

THE DIAGNOSTIC APPLICATIONS OF SALIVA— A REVIEW

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ABSTRACT: This review examines the diagnostic application of saliva for systemic diseases. As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be collected non-invasively by individuals with modest training. Furthermore, saliva may provide a cost-effective approach for the screening of large populations. Gland-specific saliva can be used for diagnosis of pathology specific to one of the major salivary glands. Whole saliva, however, is most frequently used for diagnosis of systemic diseases, since it is readily collected and contains serum constituents. These constituents are derived from the local vasculature of the salivary glands and also reach the oral cavity *via* the flow of gingival fluid. Analysis of saliva may be useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases, and endocrine disorders, as well as in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use.

Key words. Saliva, diagnosis, systemic, disease, drug, hormone.

Introduction

The most commonly used laboratory diagnostic procedures involve the analyses of the cellular and chemical constituents of blood. Other biologic fluids are utilized for the diagnosis of disease, and saliva offers some distinctive advantages. Whole saliva can be collected non-invasively, and by individuals with limited training. No special equipment is needed for collection of the fluid. Diagnosis of disease *via* the analysis of saliva is potentially valuable for children and older adults, since collection of the fluid is associated with fewer compliance problems as compared with the collection of blood. Further, analysis of saliva may provide a cost-effective approach for the screening of large populations.

Saliva can be considered as gland-specific saliva and whole saliva. Gland-specific saliva can be collected directly from individual salivary glands: parotid, submandibular, sublingual, and minor salivary glands. Secretions from both the submandibular and sublingual salivary glands enter the oral cavity through Wharton's duct, and thus the separate collection of saliva from each of these two glands is difficult (Navazesh, 1993). The collection and evaluation of the secretions from the individual salivary glands are primarily useful for the detection of gland-specific pathology, *i.e.*, infection and obstruction. However, whole saliva is most frequently studied when salivary analysis is used for the evaluation of systemic disorders. Whole saliva (mixed saliva) is a mixture of oral fluids and includes secretions from both the major and minor salivary glands, in addition to several constituents of non-salivary origin, such as gingival crevicular fluid (GCF), expectorated bronchial and nasal secretions, serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi, desquamated epithelial cells, other cellular components, and food debris (Mandel and Wotman, 1976; Fox, 1989; Sreebny, 1989; FDI Working Group 10, Core, 1992; Fig. 1). Saliva

can be collected with or without stimulation. Stimulated saliva is collected by masticatory action (*i.e.*, from a subject chewing on paraffin) or by gustatory stimulation (*i.e.*, application of citric acid on the subject's tongue; Mandel, 1993). Stimulation obviously affects the quantity of saliva; however, the concentrations of some constituents and the pH of the fluid are also affected. Unstimulated saliva is collected without exogenous gustatory, masticatory, or mechanical stimulation. Unstimulated salivary flow rate is most affected by the degree of hydration, but also by olfactory stimulation, exposure to light, body positioning, and seasonal and diurnal factors. The best two ways to collect whole saliva are the draining method, in which saliva is allowed to drip off the lower lip, and the spitting method, in which the subject expectorates saliva into a test tube (Navazesh, 1993).

Saliva has protective properties and contains a variety of antimicrobial constituents and growth factors (Zelles *et al.*, 1995; Shugars and Wahl, 1998). In addition, saliva has lubricating functions and aids in the digestion of food (Mandel, 1987). The functions of saliva and the salivary constituents responsible for these functions are summarized in Table 1.

The salivary glands are composed of specialized epithelial cells, and their structure can be divided into two specific regions: the acinar and ductal regions. The acinar region is where fluid is generated and most of the protein synthesis and secretion takes place. Amino acids enter the acinar cells by means of active transport, and after intracellular protein synthesis, the majority of proteins are stored in storage granules that are released in response to secretory stimulation (Young and Van Lennep, 1978; Castle, 1993). Three models have been described for acinar fluid secretion. These three models include the active transport of anions into the lumen and passage of water according to the osmotic gradient from the interstitial fluid into the salivary lumen (for reviews, see Turner, 1993; Turner *et al.*, 1993). The initial fluid is isotonic in nature and is derived from the local vas-

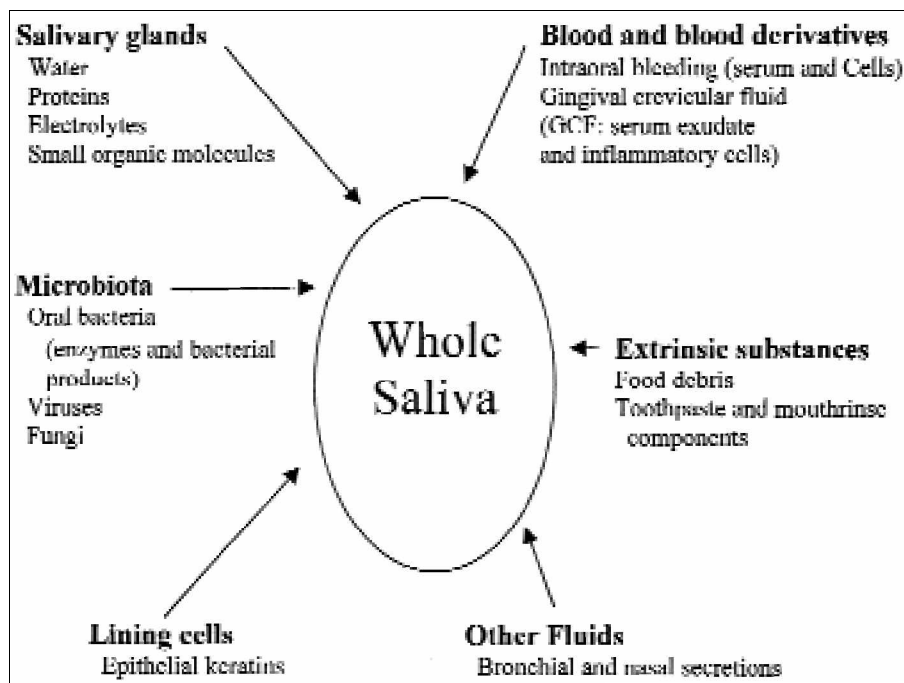


Figure 1. Components of whole saliva.

culature. While acinar cells are water-permeable, ductal cells are not. However, ductal cells actively absorb most of the Na^+ and Cl^- ions from the primary salivary secretion and secrete small

TABLE 1
The Major Functions of Saliva

Functions	Salivary Components Involved
(1) Protective functions	
Lubrication	Mucins, proline-rich glycoproteins, water
Antimicrobial	Amylase, complement, defensins, lysozyme, lactoferrin, lactoperoxidase, mucins, cystatins, histatins, proline-rich glycoproteins, secretory IgA, secretory leukocyte protease inhibitor, statherin, thrombospondin
Growth factors	Epidermal growth factor (EGF), transforming growth factor- α (TGF- α), transforming growth factor- β (TGF- β), fibroblast growth factor (FGF), insulin-like growth factor (IGF-I & IGF-II), nerve growth factor (NGF)
Mucosal integrity	Mucins, electrolytes, water
Lavage/cleansing	Water
Buffering	Bicarbonate, phosphate ions, proteins
Remineralization	Calcium, phosphate, statherin, anionic proline-rich proteins
(2) Food- and speech-related functions	
Food preparation	Water, mucins
Digestion	Amylases, lipase, ribonuclease, proteases, water, mucins
Taste	Water, gustin
Speech	Water, mucins

Adapted from FDI Working Group 10, Core (1992), and Fox (1989).

amounts of K^+ and HCO_3^- and some proteins. The primary salivary secretion is thus modified, and the final salivary secretion as it enters the oral cavity is hypotonic (Baum, 1993). The autonomic nervous system (sympathetic and parasympathetic) controls the salivary secretion. The signaling mechanism involves the binding of neurotransmitter (primarily acetylcholine and norepinephrine) to plasma membrane receptors and signal transduction *via* guanine nucleotide-binding regulatory proteins (G-proteins) and activation of intracellular calcium signaling mechanisms (for reviews, see Baum, 1987, 1993; Ambudkar, 2000).

There are several ways by which serum constituents that are not part of the normal salivary constituents (*i.e.*, drugs and hormones) can reach saliva. Within the salivary glands, transfer mechanisms include intracellular and extracellular routes. The most common intracellular route is passive diffusion, although active transport has also been reported. Ultrafiltration, which occurs through the tight junctions between the cells, is the most common extracellular route (Drobitch and

Svensson, 1992; Haeckel and Hanecke, 1993; Jusko and Milsap, 1993). In contrast, a serum molecule reaching saliva by diffusion must cross five barriers: the capillary wall, interstitial space, basal

cell membrane of the acinus cell or duct cell, cytoplasm of the acinus or duct cell, and the luminal cell membrane (Haeckel and Hanecke, 1996; Fig. 2). Serum constituents are also found in whole saliva as a result of GCF outflow. Depending on the degree of inflammation in the gingiva, GCF is either a serum transudate or, more commonly, an inflammatory exudate that contains serum constituents.

The purpose of this article is to review the literature on the diagnostic applications of saliva. Topics to be covered include analysis of saliva for the diagnosis of systemic diseases, and the monitoring of levels of hormones and drugs. Furthermore, the review will discuss some of the advantages, disadvantages, and problems associated with analysis of saliva for the diagnosis of systemic diseases.

(1) Systemic Diseases (hereditary, autoimmune, malignancy, and infectious)

Some systemic diseases affect salivary glands directly or indirectly, and may influence the quantity of saliva that is produced, as well as the composition of the fluid. These characteristic changes may contribute to the diagnosis and early detection of these diseases.

HEREDITARY DISEASES

Cystic fibrosis (CF) is a genetically transmitted disease of children and young adults,

which is considered a generalized exocrinopathy. CF is the most common lethal autosomal-recessive disorder in Caucasians in North America, with an incidence of 1 in 2500 and a carrier frequency of 1 in 25-30 of the population. The gene defect causing CF is present on chromosome 7 and codes for a transmembrane-regulating protein called the cystic fibrosis transmembrane conductance regulator (CFTR; Riordan *et al.*, 1989; Dinwiddie, 2000). A defective electrolyte transport in epithelial cells and viscous mucus secretions from glands and epithelia characterize this disorder (Grody, 1999). The CFTR is also important for plasma membrane recycling (Bradbury *et al.*, 1992). The organs mostly affected in CF are: sweat glands, which produce a secretion with elevated concentrations of sodium and chloride; the lungs, which develop chronic obstructive pulmonary disease; and the pancreas, resulting in pancreatic insufficiency (Davis, 1987). Since a large number of identified mutations in the CF gene exist, DNA analysis is not used for diagnosis of the disease. The diagnosis is derived from the characteristic clinical signs and symptoms and analysis of elevated sweat chloride values.

The abnormal secretions present in CF caused clinicians to explore the usefulness of saliva for the diagnosis of the disease. Most studies agree that saliva of CF patients contains increased calcium levels (Mandel *et al.*, 1967; Blomfield *et al.*, 1976; Mangos and Donnelly, 1981). Elevated levels of calcium and proteins in submandibular saliva from CF patients were found, and resulted in a calcium-protein aggregation which caused turbidity of saliva (Boat *et al.*, 1974). The elevated calcium and phosphate levels in the saliva of children diagnosed with CF may explain the fact that these children demonstrate a higher occurrence of calculus as compared with healthy controls (Wotman *et al.*, 1973). The submandibular saliva of CF patients was also found to contain more lipid than saliva of non-affected individuals, and the levels of neutral lipids, phospholipids, and glycolipids are elevated. These alterations in salivary lipids in CF patients may account, in part, for the altered physico-chemical properties of saliva in this disease (Slomiany *et al.*, 1982). Apparently, salivary alterations in CF patients are to a large extent due to alterations in submandibular saliva. Elevations in electrolytes (sodium, chloride, calcium, and phosphorus), urea and uric acid, and total protein were observed in the submandibular saliva of CF patients (Mandel *et al.*, 1967). Minor salivary glands are also affected. Elevated levels of sodium and a decrease in flow rate were reported for these glands in CF patients (Wiesman *et al.*, 1972). However, the parotid saliva of CF patients does not demonstrate qualitative changes as compared with that of healthy individuals. Amylase and lysozyme activity in the parotid saliva of CF patients was reported to be similar to that in healthy controls, and therefore parotid saliva cannot provide diagnostically relevant information for this disease (Blomfield *et al.*, 1976).

Decreased protease activity in saliva from CF patients was observed relative to healthy controls; however, significant overlap between the protease activity values in the two groups was detected, which makes the diagnostic significance of these findings questionable (Kittang *et al.*, 1986). Saliva from CF patients was found to contain an unusual form of epidermal growth factor (EGF). The EGF from these patients demonstrated poor biological activity compared

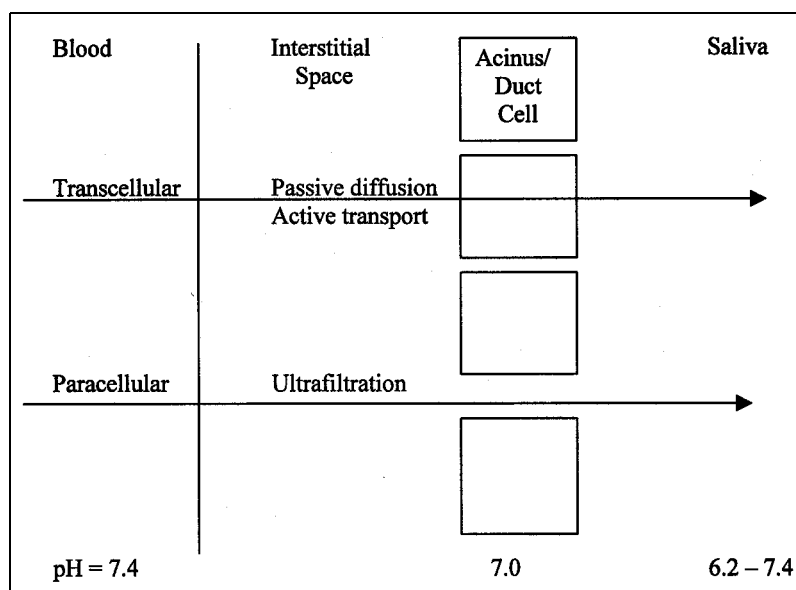


Figure 2. Transport of molecules from blood to saliva. Transport of molecules which are not part of the normal salivary secretion from serum to saliva is by the transcellular route (passive diffusion and active transport) and paracellular route (ultrafiltration) through tight junctions. (Adapted from Haeckel and Hanecke, 1996)

with EGF from healthy controls. It was suggested that this EGF anomaly might contribute to the pathology of CF (Aubert *et al.*, 1990). Further, abnormally elevated levels of prostaglandins E_2 (PGE_2) were detected in the saliva of CF patients as compared with that of healthy controls (Rigas *et al.*, 1989). However, the diagnostic and clinical importance of the EGF anomaly and elevated salivary levels of PGE_2 is difficult to interpret, since the role of EGF and PGE_2 in the pathogenesis of CF is not defined.

Most of the studies concerning the diagnostic application of saliva for CF are relatively old, and saliva is not currently used for the diagnosis of this disorder. More important perhaps than the identification of diseased individuals is the detection of carriers (heterozygotes) for the disease, which are asymptomatic and cannot be detected by salivary or other biochemical diagnostic tests. Detection of carriers will help to reduce the incidence of CF. Screening for these carriers can be performed only at the DNA level. Due to the high number of possible mutations detected in the CF gene, the utilization of DNA diagnostic techniques for the identification of carriers is difficult, and research will most likely focus on this aspect of diagnosis.

Coeliac disease is a congenital disorder of the small intestine that involves malabsorption of gluten. Gliadin is a major component of gluten. Serum IgA antigliadin antibodies (AGA) are increased in patients with coeliac disease and dermatitis herpetiformis. Measurement of salivary IgA-AGA has been reported to be a sensitive and specific method for the screening of coeliac disease, and for monitoring compliance with the required gluten-free diet (al-Bayaty *et al.*, 1989; Hakeem *et al.*, 1992). However, contradictory results were also reported. While elevated levels of serum IgA-AGA were detected in serum, this elevation was not detected in saliva (Patinen *et al.*, 1995). No obvious explanation for the difference between the two studies is apparent, since both reports were similar in both methods of patient evaluation and salivary analysis. In a

more recent study, salivary IgA-AGA produced sensitivity of 60% and specificity of 93.3% in the detection of coeliac disease. In comparison, serum IgG-AGA produced excellent sensitivity (100%) but lower specificity (63.3%). Because of the relative lower sensitivity, the authors did not recommend the use of salivary IgA-AGA for screening for coeliac disease (Rujner *et al.*, 1996).

21-Hydroxylase deficiency is an inherited disorder of steroidogenesis which leads to congenital adrenal hyperplasia. In non-classic 21-hydroxylase deficiency, a partial deficiency of the enzyme is present (Carlson *et al.*, 1999). Early morning salivary levels of 17-hydroxyprogesterone (17-OHP) were reported to be an excellent screening test for the diagnosis of non-classic 21-hydroxylase deficiency, since the salivary levels accurately reflected serum levels of 17-OHP. A high correlation ($r = 0.93$) between salivary and serum concentrations of 17-OHP was observed in both affected and healthy individuals (Zerah *et al.*, 1987).

AUTOIMMUNE DISEASES—SjÖGREN'S SYNDROME

Sjögren's syndrome (SS) is an autoimmune exocrinopathy of unknown etiology. The majority of patients are women, and the estimated prevalence of the disease in the United States is more than 1 million. A reduction in lacrimal and salivary secretions is observed, associated with keratoconjunctivitis sicca and xerostomia. The presence of these two phenomena leads to a diagnosis of primary SS. In secondary SS, a well-defined connective tissue disease (most commonly rheumatoid arthritis or systemic lupus erythematosus) is present in addition to the xerostomia and/or the keratoconjunctivitis (Schjødtt and Thorn, 1989; Thorn *et al.*, 1989). In addition to involvement of the salivary and lacrimal glands, SS may also affect the skin, lungs, liver, kidneys, thyroid, and nervous system (Talal, 1992). The diagnostic criteria for SS are still uncertain, and a single marker that is associated with all cases does not exist. The accepted procedure for the diagnosis of the salivary involvement of SS is a biopsy of the minor salivary glands of the lip. SS is characterized by the presence of a lymphocytic infiltrate (predominantly CD4⁺ T-cells) in the salivary gland parenchyma (Daniels, 1984; Daniels and Fox, 1992). A low resting flow rate and abnormally low stimulated flow rate of whole saliva are also indicators of SS (Sreebny and Zhu, 1996a). Serum chemistry can demonstrate polyclonal hypergammaglobulinemia and elevated levels of rheumatoid factor, antinuclear antibody, anti-SS-A, and anti-SS-B antibody (Atkinson *et al.*, 1990; Fox and Kang, 1992). The immunologic mechanisms involved in the pathogenesis of the disease appear also to involve B-cells (the majority of lymphomas associated with SS are of the B-cell type), salivary epithelial cells, an activated mononuclear cell infiltrate, cytokines, and adhesion molecules (Fox and Speight, 1996).

Sialochemistry may also be used to assist in the diagnosis of SS. A consistent finding is increased concentrations of sodium and chloride. This increase is evident in both whole and gland-specific saliva (Tishler *et al.*, 1997). In addition, elevated levels of IgA, IgG, lactoferrin, and albumin, and a decreased concentration of phosphate were reported in saliva of patients with SS (Ben-Aryeh *et al.*, 1981; Stuchell *et al.*, 1984). Analysis of unstimulated whole saliva was more sensitive than analysis of stimulated whole saliva for detection of these changes, since stimulation caused the elevated levels of sodium and IgA seen in SS patients to decline to the levels observed in healthy controls (Nahir *et al.*, 1987). In contrast, normal concentrations of potassium and calcium are usually found in the saliva of SS patients.

Although the amylase concentration in saliva is also normal, the production of amylase is reduced, but so is the amount of fluid. Therefore, measurement of amylase is not useful for the evaluation of salivary gland function in SS patients (Mandel, 1980). Other salivary changes associated with SS include an elevated concentration of $\beta 2$ microglobulin, although differences exist between patients (Michalski *et al.*, 1975; Swaak *et al.*, 1988). In addition, elevated lipid levels (Slomiany *et al.*, 1986) and increased concentrations of cystatin C and cystatin S have been observed (van der Reijden *et al.*, 1996). Increased salivary concentrations of inflammatory mediators—*i.e.*, eicosanoids, PGE₂, thromboxane B₂, and interleukin-6—have been reported (Tishler *et al.*, 1996a,b). Elevated levels of salivary soluble interleukin-2 receptor were also found in SS patients; however, no correlation was detected between clinical, serological, or histopathological variables and the salivary or serum levels of this receptor (Tishler *et al.*, 1999). Furthermore, elevated levels of salivary kallikrein have been found in association with SS. Again, no correlation was observed between kallikrein levels and the extent of inflammation in the labial salivary glands or the salivary flow rate (Friberg *et al.*, 1988).

SS is characterized by autoantibodies to the La and Ro ribonucleoprotein antigens. These autoantibodies have been shown to target intracellular proteins which may be involved in the regulation of RNA polymerase function (Tan, 1989). Autoantibody, especially of the IgA class, can be synthesized in salivary glands and can be detected in the saliva of SS patients prior to detection in the serum (Horsfall *et al.*, 1989). In addition to IgA, saliva has also been reported to contain IgG autoantibody, while serum contained primarily IgG and IgM autoantibody (Ben-Chetrit *et al.*, 1993). SS anti-La antibodies were primarily found in the saliva of patients whose resting and stimulated whole saliva flow rates were abnormally low. Furthermore, a strong correlation was observed between the presence of this autoantibody in serum and that in saliva. However, in some patients, the antibody was detected in whole saliva but not in serum, which suggested that the antibody is produced in the salivary glands (Sreebny and Zhu, 1996b). The deposition of this antibody within salivary gland tissue may contribute to the pathogenesis of SS. The diagnostic value of these salivary antibodies has not been determined by comparison with serum levels.

The diagnosis and early detection of SS present a serious challenge that has still not been met. Since no single salivary or serum constituent can accurately serve as a diagnostic marker for SS, the most important aspect of salivary diagnosis for this disease is evaluation of the reduced quantity of saliva. Cut-off values of 0.1 mL/min for resting whole saliva and 0.5 mL/min for stimulated saliva may be considered as indicative of salivary gland hypofunction (Sreebny and Zhu, 1996a). Nevertheless, general agreement about these cut-off values does not exist. Although variations in these cut-off values between clinicians may lead to differences in sensitivity and specificity in the diagnosis of SS, the quantitative evaluation of resting and stimulated saliva is a simple, non-invasive method of screening for patients who may have SS. Reduced salivary flow, although not pathognomonic for SS, is of clinical importance and can lead to a variety of oral signs and symptoms, such as progressive dental caries, fungal infections, oral pain, and dysphagia (Daniels and Fox, 1992). Dentists are normally the first to encounter these patients. Affected individuals should be referred for a comprehensive evaluation of the cause for the reduced salivary flow.

MALIGNANCY

Salivary analysis may aid in the early detection of certain malignant tumors. p53 is a tumor suppressor protein which is produced in cells exposed to various types of DNA-damaging stress. Inactivation of this suppressor through mutations and gene deletion is considered a frequent occurrence in the development of human cancer (Hainaut and Vahakangas, 1997; Tarapore and Fukasawa, 2000). As a result, accumulation of inactive p53 protein is observed, which in turn may lead to the production of antibodies directed against this protein (Bourhis *et al.*, 1996). These antibodies can be detected in sera of patients with different types of malignancies (Lubin *et al.*, 1995). p53 antibody can also be detected in the saliva of patients diagnosed with oral squamous cell carcinoma (SCC), and can thus assist in the early detection of, and screening for, this tumor (Tavassoli *et al.*, 1998).

Defensins are peptides which possess antimicrobial and cytotoxic properties. They are found in the azurophilic granules of polymorphonuclear leukocytes (PMNs; Lichtenstein *et al.*, 1986; Lehrer *et al.*, 1991). Elevated levels of salivary defensin-1 were found to be indicative of the presence of oral SCC. Higher concentrations of salivary defensin-1 were detected in patients with oral SCC in comparison with the defensin-1 concentration in the saliva of patients with adenocarcinoma and in healthy controls. A high-positive correlation was observed between salivary defensin-1 levels and serum levels of SCC-related antigen ($r = 0.879$; Mizukawa *et al.*, 1998).

In a recent preliminary study, elevated levels of recognized tumor markers c-erbB-2 (erb) and cancer antigen 15-3 (CA15-3) were found in the saliva of women diagnosed with breast carcinoma, as compared with patients with benign lesions and healthy controls. However, while low levels of CA15-3 were also detected in the saliva and serum of healthy individuals, erb was not detected in healthy subjects and thus appears to hold greater promise for the early screening and detection of breast cancer (Streckfus *et al.*, 2000).

CA 125 is a tumor marker for epithelial ovarian cancer. Elevated salivary levels of CA 125 were detected in patients with epithelial ovarian cancer as compared with patients with benign pelvic masses and healthy controls. A positive correlation was found between salivary and serum levels of CA 125. A further analysis of this relationship revealed that saliva demonstrated a somewhat lower sensitivity than serum (81.3% *vs.* 93.8%, respectively); however, the specificity and positive predictive value were higher for saliva *vs.* serum (88.0% *vs.* 59.8% and 54.2% *vs.* 28.8%, respectively; Chien and Schwartz, 1990).

Tumor markers that can be identified in saliva may be potentially useful for screening for malignant diseases. Salivary diagnosis may be part of a comprehensive diagnostic panel that will provide improved sensitivity and specificity in the detection of malignant diseases and will assist in monitoring the efficacy of treatment. Additional studies are certainly required to determine which salivary markers can be used for these diagnostic purposes, and to determine their diagnostic value in comparison with other, more established, diagnostic tests.

INFECTIOUS DISEASES

Helicobacter pylori infection is associated with peptic ulcer disease and chronic gastritis. Infection with this bacterium stimulates the production of specific IgG antibody. An ELISA test for the detection of IgG antibody in serum produced 97% sensitivity and 94% specificity in detection of the disease. In parallel,

saliva samples were tested for the presence of *H. pylori* DNA by polymerase chain-reaction (PCR) assay, and sensitivity of 84% was reported. The results also indicated that *H. pylori* exists in higher prevalence in saliva than in feces, and the oral-oral route may be an important means of transmission of this infection in developed countries (Li *et al.*, 1996). In another study, testing for salivary antibodies against *H. pylori* yielded sensitivity of 85%, specificity of 55%, positive predictive value of 45%, and negative predictive value of 90% (Loeb *et al.*, 1997).

A variety of other infections has also been monitored by the detection of specific antibodies in saliva. Evaluation of the secretory immune response in the saliva of children infected with *Shigella* revealed higher titers of anti-lipopolysaccharide and anti-Shiga toxin antibody in comparison with healthy controls. It was suggested that salivary levels of these immunoglobulins could be used for monitoring of the immune response in shigellosis (Schultsz *et al.*, 1992).

Pigeon breeder's disease (PBD) is an interstitial lung disease induced by exposure to antigens derived from pigeons. Measurement of salivary IgG against these antigens may assist in the evaluation of patients with this disease. A correlation coefficient of 0.58 was observed between IgG antibody levels in serum and saliva (Mendoza *et al.*, 1996). A similar correlation ($r = 0.52$) between IgG levels in saliva and serum was also reported in a more recent study (McSharry *et al.*, 1999). Furthermore, the detection of pneumococcal C polysaccharide in saliva by ELISA may offer a valuable complement to conventional diagnostic methods for pneumococcal pneumonia. Detection of this antigen in saliva demonstrated a sensitivity of 55% and specificity of 97%. The positive and negative predictive values were 0.94 and 0.73, respectively (Krook *et al.*, 1986).

Lyme disease is caused by the spirochete *Borrelia burgdorferi* and is transmitted to humans by blood-feeding ticks. The detection of anti-tick antibody in saliva has potential as a biologic marker of exposure to tick bites, which in turn may serve as a screening mechanism for individuals at risk for Lyme disease (Schwartz *et al.*, 1991).

Specific antibody to *Taenia solium* larvae in serum demonstrated greater sensitivity than antibody in saliva for identification of neurocysticercosis (100% *vs.* 70.4%, respectively). However, considering the simple and non-invasive nature of saliva sampling, it was suggested that saliva could be used in epidemiologic studies of this disease (Feldman *et al.*, 1990).

(2) Viral Diseases (exclusive of HIV)

(2A) VIRAL DISEASES (EXCLUSIVE OF HIV)

The antibody response to infection is the basis for many diagnostic tests in virology. Saliva contains immunoglobulins that originate from two sources: the salivary glands and serum. The predominant immunoglobulin in saliva is secretory IgA (sIgA), which is derived from plasma cells in the salivary glands, and constitutes the main specific immune defense mechanism in saliva. Although the minor salivary glands play an important role in sIgA-mediated immunity of the oral cavity, cells in the parotid and submandibular glands are responsible for the majority of the IgA found in saliva (Bienenstock *et al.*, 1980; Korsrud and Brandtzaeg, 1980; Nair and Schroeder, 1986). In contrast, salivary IgM and IgG are primarily derived from serum *via* GCF, and are present in lower concentrations in saliva than is IgA. Antibodies against viruses and viral components can be detected in saliva and can aid in the diagnosis of

acute viral infections, congenital infections, and reactivation of infection (Mortimer and Parry, 1988).

Saliva was found to be a useful alternative to serum for the diagnosis of viral hepatitis. Acute hepatitis A (HAV) and hepatitis B (HBV) were diagnosed based on the presence of IgM antibodies in saliva. The ratio of IgM to IgG anti-HAV antibody correlated with the time interval from onset of infection (Parry *et al.*, 1989). Further, salivary antibody levels were used for the detection of infected individuals in a school outbreak of HAV (Bull *et al.*, 1989; Stuart *et al.*, 1992). Saliva has also been utilized to detect very low levels of antibodies to HAV, which, for example, are associated with vaccine-induced immunity. Comparison of serum and saliva levels of antibody to HAV revealed excellent agreement (sensitivity = 98.7% and specificity = 99.6%; Ochnio *et al.*, 1997). Similarly, analysis of saliva provided a highly sensitive and specific method for the diagnosis of viral hepatitis B and C (El-Medany *et al.*, 1999). Analysis of oral fluid samples collected with Orasure[®] provided an excellent method for the diagnosis of viral hepatitis B and C. Sensitivity and specificity of 100% for the detection of antibodies for both diseases in oral fluid in comparison with serum antibodies were reported (Thieme *et al.*, 1992). Saliva has also been used for screening for hepatitis B surface antigen (HbsAg) in epidemiological studies. Comparing the detection of HbsAg in saliva with that in serum by means of a commercially available serological kit yielded a sensitivity of 92% and specificity of 86.8% (Chaita *et al.*, 1995).

Saliva may also be used for determining immunization and detecting infection with measles, mumps, and rubella (Friedman, 1982; Perry *et al.*, 1993; Brown *et al.*, 1994). The detection of antibodies in oral fluid samples produced sensitivity and specificity of 97% and 100% for measles, 94% and 94% for mumps, and 98% and 98% for rubella, respectively, in comparison with detection of serum antibodies for these viruses (Thieme *et al.*, 1994).

For newborn infants, the salivary IgA response was found to be a better marker of rotavirus (RV) infection than the serum antibody response. Neonatal RV infection elicited specific mucosal antibody response which persisted for at least 3 months. However, a similar systemic immune response could not be observed, possibly due to interference by maternal antibody. The authors proposed that saliva, rather than serum, can be used to monitor the immune response to vaccination and infection with RV (Jayashree *et al.*, 1988).

The shedding of herpesviruses (human herpesvirus -8, cytomegalovirus, and Epstein-Barr virus) in nasal secretions and saliva of infected patients has been reported (Blackbourn *et al.*, 1998). Other investigators suggested that reactivation of herpes simplex virus type-1 (HSV-1) is involved in the pathogenesis of Bell's palsy and reported that PCR-based identification of virus in saliva is a useful method for the early detection of HSV-1 reactivation in patients with Bell's palsy. The shed HSV-1 virus was detected in 50% of patients with Bell's palsy in comparison with 19% in healthy controls (Furuta *et al.*, 1998).

Dengue is a mosquito-transmitted viral disease. Primary infection of the virus may lead to a self-limiting febrile disease, and secondary infection may cause serious complications like dengue hemorrhagic fever or dengue shock syndrome (Burke *et al.*, 1988). Salivary levels of anti-dengue IgM and IgG demonstrated sensitivity of 92% and specificity of 100% in the diagnosis of primary and secondary infection, and salivary levels of IgG proved useful in differentiating between primary and sec-

ondary infection (Cuzzubbo *et al.*, 1998). Saliva was also found to be a reliable alternative to serum for identification of the antibody to parvovirus B 19. Sensitivity of 100% and specificity of 95% were observed for the detection of infected individuals at a primary school (Rice and Cohen, 1996).

(2b) HIV

Studies have demonstrated that the diagnosis of infection with the human immunodeficiency virus (HIV) based on specific antibody in saliva is equivalent to serum in accuracy, and therefore applicable for both clinical use and epidemiological surveillance (Malamud, 1992). Antibody to HIV in whole saliva of infected individuals, which was detected by ELISA and Western blot assay, correlated with serum antibody levels (Holmstrom *et al.*, 1990; Frerichs *et al.*, 1994). As compared with serum, the sensitivity and specificity of antibody to HIV in saliva for detection of infection are between 95% and 100% (Tamashiro and Constantine, 1994; Tess *et al.*, 1996; Emmons, 1997; Malamud, 1997). Salivary IgA levels to HIV decline as infected patients become symptomatic. It was suggested that detection of IgA antibody to HIV in saliva may, therefore, be a prognostic indicator for the progression of HIV infection (Matsuda *et al.*, 1993).

Analysis of antibody in saliva as a diagnostic test for HIV (or other infections) offers several distinctive advantages when compared with serum. Saliva can be collected non-invasively, which eliminates the risk of infection for the health care worker who collects the blood sample. Furthermore, viral transmission *via* saliva is unlikely, since infectious virus is rarely isolated from saliva (Ho *et al.*, 1985). Saliva collection also simplifies the diagnostic process in special populations in whom blood drawing is difficult, *i.e.*, individuals with compromised venous access (*e.g.*, injecting drug users), patients with hemophilia, and children (Archibald *et al.*, 1993).

Several salivary and oral fluid tests have been developed for HIV diagnosis. Orasure[®] is a testing system that is commercially available in the United States and can be used for the diagnosis of HIV. The test relies on the collection of an oral mucosal transudate (and therefore IgG antibody). IgG antibody to the virus is the predominant type of anti-HIV immunoglobulin (Cordeiro *et al.*, 1993; Gaudette *et al.*, 1994). Different oral pathologic lesions, which are relatively common in HIV-infected individuals, do not appear to influence the results (Emmons *et al.*, 1995; Gallo *et al.*, 1997). In conclusion, collection and analysis of saliva offer a simple, safe, well-tolerated, and accurate method for the diagnosis of HIV infection.

(3) Drug Monitoring

Similar to other body fluids (*i.e.*, serum, urine, and sweat), saliva has been proposed for the monitoring of systemic levels of drugs (Danhof and Breimer, 1978; Drobitch and Svensson, 1992). A fundamental prerequisite for this diagnostic application of saliva is a definable relationship between the concentration of a therapeutic drug in blood (serum) and the concentration in saliva. For a drug to appear in saliva, drug molecules in serum must pass through the salivary glands and into the oral cavity. Therefore, the presence of a drug in saliva is influenced by the physicochemical characteristics of the drug molecule and its interaction with the cells and tissues of the salivary glands, as well as by extravascular drug metabolism. Factors such as molecular size, lipid solubility, and the degree of ionization of the drug molecule, as well as the effect of salivary pH and the degree of protein binding of the drug, are important determinants of drug

availability in saliva (Drobitch and Svensson, 1992; Siegel, 1993).

Passive diffusion across a concentration gradient is thought to be the major mechanism to account for the appearance of a drug in saliva. Generally, smaller molecules diffuse more easily than larger ones. Due to the presence of the phospholipid layer of the cell membrane, lipophilic molecules diffuse more easily than lipophobic molecules. For similar reasons, non-ionized molecules diffuse more readily through lipid membranes than do ionized molecules. The pKa of the drug (the pH at which 50% of the drug molecules are ionized) and the pH gradient between plasma and saliva determine the concentration gradient on both sides of the membrane, and influence the availability of a drug in saliva (Haeckel and Hanecke, 1996). Therefore, drugs which are not ionizable, or are not ionized within the pH range of saliva, are the most suited to salivary drug monitoring. Due to their size, serum-binding proteins do not cross the membrane. Therefore, only the unbound fraction of the drug in serum is available for diffusion into saliva (Haeckel, 1993). The unbound fraction of a drug is usually the pharmacologically active fraction. This may represent an advantage of drug monitoring in saliva in comparison with drug monitoring in serum, where both bound and unbound fractions of a drug can be detected (Gorodischer and Koren, 1992). Other parameters which may influence the availability of drugs in saliva are the mechanism of drug transfer into saliva (since some drugs reach saliva in ways other than passive diffusion), salivary flow rate (increased flow rate affects salivary pH by increasing bicarbonate secretion), and drug stability in saliva.

The application of saliva for monitoring drug levels has been the subject of considerable investigation (Table 2). Saliva may be used for monitoring patient compliance with psychiatric medications (El-Guebaly *et al.*, 1981). A significant correlation ($r = 0.87$) exists between the salivary and serum lithium levels in patients receiving lithium therapy (Ben-Aryeh *et al.*, 1980, 1984). Saliva is also useful for the monitoring of anti-epileptic drugs. Salivary carbamazepine levels were found to be 38% of serum carbamazepine levels, and a positive correlation ($r = 0.89$) between salivary and serum carbamazepine levels was observed. Stimulation of salivary flow and storage of saliva for several days did not affect this correlation (Rosenthal *et al.*, 1995). In another study, salivary levels of phenobarbital and phenytoin demonstrated excellent correlations ($r = 0.98$ and 0.97 , respectively) with serum levels of these medications (Kankirawatana, 1999). A lower correlation ($r = 0.68$) was found between salivary and total serum levels of cyclosporine. Cyclosporine is a neutral lipophilic molecule that enters saliva mostly by passive diffusion, and salivary levels of this drug reflect the serum levels of free cyclosporine. Therefore, salivary cyclosporine levels may correlate better with serum levels of free, rather than total, cyclosporine (Coates *et al.*, 1988). Similarly, salivary theophylline concentration demonstrated a better correlation with serum concentration of free theophylline ($r = 0.85$) than with serum concentration of total theophylline ($r = 0.85$; Kirk *et al.*, 1994).

Saliva may also be used for monitoring levels of anti-cancer drugs. Saliva was found to be a reliable alternative to serum for the monitoring of irinotecan levels. A correlation of $r = 0.73$ between salivary and serum levels was reported (Takahashi *et al.*, 1997). Salivary analysis may be used to evaluate the cisplatin concentration in serum; however, a defined correlation between salivary and serum levels was not reported (Holding *et al.*, 1999). Conversely, serum carboplatin concentration demonstrated considerable variations and was found to be unreliable in measurements of serum carboplatin (van Warmerdam *et al.*, 1995).

Of particular interest is the use of saliva for the evaluation of illicit drug use. Following drug use, the appearance of the drug in saliva follows a time course that is similar to that of serum. In contrast, drugs appear at a later time point in urine. Nevertheless, as opposed to what is needed for the monitoring of therapeutic drugs, the presence of illicit drugs, and not their concentration, is usually sufficient for forensic purposes. One important exception is ethanol. Ethanol is not ionized in serum, is not protein-bound, and, due to its low molecular weight and lipid solubility, diffuses rapidly into saliva. Consequently, the saliva-to-serum ratio is generally about 1. A significant correlation between salivary and serum alcohol levels was reported (Penttila *et al.*, 1990). Salivary ethanol concentration may be used as an index of the blood ethanol concentration,

provided that the salivary sample is obtained at least 20 min following ingestion. This will allow for absorption and distribution of alcohol, and prevent a falsely elevated reading due to the oral route of consumption (McColl *et al.*, 1979).

Other recreational drugs that can be identified in saliva are amphetamines, barbiturates, benzodiazepines, cocaine, phencyclidine (PCP), and opioids (Cone, 1993; Kidwell *et al.*, 1998; Table 2). Saliva can also be used to detect recent marijuana use by means of radiomunoassay (Gross *et al.*, 1985). $\Delta 9$ -Tetrahydrocannabinol ($\Delta 9$ -THC), a major psychoactive component of marijuana, can be detected in saliva for at least 4 hours after marijuana is smoked (Maseda *et al.*, 1986). Furthermore, saliva can be used to monitor tobacco smoking and exposure to tobacco smoke. The major nicotine metabolite cotinine was investigated as an indicator of exposure to tobacco smoking. Cotinine is tobacco-specific and has a relatively long half-life compared with nicotine (Benowitz, 1983). Salivary cotinine levels were found to be indicative of active and passive smoking (Istvan *et al.*, 1994; Repace *et al.*, 1998). Salivary thiocyanate was also found to be an indicator of cigarette smoking (Luepker *et al.*, 1981); however, cotinine levels are considered the most reliable marker (Di Giusto and Eckhard, 1986).

(4) The Monitoring of Hormone Levels

Saliva can be analyzed as part of the evaluation of endocrine function. The factors that affect drug availability in saliva are

TABLE 2
Drug Monitoring in Saliva

Therapeutic Drugs

Antipyrine
Caffeine
Carbamazepine
Cisplatin
Cyclosporine
Diazepam
Digoxin
Ethosuximide
Irinotecan
Lithium
Methadone
Metoprolol
Oxprenolol
Paracetamol
Phenytoin
Primidone
Procainamide
Quinine
Sulfanilamide
Theophylline
Tolbutamide

Drug Abuse/Recreational Drugs

Amphetamines
Barbiturates
Benzodiazepines
Cocaine
Ethanol
Marijuana
Nicotine
Opioids
Phencyclidine

generally true also for salivary hormones. The majority of hormones enter saliva by passive diffusion across the acinar cells. Most of these hormones are lipid-soluble (*i.e.*, steroids). Small polar molecules do not readily diffuse across cells and instead enter saliva through the tight junctions between cells (ultrafiltration; Quissell, 1993; Read, 1993). The molecular-weight cut-off for ultrafiltration is 100-200. This relatively small molecular size prevents many hormones from entering saliva from serum by means of ultrafiltration. In addition, active transport does not appear to facilitate hormone transfer into saliva (Vining and McGinley, 1986). Measurements of salivary hormone levels are of clinical importance if they accurately reflect the serum hormone levels, or if a constant correlation exists between salivary and serum hormone levels. For neutral steroids which diffuse readily into saliva, salivary hormone levels represent the non-protein-bound (free) serum hormone levels. Conversely, due to their size, protein hormones do not enter saliva through passive diffusion, but primarily through contamination from serum as a result of outflow of GCF or from oral wounds. Furthermore, some steroid hormones can be metabolized in the salivary epithelial cells by intracellular enzymes during transcellular diffusion, which can affect the availability of these hormones in saliva (Quissell, 1993).

Due to their lipid solubility, steroid hormones can be detected in saliva. Salivary cortisol levels demonstrate excellent correlation with free serum cortisol levels ($r = 0.97$; Peters *et al.*, 1982; Vining *et al.*, 1983a). This high correlation is not affected by changes in concentrations of serum-binding proteins. However, the actual salivary cortisol levels are lower than the serum-free cortisol levels, possibly due to enzymatic degradation in the salivary epithelial cells during transcellular diffusion (Quissell, 1993). Salivary cortisol levels were found to be useful in identifying patients with Cushing's syndrome and Addison's disease (Hubl *et al.*, 1984), and also for monitoring the hormone response to physical exercise (Lac *et al.*, 1997) and the effect of acceleration stress (Tarui and Nakamura, 1987; Obminski *et al.*, 1997). Contrary to cortisol, salivary cortisone levels do not accurately reflect serum cortisone levels. Cortisone is a neutral steroid and therefore readily diffuses into saliva; however, cortisol is converted to cortisone by an enzyme present in the salivary glands (11 β -hydroxysteroid dehydrogenase). Thus, cortisone levels in saliva are higher than in serum and do not bear any diagnostic significance (Vining and McGinley, 1986). Other corticosteroids, like prednisone and prednisolone, also do not show a consistent correlation between serum and salivary levels, possibly due to the effect of the same enzyme (Lowe and Dixon, 1983).

Salivary aldosterone levels demonstrated a high correlation with serum aldosterone levels ($r = 0.96$), and increased aldosterone levels were found in both the serum and saliva of patients with primary aldosteronism (Conn's syndrome; McVie *et al.*, 1979). A similar high correlation ($r = 0.92$) between salivary and serum aldosterone levels was observed with the use of a solid-phase enzyme immunoassay (Hubl *et al.*, 1983). These findings were supported by an additional study ($r = 0.93$), and salivary aldosterone levels were found to be approximately one-third of serum levels (Atherden *et al.*, 1985). Testosterone and dehydroepiandrosterone have also been identified in saliva. Salivary concentrations were found to be 1.5-7.5% of the serum concentrations of these hormones

(Gaskell *et al.*, 1980). Similarly, salivary testosterone levels were detected in an additional study which proposed the use of salivary testosterone levels for the assessment of testicular function (Walker *et al.*, 1980). By a direct radioimmunoassay technique, a high correlation between salivary and serum-free testosterone concentration ($r = 0.97$) and salivary and serum total testosterone concentration ($r = 0.7-0.87$) was reported (Vittekk *et al.*, 1985). A significant correlation ($r = 0.79$) between the concentration of unbound salivary and serum testosterone was observed when hormone levels in normal and hyperandrogenic women were evaluated (Baxendale *et al.*, 1982). Monitoring salivary testosterone levels may also be useful in behavioral studies of aggression, depression, abuse, and violent and antisocial behavior (Dabbs, 1993; Granger *et al.*, 1999). However, variability in results between laboratories has been reported (Dabbs *et al.*, 1995). A high correlation between the salivary concentration of androstenedione and dihydrotestosterone and the unbound serum concentration of these hormones has also been reported ($r = 0.92$ and 0.82 , respectively; Baxendale *et al.*, 1983).

Estradiol can be detected in saliva in concentrations that are only 1-2% of serum concentrations. These concentrations are similar to the serum concentrations of free estradiol, which can diffuse into saliva. A significant correlation ($r = 0.78$) between salivary estradiol levels and serum levels of free estradiol was reported (Wang *et al.*, 1986). Salivary estradiol levels followed the same trends as serum estradiol levels during a menstrual cycle (Evans *et al.*, 1980). Furthermore, salivary estriol levels showed a very high correlation ($r = 0.98$) with serum levels of free estriol in pregnant women, and salivary estriol levels were suggested as a means for the assessment of feto-placental function (Kundu *et al.*, 1983; Vining *et al.*, 1983b). Salivary progesterone levels showed good correlation ($r = 0.47-0.58$) with serum levels during the menstrual cycle and reflected the free serum progesterone levels (Luisi *et al.*, 1981; Choe *et al.*, 1983). More recent studies supported the use of salivary diagnosis for the evaluation of clinical problems associated with these hormones. Salivary progesterone levels can be useful for the prediction of ovulation, demonstrating a correlation of 0.75 with serum progesterone levels, and salivary estradiol and progesterone levels can be used for the evaluation of ovarian function (Lu *et al.*, 1997, 1999). Decreased salivary estriol was suggested as a marker of fetal growth retardation (Lechner *et al.*, 1987). Furthermore, an increased salivary estriol-to-progesterone ratio may be a predictor of pre-term delivery (Darne *et al.*, 1987).

Insulin can be detected in saliva, and salivary insulin levels have been evaluated as a means of monitoring serum insulin levels. A positive correlation between saliva and serum insulin levels following a glucose tolerance test was reported for healthy subjects ($r = 0.52$), non-insulin-dependent diabetic patients ($r = 0.50$), and obese non-diabetic patients ($r = 0.69$; Marchetti *et al.*, 1986). Additional work by the same authors utilizing similar methods reported a better correlation between salivary and serum insulin levels in 93 healthy subjects ($r = 0.75$ in males and $r = 0.72$ in females; Marchetti *et al.*, 1988). As assessed by radioimmunoassay, a glucose tolerance test performed on nine healthy patients produced a positive correlation between salivary and serum insulin levels ($r = 0.74$). Salivary insulin levels reached maximal values approximately 30 minutes after the serum levels (90 min *vs.* 60 min; Fekete *et al.*, 1993). Other investigators also reported a similarly high cor-

relation between salivary and serum insulin levels in healthy individuals and insulin-dependent diabetic patients (0.81 and 0.91, respectively), but proposed that the use of salivary insulin levels for the evaluation of serum insulin levels could be misleading, since significant discrepancies between salivary and serum insulin levels were detected for several individuals (Pasic and Pickup, 1988). Additional studies are required to determine if salivary insulin levels should be used for the evaluation of serum insulin levels.

In general, serum and salivary levels of protein hormones are not well-correlated. These hormones are too large to reach saliva by means of passive diffusion across cells or by ultrafiltration, and the detection of these hormones in saliva is primarily due to contamination from serum through GCF or oral wounds. Therefore, serum levels of protein hormones such as gonadotrophins, prolactin, and thyrotropin cannot be accurately monitored by means of salivary analysis (Vining and McGinley, 1986, 1987).

Salivary monitoring of hormone levels has many advantages over the more conventional serum analysis. In addition to the other advantages of salivary diagnosis presented in this article, hormone evaluation often necessitates multiple sample collection in a relatively short time interval, which makes the non-invasive collection of saliva ideal for this purpose (Ellison, 1993). However, it is important to consider the possible limitations of salivary analysis for hormone evaluation. Hormones enter saliva by passive diffusion and ultrafiltration, and active transport of hormones into saliva does not exist. Therefore, mostly lipid-soluble and hormones with small molecular weight can be detected in saliva. Most hormones are protein-bound in serum, and thus salivary hormone levels represent the free hormone levels which are available for diffusion into saliva. This may provide more clinically useful information, since free serum hormone levels are the biologically active fraction of hormone in serum. For accurate results, a constant and predictable correlation must exist between salivary and serum hormone levels. However, different hormones are bound to similar serum carrier proteins, and thus changes in levels of one hormone may affect the free levels of others. For hormones that demonstrate a constant but low salivary-to-serum ratio, a sufficiently large sample volume or a more sensitive analysis method is required. In addition, many hormones exhibit marked circadian variations. Therefore, timing of saliva collection may affect the results. The salivary flow rate can also affect the concentrations of certain hormones. An increase in salivary flow rate will usually result in reduced concentrations of molecules that reach saliva by diffusion. However, the rate of diffusion of steroid hormones, particularly cortisol, is usually high enough to maintain a constant relationship between salivary and serum levels of the hormone regardless of the salivary flow rate. The concentrations of hormones that reach saliva by ultrafiltration, such as dehydroepiandrosterone sulphate, are more affected by changes in salivary flow rate. Changes in salivary flow rate may lead to changes in salivary pH. This may affect the entry into saliva of molecules according to their pka. The stability of hormones in saliva is important as well for accurate evaluation. Hormones in saliva can be degraded, among other ways, by enzymes native to saliva, enzymes derived from oral micro-organisms, and enzymes derived from leukocytes that enter the oral cavity from the gingival sulcus. In addition, molecules that reach saliva by

TABLE 3
Systemic Diseases Affecting Salivary Glands and Saliva

Affective disorder	
Autoimmune disease -	Sjögren's syndrome, rheumatoid diseases, myasthenia gravis, graft-vs.-host disease
Cancer	
Cirrhosis	
Cystic fibrosis	
HIV infection	
Hormonal disorders -	adrenal-cortical disease, diabetes mellitus, thyroiditis, acromegaly
Hypertension	
Metabolic disturbances -	malnutrition, dehydration, vitamin deficiency
Neurological diseases -	Parkinsonism, Bell's palsy, cerebral palsy, Alzheimer's disease
Renal disease	
Sarcoidosis	

Adapted from Mandel, 1990, and Fox, 1993.

passive diffusion across cells, like unconjugated steroids, may be subjected to enzymatic degradation within the salivary glands, prior to entering saliva (Vining and McGinley, 1986; Quissell, 1993; Read, 1993). These factors have to be considered when saliva is evaluated as an alternative for the evaluation of serum hormone levels.

(5) Diagnosis of Oral Disease with Relevance for Systemic Diseases

The monitoring of gland-specific secretions is important for the differential diagnosis of diseases that may have an effect on specific salivary glands, like obstruction or infection (Mandel, 1989). However, monitoring gland-specific saliva can be complicated and time-consuming. Evaluation of the quantity of whole saliva is simple and may provide information which has systemic relevance. Quantitative alterations in saliva may be a result of medications. At least 400 drugs may induce xerostomia. Diuretics, antihypertensives, antipsychotics, antihistamines, antidepressants, anticholinergics, antineoplastics, and recreational drugs such as opiates, amphetamines, barbiturates, hallucinogens, cannabis, and alcohol have been associated with a reduction in salivary flow (Sreebny and Schwartz, 1997; Rees, 1998). Reduced salivary flow may lead to oral problems like progressive dental caries, fungal infection, oral pain, and dysphagia. The reasons for such clinical findings should be thoroughly investigated, since they may be signs of an underlying systemic problem. Systemic disorders that may affect salivary glands and saliva are presented in Table 3.

Qualitative changes in salivary composition can also provide diagnostic information concerning oral problems. Increased levels of albumin in whole saliva were detected in patients who received chemotherapy as treatment for cancer and subsequently developed stomatitis. However, no difference in albumin levels in parotid saliva was observed, which implied that the salivary albumin originated from the mucosal lesions as a result of loss of epithelial barrier function. This was further supported by the fact that salivary levels of another

serum constituent, IgG, showed changes similar to those in albumin levels. The increase in the concentration of albumin in whole saliva was always detected prior to the clinical appearance of stomatitis, suggesting that albumin in whole saliva may be a marker and predictor of this complication. Therefore, the monitoring of salivary albumin can assist in the identification of stomatitis at a pre-clinical stage and enable the chemotherapy dosage to be adjusted or treatment for the stomatitis to be initiated at an early stage (Izutsu *et al.*, 1981). Furthermore, a significant negative correlation was found between normalized EGF (concentration of salivary EGF relative to total salivary protein concentration) and severity of mucositis in patients receiving radiation therapy to the head and neck. This negative correlation suggests that reduced salivary EGF levels may be important for the progression of radiation-induced mucositis (Dumbrigue *et al.*, 2000).

It has been suggested that salivary nitrate, nitrite, and nitrosamine may be related to the development of oral and gastric cancer (Tenovuo, 1986). Increased consumption of dietary nitrate and nitrite is associated with elevated levels of salivary nitrite. Higher levels of salivary nitrate and nitrite, and increased activity of nitrate reductase, were found in oral cancer patients compared with healthy individuals, and were associated with an increased odds ratio for the risk of oral cancer (Badawi *et al.*, 1998).

Saliva can be used for the detection of oral candidiasis, and salivary fungal counts may reflect mucosal colonization (Bergmann, 1996; Hicks *et al.*, 1998). Saliva may also be used for the monitoring of oral bacteria. Bacteria (including anaerobic species) can survive in saliva, and can utilize salivary constituents as a growth medium (de Jong *et al.*, 1984; Bowden, 1997). Furthermore, increased numbers of *Streptococcus mutans* and Lactobacilli in saliva were associated with increased caries prevalence (Klock *et al.*, 1990; Kohler and Bjarnason, 1992) and with the presence of root caries (Van Houte *et al.*, 1990). Saliva can serve as a vector for bacterial transmission, and also as a reservoir for bacterial colonization (Greenstein and Lamster, 1997). Detection of certain bacterial species in saliva can reflect their presence in dental plaque and periodontal pockets (Asikainen *et al.*, 1991; Umeda *et al.*, 1998). Saliva may also be used for periodontal diagnosis, due in large part to contributions from GCF. A comprehensive analysis of this topic is beyond the scope of this review and is covered elsewhere (Kaufman and Lamster, 2000). Nevertheless, the recent focus on the potential role of periodontal disease as a risk factor for cardiovascular and cerebrovascular diseases (Joshiyura *et al.*, 1998; Morrison *et al.*, 1999) and the occurrence of pre-term low-birth-weight babies (Offenbacher *et al.*, 1998) bring new importance to this aspect of salivary analysis.

Concluding Remarks

Saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes. Whole saliva contains locally produced as well as serum-derived markers that have been found to be useful in the diagnosis of a variety of systemic disorders. Whole saliva can be collected in a non-invasive manner by individuals with modest training, including patients. This facilitates the development and introduction of screening tests that can be performed by patients at home. Analysis of saliva can offer a cost-effective approach for the screening of large populations, and may represent an alternative for patients in

whom blood drawing is difficult, or when compliance is a problem (Bailey *et al.*, 1997).

This review suggests that certain diagnostic uses of saliva hold considerable promise. Monitoring of the immune responses to viral infections, including hepatitis and HIV, may prove valuable in the identification of infected individuals, non-symptomatic carriers, and immune individuals. Saliva can also be useful in the monitoring of therapeutic drug levels and the detection of illicit drug use. Further, analysis of saliva may provide valuable information regarding certain endocrine disorders.

Nevertheless, levels of certain markers in saliva are not always a reliable reflection of the levels of these markers in serum. The transfer of serum constituents which are not part of the normal salivary constituents into saliva is related to the physicochemical characteristics of these molecules. Lipophilic molecules diffuse more readily into saliva than do lipophobic molecules. Furthermore, different substances reach saliva by different mechanisms. Although passive diffusion is considered to be the most common mechanism for drugs and hormones, ultrafiltration and active transport have also been proposed for some substances. For accurate diagnosis, a defined relationship is required between the concentration of the biomarker in serum and the concentration in saliva. Normal salivary gland function is usually required for the detection of salivary molecules with diagnostic value. Salivary composition can be influenced by the method of collection and the degree of stimulation of salivary flow. Changes in salivary flow rate may affect the concentration of salivary markers and also their availability due to changes in salivary pH. Variability in salivary flow rate is expected between individuals and in the same individual under various conditions. In addition, many serum markers can reach whole saliva in an unpredictable way (*i.e.*, GCF flow and through oral wounds). These parameters will affect the diagnostic usefulness of many salivary constituents (FDI Working Group 10, Core, 1992). Furthermore, certain systemic disorders, numerous medications, and radiation may affect salivary gland function and consequently the quantity and composition of saliva (Sreebny and Schwartz, 1997; Fox, 1998). Whole saliva also contains proteolytic enzymes derived from the host and from oral micro-organisms (Chauncey, 1961). These enzymes can affect the stability of certain diagnostic markers. Some molecules are also degraded during intracellular diffusion into saliva. Any condition or medication that affects the availability or concentration of a diagnostic marker in saliva may adversely affect the diagnostic usefulness of that marker.

Despite these limitations, the use of saliva for diagnostic purposes is increasing in popularity. Several diagnostic tests are commercially available and are currently used by patients, researchers, and clinicians. Saliva is particularly useful for qualitative (detection of the presence or absence of a marker) rather than quantitative diagnosis, which makes it an important means for the detection of viral infection (especially HIV due to the non-invasive method of collection), past exposure and immunity, and the detection of illicit drug use. Saliva is also useful for the monitoring of hormone levels, especially steroids, and facilitates repeated sampling in short time intervals, which may be particularly important for hormone monitoring and avoiding compliance problems.

Due to its many potential advantages, salivary diagnosis provides an attractive alternative to more invasive, time-con-

suming, complicated, and expensive diagnostic approaches. However, before a salivary diagnostic test can replace a more conventional one, the diagnostic value of a new salivary test has to be compared with accepted diagnostic methods. The usefulness of a new test has to be determined in terms of sensitivity, specificity, correlation with established disease diagnostic criteria, and reproducibility. This review has discussed many disease markers identified in saliva. It is difficult to interpret the significance of a single report that examines levels of any particular marker. However, due to the many potential limitations of salivary diagnosis, promising results from pilot studies must be confirmed in larger, well-controlled trials.

While many questions remain, the potential advantages of salivary analysis for the diagnosis of systemic disease suggest that further studies are warranted. Definition of specific disorders that can be identified or monitored by the analysis of saliva offers the possibility of improved patient management. Consequently, we are likely to see the increased utilization of saliva as a diagnostic fluid. As a result, dentists will have greater involvement in the identification and monitoring of certain non-oral disorders.

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