REVIEWS

Salivary Glands as Test Object for Assessment of Biological Compatibility in Dentistry

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Artificial and natural materials used in dentistry include synthetic biopolymers, metals, ceramics, hydroxyapatites, and carbon. All these substances should be biologically compatible, *i.e.* produce no local inflammatory, toxic, and allergic effects and retain their functional properties during service period.

Biological compatibility of dental materials are now tested in accordance to ISO 10993-4 Standard [47]. However, this Standard does not consider function and structure of salivary glands (SG). At the same time, saliva and SG play the most important role in the maintenance of homeostasis in the oral cavity and largely determine the reaction of the oral mucosa to dentures. We found no published data on the use of glandular tissue in assessment of biological compatibility.

Here we report our experimental data on the use of SG as the test object in assessment of biological compatibility in dentistry [7-17].

Evaluation of the response of SG to dental materials allows to assess structural and functional changes in SG induced by dental materials (assessment of salivation, chemical composition, and biophysical properties of saliva), permeability of the blood-salivary gland barrier (BSB) by the excretion of metals and other ions with saliva, and corrosion of metal alloys contacting with saliva and oral fluid.

Structural alterations in salivary glands induced by biomaterials

Annually, Medline database files up to 200 papers, which report the data on structural changes in SG. As a rule, most of them are devoted to neoplastic processes in SG. Since these data are very important for clinical practice, they are analyzed, classified, and revised more accurately [20]. However, there is no universal description of benign alterations of glandular tissue.

Generally, structural changes in SG are represented by two basic morphological manifestations: hypotrophy (up to atrophy) and hypertrophy.

Atrophy of SG is observed in autoimmune thyroidites [62]. Similar process can be induced in mice by repeated injection of vitamin A. This treatment induced shrinkage of SG acini and acinar cell nuclei, degeneration of granular and striated salivary ducts, and enlargement of excretory ducts against the background of pronounced expansion of the stromal connective tissue [35].

In many cases, hypertrophy of SG is associated with intensive infiltration with eosinophils [44], lymphocytes and myoepithelial cell islets (Chagrin disease) [51]. Moreover, SG hypertrophy can be caused by cysts, secretory congestion, and acinar degeneration [46]. Essential acinar hypertrophy can be induced by chronic nicotine exposure due to long-term stimulation of β-adrenoceptors. The acinar cells are edematous and contain numerous immature secretory granules, enlarged granular endoplasmic reticulum and Golgi apparatus [54].

In some diseases (diabetes mellitus [28] and cirrhosis of the liver [49]) degenerative processes involve major salivary glands and primarily affect glandular tissue around interlobular ducts (sialoses). Various types of SG hypertrophy, the mechanisms of their development, and experimental models reproducing these pathologies are previously described in details [7].

There are only few reports on structural changes in SG induced by metals or their alloys [1,6]. We found only one report on the potency of iodine to

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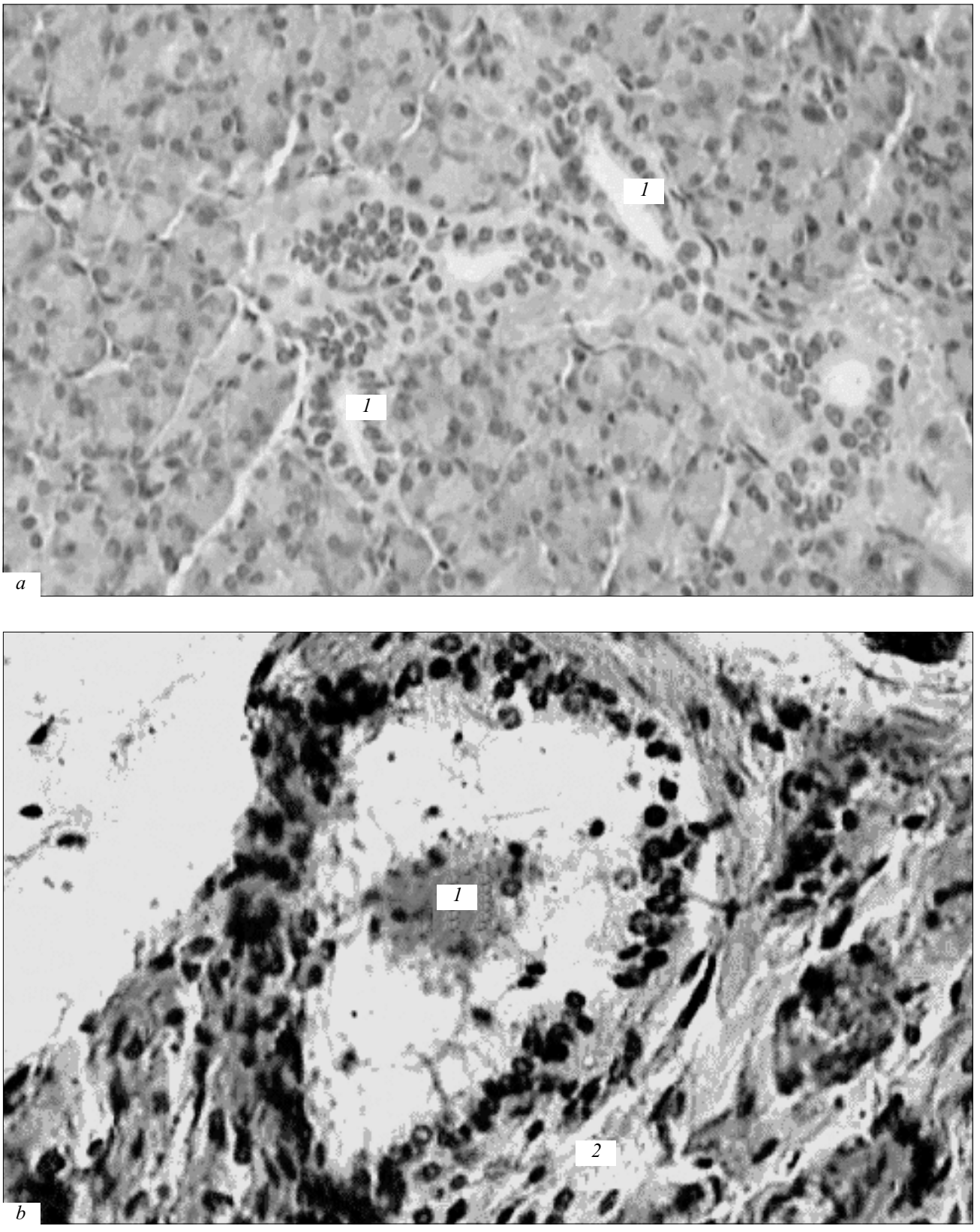

Fig. 1. Morphology of rat submandibular salivary gland in control (*a*) and 3 months after implantation of VT-14 alloy plate (*b*). Hematoxylin and eosin staining [15], ×80 (*a*), ×122 (*b*). *1*) lumens; *2*) periductal sclerosis.

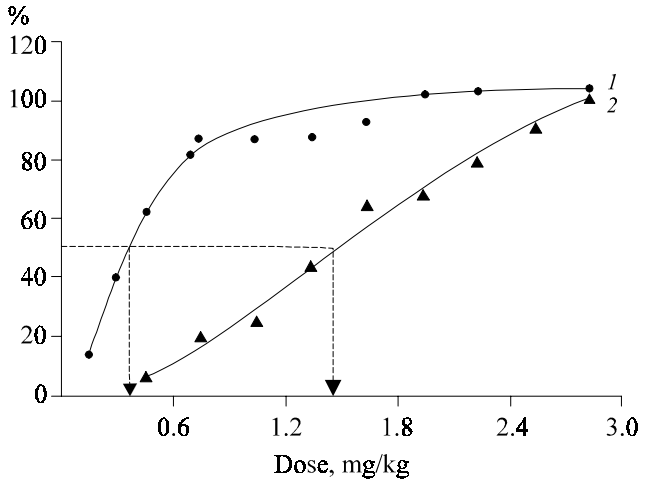

Fig. 2. Assessment of the effects of muscarinic cholinoreceptor blockers KG-62 (*1*) and methacin (*2*) by inhibition of pilocarpine-induced salivation (dose — response curve) [9]. Ordinate: inhibition of salivation. The arrows show ED_{50}.

induce sialoadenitis [58]. The appearance of mononuclear infiltrates was observed in SG and lacrimal glands in male BN rats 6-9 days after administration of mercuric chloride [63].

We studied the effect of new titanium alloy VT-14 on the structure of the major salivary glands [15]. Titanium and its alloys are widely used in dentistry as the basis of dentures and dental implants [19,33]. Titanium is biologically inert primarily due to the formation of a surface titanium dioxide film [50]. However, titanium alloys contain trace amount of metals that are not biologically inert (aluminum, vanadium, molybdenum). Thus, a comprehensive study of biological compatibility of VT-14 alloy is of practical importance.

In our experiments, sterilized VT-14 plates (700 mg) were subcutaneously implanted to the back of male rats, while to control rats medical glass particles

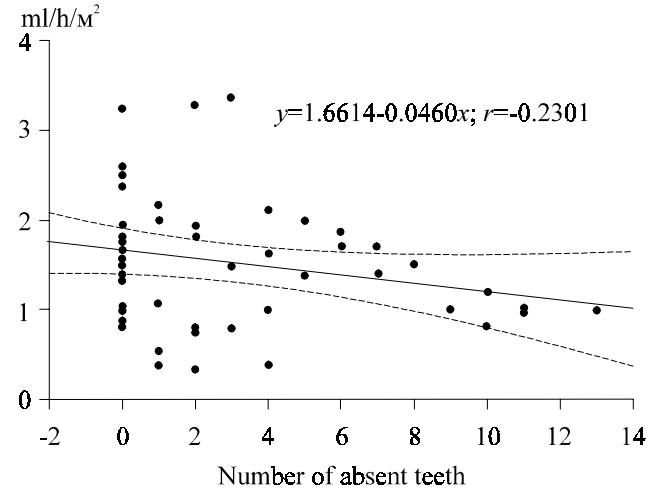

Fig. 3. Salivation rate as a function of the number of teeth. Here and in Fig. 4: ordinate shows the rate of chewing-induced salivation.

were implanted. Three months after implantation of VT-14 alloy, small degenerative alterations were detected in glandular acini, and periductal sclerosis was observed in some interlobular ducts (Fig. 1, *b*).

Therefore, glandular tissue of greater salivary glands responds to implantation of dentistry materials by structural alterations.

Functional changes in salivary glands induced by biomaterials

Assessment of the salivary function includes sialometry (salivation rate [59]) and qualitative and quantitative analysis of the saliva.

Various diseases or, most frequently, xenobiotics such as heavy metal salts (chromium [36], gold from dentures [38], mercury [40], lead and cadmium [41, 42]), many organic substances, in particular, drugs and their metabolites [21,60], as well as nitrates and nitrites [61] can induce qualitative changes in the saliva, in particular, appearance of some pathological components.

Quantitative changes in the saliva are manifested in the changes of concentration of its usual components. Conventionally, these components can be subdivided into two groups: glandular metabolites (calcium, phosphate, *etc*. [31] and transitory products transferred to saliva from other biological fluids (blood and lymph) by various transporting mechanisms, for instance, glucose [25] and urea [37]. SG produced some peptide hormones [45], while steroid hormones are transported from the blood by diffusion [26].

Assessment of salivary function (SF) was used by some investigators for ecological monitoring [22,23], monitoring of human health [2], and in pharmacology [21]. The major disadvantage of this method is the absence of standards, because salivation greatly varies under normal conditions. The individual rate of stimulated salivation can differ 30-fold [59].

We developed a method to decrease variability of SR values in physiological and clinical experiments.

Evaluation of SF in rats. This physiological model requires stimulation of glandular cells with muscarinic agonist pilocarpine, which affects glandular muscarinic cholinoreceptors subtype $M_{2\beta}$.

SF is assessed by the latency, dynamics (time-dependence of salivation during the first secretory cycle), and the mean rate of salivation. Each parameter is normalized to body weight and to conventional mean rate (10 ml/h/kg) [8]. Qualitative parameters of the saliva are taken into account. The concentration of some ions in the saliva non-linearly depends on salivation rate. We constructed normograms for some ions (Na, K, Ca, and P) and whole saliva protein [8].

Using the developed criteria of SF assessment we carried out a comparative study of pharmacological properties of substance KG-62 (muscarinic cholinoceptor blocker) and metacin. The effect of test muscarinic cholinoreceptor blockers in rats was determined by titration of the dose inhibiting pilocarpine-induced salivation [3]. The test substance blocked muscarinic receptors (Fig. 2), but this effect was 2-fold less potent than that of metacin [14].

Clinical experiments. Depending on the aim of the experiments, baseline (non-stimulated) salivation caused by spontaneous activity of minor salivary glands and stimulated salivation were studied in humans. Salivation was stimulated by chewing paraffin, activation of chemoreceptors in the oral cavity with lemon juice or vitamin C, or direct stimulation of acinar cells with pilocarpine.

We studied SF during chewing-induced salivation in practically healthy volunteers and in patients with partial dentition defects. It was established that the use of sialometric index normalized to 1 m^2 body surface significantly (by 2-fold) decreased deviation of SF sialometric data [13].

Recalculation of normalized SR to standard conventional SR yielded precise values of 13 components of mixed saliva, which were used in the assessment of SF during partial secondary adentia. A negative correlation was revealed between dentition pathology (inclusion defects) and the rate of stimulated salivation (Fig. 3). The study of SF in orthodentic patients treated with dentures made of new inert palladium-based alloy Superpal showed its high biological compatibility (Fig. 4) [18].

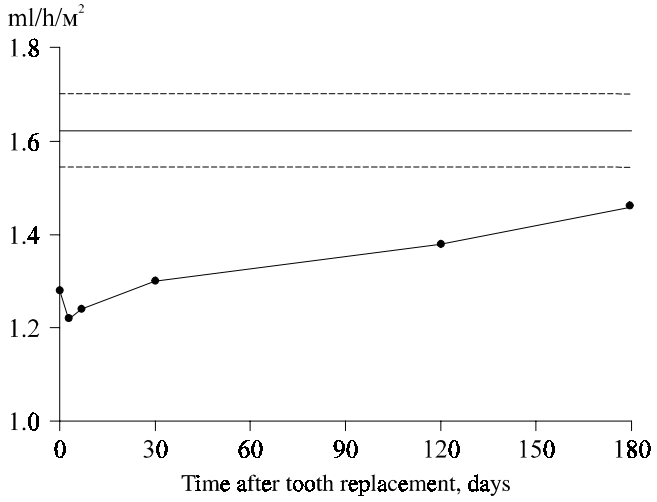

Fig. 4. Recovery of the rate of chewing-induced salivation after tooth replacement by metal crowns made of Superpal alloy. Solid horizontal line shows the mean normal value, dashed lines show 95% confidence interval.

Therefore, assessment of SF by SR normalized to 1 m^2 body surface area makes it possible to solve many problems related to the state of salivation under normal and pathological conditions.

To study biophysical properties of the saliva, not only biochemical (qualitative and quantitative), but also biophysical indices and criteria can be used. We developed a method based on the analysis of crystals formed during saliva desiccation [10-12].

In was found that different matrices determine different patterns of crystallization of saliva from the same patient. The optimal crystallization conditions and morphometric analysis are developed on the sur-

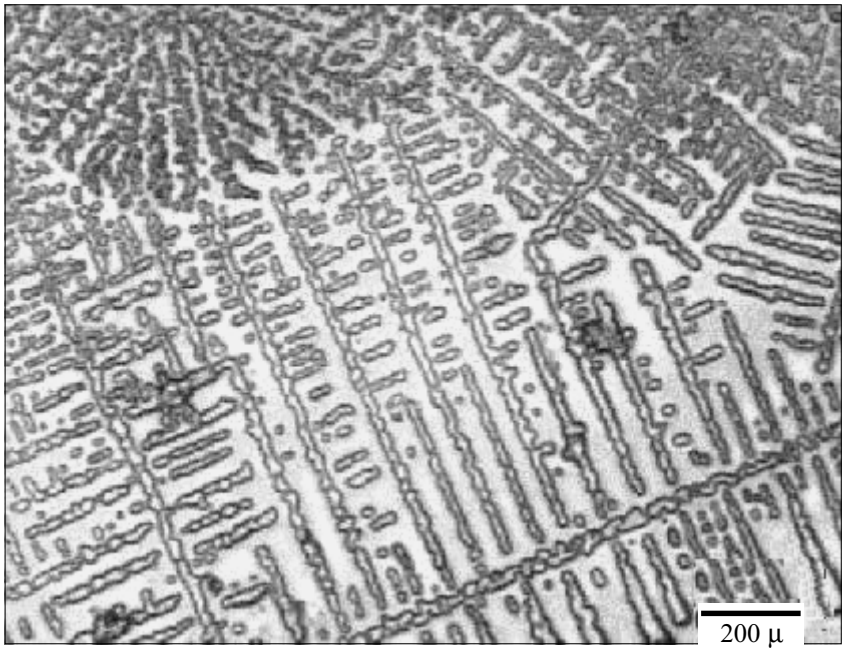

Fig. 5. Crystallization of oral fluid (mixed saliva) on plastic. Crystallogram in the center of the specimen. Fern-like structure of dendrites is clearly seen.

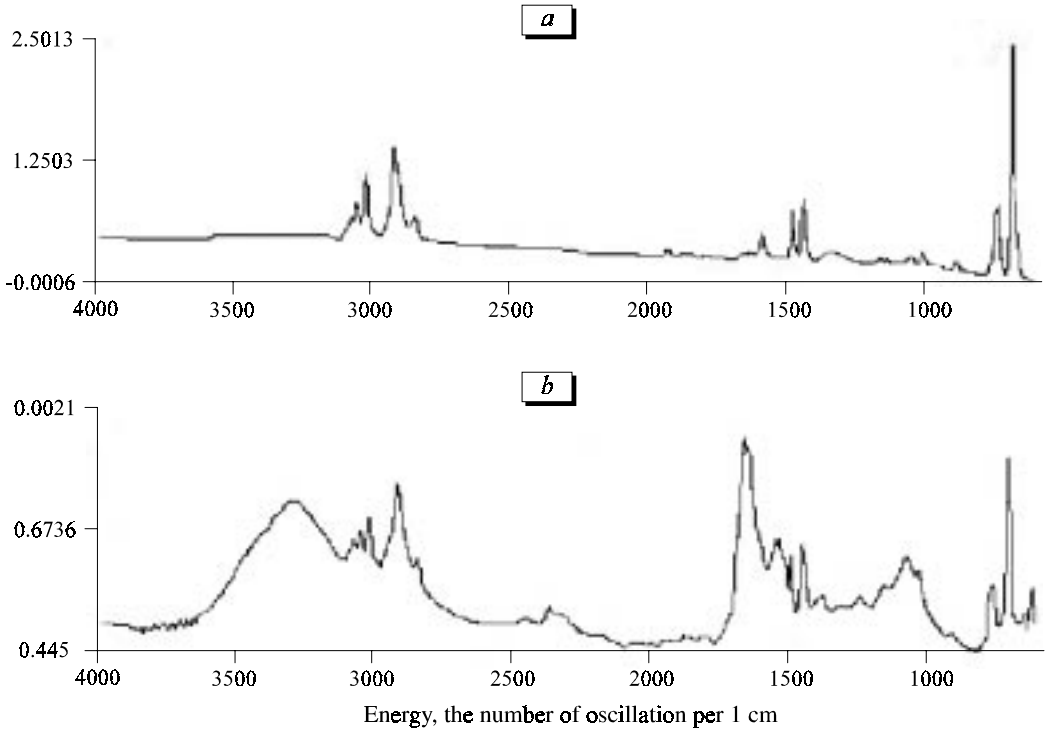

Fig. 6. Raman spectrum obtained with a Fourier spectroscope at 1.06 μ. Ordinate: intensity in arbitrary units. *a, b*) Spectra in and correspond to different parts of the crystallogram.

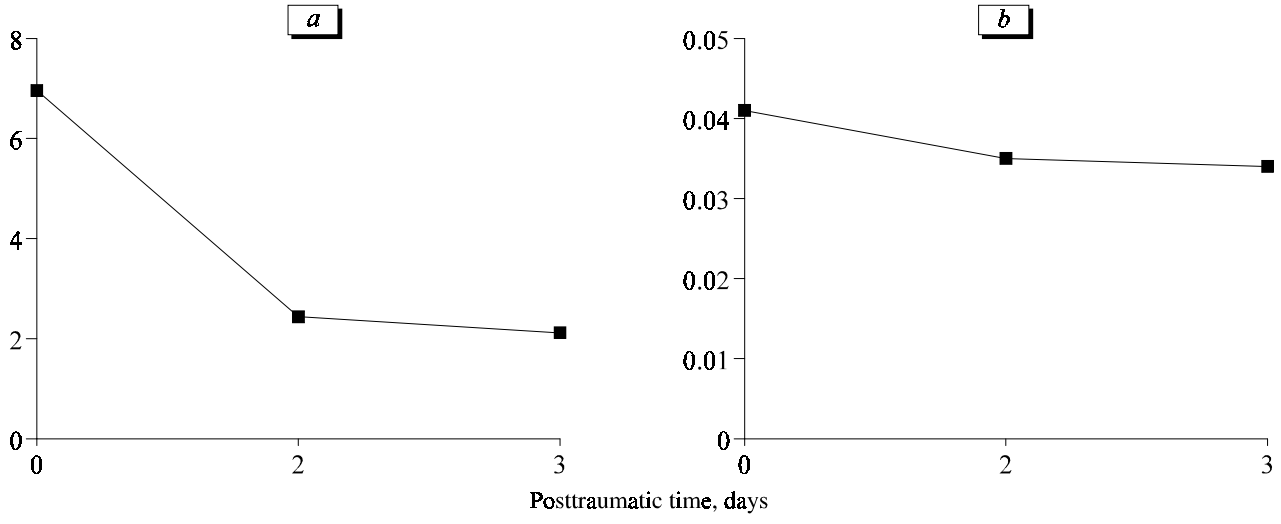

Fig. 7. Permeability of blood-saliva barrier for potassium (*a*) and sodium (*b*) ions during experimental posttraumatic inflammation. Ordinates: permeability coefficient (saliva/blood).

face of nontoxic plastic used for cell culturing (Fig. 5). The crystals formed during desiccation of oral fluid have a complex structure and consists of no less than three organic substances, in particular saliva mucin (Fig. 6).

Excretion of ions and other substances across BSB

Apart from synthesis and release of secrets, major SG can excrete some other substances, in particular, halogens [4], thus performing the excretory function. Transport of substances from blood to saliva occurs across BSB [24].

Various theories explain excretion of metals with saliva. For example, there is a view that "saliva monitoring can reflect the degree of environmental pollution" [29,41,42].

Functional state of all barriers is determined by the permeability coefficient (PC), *i.e.* the concentration ratio for a given substance at both sides of the barrier (in our case in saliva and blood). We used PC

of BSB to study functional state of SG during experimental posttraumatic sialoadenitis. In particular, we found that PC for potassium and sodium ions decreases by 65-70% and 15-17%, respectively, 2-3 days after sialotomy (Fig. 7). Since ions are excreted and reabsorbed mainly in interlobular (striated) ducts, it may be concluded that BSB is disturbed during sialoadenitis.

Resistance to corrosion is one of most important properties of the examined xenobiotics. The saliva is a biologically active medium, which actively and aggressively interacts with dentistry materials. Saliva-induced corrosion of metals and alloys was assessed both in the *in vivo* [32,43] and *in vitro* [34,48] experiments, and in many cases so-called artificial saliva was used instead of human saliva [52-57,63].

The study of SG structure and function in different pathologies leads us to a conclusion that SG can be successfully used as a test object for assessment of biological compatibility of dentistry materials.

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