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Research paper

Bone stump formation against the background of post-amputation pain syndrome

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Abstract

Introduction: Bone is the basis of the stump.

Aim: To study the impact of post-amputation pain syndrome on the nature of reparative processes in the bone residual limb.

Material and methods: Three series of experiments were performed on 45 rabbits, 15 in each with mid-third thigh amputation and muscular plasty. In series 1, 2, a perineural catheter was attached to the sciatic nerve stump during amputation, and mechanical irritation of the nerve was performed daily for 20 minutes in series 1 for 20 days. In series 2, 0.3 mL of lidocaine (1%) was injected through the catheter into the circumference of the nerve twice daily for 20 days. Series 3 is a control. The follow-up periods were 1, 3, 6 months. The study method was histological with infusion of the vessels with ink-gelatin mixture.

Results and discussion: In series 1, there was a sharp disturbance of the reparative process, which consisted in shape changes, resorption of the cortical diaphyseal plate, fractures, stump deformity, absence of bone closure plate formation, and microcirculatory disturbances. In the overall majority of experiments of series 2, the stumps retained the shape and structure characteristic of diaphysis with normalization of microcirculation. In series 3, the results of the residual limb formation were better than in series1, but worse than in series 2.

Conclusions: When the pain syndrome is resolved within 20 days after amputation, a bone stump is formed with an organotypic shape and structure characteristic of the diaphysis, the formation of a compact closure plate of mature bone tissue, normalization of the medullary tissues and blood circulation. In post-amputation pain syndrome, organotypic formation of the residual limb does not occur.

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1. INTRODUCTION

The formation of a residual limb that allows for adequate prosthetic capacity is a complex problem. This is due to the difficulty of creating favourable conditions for all residual limb tissues to heal. The very idea of the conditions required for a full reparative process and the specific parameters of the suitability of the residual limb after its completion are beyond the competence of surgeons. This is especially true for the backbone of the residual limb – its bony base. Despite a large number of studies on amputation and post-amputation pain syndrome, diseases and defects of residual limbs,[1-8] there are only single reports,[9,10] which highlight the processes of reparative regeneration at the end of the bone remnant of the future working organ. The issues of shaping the end of the residual limb in the case of concomitant post-amputation pain syndrome remain unexplored. The latter occurs as early as the first days after amputation,[11] and in many cases even before it takes place. Nerve involvement often occurs before the amputation. Prolonged inflammatory processes in pyo-inflammatory and thrombobliterative diseases, transport, domestic, industrial injuries and gunshot wounds are accompanied by damage or inflammation of nerve trunks and the occurrence of pain syndrome. Amputation with nerve transection and the possible development of ascending neuritis or end-stem or intrathoracic neuroma can also be a trigger. It is reasonable to assume that pain syndrome after limb amputation may aggravate the course of the reparative process in the bone residual limb.

2. AIM

To study the effect of post-amputation pain syndrome on the nature of reparative processes in the bone residual limb.

3. MATERIAL AND METHODS

Three series of experiments were performed on 45 rabbits, 15 in each with mid-third thigh amputation and muscle grafting. In the 1st and 2nd experimental series, a perineural catheter was brought to the sciatic nerve stump during amputation, by means of which mechanical irritation of the nerve was performed daily for 20 minutes in the 1st series for 20 days. In series 2, 0.3 mL of lidocaine (1%) was injected twice daily for 20 days through the catheter into the circumference of the nerve. Animals of series 3 served as a control. The follow-up periods were 1, 3 and 6 months.

In all experiments, the vessels were poured with ink after injecting a lethal dose of hexenal. After appropriate wiring, sections with a thickness of 15–30 μm were made, which were stained with hematoxylin and eosin and, according to van Gieson, with picrofuchsin. Enlightened sections (100–150 μm thickness) thickness were also prepared. Histological sections of nerve fibres were stained with hematoxylin-eosin and impregnated using the Bolshovsky–Gros method.

Statistical processing of the material was carried out using a nonparametric difference criterion for two conjugate populations. Using the criterion of signs, the direction of changes in each group of observations was determined. Observations having zero differences from the calculation were excluded. The number of pairs with a less frequent algebraic sign is denoted by the letter Z. The obtained value of Z is compared with tabular critical numbers of less frequently encountered signs Z_{05} and Z_{01}. The zero hypothesis was taken with $Z > Z_{05}$ and rejected at $Z \leq Z_{05}$ ($P < 0.05$) and $Z \leq Z_{01}$ ($P < 0.01$), when the differences found to be significant. The calculation of the results of the study was carried out using the software package MS Excel XP and Statistica SPSS 10.0 for Windows (license number 305147890).[12,13]

4. RESULTS

4.1. First experimental series – 15 observations

Duration of 1 month, 5 observations. All 5 observations showed a cylindrical form of the bone stump without the formation of a closure bone lamina ($P < 0.05$). In 3 observations the medullary canal was closed by dense fibrous tissue and in 2 cases partially by dense fibrous tissue and partially by a network of endosteal-formed immature bone beams (Figure 1). Between the beams is cellular-fibrous tissue with a large number of tissue cysts. In the medullary canal, a cell-poor edematous loose fibrous tissue with sinusoidal vessels and multiple tissue cysts, sometimes very large, was determined throughout the entire length. The cortical diaphyseal plate was focally thin and spongy in all cases ($P < 0.05$).

Term 3 months, 5 observations. A cone-shaped stump was obtained in 3 cases (Figure 2) and a cylindrical one in 2. In all cases, there was extensive rarefaction and spongification of the cortical diaphyseal layer ($P < 0.05$). In 2 stumps with a cone-shaped and occluded medullary canal, there was axial curvature due to extensive bone resorption, and in the 3rd one, there was a cortical layer fracture. In the cylindrical stumps, there was also massive resorption of the cortical diaphyseal layer. There was no compact bone tissue in the distal sections. Medullary tissue in all observations was replaced by dense and loose fibrous tissue with the presence of sinusoidal vessels and lymphoid plastic cells (Figure 3). The bone lamina was not formed. The medullary canal is closed by a regenerate of immature bone tissue. A large number of carcass-filled vessels passing from the medullary canal to the fibrous-tissue fringe of the stump is revealed.

Term 6 months, 5 observations. In 4 cases, a cylindrical stump was formed, with extensive resorption of the cortical diaphyseal plate and fractures in the zone of greatest resorption. Endosteal regenerate at the end of the stump is represented by bone trabeculae of varying maturity. Completeness of reparative regeneration was not noted in any of the observations ($P < 0.01$). Large branches of the feeding artery pass between the beams of the endosteal regenerate from the medullary canal into the soft tissue border. In the distal and proximal parts of the medullary canal, dilated branches

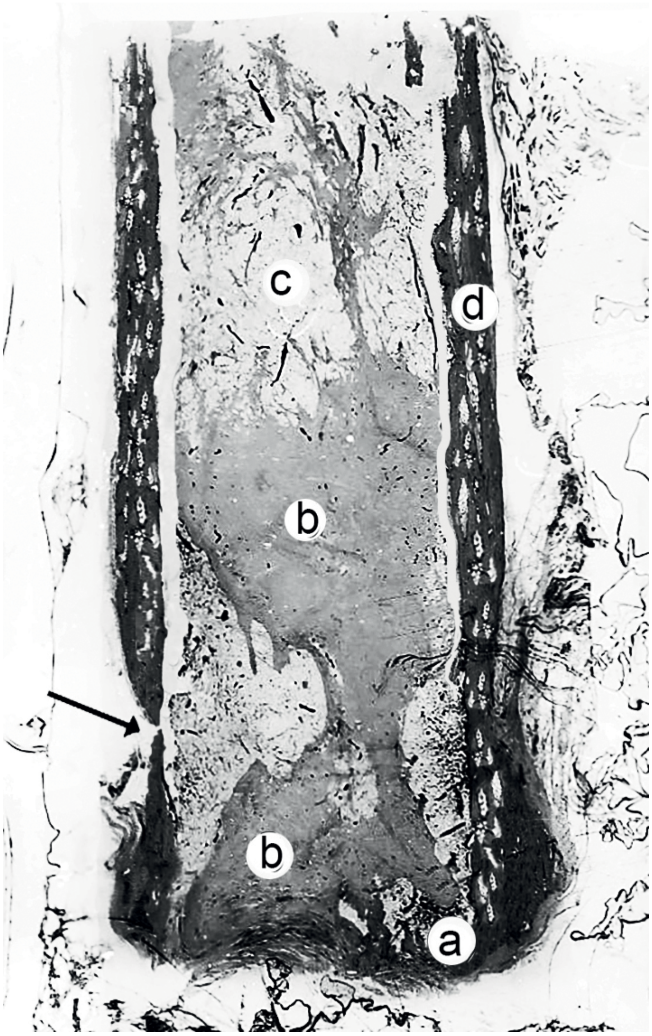

Figure 1. Histotopogram of a stump with partially formed endosteal bone regeneration (a) and partial overlapping of the medullary canal with a thick layer of dense fibrous tissue (b), fibrous tissue in the proximal part of the medullary canal (c), sharply cortical diaphyseal plate (d), cortical diaphyseal plate fracture (arrow). HE staining, magnification ×2.5.

a. nutricia, veins, sinusoidal vessels, tissue cysts. Parietal edema of the medullary contents, represented by loose fibrous tissue, is noted. In the 5th observation of this period, the diaphyseal shape was disturbed due to the formation of bone regenerate along the periosteal surface and destruction of its end (Figure 4). Near the usurized edges of the cortical diaphyseal plate, granulation tissue was revealed, turning into fibrous. In it, one can see vessels filled with ink, such as small arteries. Periosteal osteochondral growth consists of hyaline cartilage and a network of immature bone beams. In the fibrous tissue filling the lower part of the medullary canal, there are foci of ink impregnation, areas of accumulation of macrophages. The middle part of the end surface of the bone stump is occupied by fibrous tissue; closer to the cortical diaphyseal plate, small remnants of the osteone-bar structure of the bone endplate are revealed. In the lumen of the medullary canal at a considerable distance, bone beams

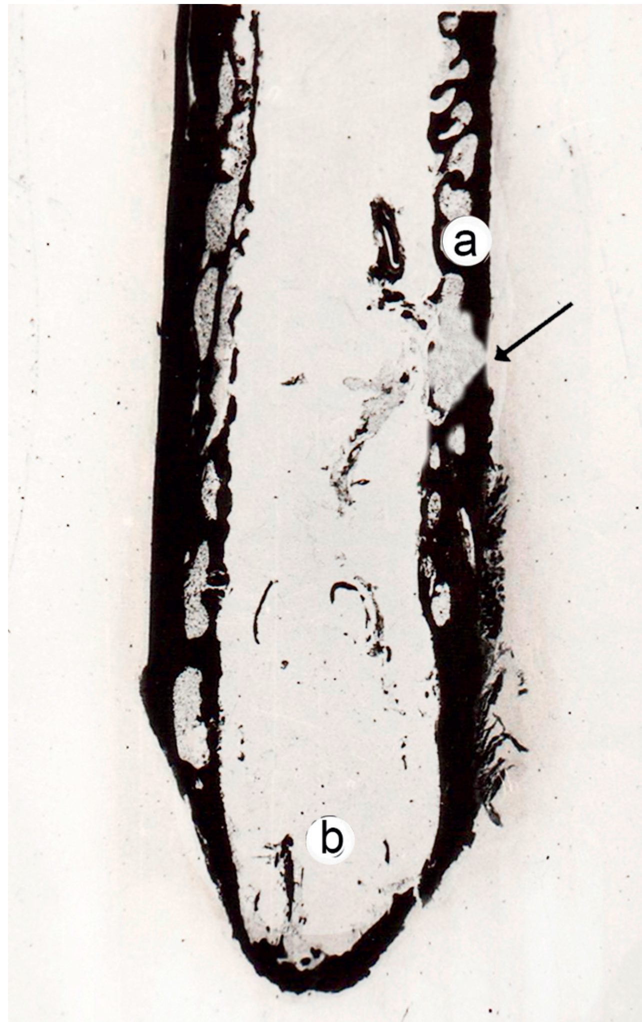

Figure 2. Histotopogram. Cone-shaped stump. Spongification of the cortical diaphyseal plate (a), cone-shaped part of the stump (b) with fracture (arrow). Van Gieson staining, magnification ×2.5.

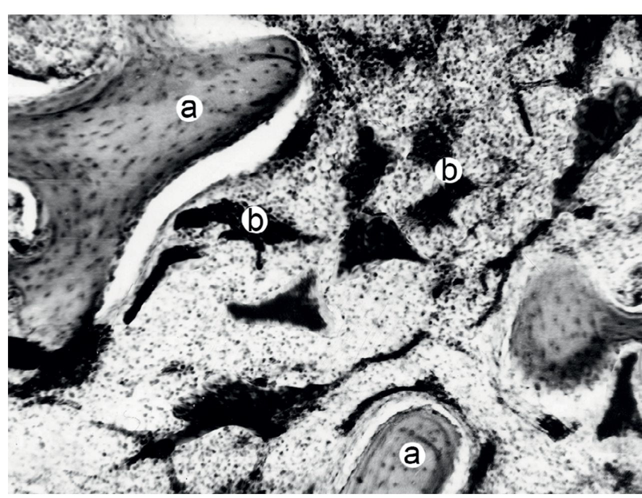

Figure 3. Microphotograph of the tissue of the end of the cone-shaped bone stump. Immature bone beams (a), sinusoidal vessels (b) in loose fibrous tissue of interbody spaces with diffusely scattered lymphoid-plasma cells. HE staining, magnification ×90.

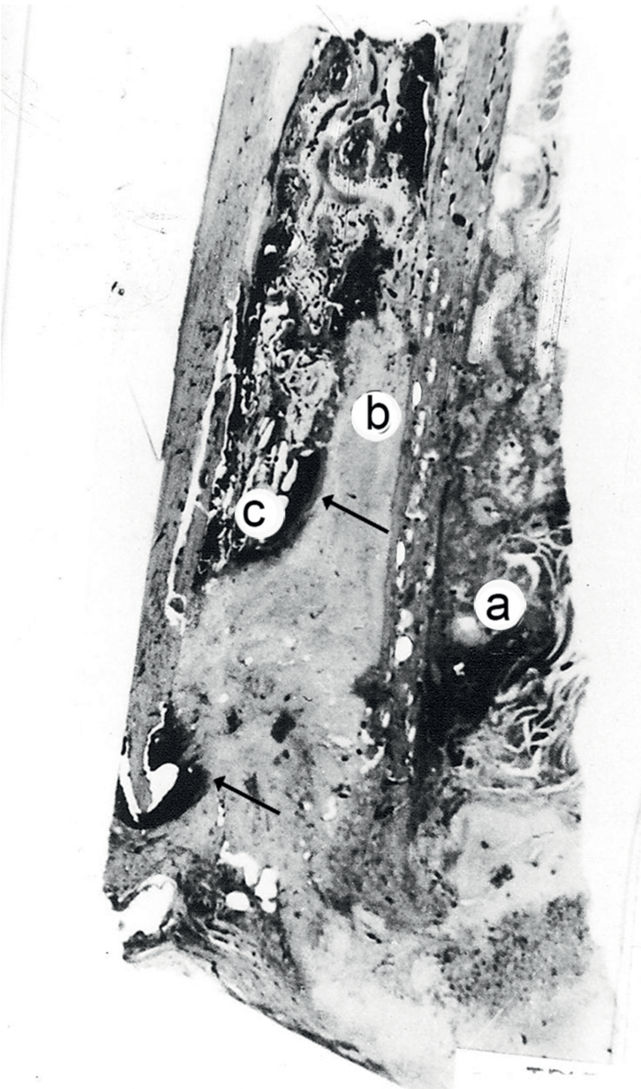

Figure 4. Histotopogram of a bone stump with resorption of the cortical diaphyseal plate and formation of periosteal regenerate (a), conglomerate of dense fibrous tissue filling the medullary canal (b), endosteal formed beams partially filling the entrance to the medullary canal (c), ink-filled vessels (arrows). HE staining, magnification ×2.5.

are visible, in the interbeam spaces – loose fibrous tissue, sinusoidal vessels, tissuecysts.

When examining the nerve and surrounding tissues, pronounced edema, degeneration of nerve fibers, infiltration of the nerve trunk with lymphocytes, arteritis and obliteration of the arteries were observed. Edema and the consequences of exudation caused rarefaction and fragmentation of nerve fibers. There was a pronounced edema of epineuria, perineuria, endoneuria, hypertrophy of lemocytes with vacuolization of their cytoplasm ($P < 0.01$).

4.2. Second experimental series – 15 observations

Term 1 month, 5 observations. In all cases, a cylindrical shape of the stump end was formed. The cortical diaphyseal

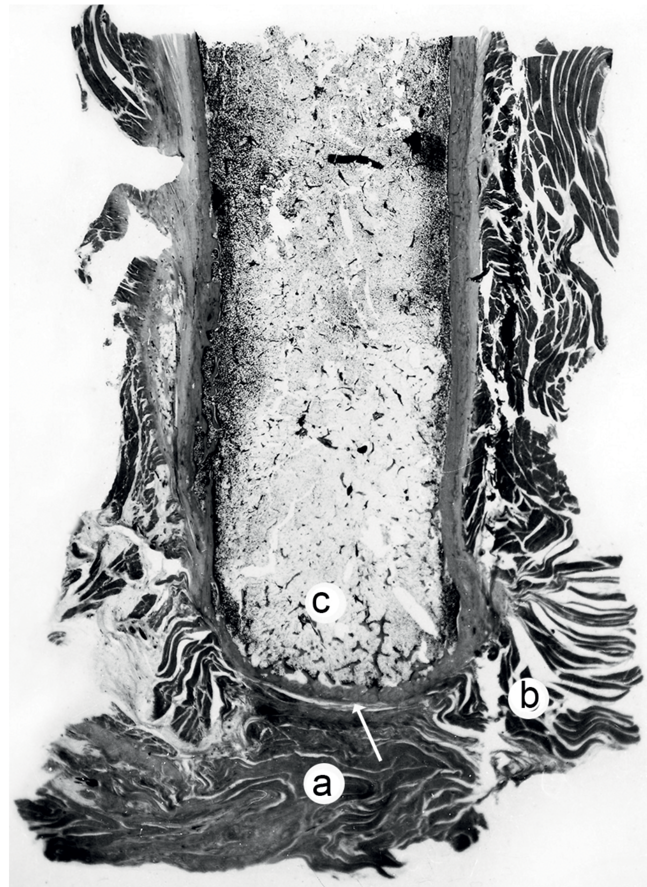

Figure 5. Histotopogram of a cylindrical stump. Laminar lamina (arrow) bordered by fibrous tissue (a), muscles adjacent to the lamina (b), endosteal formed bone beams (c). HE staining, magnification ×2.5.

layer retained the structure of compact bone. There was a slight spongification of its edges. There was a widening of part of the vascular canals. In all observations, at the end of the ossicle there was a formation of a bone lamina from not quite mature bone tissue ($P < 0.05$). The terminal section of the medullary canal was filled with fatty and partially hematopoietic bone marrow with interlayers of loose fibrous and fibroreticular tissue and the presence of sinusoidal vessels. The proximal part of the medullary canal was filled with fatty bone marrow.

Term 3 months, 5 observations. The anatomical cylindrical shape of the stump end was formed in all observations (Figure 5). In 3 cases, there was moderate osteoporosis of the cortical diaphyseal layer in the presence of the osteon-beam bone lamina. In another 2, at the level of its rarity, phyboreticular tissue with single bone beams was seen among the fatty spatial marrow in the medullary canal. Such a picture indicates a tendency for completion and attenuation of the reparative process ($P < 0.05$). In the medullary canal there was fatty bone marrow with carcass-filled vessels close to those of normal bone (Figure 6).

Term 6 months, 5 observations. In all observations, a bone stump with an organotypic shape characteristic of the diaphysis was formed ($P < 0.05$). The structure of the corti-

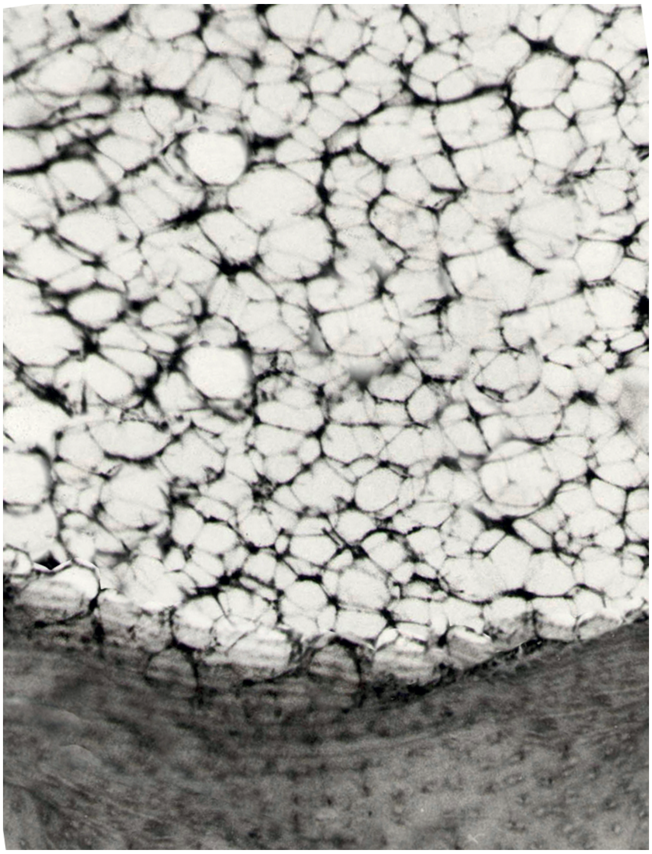

Figure 6. Microphotograph. Bone laminae of mature bone tissue. Ink-filled vessels of the microcirculatory network of the fatty bone marrow at the end of the stump. HE staining, magnification ×90.

cal layer with a unified osteon system characteristic of the normal diaphysis bone was restored. A compact thin closure plate of mature bone tissue was formed at the obolus, intraosseous microcirculation and the state of the medullary tissues were normalized. Large vessels of the bone stump were absent – reduced. The rearrangement processes were completed ($P < 0.05$). The bone acquired usual structure.

The truncated sciatic nerve had a thickening at the end. Dystrophic changes, chaotic arrangement of fibers forming a ball with growth flasks and spirals were noted at all periods.

4.3. Third (control) series – 15 observations

In this series of experiments, the results of stump formation were worse than the results of the 2nd series, but much better than the 1st. The cylindrical shape of the stump was retained in 13 out of 15 observations. Bone endplate at 1–3 months in 8 cases consisted of immature, and in 2 – of mature bone tissue. At 6 months, in 2 cases, the endplate bone tissue was mature with the completeness of the reparative process. In 2 cases of a 6-month period, the phenomena of bone resorption along the course of the vascular canals from the side of the medullary cavity and the periosteal surface progressed, leading to focal spongization, thinning of the cortical plate, and a conical change in the shape of the stump. In observations with a cone-shaped stump, there was no completeness of microcirculation and reparative regeneration. Moreover, in most cases, there was a sharp spongization of the cortical diaphyseal plate, its focal resorption. Intraosseous circulation was represented by dilated microvessels in the form of sinuses and small tissue cysts.

The nerve trunk is thickened at the end. There was a non-uniform maturation of the connective tissue. There were avascular zones formed by accumulations of thick bundles of collagen fibers.

5. DISCUSSION

Bone tissue under certain conditions is capable of regeneration, i.e. it has certain biological capabilities based on the physiological remodelling of bone tissue. To a large extent, this property is ensured by the adequacy of intraosseous circulation. Bone truncation sharply disrupts its homeostasis. Restoration of the latter after amputation at the diaphysis level implies preservation of the original shape, structure and physiology of tubular bone in the residual limb.[9,10] Therefore, when assessing the reparative process, we took into account the shape of the end section, the structure of the cortical diaphyseal plate, the presence of the closure bone plate, and the condition of tissues within the medullary canal in its proximal and distal parts.

Based on the study of preparations of 1-, 3-, 6-month period after amputation, absolutely different results of the formation of the bone stump were revealed.

In the 1st experimental series, amputation and the presence of pain syndrome due to sciatic nerve neuritis cause a violation of the microvascular network of the bone, an increase in extravascular circulation, a sharp inhibition of the proliferative activity of cellular elements, activation of resorption, which leads to spongization and atrophy of the cortical diaphyseal plate, replacement of adipose bone marrow with loose fibrous connective tissue. The process of reparative regeneration proceeds with a predominance of bone resorption ($P < 0.05$). Resorption spreads both along the vascular canals and along the surface of the cortical diaphyseal plate. There comes a restructuring of bone tissue with a pronounced spongization of the cortical diaphyseal layer. In some cases, it has a significant length. As a result, bone tissue is replaced by fibrous tissue, which contains an abundant vascular network. Due to the predominance of resorption, reparative processes are delayed or stopped, and spongization progresses. Bone crossbeams fracture. The resorption zone reveals immature bone beams resulting from the healing of fractures. The bone marrow canal was closed with a soft tissue regenerate with inclusions of the main bone vessels emerging from the bone marrow canal into the surrounding soft tissues. In the intraosseous microcirculation and in distant juices, sinusoids and tissue cysts, unusual for the diaphysis of normal bone, remained, which indicates the absence of its normalization.[9,10] Completeness of reparative processes was not observed in any observation even at 6 months, which was regarded as pathological restructuring of bone tissue ($P < 0.01$).

In experiments of the 2nd series, in the absence of pain syndrome, which was relieved within 20 days, endosteal bone regenerate was formed at the end of the stump on the basis of endosteal bone formation within 1 month ($P < 0.05$). At first it was a beam structure, and in 3 months it acquired a compact structure. In the cortical diaphyseal plate, moderately pronounced reparative processes were observed, manifested by slight resorption along the vascular canals, periosteal and endosteal surfaces, and at the end of the sawdust ($P < 0.05$). These processes did not change the shape of the stump. In the distal part of the medullary canal, adipose bone marrow with layers of loose fibrous connective tissue and a small number of sinusoidal vessels was preserved. Large vessels at the end of the stump were not detected due to obliteration. The state of microcirculation approached the state characteristic of the bone.

The unsatisfactory results obtained in some observations of the control series, where the presence of pain syndrome cannot be excluded in a number of experiments, are explained by its possible negative influence on the reparative process, which is consistent with the data.[7,8,11]

Considering that the bone is an organ providing support, and this is due to clear constants of its structure, the state of the latter in the formed stump, close to the state of a normal bone, should ensure its functional ability. The stumps obtained in the 1st series and part of the experiments of the 3rd series do not possess such properties.

The study showed the need to search for new methods of treating the nerve or adequate pain relief within 20 days after amputation of the limb, when the intensity of bone tissue remodeling is highest.

6. CONCLUSIONS

(1) In the absence of pain syndrome, the bone stump after amputation at the level of the diaphysis within 1, 3, 6 months retains the cylindrical shape, the structure of the cortical diaphyseal plate, the contents of the medullary canal traced throughout the entire length with normal microcirculation, the formation of the bone endplate and the completeness of the reparative process.
(2) The presence of post-amputation pain syndrome in the stump distorts the course of the reparative process with the development of pathological remodeling of bone tissue.
(3) The findings suggest that standard approaches to pain relief after amputation should be revised and supplemented.

Conflict of interest
None declared.

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Ethics
Experiments were performed in accordance with the principles of humane treatment of animals as set out in the European Community directives 86(609) EEC and the Declaration of Helsinki on Humane Treatment of Animals. Ethics committee approval for this study was obtained (approval No 3/2021).

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