrapolate these results to humans as these studies have often been conducted using doses of polyphenol far beyond those documented in humans (Scalbert *et al*., 2005).

Tea

Tea (*Camellia sinensis*) was originally consumed as a medicine in China 5000 years ago. Its consumption as a drink emerged in the last 3000 years with a shift from tea as a medicine to cure sickness, to a tonic to maintain good health. Medical books from the Song Dynasty (AD 960–1279) in China indicate that green tea with ginger can cure dysenteric disorders. Tea has a complex composition; the more interesting components from an oral health perspective are fluoride and polyphenols.

In the 1950s dental researchers studied the cariostatic effect of fluoride in tea (Gershon-Cohen and McClendon, 1954), Reports in experimental animals and humans suggested that green tea consumption (without added sugar) reduces dental caries (Wu and Wei, 2002; Elvin-Lewis *et al*., 1980; Mitscher *et al*., 1997). Linke and LeGeros (2003) reported a significant decrease in caries formation in hamsters receiving frequent black tea extract (4.22 ppm fluoride), even in the presence of sugars in the diet. Both green and black tea are a natural source of fluoride and an effective vehicle for fluoride delivery to the oral cavity. According to Simpson *et al*. (2001), after rinsing the mouth with tea, approximately 34% of the fluoride is retained in the mouth and shows strong binding to enamel particles where it is available for topical action. In a review of the effect of black tea on health, Gardner *et al*. (2007) concluded that there is evidence to support the contribution of tea to fluoride intakes and thus theoretical protection against caries.

In addition to fluoride, the effect of tea polyphenols on oral bacteria (Elvin-Lewis *et al*., 1980; Kashket *et al*., 1985; Hattori *et al*., 1990; Mitscher *et al*., 1997; Gardner *et al*., 2006) has been studied since the 1980s. Green tea (non-fermented) can be considered to be an important dietary source of polyphenols and particularly flavonoids, which are strong antioxidants. Flavonoids are phenol derivatives widely distributed among plants (Vison *et al*., 1995). The main flavonoids present in green tea include catechins (flavan-3-ols). Catechins refer to monomers of flavonols that have a similar composition such as catechin, epicatechin, epigallocatechin, epicatechin gallate (EGC) and epigallocatechin gallate (EGCG). These compounds are particularly abundant in green tea as the polyphenols are non-oxidized, whereas black tea, owing to the fermentation process, contains oxidized phenolic compounds such as theaflavins and thearubigins (Rahman *et al*., 2006; Cabrera *et al*., 2006).

Studies conducted over the last 30 years have shown that (–)-epigallocatechin gallate (EGCG) and (–)-epicatechin gallate (ECG), can inhibit the growth of a wide range of Gram-positive and Gram-negative bacterial species with moderate potency. Evidence is emerging that these molecules may be useful in the control of common oral infections, such as dental caries and periodontal disease. Extracts of green tea and black tea, and polyphenol mixtures, show appreciable inhibition in the synthesis of insoluble glucan from sucrose by *S. mutans* glucosyltransferase (Hattori *et al*., 1990). Crude tea polyphenolic compounds were found to inhibit effectively the attachment of *S. mutans* to saliva-coated hydroxyapatide discs and rats infected with *S. mutans* and then fed with a cariogenic diet containing green tea polyphenols had significantly lower caries scores (Otake *et al*., 1991). Among the tea catechins tested, EGCG and ECG showed the most potent inhibition of the glucosyltransferase activity. Supplementing the drinking water of rats with 0.1% green tea polyphenols along with a cariogenic diet also significantly reduced total fissure caries lesions (Wu and Wei, 2002).

The effect of tea on salivary amylase has also been studied. Amylase hydrolyses food starch to low molecular weight fermentable carbohydrates which are then available for metabolism by plaque bacteria. Zhang and Kashket (1998) found that rinsing with boiled tea inhibits amylase in human saliva, reducing maltose release by up to 70% and thus lowering the cariogenic potential of starch-containing food. Black tea had a greater inhibitory effect than green tea and when the tannin was removed the inhibitory effect was lost.

The mechanisms by which catechins achieve their beneficial effects is still not entirely clear (Clarke and Mullin, 2008); however, there is mounting evidence that they may work through a combination of both an antioxidant effect and an alteration of intracellular signalling. Catechins, particularly EGCG, are effective free radical scavengers *in vitro* (Heijnen, 2001); however, Williams *et al*. (2004) suggest that these compounds may play a minor role as conventional hydrogen-donating antioxidants *in vivo* owing to low circulating levels and rapid metabolism and may exert modulatory actions in cells through their actions on protein kinase and lipid kinase signalling pathways.

Tea appears to have a positive effect on oral health through a number of different mechanisms, mediated by a variety of constituents. However, there is little high-quality evidence on the effects of tea on oral health. Much of the research is carried out *in vitro* or using animal models. More, carefully designed, human randomized controlled clinical trials are needed to fully explore and quantify these effects. Standardization in tea research is important as not only do teas vary according to how they are processed, but the geographic location of cultivation also has an impact on tea properties. Henning *et al*. (2003) reported a large variation in catechin content among various brands and types of tea and suggested that this variation is not taken into consideration in most of the reported epidemiological studies.

Standardization of the product to be tested in future studies and a thorough elucidation of the mechanism of action are recommended.

Cranberries

Cranberry fruit (*Vaccinium macrocarpon* Ait, Ericaceae) is another rich source of polyphenols, especially flavonoids (Cunningham *et al*., 2004; Vvedenskaya and Vorsa, 2004). Its juice and hydroalcoholic crude extracts have been shown to disrupt the formation of biofilms of oral bacteria and bacterial adherence to apatitic surfaces *in vitro* (Weiss *et al*., 2002; Steinberg *et al*., 2004; Yamanaka *et al*., 2004; Duarte *et al*., 2006; Koo *et al*., 2006). Biological activity against *S. mutans* has been demonstrated. Duarte *et al*. (2006) examined the influence of extracts of flavonols, anthocyanins and proanthocyanidins as well as crude extract from cranberry on virulence factors involved in *S. mutans* biofilm development and acidogenicity. The study was carried out on a single species biofilm and showed that biofilm development and acidogenicity were significantly affected by topical applications of proanthocyanidins and flavonols ($p < 0.05$). Proanthocyanidins and flavonols are the active constituents of cranberry which affect the virulence of *S. mutans*. The study provided useful information on the possible mechanism of action of these polyphenols on *S. mutans* which according to the study may involve several routes. The authors proposed at least three routes: (1) inhibition of insoluble glucans synthesis by surface-adsorbed glucosyltransferase (GTF) B and C, which affects the ability of *S. mutans* to adhere to the tooth; (2) inhibition of the proton-translocating F-ATPase activity which is necessary to maintain the difference in pH across the cell membrane; this difference is essential for the optimum function of glycolysis; and (3) disrupting acid production. Another study by Gregoire *et al*. (2007) examined the individual effects of different flavonoids and concluded that the biological activity of cranberry is related to additive effects of various moderately active polyphenols present in the whole fruit rather than a single bioactive compound. Further research is required to explore fully these compounds for their anti-biofilm and anticariostatic properties *in vivo*.

Cocoa

Extracts from cocoa mass have shown some anticariogenic potential, but its anticaries activity is not strong enough to suppress the cariogenic activity of sucrose (Ooshima *et al*., 2000a). However, the cocoa bean husk, a waste material generated in the chocolate industry has been shown to possess two types of cariostatic substances, one showing anti-GTF activity and the other antibacterial activity (Ooshima *et al*., 2000b; Osawa *et al*., 2001). *In vitro* studies (Percival *et al*., 2006), found that pretreatment of artificial saliva-coated surfaces with cocoa polyphenol pentamer could reduce biofilm formation by *S. mutans* and *S. sanguinis* and inhibit acid production by *S. mutans*. This inhibitory effect could be maintained for up to 24 hours in the absence of sucrose. The authors concluded that, although the reductions

were small in magnitude, the repeated exposure of developing biofilms to cocoa polyphenols could result in biologically significant inhibition of plaque formation over time.

24.9.4 Other foods

Other polyphenol-containing foods have also been tested for their caries protective potential, for example, grapes, which are a rich source of potentially bioactive polyphenols. The effect of phenolic extracts prepared from several red wine grape varieties and their fermented byproduct of wine-making (pomace) on some of the virulence properties of *S. mutans* has been reported (Thimothe *et al*., 2007). Grape phenolic extracts, especially from pomace, were found to be highly effective against specific virulence traits of *S. mutans* despite major differences in their phenolic content.

A comparison of the minimum inhibitory concentrations (MIC) of commercially available and 70% aqueous propanone (P70) extracts from plants chosen for polyphenol content on *S. mutans* and other bacteria was carried out by Smullen *et al*. (2007). The lowest MICs were for the P70 extracts of red grape skin (0.5 mg ml), green tea and sloe berry skin (2 mg ml). The commercial extracts generally had a lower activity with a minimum MIC of 2 mg ml for tea extracts, grape seed extracts and pynogenol (extract of maritime pine). All other extracts had MICs of ≥4 mg ml. Unfermented cocoa had greater antimicrobial activity than fermented cocoa and the activity of the fractionated extract increased with the extent of epicatechin polymerization. Epicatechin polymer had an MIC of 1 mg ml and a minimal bacteriocidal concentration (MBC) of 64 mg ml.

24.10 Plant products and periodontal disease

Plaque control is a corner-stone of prevention and treatment of periodontal disease. It is reasonable to suggest that agents that inhibit coaggregation among oral bacteria may disturb the development and maturation of dental plaque and could, therefore, potentially improve plaque control (Weiss *et al*., 1998). Cranberry juice and tea are two plant products which have been studied for their effect on inhibiting microbial adhesion to hard and soft tissues.

24.10.1 Cranberries

The potential for altering the subgingival microflora was demonstrated by Weiss *et al*. (1998) who demonstrated the anti-coaggregating effect of a high molecular weight cranberry constituent on bacterial strains from the human gingival crevice. The constituent reversed the coaggregation of 58% of the 84 coaggregating bacterial pairs tested. It acted preferentially on pairs in

which one or both members are Gram-negative anaerobes frequently involved in periodontal diseases. Effective disruption of the subgingival microbiota in this way has implications for the development of a means of conservative control of periodontal diseases.

The effect of identified plant polyphenols on periodontal pathogens has also been investigated. Bodet *et al*. (2008) investigated the use of a proanthocyanidin-enriched cranberry fraction, prepared from cranberry juice concentrate, on inflammatory mediator production by gingival fibroblasts stimulated by the lipopolysaccharide (LPS) of *A. actinomycetemcomitans*. They found that the inflammatory mediators produced by fibroblasts were inhibited by treatment with the cranberry fraction. This study suggests that cranberry juice contains molecules which may have applications for the development of new host-modulating therapeutic strategies in the adjunctive treatment of periodontitis.

The effect of tea polyphenols has been considered in a number of studies but hop bract polyphenols (HBP) have also been found to have a positive effect. HBP is suggested to be a potent inhibitor of cellular inflammatory responses induced by *P. gingivalis* vesicles and may be useful for the prevention and/or attenuation of periodontitis (Kou *et al*., 2008). *P. gingi*valis, a Gram-negative anaerobic rod, has been implicated in the development and progression of periodontitis (Lamont and Jenkinson, 1998). Agents which suppress it are therefore seen as having a benefit on the prevention or control of periodontal disease. In other *in vitro* cell tests Inaba *et al*. (2008) found HBP to be a potent inhibitor of cellular PGE2 production induced by *P. gingivalis* and they suggested that HBP may be useful for the prevention and attenuation of periodontitis.

24.10.2 Tea

The effect of tea on periodontal disease has also been studied. In a review of studies published between 1990 and 2004, Gardner *et al*. (2007) found little evidence to support the effect of black tea on dental plaque inhibition. Sakanaka *et al*. (1996) reported that green tea polyphenols, especially EGCG completely inhibited the growth and adherence of *P. gingivalis* onto the buccal epithelial cells at concentrations of 250–500 μg/mL. In a later study (Sakanaka and Okada, 2004) they demonstrated the effect of green tea polyphenolic compounds on the production of toxic end metabolites of *P. gingivalis*. Green tea polyphenols completely inhibited the production of *n*-butyric acid and propionic acid at a concentration of 1.0–2.0 mg/mL in a general anaerobic medium.

A further mechanism was observed by Okamoto *et al*. (2004) who suggest that (−)-epigallocatechin and (−)-gallocatechin may have the potential to reduce periodontal breakdown as a result of the potent proteinase activity of *P. gingivalis* because of its ability to inhibit the activity of the organisms cysteine proteinases. Polyphenols from green tea have also been shown to

inhibit phosphatase activity by *P. intermedia* (Okamoto *et al*., 2003). Another mechanism for prevention of periodontal destruction was suggested by Yun *et al*. (2007) who reported that EGCG could prevent alveolar bone resorption by inhibiting osteoclast survival through the caspase-mediated apoptosis. In addition, an effect of tea polyphenols in stimulating periodontal ligament fibroblasts has been demonstrated, suggesting a further role for tea in promoting periodontal health (He *et al*., 2008).

Research on the use of polyphenol compounds to prevent or control periodontal disease is in its infancy. Published reports to date provide encouraging results and, although Sakanaka and Okada, (2004) suggested that continuous application of tea polyphenols on a daily basis can be considered to be a useful and practical method for the prevention of periodontal diseases, extensive product development and human studies are required in these promising areas before such a generalization can be made.

24.11 Plant products and oral cancer

Green tea polyphenols are found to induce apoptosis (programmed cell death) in many types of tumour cells, including oral cancer cells (Hsu *et al*., 2002). The effect leaves normal cells untouched and the research suggests that regular consumption of green tea may be of use in the prevention of oral cancer.

24.12 Regulation of functional foods for oral health

Food claims for oral health benefits are subject to the same considerations as claims for general health and have experienced similar incentives as well as obstacles. Historically, the promotion of health claims for foods has been impeded by incompatibility with medicinal product legislation which defines products intended for the alleviation, prevention and treatment of diseases as drugs (Asp and Bryngelsson, 2008). There are many vested interests in the development and effective regulation of the functional food market. Adequate regulation of health claims on the basis of solid scientific evidence to support the health benefit of the food is essential to support appropriate use of the food by consumers. Given valid information, consumers may choose foods suited to their own particular health needs.

Effective disease risk reduction and prevention through consumption of functional foods has public health implications as the health and well-being of the population is reduced and the incidence or severity of target diseases decreases. National productivity may be increased through an increase in productivity of a healthier population. Effective regulation drives scientific endeavour to develop effective functional foods and to demonstrate this effectiveness through well-conducted research. Commercial interests benefit

from consumer confidence in scientifically supported claims. A quality cycle is established by effective regulation with benefits for consumers, producers, public health, the economy and the state.

As the food industry forges ahead with research on functional foods, regulators have wrestled with the complex area of regulation. In most developed economies, the use of medicine is highly regulated and well controlled for safety reasons. As foods are developed which target specific diseases, the boundary between food and medicine becomes less clear and issues of safety and appropriate dosage are raised. Unlike medicine, it can be difficult to control the use or over-use of a particular food. The indications or contraindications for use are further considerations as it is now up to the consumer to decide whether the use of a functional food is warranted or appropriate. In the case of medicine, manufacture and storage are also tightly controlled, a requirement that is reflected in the high price of many medicines. The food industry is also well regulated but to a lower level, and regulation may be required to ensure the stability of active ingredients over the shelf-life of a food. Thus, there are many potential beneficiaries of foods with enhanced health benefits. However, the manipulation of foods in this way increases the need for regulation to minimize the risks and maximize the benefits for the consumer. Good regulation will also underpin the development and promotion of high-quality effective functional foods.

The Japanese were early innovators in research and regulation for functional foods. In 1984 the Japanese Ministry of Education initiated research and development projects on the nutrition, sensory and physiological functionalities of foods. Those with physiological functions were referred to as functional foods (Yamada *et al*., 2008). The Japanese Ministry of Health, Labour and Welfare (MHLW) in 1991 was the first to introduce legislation (Nutrition Improvement Law, 1991) to establish a regulatory system to review and approve health claims on food labels in order to regulate the marketing of functional foods.

The current Japanese system for regulation of health foods is called Food with Health Claims and is made up of two categories (1) Food with Nutrient Function Claims (FNFC) and (2) Food for Specified Health Uses (FOSHU). The term functional foods is not used and functional foods are referred to as FOHSU. FOHSU are foods that contain dietary ingredients that have beneficial effects on the physiological functions of the human body, to maintain and promote health and improve health-related conditions. 'Reduction of disease-risk' claims are not allowed, except for calcium and folic acid. Safety is examined by The Food Safety Commission and effectiveness is evaluated by The Pharmaceutical Affairs and Food Sanitation Council. Subsequently, the MHLW individually approves claims, which allows the manufacturer to carry the claim and special FOSHU logotype officially on their product (Yamada *et al*., 2008). The FOHSU list is available in Japanese and in August 2008 listed 797 approved foods.

An example of a food with an oral health claim is a chocolate containing isomaltulose and tea polyphenol. The claim states that, since the sweetner isomaltulose and tea polyphenol do not cause tooth decay, the chocolate is unlikely to cause tooth decay. There is also an addendum that eating the chocolate does not cure tooth decay. Isomaltulose is a disaccharide non-cariogenic product of sucrose. Another claim relates to chewing gum which contains calcium-bound phosphoryl oligosaccharides (POs-Ca), a soluble form of calcium. The approved claim says that, since the product contains POs-Ca, this product adjusts the oral environment in such a way that it is likely to remineralize teeth and makes teeth strong and healthy. Chewing gum containing CPP–ACP is approved and the claim translates as 'Since this product contains CPP–ACP which reduces demineralization that is the start of decayed tooth, and fortifies remineralization and acid resistance of the site, this product makes teeth strong and healthy'. The standard of intake per day which accompanies the claim is for use four times a day for 20 minutes. Further claims include products containing fluoride. The Bureau of Social Welfare and Public Health, Tokyo Metropolitan Government lists ingredients of FOSHU according to ten categories. Two of these categories relate to oral health and the entries are as follows:

- Food unlikely to cause decayed teeth: maltitol, palatinose, tea polyphenol, reduced palatinose, erythritol;
- Food to help dental health: CPP–ACP, xylitol, maltitol, calcium phosphate, fukuronori (*Gloiopeltis furacata*) extract (funoran), reduced palatinose, $CaHPO_4$.

In the United States, the Food and Drug Administration (FDA) is responsible for assuring that foods sold in the United States are safe, wholesome and properly labelled. The Federal Food, Drug, and Cosmetic Act (FD&C Act) and the Fair Packaging and Labeling Act are the Federal laws governing food products under FDA's jurisdiction. The FDA (2008) publishes guidance for industry on the requirements for food labelling. This guidance includes requirements for labelling regarding health claims. Health claim means any claim made on the label or in labelling of a food that expressly or by implication characterizes the relationship of any substance to a disease or health-related condition. Health claims are limited to claims about disease risk reduction and cannot be claims about the diagnosis, cure, mitigation or treatment of disease. Health claims are required to be reviewed and evaluated by FDA prior to use. An example of an authorized health claim is: 'Frequent between-meal consumption of foods high in sugars and starches promotes tooth decay. The sugar alcohols in [name of food] do not promote tooth decay'.

It is a requirement that the food be sugar free and when a fermentable carbohydrate is present; the food must not lower plaque pH below 5.7. The sweeteners eligible to be used in foods making this claim are:

- sugar alcohols: xylitol, sorbitol, mannitol, maltitol, isomalt, lactitol, hydrogenated starch hydrolysates, hydrogenated glucose syrups, erythritol, or a combination of these;
- sugar: D-tagatose;
- non-nutritive sweetener: sucralose.

In the case of new health claims, applications must be made to the FDA. All of the publicly available scientific evidence to support the claim, as well as appropriate expert opinion about the validity of the claim, must accompany the application. The Food and Drug Administration (FDA) criteria for 'significant scientific agreement' are available in a guidance document (FDA, 1999).

In the USA health claims can also be authorized based on an authorative statement made by a federal scientific body as is the case with water fluoridation. Bottled water with between 0.6 and 1.0 mg/L fluoride can carry the following claim: 'Drinking fluoridated water may reduce the risk of [dental caries or tooth decay]'.

'Qualified health claims' were introduced for foods in 2003. The system allows the manufacturer to put a qualified health statement on the label, for example 'Scientific evidence suggests but does not prove…'. There are three different levels of qualified health claims and the unqualified health claim makes a fourth level. Web-based research involving over 5642 adults in 2004 (International Food Information Council Foundation, 2005) indicated that consumers have difficulty distinguishing between the four levels of scientific evidence, especially with language-only claims. Overall, the US system has been subject to some criticism as the process is lengthy and expensive and has been found not to be particularly useful as the labelling system including the 'qualified health claims' is not well understood by consumers and may in fact be misleading (Hasler, 2008).

The EU (2007) adopted community rules on the use of nutrition and health claims for foods to standardize provisions relating to such claims, thereby removing impediments to free movement of foods across the EU and equalizing conditions of competition. Full implementation of the regulation (2010) includes a publicly available list of permitted and rejected claims.

The regulation defines its terms and 'health claim' is defined as 'any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health'; 'reduction of disease risk claim' means any health claim that 'states, suggests or implies that the consumption of a food category, a food or one of its constituents significantly reduces a risk factor in the development of a human disease'. Authorization of health claims is the responsibility of the European Food Safety Authority which also presides over the wording to be used in the claim. The regulation makes provision for accelerated authorization for those health claims which are based on newly developed scientific evidence, in order to

stimulate innovation. The regulations require that 'a nutrition or health claim should not be made if it is inconsistent with generally accepted nutrition and health principles or if it encourages or condones excessive consumption of any food or disparages good dietary practice'. The regulation also alludes to the need to ensure bioavailability of sufficient quantities of the active component in a quantity of the food that can reasonably be expected to be consumed.

An application to the Panel on Dietetic Products, Nutrition and Allergies to deliver an opinion on the scientific substantiation of a health claim related to dairy foods (milk and cheese) and dental health, received a negative judgment. The panel found that 'on the basis of the data presented, a cause and effect relationship has not been established between the consumption of milk or cheese and dental health in children' (EU, 2008).

The EU supported a 'Concerted Action' project under the 5th Framework programme called The Process for the Assessment of Scientific Support for Claims on Foods (PASSCLAIM) project (Aggett *et al*., 2005; Asp and Bryngelsson, 2008). The project was coordinated by the International Life Sciences Institute, ISLI Europe and its objective was to define a set of generally applicable criteria for the scientific substantiation of claims about foods. The project called upon the expertise of countries that had applied voluntary codes of practice to health claims for foods, with scientific evaluation. The group established a scientifically robust tool for evaluating the quality of the data submitted in support of health claims on foods. It is useful in assisting applicants for a health claim to prepare their supporting dossiers as well as in aiding agencies responsible for evaluating the scientific evidence for the claim. The importance of human interventional studies with evidence from more than one RCT is a key issue in PASSCLAIM and, whilst it is desirable to elucidate the mechanisms for the effects, it is not essential to know them (Asp and Bryngelsson, 2008). Whilst PASSCLAIM is not a requirement, it involved over 160 scientists in its development and provides a robust set of criteria to evaluate the validity of a health claim. It provides a useful tool for researchers and applicants for a health claim as well as for those responsible for evaluating the scientific evidence for the claim.

The situations described in Japan, the United States of America and the European Union indicate a global diversity in establishing health claims for foods. The pronounced heterogeneity in the relative permissiveness of these and other regions including China, Australia–New Zealand and Canada in establishing health claims for foods emerged at an international symposium at the Canadian Nutrition Congress in 2007 (Jones *et al*., 2008). The aim of the symposium was to provide a global perspective of the state of knowledge concerning health claims about foods and, through examination of existing legislative models globally, to promote the goal of a common format which could serve as middle ground for emerging jurisdictions that want to

set standards of evidence for food health claim policy development (Jones *et al*., 2008).

Technological progress has facilitated the identification of new products with potential for development as functional foods that are protective against oral diseases. Further progress will be needed to optimize these benefits and capture the synergies among mineral content of foods, probiotics, antimicrobial peptides and plant extracts. Scientific development has been followed by the development of regulations to protect the consumer and to encourage safe and effective innovation in food science.

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