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Saliva and Its Use as a Diagnostic Fluid

Saliva is the familiar fluid present in the mouths of humans and some animals, which serves to moisten and lubricate the mouth. In addition, it contains enzymes that begin the process of digesting food, it aids our sense of taste, and it helps cleanse and protect the teeth, gums, and other tissues inside the mouth. (1-4)

The Salivary Glands

Human saliva is produced by glands in various locations in and around the mouth. Three primary glands occur in pairs located symmetrically on both sides of the head: the parotids, the submandibulars (also known as the submaxillarys), and the sublinguals. In addition to the primary glands, there are also hundreds of smaller

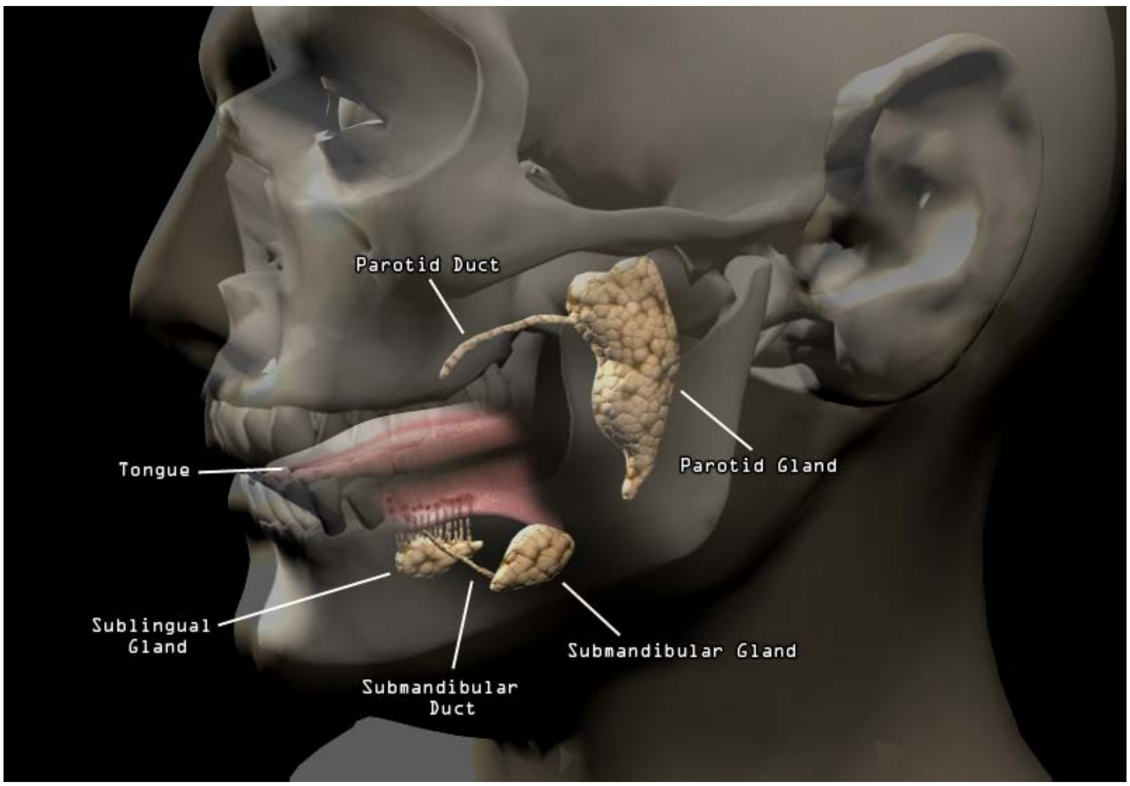

Figure 1. Locations of primary salivary glands.

glands located in the lips, cheeks, tongue, and palate. Although the parotid glands are the largest in size, they produce only about 20% of the total saliva in the unstimulated rest state, and the minor glands and the sublinguals together contribute only about an additional 15%. The submandibular glands are by far the most active in the unstimulated state, and they are estimated to produce about 65% of the total rest volume. (3) (14)

Salivary Gland Structure

The primary salivary glands are composed of numerous clusters of 15 to 100 secretory cells arranged in globular or tubular configurations. These clusters are called acini (singular acinus.) The acini open into ducts, which merge to carry the saliva towards the mouth. Ductal cells also transport electrolytes in and out of saliva, and they can participate in secretory activity to a limited degree. The acini and ducts are surrounded by myoepithelial cells, which can contract to help accelerate saliva flow. (5)

Acini are composed principally of two types of secretory cells, serous and mucous, which are both specialized for the production of large quantities of proteins. Serous cells produce a thin, watery saliva containing the digestive enzyme α-amylase. Mucous cells produce a thicker saliva rich in large glycoproteins known as mucins, which help lubricate the mouth and aid in swallowing food. The proportions of serous and mucous cells are different in the various salivary glands, and each gland secretes a saliva that reflects the cellular makeup of its acini. (6,7) (See Table 1.)

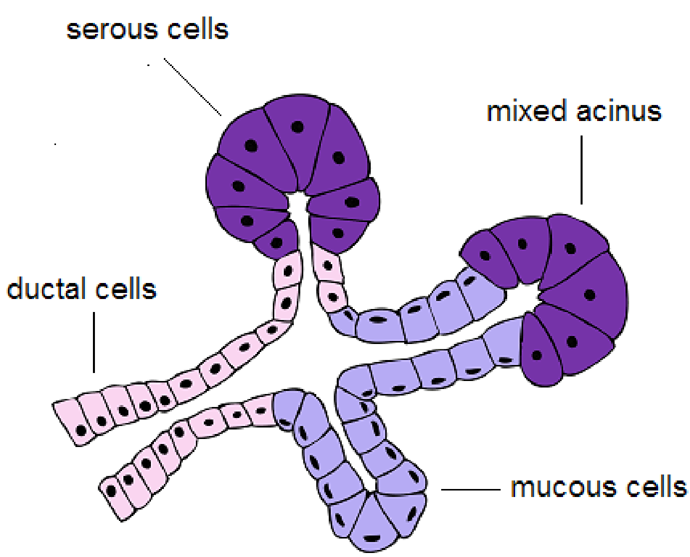

Figure 2. Cross section of a non-realistic salivary gland, showing three types of acini.

Table 1. Salivary Gland Secretions

Gland type	Saliva type
Parotid, and Von Ebner's (on the tongue)	Serous
Submandibular	Mixed, more serous than mucous
Sublingual	Mixed, but mostly mucous
Most minor	Mucous

The Composition of Saliva

Saliva is principally a mixture of water and electrolytes; both pass into the acini from a dense network of capillaries that surround the salivary glands. The initial product secreted by the cells in the acini has concentrations of sodium, potassium, chloride, and bicarbonate ions similar to plasma. As the saliva passes through the ductal regions of the glands, sodium and chloride ions are absorbed and additional potassium and bicarbonate ions are secreted. (See figure 3.) The total ionic concentration of the final product is lower than that of plasma. (7,8)

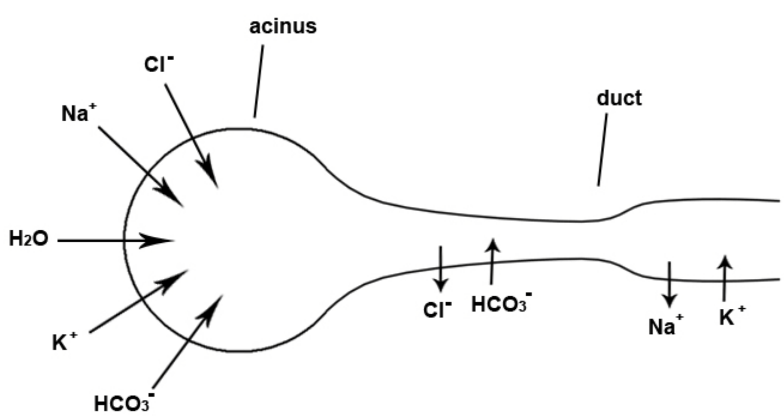

Figure 3. Ion exchanges during saliva production.

Ionic concentrations change as saliva production is stimulated, however, and concentrations of sodium, chloride, and bicarbonate ions all increase with accelerated flow. As bicarbonate levels increase, the pH of saliva changes from slightly acidic (6-7) to slightly basic (around 8). (7,8) Changes in the pH of the saliva can be a concern for saliva testing because pH can have an effect on the amount of ionic charge present on certain drugs or other compounds. The presence of these charges can affect the ability of the compound to diffuse through neutral lipid membranes and be present in saliva. (9)

Saliva also contains organic compounds that are synthesized primarily in the cells of the acini, and also to a lesser extent by some ductal cells. These organic products are mostly proteins or peptides, including enzymes, mucins, lactoferrin, lysozyme, cystatins, and histatins. (10-12) Nutrients needed for the synthesis of these compounds pass from the capillaries surrounding the glands into the cells, either by simple diffusion, or by active transport mechanisms. (7) The presence or concentrations of these proteins may vary substantially in different glandular salivas due to differences in the cellular makeup of the glands. (3,13) The organic and inorganic components of saliva serve a wide range of functions. Some of the more important of these are summarized in the Table 2. (14)

Whole Saliva

The whole saliva that pools on the floor of the mouth is a mixture of the fluids secreted by all of the various saliva glands, and it may also contain the following components in varying degrees: (9)

- Bronchial and nasal secretions
- Fluid that comes from the junctions between gums and teeth (gingival crevicular fluid or GCF)
- Blood and serum from wounds in the mouth, including the gums if they are not healthy
- Micro-organisms (bacteria, viruses, fungi) and products derived from them, including enzymes
- Assorted cellular components and food debris

The Control of Saliva Secretion and Composition

Saliva production changes throughout the course of the day. It is greatest during the waking hours and diminishes greatly during sleep. (15) Various stimuli including taste, smell, and chewing motions of the jaw greatly increase saliva flow. (2,15,16) Control over saliva production is shared by the sympathetic and parasympathetic branches of the autonomic nervous system, which work together in a complex relationship. The parasympathetic system is largely responsible for increases in fluid secretion by the salivary glands, but the sympathetic system also plays a smaller role. Both systems can signal the myoepithelial cells in the salivary glands to contract, increasing the flow of saliva. (17)

Table 2. Functions of Saliva Components.

Mucins	Lubricate food Protect teeth against acid Help protect against bacteria, viruses, fungi
Digestive Enzymes	α-Amylase – digests starches Lipase – digests fats Protease – digests proteins
Lysozyme Peroxidases Lactoferrin Histatins Cystatins	Anti-bacterial agents
Secretory Immunoglobulin A Histatins Cystatins	Anti-fungal, anti-viral agents
Bicarbonate ions Phosphate ions Proteins	Help protect teeth and soft tissues against acidic conditions
Calcium ions Phosphate ions Proline-rich proteins	Help maintain mineral content of tooth enamel

Concentrations of some components in whole saliva can be altered because of differing flow rates from the principal glands. While in the unstimulated rest state, the parotid glands contribute only a relatively small proportion of the total mix, and the viscous, mucin-rich saliva from the minor, sublingual and submandibular glands predominates. When stimulated, however, the parotid glands disproportionately increase their output of watery saliva, effectively lowering the concentration of mucins in the mixed saliva. (14)

Control over the secretion of the salivary proteins that are synthesized in the salivary glands is largely handled by the sympathetic nervous system, but the parasympathetic system is also involved. These proteins include mucins and digestive enzymes, such as α-amylase and lipase, which are stored in small granules within the cell; these components can be quickly released into saliva in response to stimulation from taste, smell, and chewing. (17) Physical and psychological stress have also been shown to affect the secretion of salivary proteins. (18,19) Recent investigations have explored the use of salivary α-amylase as a biomarker of stress, and there has been much interest in its ability to serve as a convenient and non-invasive measure of sympathetic nervous activity. (20-27) However, due to the role that the parasympathetic nervous system also plays in the control of protein secretion, a recent paper has questioned the ability of salivary α-amylase to serve exclusively as a sympathetic marker. (28)

The Movement of Extra-Glandular Substances into Saliva

In addition to the molecules that are produced locally in the saliva glands, there are some that pass into saliva from outside the salivary glands. These include drugs, drug by-products, hormones, and some proteins. The presence of these substances in saliva has spurred research into its use as a diagnostic fluid, especially in view of the relative ease and safety of collection it offers when compared to more traditional diagnostic fluids such as blood and urine. (9)

Many of the substances that circulate in the bloodstream can pass from blood into saliva by unaided, or passive, diffusion. As described above, the capillaries surrounding the salivary glands are quite porous for many substances. Materials can pass from the blood system into the interstitial space surrounding the glands and then make their way through the membranes of acinar or ductal cells. The ability of a molecule to diffuse passively through cell membranes depends partly on its size and partly on how much electrical charge it carries. If a molecule is polar in nature, or if it separates into charged ions while in solution, it will have a hard time passing through the membranes, which are made out of neutral fatty compounds called phospholipids. Steroid hormones are relatively small in size, and most of them are fatty, non-polar compounds, so they tend to pass relatively easily by diffusion. Other molecules such as the large protein hormones, or hormones or drugs that are bound to large carrier proteins while in the bloodstream, are too big to enter by this route. (29)

A second pathway used by molecules to enter saliva is by filtering through the tight spaces between acinar or ductal cells. In order to do this they must be relatively small. Sulfated steroids such as dehydro-epiandrosterone sulfate (DHEA-S) and estriol sulfate, which are not able to pass through the fatty cell walls because of their electrical charges, were formerly thought to enter saliva by this route. These molecules are too large to enter easily by this pathway, however, and this was thought to limit the amounts that could enter saliva. (29) More recent research has identified a large family of organic anion transport polypeptides (OATP) that actively transport molecules such as DHEA-S across membranes. It therefore seems possible that such a mode of entry into the saliva glands may exist for DHEA-S as well. (30-31) Compounds such as DHEA-S are slower to migrate into saliva than the neutral steroid hormones, and when saliva output is stimulated they may move too slowly to keep up with the accelerated flow rates, causing concentrations in saliva to drop. (29)

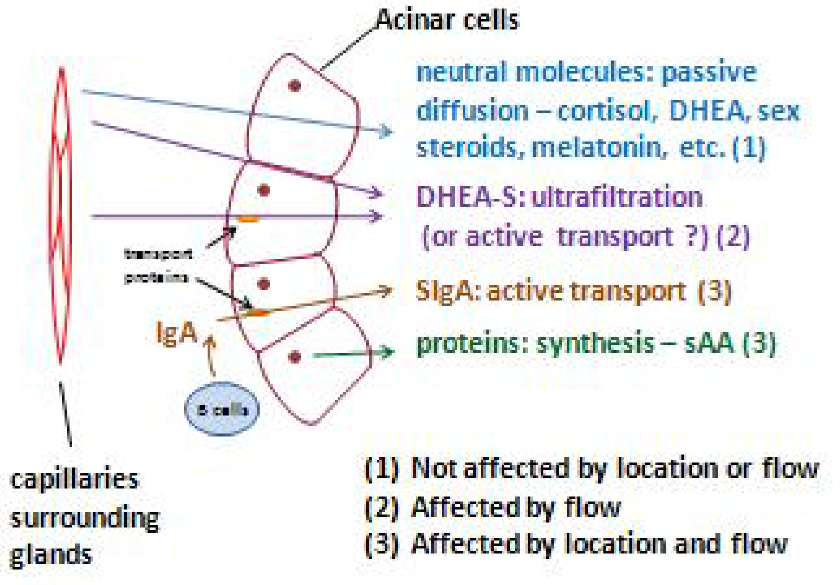

Figure 4. Movement of biomarkers into saliva.

Blood components can also gain entry into saliva from the outflow of the serum-like gingival crevicular fluid (GCF) from the gums, or from small injuries or burns in the mouth. GCF is believed to be a major route by which certain molecules, which would ordinarily be too large to pass by either diffusion or filtration, can find their way from serum into saliva. (29,32) Small amounts of oral mucosal transudate (OMT), a serum-derived fluid that passes through oral mucosal surfaces, also mix into whole saliva. (33)

Another substance that originates outside the saliva glands is secretory immunoglobulin A (SIgA), but salivary SIgA is not derived from circulating IgA . Rather, polymeric IgA is secreted by B-lymphocyte cells close to the salivary cells, then bound and transported across the cells by a Polymeric Immunoglobulin Receptor

(PIgR), and finally released into salivary secretions as SIgA. (10,34) It has been shown that secretion of SIgA is increased by nervous stimulation of the saliva glands, but the details of the nervous control of production and transport are not fully understood. (35,36) Saliva flow rates are also affected by stimulation, and this effect appears to be greater than the increase in the secretion rate. SIgA concentrations in saliva are known to decrease as saliva flow is stimulated. (37)

The Use of Saliva Testing for Hormones

Due to the ease with which saliva can be collected, it is an appealing medium for hormone studies that require multiple samples to be taken over the course of the day. In addition to simply being more convenient, saliva testing can actually be preferable to serum testing in several ways. First, for hormones such as cortisol that reflect stress levels, the collection of a saliva sample is much less invasive and stress-inducing than blood collection. Using saliva as a testing medium should therefore help avoid measurement of reaction to the collection process itself. Secondly, measurement of steroid hormone levels by salivary testing is actually preferable to serum measurement because the presence of specific and non-specific binding proteins in serum complicates attempts to measure the levels of active hormones. In the bound form, the hormone is not biologically active, and it is also too large to pass into saliva. Only a small, unbound fraction of the hormone is available to diffuse into the saliva, and for this reason salivary steroid hormone levels are consistently lower than in serum. The low level of a hormone measured in saliva is believed to be a direct measure of the biologically active, free fraction in serum. (38)

One of the most-studied steroid hormones is cortisol, and it has been demonstrated that salivary cortisol levels have a steady and predictable relation to the free, unbound cortisol levels in serum. It has also been shown that the rate of equilibrium of cortisol between blood and saliva is rapid, which helps insure that cortisol levels in saliva do accurately reflect the free-serum levels regardless of the degree of stimulation of the saliva glands. (39) Commercials kits for assaying cortisol levels in saliva are used to identify patients with Cushing's Syndrome and Addison's Disease, as well as in a wide range of bio-behavioral and stress-related studies. (23,24,26,40)

Other steroid hormones have been studied in saliva, and a number, including progesterone, testosterone, the various estrogens, and common precursor molecules such as androstenedione, have also been shown to have stable relationships between free-serum and saliva levels and rapid migration rates. (41,42) Like cortisol, the levels of these hormones measured in saliva are lower than in serum, and for some like estradiol and testosterone the saliva levels can be very low, requiring assay methods with very high sensitivity. Salivary sex steroids are increasingly being used in behavioral, developmental, and aging studies, as well as in clinical and research applications related to reproduction. (43-52.)

The Growing Use of Salivary Testing

Given the ease with which saliva can be collected in non-laboratory settings, it is an ideal medium for use in the field to monitor drug use or to screen for various diseases. (33). Cotinine, a metabolite of nicotine, is widely used to assess tobacco use and in studies on the effects of smoking on health. (53-55) Another notable development is the use of saliva test kits to check for the presence of antibodies to the HIV virus. (56,57)

It is also becoming increasingly clear that saliva contains low levels of many more substances than had been previously realized, and that some of these may have diagnostic potential. The UCLA Human Salivary Proteome Project, funded by the National Center for Dental and Craniofacial Research, has already identified more than 1000 proteins in the saliva of healthy individuals, and many groups are now studying the saliva of individuals with various diseases, looking for substances that could be used for screening and diagnostic purposes. (58,59) Studies have already reported encouraging results, such as the identification of RNA molecules and other biomarkers in saliva that are associated with cancers–both in the oral cavity and elsewhere in the body–which could lead to practical tests for the disease in the near future. (60-64) Salivary biomarkers related to cardiovascular disease and periodontal disease are also being actively studied. (65-70) As additional substances of interest are discovered in saliva, methods to assay their presence quickly and efficiently will need to be developed and made commercially available.

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