УДК 616.8-009.62+616.833-002]:616.71-089.873](045)

DOI: http://dx.doi.org/10.15674/0030-598720221-220-25

# The influence of post-amputation pain syndrome and intraosseous main vessels on the formation of limb bone stump

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*Until now, remains quit high a percentage of unsatisfactory results of amputations. At the same time, a special place is occupied by the healing of the bone remnant — the main supporting element of the stump. Objective. To study the influence of post-amputation pain syndrome and intraosseous main vessels on the nature of reparative processes in the bone stump. Methods. Three series of 15 experiments were conducted on 45 rabbits with amputation of the femur in the middle third and muscle plastic surgery. In the 1st and 2nd series, during amputation, a perineural catheter was brought to the stumps of the sciatic nerve. With its help, animals were subjected to the following daily for 20 days: 1st series — mechanical irritation of the nerve, 20 minutes; 2nd — injection of 0.3 ml of 1 % lidocaine twice a day into the surrounding area of the nerve. Animals of the 3rd series were the control. In 1, 3, 6 months. a histological examination was performed with the pouring of carcass (gelatin mixture) into the vessels. The results. In the 1st series, there was a sharp violation of the reparative process: a change in the shape and loosening of the cortical diaphyseal plate, fractures and deformation of the stump, a significant expansion of the feeding artery and its branches with perforation of the endosteal regenerate, the absence of formation of the bone closing plate, a violation of the microcirculation. In the animals of the 2nd series, the stumps mostly kept the shape and structure characteristic of the diaphysis with normalization of macro- and microcirculation. In the 3rd series, the results of stump formation were better than in the 1st, but worse than in the 2nd. Conclusions. If the pain syndrome subsides after amputation, a bone stump is formed with an organotypic shape and structure characteristic of the diaphysis, normalization of the state of bone marrow tissues and blood circulation. The rapid and complete formation of the bone closing plate contributes to the reduction of the branches of the feeding artery and prevents the functional depressurization of the bone marrow cavity. In the presence of post-amputation pain syndrome, there are significant violations of the reparative process with the formation of an inferior bone closing plate, its penetration by the branches of the feeding artery without the organotypic formation of the stump.*

*Дотепер залишається високим відсоток незадовільних результатів ампутацій. При цьому особливе місце займає загоєння кісткового залишку — основного опорного елемента кукси. Мета. Вивчити вплив післяампутаційного больового синдрому та внутрішньокісткових магістральних судин на характер репаративних процесів у куксі кістки. Методи. На 45 кролях проведено три серії дослідів по 15 у кожній з ампутацією стегнової кістки в середній третині та м'язовою пластикою. У 1-й і 2-й серіях під час ампутації до сідничного нерва кукси підводили периневральний катетер. За допомогою нього тваринам протягом 20 днів щодня здійснювали: 1-ша серія — механічне подразнення нерва, 20 хв; 2-га — введення в оточення нерва двічі на добу 0,3 мл 1 % лідокаїну. Тварини 3-ї серії були контролем. Через 1, 3, 6 міс. виконано гістологічне дослідження з наливанням у судини туші (желатинової суміші). Результати. У 1-й серії відбулося різке порушення репаративного процесу: зміна форми та розсмоктування коркової діафізарної пластинки, надломи і деформації кукси, значне розширення живильної артерії та її гілок із перфорацією ендостального регенерату, відсутність формування кісткової замикальної пластинки, порушення мікроциркуляції. У тварин 2-ї серії здебільшого кукси зберігали характерну для діафіза форму і структуру з нормалізацією макро- та мікроциркуляції. У 3-й серії результати формування кукси були кращими, ніж у 1-й, проте гіршими, ніж у 2-й. Висновки. За умови купування больового синдрому після ампутації формується кукса кістки з характерною для діафіза органотиповою формою і структурою, нормалізацією стану кістковомозкових тканин і кровообігу. Швидке та повноцінне формування кісткової замикальної пластинки сприяє редукції гілок живильної артерії та попереджує функціональну розгерметизацію кістковомозкової порожнини. За наявності післяампутаційного больового синдрому виникають значні порушення репаративного процесу з формуванням неповноцінної кісткової замикальної пластинки, пенетрацією її гілками живильної артерії без органотипового формоутворення кукси. Ключові слова. Ампутація, больовий синдром, неврит, репаративна регенерація, живильна артерія*

**Key words.** Amputation, pain syndrome, neuritis, reparative regeneration, feeding artery

## Introduction

Stump formation, which provides the possibility of adequate prosthetic repair, is a challenging problem. It is caused by the difficulties of creating favorable conditions for the healing of all tissues. The idea of the conditions necessary for the full course of reparative processes, and the specific parameters of the suitability of the stump after their completion, are beyond the competence of surgeons. This especially applies to the skeleton of the stump — its bony base. Despite the large number of studies dealing with amputations and post-amputation pain syndrome, diseases and deformities of the stump [1–8], only isolated reports [9, 10] highlight the processes of reparative regeneration at the end of the bone fragment. The issue of shaping the end of the bone stump in the case of concomitant post-amputation pain syndrome remains unexplored. The latter occurs immediately after amputation [11], and in many cases even before it is performed. Involvement of nerves in its development most often occurs even before amputation. Prolonged inflammatory process in purulent-inflammatory and thrombo-obliterating disorders, transport, household, industrial injuries and gunshot wounds are accompanied by a damage or inflammation of nerve trunks with pain syndrome. Amputation with transection of nerves and possible development of ascending neuritis, terminal or intratruncal neuroma can also be a trigger. We assume that the pain syndrome after amputation can negatively affect the course of the reparative process in the bone stump.

The role of the feeding artery system, which is one of the muscle branches, penetrating the bone in the middle of the diaphysis, dividing into proximal and distal vessels and branching into many smaller vessels in the medullary canal, remains unexplored. The latter in the form of pre- and post-capillaries through the system of Volkmann channels penetrate to the inner layers of the cortical plate, and through the channels of osteons in the form of capillaries are distributed along the bone and feed the inner two-thirds of the thickness of the cortical layer of the diaphysis [12, 13].

*Purpose:* to study the influence of post-amputation pain syndrome and intraosseous main vessels on the nature of reparative processes in the bone stump.

## Material and methods

Three series of experiments were conducted on 45 rabbits with 15 animals in each group with amputation of the femur in the middle third and muscle plastic surgery. In the 1st–2nd series, during the amputation, a perineural catheter was brought to the stump of the sciatic nerve, and for 20 days, the following were performed: 1st series — mechanical stimulation of the nerve for 20 minutes; 2nd series — administration of 0.3 ml of 1 % lidocaine twice a day, 3rd series — control group. Observation periods — 1, 3, 6 months.

Blood vessels in all the rabbits were filled with ink after the introduction of a lethal dose of hexenal. After histological processing, sections with a thickness of 15–30 µm were made, which were stained with hematoxylin and eosin and picrofuchsin according to Van Gieson. Histological sections of nerve fibers were stained with hematoxylin-eosin and impregnated according to Bilszovsky-Gross.

The study was approved at the meeting of the Bioethics Committee of the Research Institute of Rehabilitation of Persons with Disabilities of M. I. Pirogov Vinnytsia National Medical University (Protocol No. 1 of 11.02.2022). The experiments were performed in accordance with the principles of humane treatment of animals, in accordance with the directives of the European Community and the Helsinki Declaration on Humane Treatment of Animals.

## Results and their discussion

*1st experimental series (n = 15).* A month after the operation, a cylindrical bone stump without the formation of a bone locking plate was observed in all the animals. In 3 animals, the bone marrow canal was closed by dense connective tissue, in 2 animals it was loose and dense, partly by a network of endosteally formed immature bone trabeculae (Fig. 1). Between the trabeculae there was a cell-fibrous tissue with a large number of cysts. Cell-poor loose connective tissue with signs of edema, sinusoidal vessels, and multiple tissue cysts, sometimes very large, were identified in the bone marrow canal along its entire

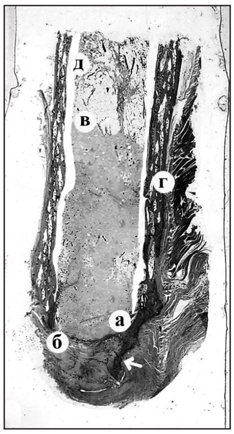


**Fig. 1.** Histotopogram. Stump with partially formed endosteal bone regenerate (a) and partial covering of the bone marrow canal by a thick layer of dense connective tissue (b), loose connective tissue in the proximal part of the bone marrow canal (c), sharply spongy cortical diaphyseal plate (d), swelling of the tissues of the bone marrow canal (f), the rest of the cortical diaphyseal plate (arrow). H&E, x 2.5

length. The cortical diaphyseal plate was sometimes thin and spongy.

In *3 months* 3 animals were found to have a cone-shaped bone stump (Fig. 2), 2 animals were shown to have a cylindrical bone stump. A significant rarefaction and spongization of the cortical diaphyseal layer was determined. In two stumps with a cone-shaped shape and overgrowth of the bone marrow canal, the axis was bent due to significant resorption of bone tissue, and in the third one, there was a fracture of the cortical layer. In stumps with a cylindrical shape, massive resorption of the cortical diaphyseal layer also occurred. There was no compact bone tissue in the distal parts. The medullary tissue in all cases was replaced by dense and loose connective tissue with the presence of sinusoidal vessels and lymphoid plasma cells (Fig. 3). The bone locking plate was not formed. The medullary canal was closed by regenerate from immature bone tissue. There were a large number of wide vessels filled with ink, passing from the medullary canal to the tissues around the stump.

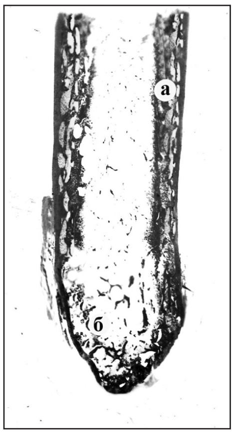


**Fig. 2.** Cone-shaped stump: spongization of the cortical diaphyseal plate (a), cone-shaped part of the stump (b). H&E, x 2.5

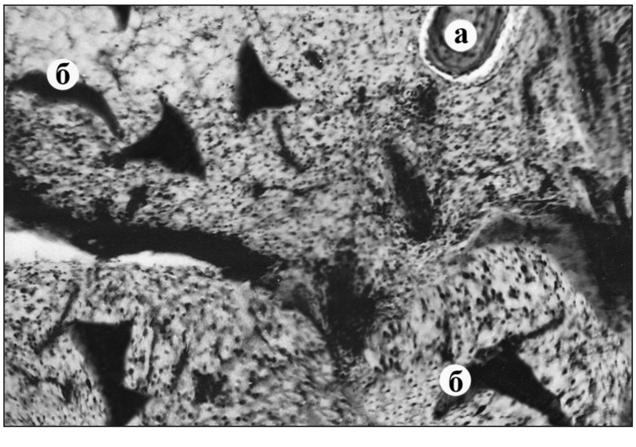


**Fig. 3.** Photomicrogram. Tissue at the end of cone-shaped stump of the bone: immature bone trabeculae (a), vessels of the sinusoidal type (b) in the loose connective tissue of intertrabecular spaces with diffusely scattered lymphoid-plasma cells. H&E, x 90

In *6 months* cylindrical stumps were formed in 4 rabbits, with significant resorption of the cortical diaphyseal plate and fractures in the area of greatest resorption. The endosteal regenerate at the end of the stump was bone trabeculae of varying degrees of maturity. Completion of reparative regeneration was not indicated in any case. Between the trabeculae of the endosteal regenerate, large branches of the feeding artery passed from the bone marrow canal to the soft tissue environment of the stump. They were significantly expanded, forming large vascular conglomerates (Fig. 4). In the distal part of the bone marrow canal, there were branches of *a. nutricia*, venous sinus, vessels of the sinusoidal type, tissue cysts. There was a parietal edema of the medullary contents, represented by loose connective tissue. In the fifth case, the shape of the diaphysis was disturbed due to the formation of the periosteal surface of the bone regenerate and the destruction of the end of the bone. Granulation tissue was located near the worn edges of the cortical diaphyseal plate, which was gradually replaced by connective tissue. It contained ink-filled vessels of small artery type. Periosteal osteocartilaginous growth consisted of hyaline cartilage and a network of immature bony trabeculae. Fibrous tissue that filled the lower part of the medullary canal contained cells of infiltration with ink and areas of accumulation of macrophages.

The middle part of the end surface of the bone stump was was shown to comprise connective tissue, with small remnants of the osteon-trabecular structure of the bone closing plate near the cortical diaphyseal plate. Bone trabeculae were observed at a considerable distance in the lumen of the bone mar-

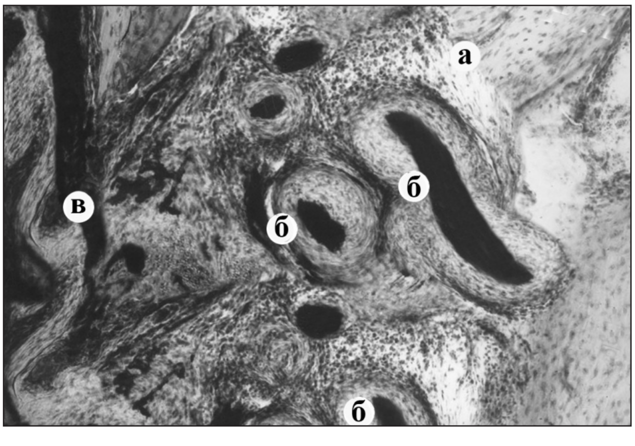


**Fig. 4.** Endosteal regenerate (a) at the end of the stump, numerous branches of the feeding artery filled with ink (b), venous sinus (c). Hematoxylin and eosin, coll. 90

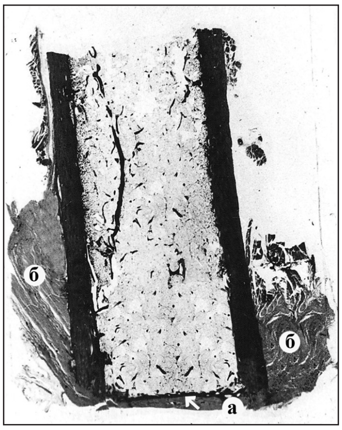


**Fig. 5.** Cylindrical coccyx: locking bone plate (arrow), bordered by fibrous tissue (a), muscles adjacent to the plate (b). H&E, x 2.5

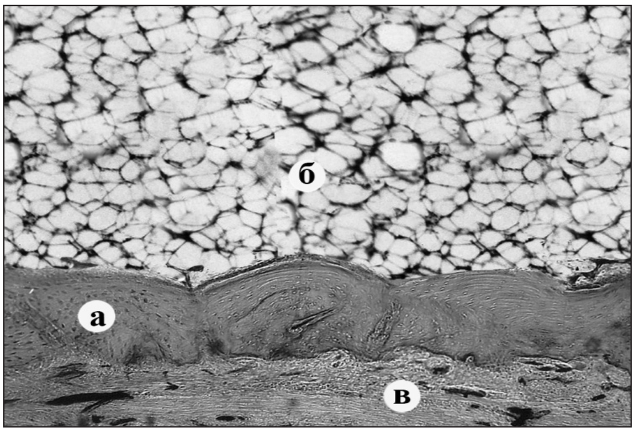


**Fig. 6.** Bone closing plate of mature bone tissue (a), vessels filled with ink of the microcirculatory network of fatty bone marrow at the end of the stump (b), dense connective tissue. H&E, x 90

row canal, loose connective tissue, sinusoidal vessels, tissue cysts, and wide openings of the feeding artery branches were observed in the intertrabecular spaces.

During the examination of the nerve and adjacent tissues, severe swelling, degeneration of nerve fibers, infiltration of the nerve trunk by lymphocytes, arteries and obliteration of arteries were observed. Edema and the effects of exudation caused thinning and fragmentation of nerve fibers. Severe swelling of the epineurium, perineurium, endoneurium and hypertrophy of Schwann cells (lemocytes) with vacuolization of their cytoplasm were found.

*2nd experimental series (n = 15).* One month after the operation, all rabbits had a cylindrical shape at the end of the stump. The cortical diaphyseal layer preserved the compact bone structure with slight sponginess of its marginal parts. There was an expansion of the lumen of a part of the vascular channels. In all cases, a bony locking plate was formed from immature bone tissue at the end of the cut bone. The terminal part of the medullary canal was filled with fatty and partially hematopoietic bone marrow with layers of loose connective and fibroreticular tissues with the presence of sinusoidal vessels. The proximal part of the bone marrow canal was filled with fatty bone marrow.

In *3 months* the anatomical cylindrical shape of the end of the stump was formed in all 5 animals (Fig. 5). In three cases, moderate osteoporotic changes in the cortical layer of the diaphysis were found in the presence of a bony closing plate of the osteon-trabecular structure. In two rabbits, fibroreticular tissue with single bone trabeculae was located in the medullary canal at the level of its rarefaction among fatty bone marrow. The described histological picture indicates a tendency towards completion and attenuation of the reparative process. In the medullary canal, there was fatty bone marrow with vessels filled with ink, similar in structure to the vessels of normal bone (Fig. 6).

In *6 months* a bone stump with an organotypic shape typical for the diaphysis was formed in all 5 animals. The reconstruction processes were mostly completed. The bone acquired the usual structure. The structure of the cortical layer with the osteon system, typical for normal bone diaphysis, was restored. At the end of the cut, a compact, thin closing plate of mature bone tissue was formed; intraosseous microcirculation and the state of bone marrow tissues were normalized. Major vessels at the end of the bone stump were absent, which indicated their reduction.

The severed sciatic nerve had a thickening at the end. In all stages, there were dystrophic changes, chaotic arrangement of fibers that form a ball with growth bulbs and spirals.

*3rd (control) series (n = 15).* In the rabbits of this series, the results of stump formation were worse than in the 2nd, but significantly better compared to the 1st series. The cylindrical shape of the stump was preserved in 13 out of 15 animals. In 8 cases, bone locking plate consisted of immature bone tissue 1 and 3 months after surgery, and in 2 cases it consisted of mature bone tissue. After 6 months in two rabbits, the bone tissue of the locking plate was mature, the reparative process was completed. In two animals 6 months after surgery the signs of bone tissue resorption progressed along the vascular channels from the endosteal part of the bone marrow cavity and the periosteal surface, which led to focal spongization, thinning of the cortex and cortical plate, and a cone-like change in the shape of the stump.

In observations with a cone-shaped stump, there was no completeness of microcirculation and full-fledged bone reparative regeneration. At the same time, sharp spongization of the cortical diaphyseal plate and its focal resorption were determined in most animals. Intraosseous circulation was represented by expanded branches of the feeding artery and microvessels in the form of sinuses. Small cyst-like cavities predominated.

The nerve trunk ended with a thickening at the end. The revealed connective tissue had a different organization — from loose to dense. There were areas without blood vessels, formed by clusters of thick bundles of collagen fibers.

Summing up, it should be noted that bone tissue is capable of recovery under certain conditions, i. e. it has certain biological capabilities, which are the basis of its physiological reconstruction. To a large extent, this property is provided by the adequacy of intraosseous circulation. Truncation of the bone sharply disrupts its homeostasis, the restoration of which after amputation at the level of the diaphysis means preservation of the initial shape, structure and physiology of the bone by the stump [9, 10]. Therefore, it is very important that the shape and structure of the bone stump during reparative regeneration are preserved and the closure of the bone marrow cavity is restored as soon as possible. This is necessary for the normalization of intraosseous hemocirculation, since its violation leads to dystrophy of bone tissue.

*Discussion*

Based on the study of histological samples from different experimental series 1, 3, 6 months after amputation, completely different results of bone stump formation were found. In particular, in the 1st experimental series, amputation and pain syndrome due to neuritis of the sciatic nerve caused a violation of the microvascular network of the bone, an increase in extravascular circulation, a sharp suppression of the proliferative activity of cellular elements, activation of resorption, which resulted in spongization and atrophy of the cortical diaphyseal plate, replacement of fatty bone marrow loose connective tissue. Processes of bone tissue resorption prevailed over bone formation. Resorption occurred both along the vascular channels of the cortex and in the endosteal and periosteal areas, which was accompanied by its spongization. In four animals, the spongization zone had a considerable length. As a result of a violation of reparative osteogenesis, bone tissue was replaced by a connective tissue with a branched vascular network. As a result of increased pressure on the stump, fractures of bone trabeculae occurred, in addition, immature trabeculae from coarse-fiber bone tissue were formed.

Violations of reparative process at the end of the stump resulted in incomplete closure of the bone marrow canal. This condition is an obstacle to a powerful blood flow in the intraosseous major vessels. Under its influence, the latter become tortuous and form vascular conglomerates. They, in turn, occupying the lumen of the bone marrow canal, create a mechanical obstacle to restore the closure of the bone marrow cavity, necessary for normalization of intraosseous microcirculation. In the intraosseous microcirculation, sinusoids and tissue cysts, which are not typical for the diaphysis of a normal bone, were preserved even in the long term, which indicated the absence of its normalization [9, 10]. Reparative processes were not complete in any case even for a period of 6 months, which is classified as pathological development of bone tissue.

In the animals of the 2nd series, in the absence of a pain syndrome, as a result of the administration of anesthetic for 20 days, bone regenerate was formed at the end of the stump as early as a month after amputation due to endosteal bone formation. Initially, it was represented by trabecular bone, and in the observation period of 3 months acquired a compact structure. Cortical diaphyseal plate was found to undergo moderate reparative processes, characterized by a decrease in resorption cavities along the vascular channels, on the periosteal and endosteal surfaces and at the end of the cut due to bone formation. These processes did not change the shape of the stump. Fatty bone marrow with layers of loose connective tissue and a small number of sinusoidal vessels was preserved in the distal part of the bone marrow canal. It should be emphasized that large vessels at the end of the stump were not detected due to obliteration.

As for histological structure, the vascular bed approached the condition typical for a bone.

Unsatisfactory results obtained in some cases of the control series, where it was impossible to exclude the presence of a pain syndrome, can be explained by its negative impact on the reparative process, which is consistent with the data of other authors [7, 8, 11].

If we take into account that bone is an organ that provides support, and this is determined by clear constants of its structure, the state of the latter in the formed stump, close to the state of normal bone, should ensure its functional capacity. The stumps obtained in the 1st series and part of the experiments of the 3rd series did not have such properties.

The conducted study showed the need to find new ways of treating the nerve or adequate analgesia at least 20 days after limb amputation, when the intensity of bone tissue remodeling is the highest.

## Conclusions

Provided that the pain syndrome is stopped after amputation, a bone stump is formed with an organotypic shape and structure characteristic of the diaphysis, normalization of the state of bone marrow tissues and blood circulation. The rapid and complete formation of the bone closing plate contributes to the reduction of the branches of the feeding artery and prevents the functional depressurization of the bone marrow cavity. In the presence of post-amputation pain syndrome, there are significant violations of the reparative process with the formation of an inferior bone closing plate with penetration by its branches of the feeding artery and the impossibility of organotypic formation of the stump. The obtained results indicate that standard approaches to analgesia after bone amputation should be reviewed and supplemented.

**Conflict of interest.** The authors declare no conflict of interest.

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The article has been sent to the editors 15.02.2022

# THE INFLUENCE OF POST-AMPUTATION PAIN SYNDROME AND INTRAOSSEOUS MAIN VESSELS ON THE FORMATION OF LIMB BONE STUMP

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