The importance of living metal ions in saliva and teeth

Introduction

Saliva is the mixed glandular secretion which constantly bathes the teeth and the oral mucosa. On the other hand, Salivary fluid is an exocrine secretion, consisting of approximately 99% water, containing a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins, represented by enzymes, immunoglobulins and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides of importance to oral health. There are also glucose and nitrogenous products, such as urea and ammonia. The components interact and are responsible for the various functions attributed to saliva. So, Chemical digestion: breaks down starch by the function of "salivary amylase". On the other hand, when there is a muscular activity, or some olfactory stimulation is there (food) it will also result in the production of saliva (stimulated) produced from all the sides of face which is the most part of the average production of saliva per day.

Components interact in the following general area:

(1) bicarbonates, phosphates, and urea act to modulate pH and the buffering capacity of saliva.

(2) macromolecule proteins and mucins serve to cleanse, aggregate, and/or attach oral microorganisms and contribute to dental plaque metabolism.

(3) calcium, phosphate, and proteins work together as an antisolubility factor and modulate demineralization and remineralization.

(4) immunoglobulins, proteins, and enzymes provide antibacterial action.

Composition of saliva

Its composition is 96 wt.% inorganic material and 4 wt.% organic material and water. In dentin, the inorganic material represents 70 wt.%. This inorganic material is mainly composed by a calcium phosphate related to the hexagonal hydroxyapatite, whose chemical formula is Ca10(PO4)6·2(OH).

The composition of saliva varies, depending on whether it is stimulated or unstimulated (resting). Stimulated saliva is secreted in response to masticatory or gustatory stimulation, or to other less common stimuli such as vomiting center.

While unstimulated saliva is the mixture of secretions which enter the mouth in the absence of exogenous stimuli. The submandibular gland secretes the greatest proportion of the unstimulated saliva (69%) followed by the parotid, sublingual and minor glands.

On the other hand, Saliva is composed of about 99.5% water and 0.5% solid of which about half is inorganic (chloride, bicarbonate, phosphate, sodium, calcium, potassium, trace elements, and dissolved carbon dioxide, oxygen, and nitrogen) and about half is organic (proteins, vitamins, lipids, hormone like substances, free amino acids, urea, as well as microbial and shed cells, and antimicrobial substances).

Saliva Function

Saliva plays a significant role in the protection of the intraoral structures against injuries caused by various pathogenic microbes, mechanical or chemical irritants.

The functions of the saliva:

- Defensive/buffering capacity
- Remineralization of teeth
- Restoration of soft tissues
- Lubrication capacity
- Digestion
- Antimicrobial capacity

The most important ions for maintaining the ionic strength of saliva are sodium, potassium and chloride.

1- Flow rate: It is the main factor affecting the composition of saliva. The concentration of constituents varies between stimulated and unstimulated flow rate of saliva. As the flow rate increases, the pH and other constituents like protein, sodium, calcium, chloride and bicarbonate rise. While other constituents fall (magnesium, potassium and phosphate).

2- Glandular source: The parotid glands normally contribute 20% of the total volume of unstimulated saliva, while at high flow rate the parotid glands contribute 50% of the whole saliva.

3- Duration of stimulation: The saliva collected for two minutes will have a different composition from saliva collected for 10-15 minutes. Bicarbonate, protein and calcium concentration increases with duration of stimulation whereas decreased chloride.

4- Nature of stimulus: The taste of salts stimulated much the higher protein content. Acid is the most potent stimulus for salivary secretion and leads to production of an alkaline saliva. The pH of saliva is dependent mainly on the flow rate and is independent of the nature of the stimulus. The secretion of salivary glands is mainly controlled by parasympathetic impulses from the salivary nuclei. Sympathetic impulses are more likely to influence salivary composition by increasing exocytosis from certain cells.

5- Others like medications (Antidepressants, diuretics, antihistamines and narcotics), various diseases (Autoimmune diseases, diabetes mellitus, salivary glands stones, malnutrition) and exercises.

Influence of saliva on dental caries

The presence of saliva is important for the health of both oral soft and hard tissues. The complexity of physical and chemical composition of salivary secretions performs a considerable number of protective functions in addition to other functions. The influence of saliva on the caries process is fundamental; the anti-caries effects of saliva can be categorized as static or dynamic. The static effects are those which may be assumed to be exerted continuously, throughout the day and include effects on bacterial composition of plaque through antibacterial or metabolic factors, protective effects of pellicle formation, and effects of salivary ions in maintaining a supersaturated environment for the tooth mineral. Dynamic effects, on the other hand, are those that are mobilized over the time-course of the Stephan curve. These include the clearance of the carbohydrate challenge and of the acid products of plaque metabolism, and the alkalinity and buffering power to restore plaque pH towards neutrality.

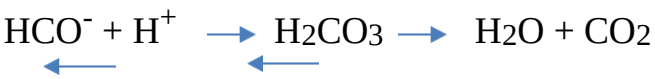
These dynamic effects are related to the level of salivary stimulation, and are thus activated during eating or drinking, i.e., approximately when their action is required.

1- Saliva enhances the clearance of cariogenic microorganisms from the mouth, not only by its flushing effect, but also by bacteria agglutination.

2- Saliva may remineralize early carious lesion by its calcium and phosphate ions. Remineralization is remarkably enhanced by the presence of fluoride.

3- Inorganic components of saliva (calcium, phosphate, fluoride) increase the resistance of hydroxyapatite against cariogenic challenge and enhance the saturation of plaque fluid with respect to the tooth surface structure.

4- Salivary buffering effect (bicarbonate, phosphate and some protein systems which not only have a buffer effect but also provide ideal conditions for automatically eliminating certain bacterial components that require a very low pH to survive). The buffering systems in saliva (buffer is a solution that tend to maintain constant pH) have different pH ranges of maximal buffering capacity, the phosphate and bicarbonate systems have pH of 6.8 - 7.0 and 6.1 - 6.3 respectively. Bicarbonate may buffer against pH fall, thus plays an important role in the protection against dental caries. The bicarbonate found in saliva is present as a result of carbohydrate metabolism, through anaerobic glycolysis and the citric acid cycle. When an acid is added bicarbonate release a weak carbonic acid, which rapidly decompose to water and carbon dioxide that leave the solution. The increase in the carbonic acid concentration lead to more carbon dioxide escape from saliva making it possible for more bicarbonate to bind to hydrogen ions (H+), which will end in the removal of the acid.



The phosphate buffer plays an essential role when salivary flow is low. At a pH greater than 6 the saliva is supersaturated with phosphate with regard to hydroxyapatite (HA) which forms the dental enamel. When the pH falls below the critical level (5.5) the HA begins to dissolve, freeing phosphates that attempt to restore the pH balance. Above this value, enamel tends to remineralize. Certain proteins, such as histatins or sialin, as well as certain alkaline products generated by the metabolic activity of bacteria on amino acids, peptides, proteins and urea, are also important for controlling the pH of the saliva. Maintenance of physiological pH at mucosal cells and tooth surface is an important function of salivary buffers. Hydrogen ion concentration is expressed in pH units; it is equal to the negative logarithm of the hydrogen ion concentration. The value of salivary pH varies is in the range of 6.7 and 7.4.

General Properties of enzymes

Enzymes are protein catalysts for chemical reaction in biological systems. They increase the rate of chemical reactions taking place within living cells without changing themselves.

Nature of Enzymes

Most enzymes are protein in nature. Depending on the presence and absence of a non-protein component with the enzyme enzymes can exist as, simple enzyme or holoenzyme.

1. Simple enzyme: It is made up of only protein molecules not bound to any nonproteins. Example: Pancreatic Ribonuclease.

2. Holo enzyme is made up o protein groups and non-protein component.

A- The protein component of this holo enzymes is called apoenzyme

B- The non-protein component of the holo enzyme is called a cofactor.

If this cofactor is an organic compound, it is called a coenzyme and if it is an inorganic groups it is called activator. (Fe 2+, Mn 2+, or Zn 2+ ions).

If the cofactor is bound so tightly to the apoenzyme and is difficult to remove without damaging the enzyme it is sometimes called a prosthetic group

Metal ions in enzymes

Many enzymes require metal ions like ca^{2+}, K^{+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Co^{2+} for their activity.

Metal-activated enzymes-form only loose and easily dissociable complexes with the metal and can easily release the metal without denaturation. Metalloenzymes hold the metal tightly on the molecule and do not release it even during extensive purification.

Metal ions promote enzyme action by:

- **A.** Maintaining or producing the active structural conformation of the enzyme (e.g., glutamine synthase)
- **B.** Promoting the formation of the enzyme-substrate complex (Example: Enolase and carboxypeptidase A.)
- **C.** Acting as electron donors or acceptors (Example: Fe-S proteins and cytochromes).
- **D.** Causing distortions in the substrate or the enzyme Example: phosphotransferases).

Salivary amylase is a glucose-polymer cleavage enzyme that is produced by the salivary glands. It comprises a small portion of the total amylase excreted, which is mostly made by the pancreas. Amylases digest starch into smaller molecules, ultimately yielding maltose, which in turn is cleaved into two glucose molecules by maltase. Starch comprises a significant portion of the typical human diet for most nationalities.

Important role of saliva

Saliva has many crucial roles in promoting health, including

1. protecting the oral cavity and facilitating eating.
2. Within the mouth, saliva hydrates mucosal tissues, removes cell and food debris, buffers oral pH.

3. lubricates the oral cavity aiding mastication and preventing dental wear, forms food boli to assist swallowing,
4. protects against teeth demineralization, has antimicrobial activity and prevents infections, and closes wounds while stimulating healing.
5. Saliva also plays essential roles in food perception and digestion. The exact mechanisms of digestion remain unclear.
6. For taste, the physical and compositional characteristics of saliva facilitate perception.

Properties of Enzyme

A. Active site

Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid chains that create a three-dimensional surface complementary to the substrate.

The active site binds the substrate, forming an enzyme-substrate (ES) complex. ES is converted to enzyme-product (EP); which subsequently dissociates to enzyme and product.

B. Catalytic efficiency/ Enzyme turnover number

Most enzyme- catalyzed reactions are highly efficient proceeding from 103 to 108 times faster than uncatalyzed reactions. Typically each enzyme molecule is capable of transforming 100 to 1000 substrate molecule in to product each second.

C. Specificity

Enzymes are specific for their substrate. Specificity of enzymes are divided into:

a. Absolute specificity:- this means one enzyme catalyzes or acts on only one substrate. For example: Urease catalyzes hydrolysis of urea but not thiourea.

b. Stereo specificity- some enzymes are specific to only one isomer even if the compound is one type of molecule.

D. Regulation

Enzyme activity can be regulated- that is, enzyme can be, activated or inhibited so that the rate of product formation responds to the needs of the cell.

E. Zymogens (- inactive form of enzyme)

Some enzymes are produced in nature in an inactive form which can be activated when they are required. Such type of enzymes are called Zymogens (Proenzymes).

Many of the digestive enzymes and enzymes concerned with blood coagulation are in this group.

F. Isoenzymes (Isozymes)

These are enzymes having similar catalytic activity, act on the same substrate and produces the same product but originated at different site and exhibiting different physical and chemical characteristics such as electrophoretic mobilities, amino acid composition and immunological behavior.

Example: LDH (Lactate dehydrogenase) exists in five different forms each having four polypeptide chains. H= Heart and M=Muscle.

Dental caries development

Dental caries: is a chronic disease involving a large number of populations. The term dental caries (tooth decay) is used to describe the results – the signs and symptoms – of a localized chemical dissolution of the tooth surface caused by metabolic events taking place in the biofilm (dental plaque) covering the affected area. It is a disease of dental calcified tissue , a multi-factorial disease characterized by "demineralization of the mineral components and dissolution of the organic matrix".

The caries process affects the mineralized tissues of the teeth, enamel dentine and cementum and caused by the action of micro-organisms on fermentable carbohydrates in the diet.

The disease is often described as progressive disease, if it is not treated may expand in size and progress to the pulp leading to pulp inflammation thus pain, discomfort, and the end result will be loss of vitality then loss of tooth

Carious process is complicated process, there should be interaction of several etiological and predisposing factors for caries t occur. This may be explained the variation in the caries susceptibility of individuals to caries process

Dental caries process is the result of an interaction of the following:

1- Host
2- Plaque.
3- Diet

Tooth Morphology: such as presence of deep pits and fissures with a sharp cusp,

1- Tooth Morphology: Dental caries lesions may develop at any tooth site in the oral cavity where a biofilm develops and remains for a period of time. Such sites include pits, grooves and fissures in occlusal surfaces, especially during eruption, approximal surfaces cervical to the contact point/area and along the gingival margin. Insertion of foreign bodies to the dentition (e.g. fillings with inappropriate margins, dentures, orthodontic bands) may also result in such 'protected' sites.

Morphology of tooth are mostly occurred due to hereditary factors while in the same time there is some environmental factors which affected the type of pits and fissure like the presence of some trace elements in the environment especially strontium which make effect on tooth morphology and make the cusps more rounded and shallower pits and fissures, these areas are relatively protected from mechanical influence from the tongue, the cheeks, abrasive foods and, not least, tooth brushing. These are the sites where lesion development is more likely to occur because the biofilm is allowed to stagnate there for prolonged time.

Sites on the tooth, which favor plaque retention and stagnation, are prone to decay: these are:

1. Enamel Pits and fissure
2. Proximal enamel surfaces.
3. Cervical margin of teeth.
4. Exposed root surface because of gingival recession
5. Deficient or over hang restoration (recurrent caries).
6. Tooth surfaces adjacent to denture and bridges

The relationship between Gibbs free energy and demineralization Fluoride and tooth decay presentation include toothpaste and fluoride varnishes

There are elements in the teeth are Zinc, copper, strontium, magnesium and fluoride, - - etc.

These elements may incorporate the enamel crystal in substitutions with one of its major elements as for example substitution of Ca ions by Mg, (Ca_9Mg $(PO_4)6F_2$, or substitution of hydroxyl group by fluoride ion and formation hydroxy appetite crystals $Ca_{10}(PO_4)6F_2$. Certain elements (zinc, fluoride, iron, chloride) accumulate in the enamel surface, while others are sparse in surface as compared with subsurface enamel. Changes of the enamel (decrease in density and permeability, an increase in fluoride content) occur with age.

Some of these elements may incorporate either in pre-eruptive stage including all layers of enamel and dentin or in post eruptive stage involving the outer enamel surface only.

Some of these elements may increase the resistance of teeth to dental caries as fluoride ions, tin, zinc, strontium and molybdenum, while others increase susceptibility to dental caries as magnesium, however the role of other elements may not well substantiate as K, Mn and Al

The organic constituents and water of both enamel and dentin may act as a diffusion pathway for bacterial acids increasing the tooth destruction. In other way, they permit the penetration of ions for physiological remineralization-demineralization process. Such voids in enamel as well as proteins act as a caution for intense biting pressure to prevent fracture.

Dynamics Process of De-/Remineralization

Dental caries is a disease that is manifested as a dynamic process of de/remineralization in the mouth (Enamel sieve concept). Demineralization is a continual imbalance between pathological and protective factors that results in the dissolution of apatite crystals and the net loss of calcium, phosphate, and other ions from the tooth. The first stage of demineralization is occurring at the atomic level far before it can be seen visually as gross demineralization. During this step, fermentable carbohydrates are metabolized by bacteria in dental plaque to produce organic acids. The acids diffuse into the dental hard tissue through the water among the crystals and could reach a susceptible site on a crystal surface. Calcium and phosphate are dissolved into the surrounding aqueous phase between the crystals. This is considered as the first step in the continuum of the dental caries process which can eventually lead to cavitation.

The oral fluids (saliva, biofilm fluid) have calcium (Ca) and phosphate (P) insupersaturated concentrations with respect to the mineral composition of enamel.

At physiological conditions (a neutral pH of 7), low ion concentrations are sufficient to keep dental hard tissues in equilibrium. If the pH drops because ofacid produced by the dental plaque, higher ion concentrations are needed toprevent dissolution of dental hard tissue. Calcium (Ca) and phosphate (P) ions are continually deposited on the enamel surface or are redeposit in enamel areas where they were lost. At a pH of 5.5, under saturation begins, that is, the calcium and phosphate ion concentrations in the plaque fluid are not sufficient to maintain the enamel in stable equilibrium; thus, the enamel starts to dissolve. The term “remineralization” is used to described mineral gain. Remineralization isthe body’s natural repair process for subsurface non-cavitatedcarious lesions. In the process of remineralization, calcium and

phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain.

De-/remineralization cycles continue in the mouth as long as there are factors including cariogenic bacteria, fermentable carbohydrates, and saliva present. The balance between pathological factors and protective factors determines whether demineralization or remineralization is proceeding at any one time.

The development of carious lesion ocuurs in three stages:

-The earliest stage is the incipient lesion; macroscopically evidenced on the tooth surface by the appearance of an area of opacity (the white spot lesion), which is accompanied by histologic changes of the enamel at the microscopic level and is well established with a number of recognizable zones.

- The second stage includes the progress of the demineralization front toward the dentino-enamel junction and/or into the dentin; the affected dentin displays discoloration from brown to dark brown or black, microscopic changes of dentil showed different zones.

- The final phase of caries development is the development of the overt, or frank lesion, which is characterized by actual cavitation.

Role does fluoride play in preventing tooth decay

Mechanism of fluoride action in caries prevention

Fluoride treatment regimens have been developed to prevent dental caries. Systemic fluoride is easily absorbed and is taken up into the enamel during the period of pre-eruptive tooth formation. The predominant beneficial cariostatic effects of fluoride in erupted teeth occur locally at the tooth surface. This could be achieved by fluoridated toothpaste, fluoride-containing water, fluoridated salt, etc. maintaining elevated intra- oral fluoride levels of the teeth, dental biofilm and saliva throughout the day.

Dental enamel is a calcified tissue that forms the outer protective covering of the anatomical crown of a tooth. Enamel once formed cannot be biologically repaired or replaced2. The oral cavity continuously goes through cycles of demineralization and remineralization.

Loosing minerals from the tooth after an acidic encounter is called demineralization, whereas restoration of these minerals back into the tooth structure is called remineralization.

During demineralization, the enamel surface becomes rough and rugged upon acidic encounter. Thus throughout the life of a tooth, there are enamel demineralization/remineralization cycles that dictate the extent of mineral balance and tissue integrity or degradation3. Human saliva has a buffering role and acts as a carrier of essential ions that can bring a constructive change in the structure of enamel, promoting remineralization.

Structure of dental enamel

Dental enamel is composed of 96% inorganic material, 3% water, and 1% organic matrix6. The inorganic component of dental enamel is hydroxyapatite (HAP) crystal and human enamel is a hard, acellular, and avascular tissue.

What minerals are in saliva?

The main components of saliva electrolytes are **sodium, calcium, copper, magnesium, bicarbonates, and organic phosphates**. Increase in calcium level in the remineralization solution may enhance the deposition velocity of minerals in the caries lesion

What is composition of saliva?

Saliva is composed of a variety of electrolytes, including **sodium, potassium, calcium, magnesium, bicarbonate, and phosphates**. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia

Is there calcium in saliva?

The calcium level of whole saliva had a **median of 1.23 mMol/l**. Subjects with calcium level below the median were categorized as 'low', while those with higher values formed the 'high' salivary calcium group. There were more men than women in the 'High' salivary calcium group (p=0.025)

How many enzymes are in saliva?

The mouth and esophagus themselves **don't make any enzymes**, but saliva, produced in the salivary glands and excreted into the mouth, and down into the esophagus, contains several important enzymes such as amylase, lysozyme and lingual lipase

What is the name of enzyme in your saliva?

amylase

Saliva contains special enzymes that help digest the starches in your food. An enzyme called **amylase** breaks down starches (complex carbohydrates) into sugars, which your body can more easily absorb.

What are the components of saliva and their functions?

Organic and inorganic constituents of saliva

Salivary component	Function
Lysozyme	Hydrolysis of cell membrane
Mucins	Digestion, lubrication, and pellicle formation
Protease	Digestion
Water	Mucosal integrit

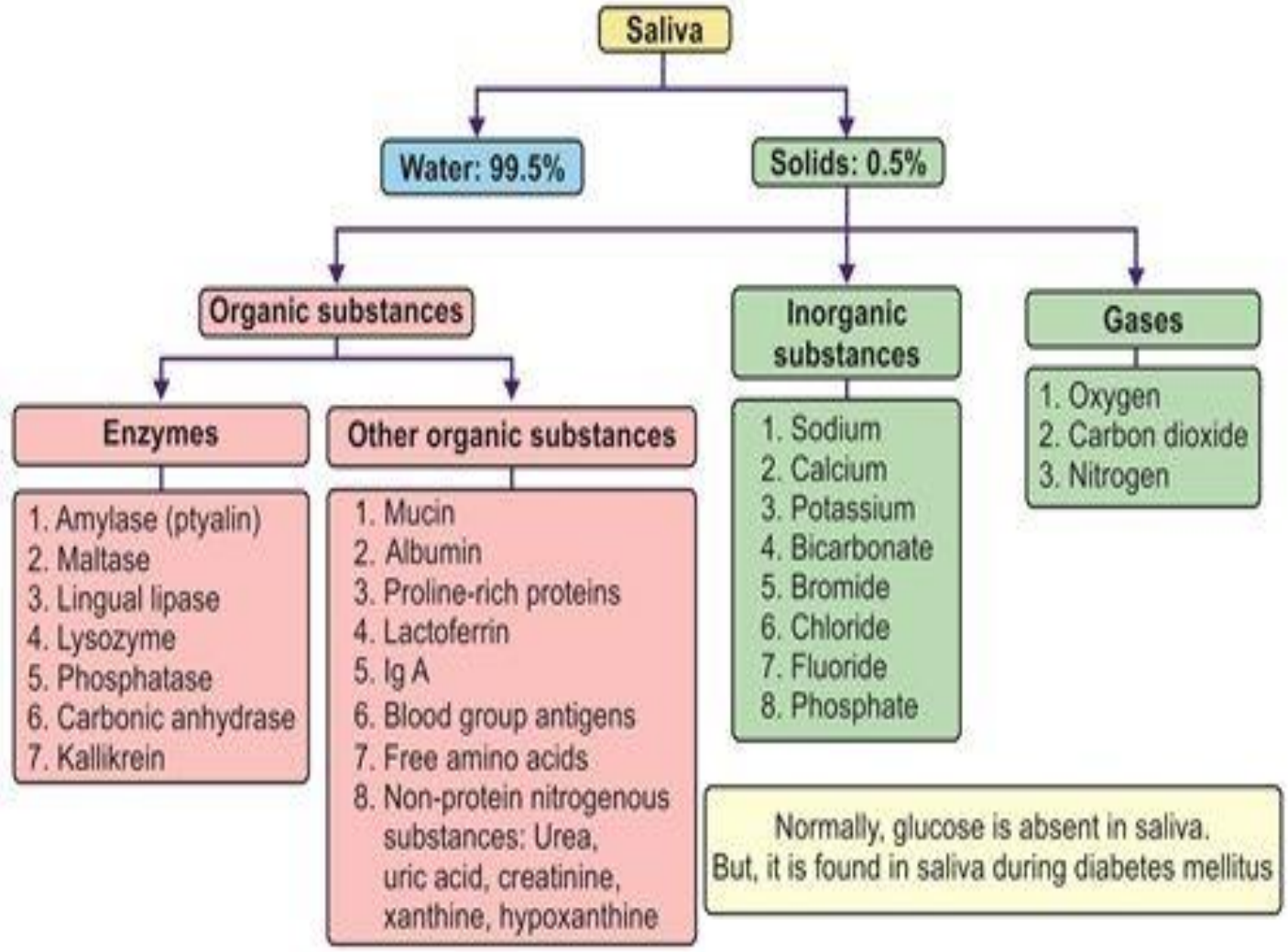
What is the test for calcium?

Serum calcium is a blood test to measure the amount of calcium in the blood. Serum calcium is usually measured to screen for or monitor bone diseases or calcium-regulation disorders (diseases of the parathyroid gland or kidneys)

What are the components of saliva?

About 98 to 99% of saliva is water and the rest of it contains organic and inorganic substances. The main inorganic components present in saliva are electrolytes like sodium, potassium, calcium, magnesium, chlorides, phosphates and sulfates. The organic components of saliva are **mucus and enzymes**

Flow Chart for saliva



What is the relationship between dental caries and fluoride?

Dental caries increased with increasing severity of dental fluorosis, both in moderate- and high-fluoride areas. Thus, a positive relationship between dental caries and dental fluorosis was observed across various tooth types, in both areas
Fluoride is one of the most powerful minerals to help prevent tooth decay **by making the tooth enamel more resistant to those attacking acids**. It can also actually reverse very early decay

What is demineralization and remineralization?

Demineralization is **the process of removing minerals ions from HA crystals of hard tissues**, for example, enamel, dentin, cementum, and bone. Restoring these mineral ions again to the HA crystals is called remineralization

What is remineralization process?

Saliva cleanses our teeth and reduces levels of acidity. And our saliva constantly washes important minerals over our teeth. **Calcium and phosphate ions rebuild and strengthen molecules where** demineralization has taken place. This process is called remineralization.

What is remineralization in dentistry?

Remineralization is **a natural tooth repair process**. Your body takes calcium and phosphate minerals from your saliva and deposits them in your enamel. Enamel is the protective outer layer of your teeth. Your teeth lose minerals in a process called demineralization, which happens as you eat and drink throughout the day

What information does Gibbs free energy give about a reaction?

In other words, ΔG is the change in free energy of a system as it goes from some initial state, such as all reactants, to some other, final state, such as all products. This value tells us **the maximum usable energy released (or absorbed) in going from the initial to the final state**.

What is fluoride toothpaste?

Fluoride is **a chemical commonly added to toothpaste to help prevent tooth decay**. In many countries, it's also added to the water supply for this reason. However, many people are concerned about the potential harms from excessive fluoride intake

How much fluoride is in toothpaste?

Concentrations of fluoride in toothpaste sold in the United States range from **1,000–1,500 ppm**. Most people report brushing their teeth at least once per day, but more frequent use can offer additional protection

What is the relationship between water fluoridation and the incidence of dental caries?

Conclusions: Tooth decay is strongly associated with social deprivation. The findings confirm that the implementation of water fluoridation has halved tooth decay in 5-year-old children and that **the dental caries divide between rich and poor is reduced**

What minerals increase the stability of calcium in tooth enamel?

The conducted enamel analysis has shown that the **magnesium content** increases with growing calcium content at a depth between 150 and 900 μm. It means that the content of one of those minerals directly influences the content of the other one

Why does fluoridation make teeth more resistant to decay?

Fluoride prevents tooth decay by **making the enamel more resistant to the action of acids**. They and accelerate the buildup of healthy minerals in the enamel, further slowing the occurrence of decay. Studies even show that in some cases, fluoride can stop already started teeth decay