Specific Features of Bone Tissue Regeneration after Replacement of the Defect with a Synthetic Implant

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A synthetic implant is constructed on the basis of a biopolymer (collagen) with inorganic salts (hydroxysulfates and hydroxyphosphates of Ca, Mg, and Al) introduced into the collagen in different ways. Regeneration is monitored by roentgenophase, differential thermal, and chemical analyses, computerized tomography, and by x-ray and morphological methods. It is shown that the composition of the salt component and the salt:collagen ratio do not significantly affect the regeneration.

Key Words: *implant; collagen; bone tissue; regeneration*

The dynamics of repair of injured bone regions replaced by artificial implants is an important factor in the full regeneration of the bone defect.

A study of the mechanism of this process, which is crucial to the improvement of synthetic prosthesis materials and to the monitoring of the regeneration dynamics, is extremely difficult to carry out.

Repair of bone tissue is unique among the various regeneration processes in the organism. Skeletal tissue is a multicomponent system composed of both inorganic and organic substances. In the diagram of possible compositions (assuming there to be three basic components, namely, hydroxyapatite, protein, and water) normal bone tissue resides in the hatched area (Rosebaum's triangle, Fig. 1, *a*). It is to be pointed out that the area of compositions on the diagram (Fig. 1, *a*) reflects much more information than the usual crystal structural motif of the initial components. In fact, this is a framework of rather complex structure which contains multicomponent biological elements, i.e., cells (osteocytes, osteoblasts, osteoclasts) that are continually producing and at the same time resorbing bone tissue [6,7].

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Evidently, bone regeneration is a multistage process which results in the formation of a multicomponent and continually changing (though outwardly appearing unchanged) system. This system exists and functions normally under normal conditions, i.e., homeostasis. It will be here recalled that such a state of a system is defined as steady-state in thermodynamics [2].

Bone tissue regeneration can be monitored using one of the parameters of the steady state of the $Ca_5(OH)(PO_4)_3$-protein-H_2O system. In the course of noninvasive monitoring, the tissue density in the area of growth may serve as a useful parameter, evaluated by densitometry.

It has been shown that a bone tissue defect can be replaced with demineralized allogeneic bone [5], carbon plastics [3], insoluble salts (in particular, hydroxyapatite), and other organic and inorganic materials (e.g., collagen). Use of ground bone as well as of demineralized bone is complicated due to both hygienic and juridical considerations. Carbon plastics do not biodegrade fast enough, thus making it impossible to achieve complete restoration of the functional properties of the injured region. Hydroxyapatite either encapsulates or disperses, depending on how it is obtained. Collagen also lacks the necessary mechanical strength.

Recently the use of implants made of composite materials has been investigated. The materials

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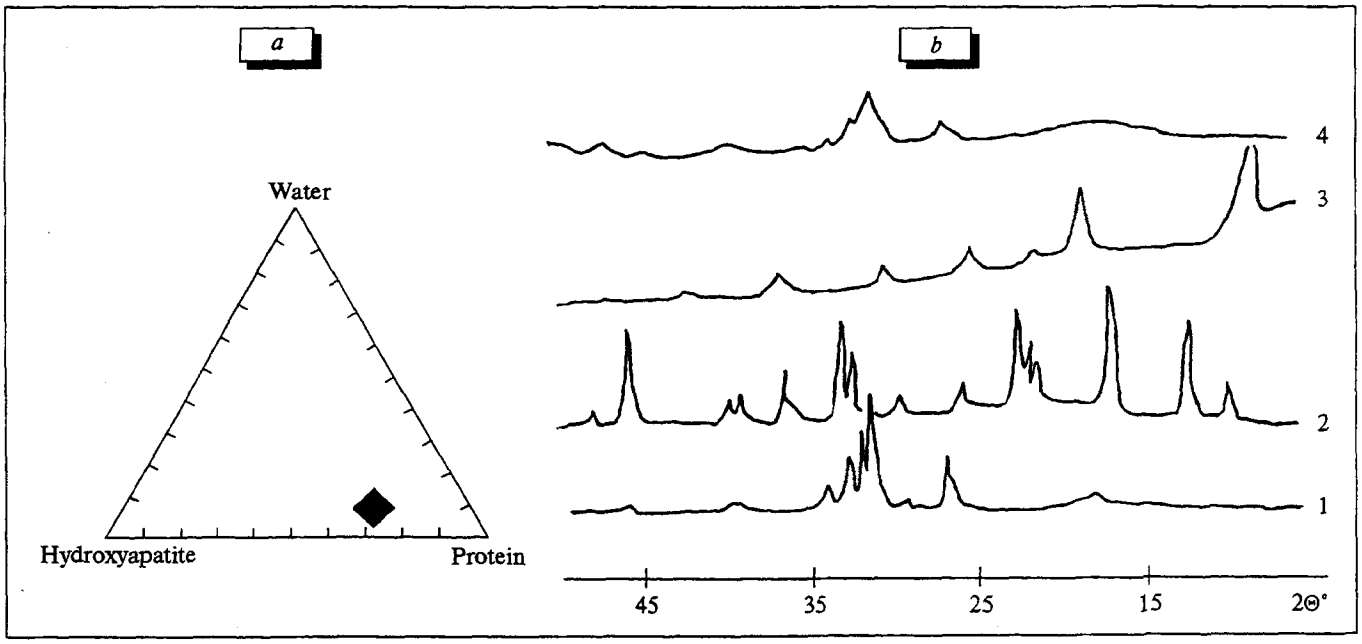

Fig. 1. Characteristics of bone tissue and implant components. *a*) Rosebaum's triangle (hatched region represents composition of native bone); *b*) diffractograms: *1*) bone tissue; *2*) $Mg_nAl_m(OH)_p(SO_4)_q \times xH_2O$; 3) $nMg(OH)_2 \times MgSO_4 \times xH_2O$; *4*) $Ca_5(OH)(PO_4)_3$ (hydroxyapatite).

consist of collagen and inorganic salts cross-linked with a third component, e.g., succinic anhydride [4], or collagen cross-linked with apatite by means of ultrasound treatment [1].

MATERIALS AND METHODS

The implant studied here consisted of collagen (allo- and xenogeneic) mixed with coprecipitated inorganic Ca, Mg, and Al salts of the following structure: $Ca_5(OH)(PO_4)_3$, $Ca_4H(PO_4)_3$, $nMg(OH)_2 \times \times MgSO_4 \times xH_2O$, and $Mg_nAl_m(OH)_p(SO_4)_q \times xH_2O$. The salts were introduced into the collagen by mechanical mixing, coprecipitation of protein from the solution, and electrophoretically. The degree of ionic replacement in the specimens was recorded by the method of differential thermal analysis (DTA), according to the thermal stability of the implant. The homogeneity of inorganic component distribution within the collagen was checked by computerized tomography. The individuality of the inorganic component was confirmed by roentgenophase analysis (RPA) and infrared spectroscopy (IRS). Chemical analysis of the specimens was also conducted.

Implants were sterilized with 0.5% formalin or gamma-radiation (25 kGy).

Cylindrical implants (6 mm in diameter and 10 mm long) in which 50-70% of the weight was made up by inorganic compounds were placed according to the method elaborated by us earlier into the trepanation lumen of the tibial metaepiphysis and/or mandible of a dog. The implant passed through the cortical layer perpendicular to the bone axis from both sides. The operation was performed under intravenous thiopental or intramuscular phenazepam-calypsol anesthesia. In the control group the trepanation channels were left empty.

Biotransformation of immplants was followed up by survey and magnified roentgenography, infrared photography, computerized tomography, DTA, RPA, IRS, and by radiological and morphological methods. Experiments were carried out on 30 dogs (2-7-year-old outbred animals weighing 6-10 kg). A total of 200 implantations were performed. In addition to the *in vivo* control of regeneration, the process was monitored by *in vitro* methods. Three, 6, 9, 12, and more months after the operation euthanasia was performed by an overdose of a narcotic, and an autopsy was made of the regeneration areas.

RESULTS

Figure 1, *b* presents diffractograms of certain inorganic components introduced into the implant, and a diffractogram of the bone tissue from the operated region after the completion of regeneration. Changes in the diffraction picture (even with the use of synthetic hydroxyapatite) confirm implant biotransformation into bone tissue.

The data of thermal analysis of implant specimens show that the temperature of dehydration of unbound water (depending on the method by which the material was obtained) varies from

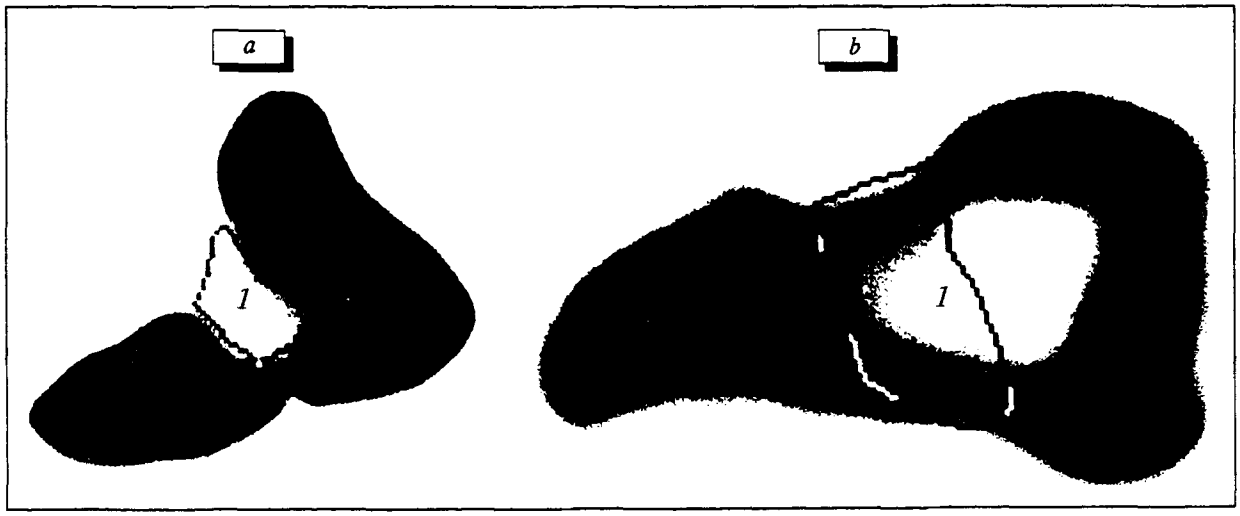

Fig. 2. Tomograms of trepanation channels filled with implant. No formation (*a*) and formation (*b*) of a cortical layer. *1*) site of implantation.

110°C to 210°C in the following series: mechanical mixture - coprecipitation from solution - electrophoretic introduction of salts into collagen. These differences evidently influence the nature of regeneration.

The results of computerized tomography of the surgical area at different times after implantation are presented in Fig. 2. The diagrams in Fig. 3 show the changes of tissue density in the region of the implant as a function of time. Curve *1* reflects the relationship between the density of newly formed tissue in the operated region and the duration of the regeneration process in the cases of implants with a different salt composition but an approximately equal (as in native bone) salt to collagen ratio. The horizontal line *5* represents the borderline of density transition (in Hounsfield units) from soft tissue to bone tissue structures. The threshold level estimated by computerized tomography is arbitrarily taken as 100. The implants were prepared so that their density was equal to or slightly exceeded that of bone tissue (110-112).

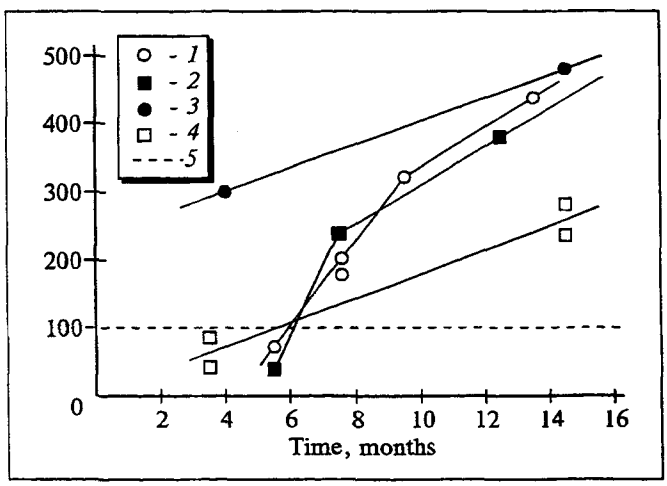

Fig. 3. Curves of bone tissue density within the implantation region as a function of the time postoperation. *1*) collagen–salt implant; *2*) collagen implant; *3*) regeneration with cortical layer formation; *4*) regeneration without cortical layer formation; *5*) borderline of soft tissue to bone tissue transition. Ordinate: density on x–ray, relative units.

The chosen initial implant density made it possible to follow up the regeneration process according to the changes of density occurring during biodegradation.

It should be noted that the points on the diagram (Fig. 3, curves *1* and *2*) more or less lie on one straight line and can be approximated into a linear relationship described by the equation $y=36.6x-62.0$.

Thus, the inorganic salt composition does not have a marked influence on the nature of regeneration. The curve corresponding to the implants lacking a salt component practically coincides with that corresponding to the mineralized implants. This suggests that the normal function of bone cells depends little on the composition of the water-insoluble inorganic component. Therefore, it is possible to vary the implant composition within a broad range, i.e., from admixture-free collagen to a complex containing 70% of inorganic salts.

It is also important to note that the diagrams depicting changes of tissue density in the region of implantation and reflecting the presence (curve *3*) and absence (curve *4*) of a cortical layer are almost parallel. Thus, the range of density values of the tissue forming in the regeneration region in early (2-3 months) and late (9-12 months) periods determines the growth zone of the densest cortical layer (the area between curves *3* and *4*). Comparative analysis of these curves (Fig. 3) shows that early-stage bone regeneration and the

formation of its densest and strongest form at the late stage do not depend much on the implant composition. The key factor is the time required for the formation of a healthy region of native bone replacing the implant volume.

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Morphological Analysis of Protease Treatment of Damaged Nerves

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Intravitam phase-contrast microscopy of individual isolated myelin fibers of rabbit tibial nerves shows that 40-min treatment with 0.2% pronase solution is harmless to intact myelin fibers. In injured nerves such treatment causes sharply expressed proteolysis and a rapid process of wallerian degeneration with ruptures of the axial cylinders and the formation of degeneration ovoids. The findings prove the feasibility and desirability of early primary protease treatment of nerve wounds.

Key Words: *degeneration of nerve fibers; protease treatment of nerves; regeneration*

The problem of hastening nerve regeneration is not a new one. Attempts at modifying the nerve suture, the use of natural and artificial tubular structures as growth guides, and the use of growth factors and other chemical and physical stimulants [3-7] have not yielded the expected results, evidently because of insufficient knowledge about the general biological processes developing during nerve damage. At present we are trying to develop a method for speeding up the regeneration of an injured nerve after protease treatment. Protease treatment of nerves is believed to have a number of advantages: it promotes faster resorption of degradation products, reduces the number of nerve membrane phagocytes capable of migrating to the site of injury, and exerts neurotropic, vasotropic, and antibacterial effects. The aim of the present study was to assess the possible speeding up of autolysis and resorption of degenerating nerve fibers and to analyze the conditions under which secondary protease injury to intact fibers is precluded.

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MATERIALS AND METHODS

Two series of experiments were carried out. In group 1 (8 rabbits) both tibial nerves were cut at

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