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Experimental study of the parameters of human saliva as a mineral-forming medium

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Research conducted in the normal saliva and dental calculus formation. It is shown that changing the chemical composition of saliva creates conditions favorable for the formation of hydroxyapatite. Features of the elemental composition of saliva in lithogenesis in an oral cavity were installed.

Key words: pathogenic mineralization, saliva, dental calculus, chemical composition, the experiment, trace elements

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Calculi formation in an oral cavity is a fairly common disease, the causes of which are currently not well established. Several authors point to the important role of saliva in the formation of dental calculus [*Borowski, 1991; Galiulina, 1988, 2000; Leontiev, 1991*].

The normal composition of saliva is well understood. However, studies of saliva composition in conditions of calculi formation in an oral cavity are rare. It is interesting to determine the content of macro-and microelements in normal human saliva, as well as in terms of calculi formation in an oral cavity, and to identify features of the environment of dental calculus formation. Therefore, the aim of this study was to determine the basic parameters of saliva in normal conditions and calculi formation.

Materials and Methods. The material used in the study was supernatant saliva of people with dental plaque and the healthy, selected as the control group (a total of 250 samples). Saliva was collected in the morning on an empty stomach before brushing teeth, centrifuged at 3000 rev / min. The following parameters were determined in all samples of saliva: pH, pK, pNa, the concentration of calcium, phosphorus, protein, and type of microcrystallization. Such parameters as pH, pK, pNa were measured by direct potentiometry using ion-selective electrodes. Protein was determined photometrically by the method of Benedict. Inorganic phosphorus in the saliva were determined by the method of Leontiev V.K. [*Leontiev, 1976*], the total concentration of calcium – by complexometric titration. Mathematical data processing was carried out using the statistical package STATISTICA 6.0 (Stat Soft Inc. USA).

The elemental composition of the saliva samples was determined by atomic emission spectroscopy with inductively coupled plasma (ICP-AES). The measurements were made on the spectrometer OPTIMA 2000 DV (Perkin Elmer, Germany). Processing of the results was performed using the software of the spectrometer. The method of calibration curve is used for a quantitative calculation.

Results and discussion. Previously, using XRD and IR spectroscopy [*Belskaya, 2009*] was established that the mineral component of dental calculus inhabitants of Omsk represented carbonate-containing hydroxyapatite, as an impurity found brushite (5–10%). It is known that brushite crystallizes at lower pH than the apatite [*Belskaya, 2008*]. The presence of brushite and apatite in one sample shows significant variations in the saliva pH in the process of formation and growth of dental calculus.

The research of samples by the method of XRF-SR was conducted to identify and quantify the content of the maximum possible number of trace elements in dental calculus [*Golovanova, 2006*]. All the samples revealed the presence of 29 trace elements, whose content varies in a fairly wide range from 10^{-4} to 10^{-2} wt.% (Table 1).

On the content of the elements defined in the dental calculus, can be divided into three groups:

1) containing $10^{-3} \div 10^{-2}$ wt.% - Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Br, Sr, Ba.
2) containing $10^{-4} \div 10^{-3}$ wt.% – Ga, Rb, Zr, Mo, Ag, Sn, I, La, Ce.
3) containing $< 10^{-4}$ wt.% – As, Se, Y, Nb, Cd, In, Sb, Te, Cs.

All these elements are essential, ie, vital. The wide range of individual fluctuations in the content of the elements determined, apparently, the balance of trace elements and features of the metabolic processes in the human body.

Table 1. Trace element composition of dental calculus according to XRF-SR, $\cdot 10^{-4}$ wt.%

Element	Range of the individual fluctuations	Mean value	Element	Range of the individual fluctuations	Mean value
Ti	5.8–226.9	76.2	Zr	0.3–74.9	3.6
V	7.4–117.5	42.7	Nb	0.50–1.30	0.90
Cr	17.3–319.5	70.6	Mo	0.3–4.9	1.39
Mn	7.0–45.6	24.1	Ag	0.35–6.00	2.08
Fe	1.4–417.5	81.9	Cd	0.3–1.0	0.63
Ni	1.0–47.1	16.0	In	0.313–0.794	0.532
Cu	1.7–74.4	15.3	Sn	0.3–4.4	1.5
Zn	1.0–880.9	251.9	Sb	0.3–2.6	0.6
Ga	0.3–11.7	3.0	Te	0.27–2.10	1.0
As	0.26–5.10	0.84	I	0.5–34.0	4.4
Se	0.3–1.4	0.87	Cs	0.6–1.4	0.96
Br	0.6–816.9	34.7	Ba	5.2–312.0	40.9
Rb	0.3–11.2	1.35	La	0.6–16.0	7.6
Sr	37.9–177.3	88.2	Ce	0.3–333.6	30.1
Y	0.27–2.10	0,87			-

We can assume that most of the elements in the first group included of the crystal structure of apatite isomorphic to: Sr, Ba – in the position of Ca; Ti, V, Cr – in the tetrahedral position of P. In addition, since most identified in this group elements (Zn, Cu, Ni, Fe, etc.) are good complexing agents, they can form stable complexes with the organic component of the calculus. The elements of the second and third groups received the body mainly through food and accumulate in the calculi due to their ability to concentrate in the bone tissue, particularly in the areas of growth (eg, Ga, Sn, etc.).

The next phase was investigated the chemical composition of the saliva of patients with dental calculus. It is shown that under conditions of calculi formation in an oral cavity is a shift of pH to the alkaline side (table 2). It is at these pH values are optimal conditions for the formation of hydroxyapatite – the main mineral component of human dental calculus [*Belskaya, 2009*]. Simultaneously, the total mineralization (the concentration of ions of phosphorus, sodium, potassium) increased, but decreased the protein content.

Table 2. Comparative characteristics of the saliva of people with different oral health

Rate	Control group n=47; t=1,96	Dental calculus n=31; t=1,96
pH	6.80±0.11	7.04±0.12
C (Na), g / l	0.30±0.04	0.38±0.08
C (K), g / l	0.72±0.05	1.15±0.13
Total calcium, g / l	0.051±0.004	0.055±0.005
Phosphorus, g / l	0.16±0.01	0.20±0.02
Protein, g / l	1.73±0.24	1.39±0.39

It is known that saliva is a structured biological fluid, the entire volume is distributed between the micelles – colloid formation [*Leontiev, 1991*]. Their nuclei are composed of calcium phosphate molecules and are surrounded by water and protein membranes.

$\{[m(Ca_3(PO_4)_2]\ nHPO_4^{2-}(n\text{-}x)Ca^{2+}\}^{2x-}xCa^{2+}$

Alkalization of saliva, contributing to higher of PO_4^{3-} ions content leads to a change in the composition of the micelles and their destruction (fig. 1):

$\{[mCa_3(PO_4)_2]\ nPO_4^{3-}3/2(n\text{-}x)/2Ca^{2+}\}^{3x}\ 3/2\ xCa^{2+}$

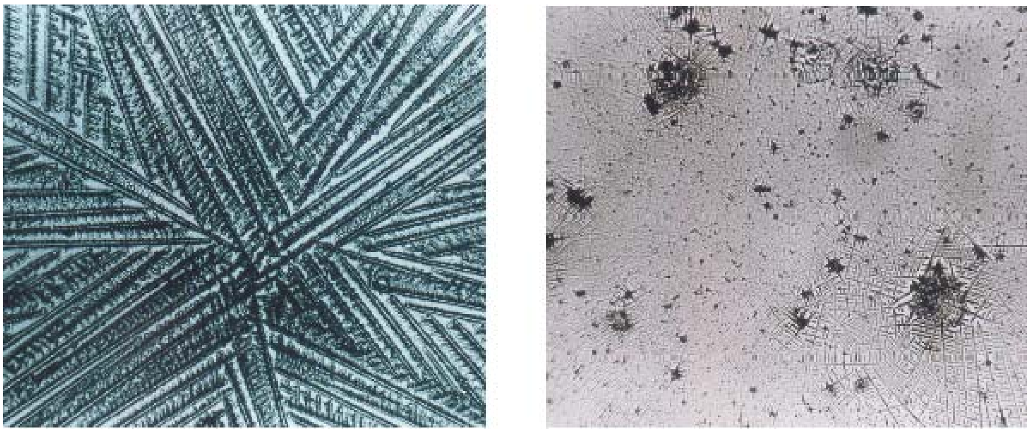

Fig. 1. Violation of the structural properties of saliva in the formation of calculi

Upset micellization process due to the fact that the ions Ca^{2+} and HPO_4^{2-}, cannot simultaneously be in the adsorbed layer, since it is formed a law soluble compound $Ca_3(PO_4)_2$. In this case, with the participation of microorganisms, exciting the precipitated particles $Ca_3(PO_4)_2$ and transferring them to the rough surface of the enamel, creating favorable conditions for the formation of calculi.

Using ICP-AES revealed that the content of zinc and iron increases in saliva in calculi formation (Table 3).

Table 3. Characteristics of trace element composition of saliva, mg / l

Element	Control group	Dental calculus
Zn	0.476±0.183	1.082±1.010
Cu	0.342±0.314	0.054±0.033
Fe	0.278±0.041	0.399±0.185
Mn	0.050±0.014	-
Al	0.705±0.094	-

The correlation analysis was made in order to establish the correlation between composition of dental calculus and saliva. In those experiments, n = 10, significance level of f = 0.01, confidence level of P = 0.99 presence of linear correlation was considered proven if the correlation coefficient in excess of the value of r = 0.65 (Table 4).

Table 4. The correlation coefficients between the parameters of the composition of saliva and dental calculus

The parameters of saliva	The composition of dental calculus
pH	V (r = -0.69), Fe (r = -0.68), Ni (r = -0.68), **Ag (r = 0.68)**
Viscosity	**Br (r = 0.75)**
Potassium	**V (r = 0.72), Fe (r = 0.71), Ni (r = 0.71), Zn (r = 0.66),** Ag (r = -0.66)
Calcium	**Ba (r = 0.78)**, Mn (r = -0.82), Rb (r = -0.68), Ag (r = -0.68)
Phosphorus	Br (r = -0.73), Rb (r = -0.88), Ag (r = -0.88), **Sr (r = 0.74), Ba (r = 0.65)**
Ca/P	Mn (r = -0.93), **I (r = 0.68)**
Na/K	**Rb (r = 0.66), Ag (r = 0.89)**

As can be seen from these data, there is a positive correlation between the viscosity of saliva and bromine, it can be explained by the fact that bromine is more accumulated in the organic component of dental calculus, and organic matter content, in turn, correlates with the viscosity of saliva.

Relatively high values of correlation coefficients of calcium and phosphorus in the saliva, as well as barium and strontium in the composition of dental calculus, can be explained by the proximity of the ionic radii of these elements ($r(Ca^{2+})$ = 0.100±0.003, $r(Ba^{2+})$ = 0.137±0.005, $r(Sr^{2+})$ = 0.116±0.003 nm) and the ability to isomorphic substitution in the crystal structure of hydroxyapatite – the main mineral component of human dental calculus. Similar patterns are revealed for potassium in the saliva and the ions of iron, zinc, nickel and vanadium in the composition of dental calculus. Since potassium ions are in the hydration shell of the micelles of calcium phosphate, in the process of formation and growth of calculus may be substituted by ions coming into the oral cavity, and the concentration of potassium ions in the saliva with the natural increase.

The negative correlation between the content of vanadium ions, iron and nickel in the composition of dental calculus, and the pH of saliva due to the fact that with increasing pH the predominant form of existence of these ions in solution is anionic and the ability to isomorphic substitution decreases.

Conclusion. Based on the experimental data can distinguish the changes occurring in the saliva of a person in calculi formation in the oral cavity:

1. Under the conditions of calculi formation in the oral cavity is a shift of pH to the alkaline side, which creates favorable conditions for the formation of hydroxyapatite.
2. In the formation of dental calculus in saliva decreased the content of calcium ions, but increases the content of phosphorus and electrolyte components – sodium and potassium ions.
3. A decrease in protein content in the saliva of patients with dental calculi, indicating that violation of the structural properties of saliva.
4. It was established that under conditions of calculi formation in the mouth increases the content of zinc and iron.

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