

Реферати

**ИЗУЧЕНИЕ ВОЗМОЖНОГО УЛЬЦЕРОГЕННОГО  
И МЕСТНОРАЗДРАЖАЮЩЕГО ДЕЙСТВИЯ  
БЕНФУРАМА**

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Пonomarenko О.В., Гаркуша И.В.**

Проведено исследование возможного ulcerогенного и местнораздражающего действия бенфурама, который в дозе 50 и 100 мг/кг не вызывал повреждения слизистой оболочки желудка и двенадцатиперстной кишки. Слабое ulcerогенное действие бенфурама проявилось лишь в дозе 200 мг/кг, он вызывал повреждение слизистой оболочки желудка у одного животного, что составляет 10%. Язвенный индекс равняется 0,02, что в 10 раз меньше, чем при введении ацетилсалициловой кислоты. В отличие от ацетилсалициловой кислоты бенфурам не потенцирует ulcerогенное действие этанола. Введение бенфурама животным в течение 14 суток не вызывает повреждения слизистой желудка и двенадцатиперстной кишки. У кролей бенфурам не вызывал отека век, помутнения роговицы, слезотечения, экземы, токсикодермии и конъюнктивитов. Не наблюдали также изменений со стороны слизистой конъюнктивы глаз и диаметра зрачка спустя сутки после закапывания.

**Ключевые слова:** ulcerогенное, местнораздражающее действие, бенфурама

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**STUDY OF POSSIBLE ULCEROGENIC  
AND LOCALLY IRRITATING ACTION  
OF BENFURAM**

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A possible ulcerogenic and locally irritating effect of benfuram was investigated, which did not cause damage to the mucous membrane of the stomach and duodenum in a dose of 50 and 100 mg / kg. The weak ulcerogenic effect of benfuram was manifested only in a dose of 200 mg / kg, it caused damage to the gastric mucosa in one animal, which is 10%, the UI is 0.02, which is 10 times less than with acetylsalicylic acid. In contrast to acetylsalicylic acid benfuram does not potentiate the ulcerogenic effect of ethanol. The introduction of benfuram to animals for 14 days does not cause damage to the gastric mucosa and duodenum. In rabbits, benfuram did not cause eyelid edema, corneal opacity, lacrimation, eczema, toxicodermia and conjunctivitis. No changes were observed from the mucosa of the conjunctiva of the eyes and the diameter of the pupil a day after instillation.

**Key words:** ulcerogenic, locally irritating effect, benofuram.

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**DETERMINATION OF THE MINERALIZATION ZONES IN THE HARD TOOTH TISSUES**

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The enamel and dentin caries is well elucidated in the contemporary publications. However, the role of functional state of the organic (pellicula) and mineral components of the surface layer of the enamel, which provide a complete metabolism in hard tooth tissue, is not fully studied to date. The teeth of different classes, not affected by fluorosis and extracted on orthodontic and surgical indications in patients aged 28 to 60 years, have been studied. Electron microscopic studies of the detached crowns of the teeth have been carried out for a detailed study of the elements of the surface structure of the enamel. To study the process of mineralization in the dentine structures the roots of the teeth were embedded into citrate buffer solution, proposed by our staff. The use of the proposed citrate buffer solution enables the detailed analysis and study of the hard tooth tissues mineralization. The suggested technique contributes to the efficacy of the detection of crystal structure in the dentinal canaliculi to clarify accurately its functional properties.

**Keywords:** dentine, mineralization, pellicula.

The enamel and dentin caries is well elucidated in contemporary publications. Most authors are convinced that the basis of the pathogenesis of the initial processes of dental caries is demineralization in the superficial layers of the enamel and dentine. However, we hypothesize, that the role of functional state of the organic (pellicula) and mineral components of the superficial layer of the enamel, which provide a complete metabolism in hard tooth tissue, is not fully studied to date.

Regardless of the presence of structural-functional barriers of biomineralization, due to the first contacts of salivary fluid, the pellicula appears, represented by the thin film of salivary glycoproteids that are formed during 20-30 minutes. It has a selective permeability and ensures the diffusion process, mainly of calcium ions, into the tooth enamel and protects it from the damaging action of chemical factors. Salivary glycoproteids, precipitated in the pellicula in the form of levans and dextrans, contain the glycosidase enzyme, which destroys the glucoside relationships. Bacterial glucosyltransferases contribute to the formation of the abovementioned sticky glucans that assist in the formation of pellicula. In addition, adhesin, a protein, excreted by Streptococcus, is of significant functional importance, since it ensures hydrogen bonds of calcium ions, which are located in the salivary micella. Moreover, non-organic substances (94-97%), represented by the calcium compounds and other metals, phosphates,

carbonates, fluorides, etc., are essential in the structures of the enamel. The latter are represented by the crystals of various sizes and shapes. Some of these substances can be impregnated into the pellicula depth and supplement its protective role against the effect of external factors. Organic compounds are accounted for 1,2-2%, and 0.1% of which are calcium citrates and other metals. The dentine is composed of non-organic (70%), organic (17%) substances and water (13%) [2, 5, 8].

**The purpose** of the study was to clarify the functional role of the organic (pellicula) and mineral (crystals) components in the metabolic processes of the enamel and dentin.

**Materials and methods.** The teeth of different classes, not affected by fluorosis and extracted on orthodontic and surgical indications in patients aged 28 to 60 years, have been studied.

The 36 extracted teeth of different classes were mechanically scraped from residual blood and fixed in 10% neutral formalin solution. After fixation the teeth was cleansed with running water. Subsequently, the crown of each tooth was detached from the root along its neck, cutting the latter transversally by the fine-grained diamond disc (up to 100 rpm) under the water cooling.

Electron microscopic studies of the detached crowns of the teeth have been carried out for a detailed study of the elements of the surface structure of the enamel. The samples (36 crowns) have been studied using the scanning electron microscope (SEM) REM-100 with the following specifications: acceleration voltage of 20 kV; current (0.5-0.7)  $\mu\text{A}$ , increasing from 50 to 3000 times. The sample for the study was prepared in accordance with the conventional technique. To study the process of mineralization in the dentine structures the detached roots of the 18 teeth (molars, premolars) have been used, which were embedded into citrate buffer solution, proposed by our staff [1] and kept in the thermostat at 36°C for 18 hours. Thereafter, all samples were thoroughly cleansed with water during 10 minutes and dried. The cut-off surface of the roots of the teeth was examined using the binocular magnifier (MBS-9) and the images of the resulting data were made by the digital camera. Another 18 teeth (incisors, canines, premolars) have been used at the second stage of the study. They were preparing for the study in a similar way. However, in this case, each root of the tooth was cut lengthwise in halves, polished on the special glass, washed with water and drained. The thick slices, selected for the analysis were stained histochemically with Schiff reagent and studied using the binocular magnifier (MBS-9). The images of the resulting data were made by the CanonA 590 digital camera.

**Results of the study and their discussion.** According to the current presentations, the insoluble nucleus of calcium phosphate  $\text{Ca}_3(\text{PO}_4)_2$  is in the core of micella. Molecules of hydroxyapatite  $(\text{HPO}_4)^{2-}$ , containing brushite that forms the reabsorption layer, are sorbed on the surface of the nucleus. Superiorly, there is a diffuse layer of micella, where  $\text{Ca}^{2+}$  calcium and  $\text{Mg}^{2+}$  magnesium ions are acted as counterions, forming the monetite crystal together with  $\text{Na}^+$  sodium ion. The mucin binds a significant amount of water between micellas. Due to the surface microflora, which facilitates the formation of acidic medium, the charge of micella is reduced by half, since the monohydrophosphate ions are bound with  $\text{H}^+$  hydrogen protons and  $\text{H}_3\text{PO}_4$  appear instead of  $(\text{HPO}_4)^{2-}$  monohydrophosphate. This reduces the stability of micella, and the micella ions of dihydrogen phosphate are not involved in the process of biomineralization of the enamel. In the alkaline medium the number of phosphate ions increases that are bound with calcium, forming the insoluble in water  $\text{Ca}_3(\text{PO}_4)_2$  calcium phosphate salts in the form of dental calculus [3, 7].

Our studies have shown that in normal conditions the pellicula consists of salivary micella, in the center of which there is a large orbicular white crystal, obviously, represented by  $\text{Ca}_3(\text{PO}_4)_2$  calcium phosphate. Twisted processes of salivary micella are located around the core nucleus. In the places of their connection poorly circumscribed coccal microorganisms are found. As far as moving from the micella's nucleus the tapered crystals appear, penetrating into the proteinaceous matrix of micella. In its configuration these crystals resemble the structure of brushite.

Brushite is an acidic aqueous calcium phosphate  $(\text{CaHPO}_4)2\text{H}_2\text{O}$ . Its percentage (Ca – 32,58%,  $\text{P}_2\text{O}_5$  – 41,25%,  $\text{H}_2\text{O}$  – 26,17%) corresponds to the form of monocellular syngony in the form of prismatic crystals. The mineral is named after American scientist G. Brush, who studied the origin and essence of the crystals. In the natural environment this mineral is often found.

The electron microscopic studies show that pits and fissures contain light crystals of rhomboid or triangular shape, obviously, represented by brushite.

To confirm the presence of brushite, the X-ray microradiographical analysis was carried out to determine the calcium to phosphorus ratio, which constituted 1.3. This value corresponds to the structural formula of brushite  $(\text{CaHPO}_4)2\text{H}_2\text{O}$ . Apparently, the presence of light orbicular and fine crystals on the electron diffraction patterns indicates about the possible conversion of brushite into insoluble  $\text{Ca}_3(\text{PO}_4)_2$  calcium phosphate. Our studies show that the sizes of crystals, impregnated into the depth of pellicula, may vary. The crystal of brushite is almost completely embedded into the depth of pellicula; the micella

processes are well-defined along its perimeter. In other cases, the crystals can be attached to the pellicula only by its main body. The micella processes of various orientation and length are noted along the crystal's perimeter.

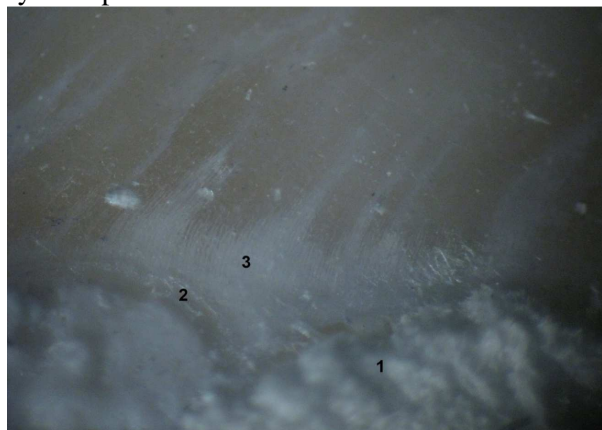


Fig. 1. Transverse section of the tooth crown in the dental pulp area. Epimicroscopy of the native slice. Magnification:  $\times 56$ : 1 – stratification of the calcium citrate in the zone of pulp chamber; 2 – zone of circumpulpar dentin; 3 – wide bundles of the zone of monopedic dentinal canaliculi.

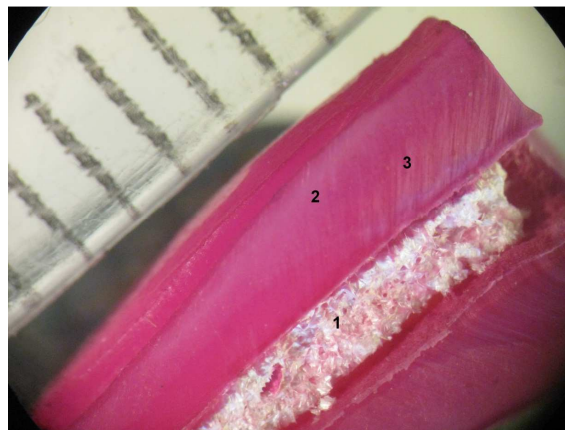


Fig. 2. Root canal of the incisor, filled with calcium citrate. PAS stain. Magnification:  $\times 10$ : 1 – calcium citrate in the root canal; 2 – dentin of the root canal; 3 – zone of citrate buffer-impregnated dentinal canaliculi.

The mineralization degree of dentiny has been studied at its different areas from the pulp to mantle, using the suggested technique. First of all, the transverse sections of the impregnated teeth at the level of the precervical area have been studied. It should be noted that in cases where the dentinal canaliculi were located parallel to the shear section, the layers of calcium citrate were well-defined. In this way, on the smooth surface of the dentine the passage of dentinal canaliculi, filled with calcium citrate in the form of white fine lines was observed. They are originated from the zone of circumpulpar dentin with the wide bundles, splitting subsequently and disappear in its depth (fig. 1). Notably, the pulp chamber contains well-defined significant stratifications of citrate across the entire investigated surface.

The next set of studies of the cross-half-cut and PAS stained roots encompassed the analysis of the state of the dentinal canaliculi along the entire length of the roots of incisors, canines and premolars.

The study of the process of impregnation of the incisor's root canal by the citrate buffer showed a significant filling of the entire root canal with calcium citrate (fig. 2).

Noteworthy, on the surface of the dentine of the root transverse section the white stripes are well-defined and tangentially directed to the surface of the canal and are comprised from numerous dentinal canaliculi, impregnated by citrate buffer.

Figure 3 presents the detailed view of such arrangement. In this case on the surface of the slice the numerous well-defined fine white lines are identified, which tangentially pass almost through the entire depth of the root wall with parallel stripes and end its passage at the zone of the mantle dentine.

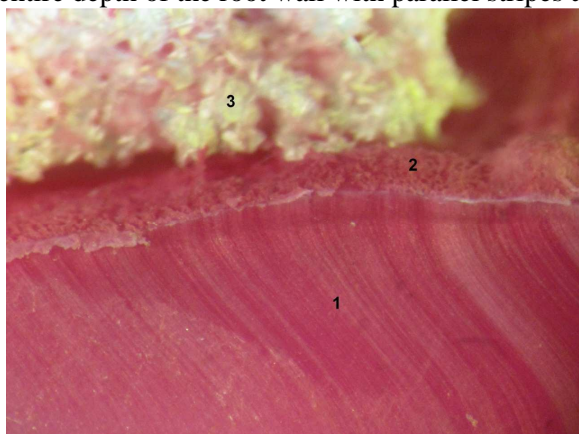


Fig. 3. Selected area of the transverse section of the incisor's root canal, filled with calcium citrate. 1 – white stripes of the dentinal canaliculi, joined into bundles; 2 – circumpulpar dentin; 3 – crystals of calcium citrate in the root canal. PAS stain. Magnification:  $\times 56$ .

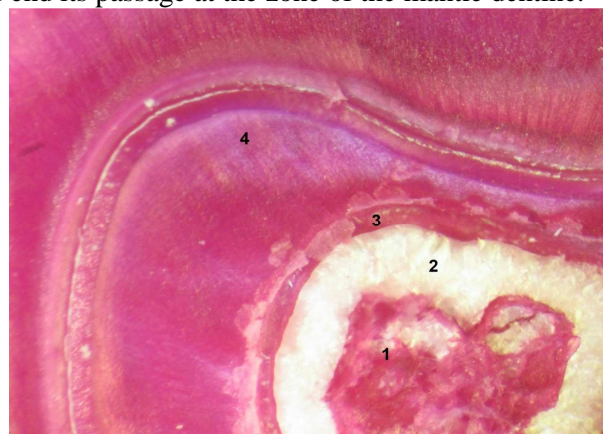


Fig. 4. Transverse section of the root of the tooth. PAS stain. Magnification:  $\times 32$ : 1 – section of the dental pulp; 2 – calcium citrate; 3 – circumpulpar dentin; 4 – zone of citrate buffer-impregnated dentinal canaliculi.

The similar picture is observed during the study of the transverse section of the root. Thereafter, it has been established that in the central part of the root canal there is a pulp, separated from the circumpulpar dentin by the thick layer, which was formed by the calcium citrate. Radially from the pulp

the dentinal canaliculi in the form of white stripes are well-defined, which penetrate wavelike into the depth of the dentine in the direction to its mantle zone (fig. 4).

### Conclusion

The use of the proposed citrate buffer solution enables the detailed analysis and study of the hard tooth tissues mineralization. The technique contributes to the efficacy of the detection of crystal structure in the dentinal canaliculi to clarify accurately its functional properties.

Apparently, the features, detected using the citrate buffer, can be applied as the alternative to histochemical studies of the hard tooth tissues.

*Prospects for further research will encompass the comparative studies of the possibilities of the proposed technique and immunohistochemistry in the study of the hard tooth tissues.*

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### Реферати

#### ВИЗНАЧЕННЯ ЗОН МІНЕРАЛІЗАЦІЇ ТВЕРДИХ ТКАНИН ЗУБА

Костиренко О.П., Винник Н.І., Гасюк А.П., Коптев М.М., Проскурня С.А.

Карієс емалі та дентину широко висвітлений у сучасних літературних джерелах. Проте, недостатньо з'ясовано залишається роль функціонального стану органічних (пелікула) та мінеральних компонентів поверхневого шару емалі, які забезпечують повноцінний обмін твердих тканин зуба. Матеріалом для дослідження стали зуби різних класів, які не були уражені флюорозом і видалені за ортодонтичними та хірургічними показаннями в пацієнтів від 28 до 60 років. Із метою детального вивчення елементів поверхневої структури емалі було проведено електронномікроскопічні дослідження відділених коронок зубів. Для дослідження процесу мінералізації в структурах дентину корені зубів занурювали в розчин запропонованого нашими співробітниками цитратного буферу. Використання запропонованого розчину цитратного буферу дало можливість детально досліджувати й вивчати процес мінералізації твердих тканин зуба. Дана методика вказує доцільність та ефективність виявлення кристалічних структур в дентинних каналцях із метою детальнішого з'ясування їхніх функціональних властивостей.

**Ключові слова:** дентин, мінералізація, пелікула.

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#### ОПРЕДЕЛЕНИЕ ЗОН МИНЕРАЛИЗАЦИИ ТВЕРДЫХ ТКАНЕЙ ЗУБА

Костыренко А.П., Винник Н.И., Гасюк А.П., Коптев М.Н., Проскурня С.А.

Карієс емалі та дентина широко освітлен в сучасних літературних джерелах. Однак, недостатньо висвітленою залишається роль функціонального стану органічних (пелікула) та мінеральних компонентів поверхневого шару емалі, які забезпечують повноцінний обмін твердих тканин зуба. Матеріалом для дослідження стали зуби різних класів, які не були уражені флюорозом і видалені за ортодонтичними та хірургічними показаннями у пацієнтів від 28 до 60 років. Із метою детального вивчення елементів поверхневої структури емалі було проведено електронномікроскопічне дослідження відділених коронок зубів. Для дослідження процесу мінералізації в структурах дентина корені зубів занурювали в розчин запропонованого нашими співробітниками цитратного буферу. Використання запропонованого розчину цитратного буферу дало можливість детально досліджувати й вивчати процес мінералізації твердих тканин зуба. Дана методика вказує на доцільність та ефективність виявлення кристалічних структур в дентинних каналцях з метою більш детального висвітлення їх функціональних властивостей.

**Ключевые слова:** дентин, мінералізація, пелікула.

Рецензент Єрошенко Г.А.