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Research paper

Changes in the structural organization of bone after amputation

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Abstract

Introduction: Despite a considerable amount of work on limb amputations, questions of the mechanism and conditions for the occurrence of pathological rearrangement in the bone stump and ways to prevent it remain unexplored.

Aim: To study the nature of changes in the structural organization of bone stump after amputation.

Material and methods: Nine series of experiments were conducted on 129 rabbits with amputation of the thigh and closure of the filing with fascia, muscles with varying degrees of tension and a bone plate. Duration of observation is 1, 3, and 6 months. The research method is histological with a filling of blood vessels with a mascara-gelatin mixture.

Results and discussion: Tight closure of the bone marrow cavity and uniform muscle tension during plastic surgery in the I–III series of experiments make it possible to obtain cylindrical stumps with the formation of a bone closure plate and the completion of the reparative process. In the majority of observations of the IV–IX series, significant violations of the structural organization of the bone occurred in the form of conical, club-shaped, swollen stumps, creeping fractures.

Conclusions: The rapid completion of the reparative process and the normalization of intraosseous circulation with a locking bone plate, while maintaining normal bone marrow tissue, is possible only with a dense closure of the filing. The lack of tight closure of the bone marrow canal and uneven muscle tension cause a violation of regeneration with changes in the structural organization of the bone. These changes are manifested by a thickening of the bone diameter, axis curvature, creeping fractures.

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1. INTRODUCTION

The bone undergoes a programmed process of physiological reconstruction, which is carried out by parallel processes of resorption and deposition of newly formed bone tissue. With a functional load in the bones, elastic deformations occur, causing a hydrodynamic effect that contributes to normal intraosseous microcirculation. The mismatch of microcirculation with the metabolic needs of bone tissue leads to the activation of physiological rearrangement processes.[1] With changes in the stereotype of the functional load, the processes of physiological reconstruction proceed with the predominance of osteoresorption or osteogenesis until a new stable circular-metabolic correspondence is established that is adequate to the newly established stereotype of the functional load. In this regard, it seemed appropriate to study experimentally the effect of limb amputation at the diaphysis level on the structural state of the remaining stump. In addition to theoretical interest, this could be of applied value, because, despite the long-standing amputations and numerous publications on this problem,[2–11] questions of changes in the structural organization of the bone stump remain unlit.

2. AIM

To study the nature of changes in the structural organization of bone stump after amputation.

3. MATERIAL AND METHODS

Nine series of experiments were performed on 129 rabbits with amputation of the thigh and plastic measures. In the main series, the method of myodesis was applied – attaching the crossed muscles to the end of the filing through the drilled holes and tightly suturing their ends without taking into account muscle tension. In series II, the muscles were fixed in tension of 916–962 mcN (optimal). In series III, the filings of bones were closed with a thin cortical plate tightly fitted to the opening of the opened bone marrow cavity. In series IV, muscles were fixed in tension of 980–1100 mcN, in V – in tension of 1100–1200 mcN, in VI – in tension of 650–800 mcN, fascioplastic closure of filing was performed in VII, muscle plasty was performed with suturing of antagonist muscles under the filing, in the IX series, in addition to muscle grafting, in the postoperative period, one of the muscles was electrically stimulated with a current of 3–12 mA, a pulse duration of 5–10 ms, a modulation frequency of 24–36 imp/min, a pulse frequency of 30–100 Hz, daily 10–15 minutes, within 19–22 days. Duration of observation was 1, 3, 6 months. The research method was histological, with a filling of the vessels with a mascara-gelatin mixture. Before removing from the experiment, 50 000 units of heparin were injected intra-arterially in physiological saline. After 15 minutes, the animal was killed by rapid intravenous administration of thiopental sodium and the abdominal aorta was ligated. Below the ligature, a cannula from the system for intra-arterial injection was introduced, it was fixed in the lumen and vessels were filled with a 10% mascara-gelatin mixture. The limbs were articulated, the muscles were removed. There was a thin layer of tissue surrounding the bone. A longitudinal frontal section of the femoral stump was made. After decalcification in a 15% solution of nitric acid, it was poured into celoidin. Sections with a thickness of 15–20 mcm were stained with hematoxylin and eosin and according to Van Gieson. At the same time, enlightened preparations were made. The thickness of the sections was 90–100 mcm.

4. RESULTS

The first and third series (experimental) had 49 observations. In all cases, after 1 month, along the frontal surface of the end of the stump formed a locking bone plate.The constituent bone tissue in most cases after amputation was mature after 3 months. The bone stump, like the diaphysis of the tubular bone, was cylindrical (Figures 1 and 2) with normalization of intraosseous circulation and completeness of reparative processes. The slight focal resorption of the edges of the sawn diaphyseal plate or the formation of small periosteal regenerates in some cases was associated with underestimation of the tension of the muscles fixed to the edge of the filing and did not affect the main properties

Figure 1. A histotopogram of a cylindrical stump with a cortical diaphyseal plate of uneven thickness (a), its moderate rarefaction. Arrow indicates cortical bone locking plate. Hematoxylin and eosin staining, magnification ×6.

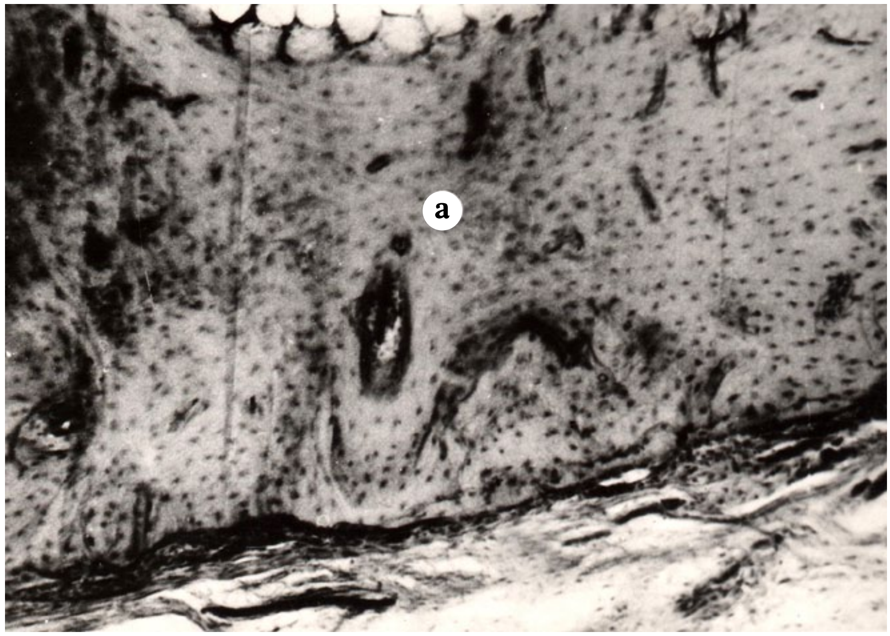

Figure 2. Microphotography: the closing cortical plate from mature compact bone tissue (a). Hematoxylin and eosin staining, magnification ×90.

Figure 3. A histotopogram of the stump of the bone with almost complete resorption of the ends of the cortical diaphyseal plate and the formation of endosteoperioste regenerate (a) on this site. Hematoxylin and eosin staining, magnification ×6.

Figure 4. A histotopogram of a stump of irregular shape with an angular deviation of the end from the vertical axis. periosteal bone regenerate (a), bordering fibrous tissue plate (b), fibro-bone regenerate, closing the bone marrow canal of sawn bone (c). Hematoxylin and eosin staining, magnification ×6.

of the formed stump. These circumstances required special experimental testing to clarify the optimal muscle tension, which was carried out in the II, IV, V, VI series of experiments.

In all observations of series IV with a muscle tension of 980–1100 mcN, VIII – with stitching of antagonist muscles over the filing, in IX, where muscle stimulation was performed, and two observations of series VII with fascioplastic

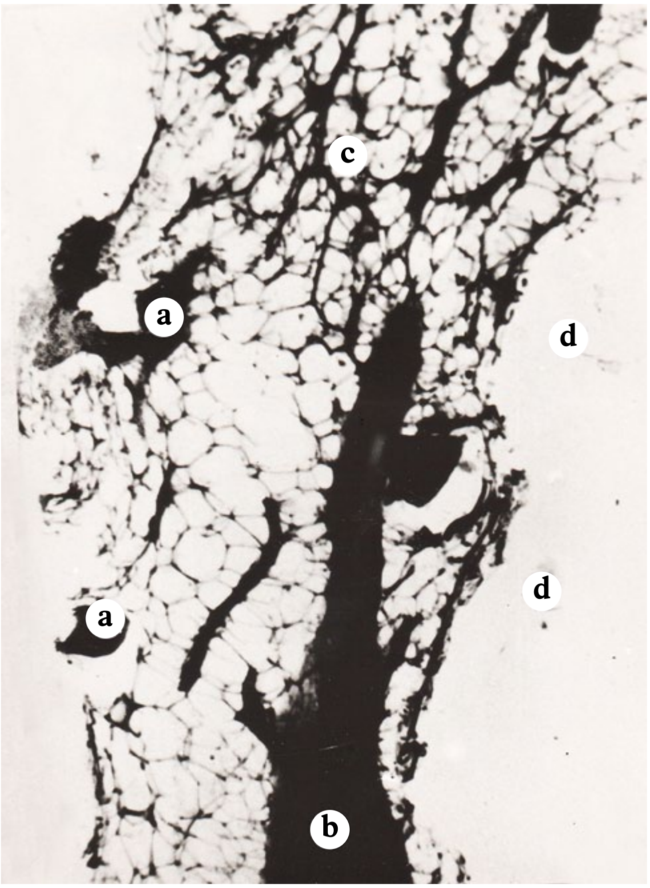

Figure 5. Micrograph. Bone marrow away from filing: ink-filled sinusoids (a), ordinary vessels of the microvasculature (b), a large branch of the feeding artery (c), large tissue cysts (d). Hematoxylin and eosin stain, magnification ×78.

Figure 6. Histotopogram of a stump with a cone-shaped end. Rarefaction, thinning and breaking in separate sections of the cortical diaphyseal plate. Hematoxylin and eosin stain, magnification ×6.

closure of filing, stumps with a sharply expanded diameter due to periosteal and endosteal regenerates merging with them, thickened due to 'bloating' end and angular deviation of the end from the longitudinal axis (Figures 3 and 4).

In a number of observations, a significant part of the lumen of the medullary canal from the edge of the stump was filled with beams of endosteal bone formation of varying degrees of maturity. In some, these beams in some areas were located below the level of the cortical diaphyseal plate due to its partial focal resorption. Significant thinning of the cortical diaphyseal plate was observed over a large length from the end of the stump. In other areas, its spongization was noted. In the resorption zone of the cortical diaphyseal plate there were remnants of the plate and immature bone beams. In the inter-beam spaces, vessels filled with mascara – small branches of the feeding artery – were revealed. The ends of the stumps were bordered by fibrous tissue. In another part of the observations, beams of endosteal bone formation were also located in the area of the resorbed edge of the filing of the cortical diaphyseal plate, forming a rounded end of the stump. In the inter-beam spaces, fibrous tissue was revealed with mascara-filled small branches of the feeding artery, as well as edematous edematous loose fibrous tissue soaked with mascara. The connecting bone plate is not formed. In the proximal part of the medullary canal, there is an edematous adipose marrow with wide areas of carcass-soaked edematous loose fibrous connective tissue and many tissue cysts. Mascara-filled expanded gaps of the feeding artery were revealed (Figure 5). In all observations, the end of the stump of the bone was bordered by fibrous cartilage and fibrous tissue. In four observations of 3–6-month periods in places of uneven thinning of the cortical diaphyseal plate, its breaks were observed.

In the V and part of the observations of the VI and VII series, where either very strong or insufficient muscle tension was carried outwith plastic conical stumps formed. The morphological picture was similar. On the histotopograms, conical deformity of the stump of the bone, osteoporosis, pronounced rarefaction of the cortical diaphyseal plate with its spongization and thickening were revealed (Figure 6). In some observations, its massive resorption and fusion of the bone marrow canal occurred with a violation of the structure of the diaphysis. The walls of the narrowed canal consisted of immature bone tissue. In the cortical diaphyseal plate of the cone-shaped part of the stump, elongated bone beams forming it were revealed. In its proximal sections, along with focal resorption and the presence of immature bone beams in 4 cases, its breaks occurred. The marrowy

Figure 7. Microphotograph. Endostal bone regenerate near the end of the residual limb with multiple lances of the feeding artery filled with ink and branches of the venous sinus. Tinction is made by hematoxylin and eosin, magnification ×78.

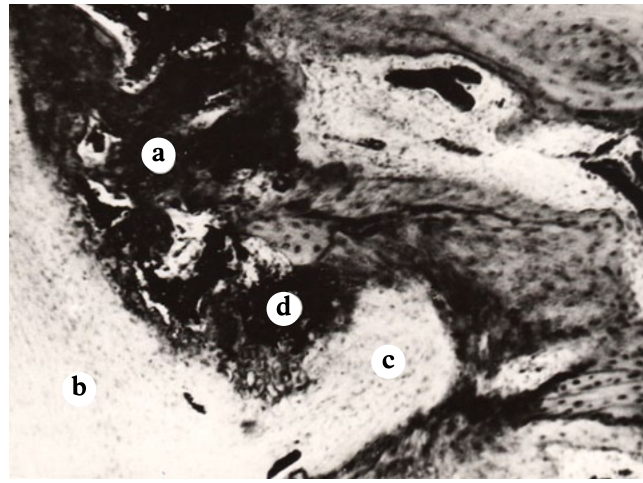

Figure 8. Micrograph of the butt portion of the stump: immature bone structures that form the cortical closure layer (a), with the participation of cartilaginous tissue (b), fibrous cartilage and fibrous tissue bordering the end surface of the stump (c), sinusoidal vessels filled with mascara in the inter-beam spaces (d). Hematoxylin and eosin staining, magnification ×90.

canal at this level was filled with sharply edematous loose fibrous tissue with many sinusoidal vessels, tissue cysts and the presence of lymphoid-plasma clusters. Mascara-filled expanded gaps of the branches of the feeding artery were revealed (Figure 7). Among the structures of endosteal bone formation that close the medullary canal, foci of immature bone tissue were revealed (Figure 8). In the proximal part due to porosity of the vascular wall, the bone marrow contents were stained with mascara.

In a series of observations of the IV–IX series, breaks and complete fractures of the cortical diaphyseal plate were revealed. They arose in the preparations of 3 and 6 month observations and were localized in the proximal section at a distance of 3–5 cm from the end of the stump. The direction of the kink line in most cases was oblique. They arose more often in cone-shaped and club-shaped stumps with impaired reparative regeneration processes. In two cases, breaks were in stumps without disturbance of these processes. The fracture occurred at the site of focal osteoclastic resorption and uneven thickening of the cortical diaphyseal plate. Fusion in some cases occurred mainly due to fibrous and chondroid tissue, and in others due to mixed fibrous-bone tissue from osteogenic and newly formed bone tissue. As a rule, the medullary canal opposite the fracture or fracture was filled with edematous loose fibrous tissue with a large number of sinusoids, tissue cysts, carcass-filled branches of the feeding artery.

5. DISCUSSION

After amputation with a tight closure of the medullary canal and therefore with minor changes in the intraosseous vasculature, at the end of the canal, based on a slightly pronounced endosteal bone formation, a closure bone cortical plate soon formed (by the 1st month). It began from the inner surface of the cortical diaphyseal plate and was located horizontally, creating the base of the stump along with the latter. Sometimes, with a slight resorption of the edges of the cortical diaphyseal plate, some obliquity of the base was revealed. We were convinced that such a quick restoration of the closure of the bone marrow cavity and the completion of reparative processes with the formation of the organotypic structure of the stump is possible only with a certain level of intraosseous circulation. It, in turn, is due to the normal value of intraosseous pressure, which is ensured by plastic closure of the medullary canal during amputation and the creation of a high level of blood supply in the developing bone. The state of the diaphyseal plate changed insignificantly: moderately expressed reparative processes with a slight resorption of bone substance were observed in it, mainly along the vascular channels and also along the periosteal and endosteal surfaces and in the filing region, however, the main contours of the tubular bone and the characteristic structure of the compact bone in its cortical the layer remained unchanged. Post-traumatic restructuring processes, including reparative regeneration at the end of the stump, only to a certain extent changed the state of the tissue of the bone marrow canal. In the proximal stump, the adipose bone marrow was mainly preserved, layers of loose fibrous tissue with tissue cysts and a small number of sinusoidal vessels appeared. The formation of a bone cortical closure plate, which began, as already noted, from the inner surface of the cortical diaphyseal plate at the end of the filing, occurred by replacing a small area of the adipose bone marrow with fibroreticular osteoblastic tissue. Initially, beams of endosteal bone formation appeared on this site; later, a plate of compact bone was formed. There were almost no bone formation processes along the endostal surface in the proximal stump, as well as the appearance of periosteal regenerates with this type of healing. In those cases when the muscle tension was slightly higher and microcirculation was slightly disturbed, this caused a somewhat more intense resorption of the bone substance of the cortical diaphyseal plate. Not densely spaced bone beams were revealed in the proximal part of the medullary canal; insignificant periostally formed bone regenerates were determined closer to the end of the stump. In them reparative processes that were perfect were completed rather early, the structure of the bone stump was stable, the stump was approaching organotypic.

The state of the vessels in such a cult, determined on total preparations with mascara, by the end of the first month approximately corresponded to the intraosseous hemocirculation of the normal diaphysis; at the end of the stump, the vessels were defined as small branches among the adipose bone marrow. Larger branches were not detected here, obviously, in connection with their obliteration.

In stumps, the shape of which differed from that of the normal diaphysis, the closure of the medullary cavity occurred by overgrowing the medullary canal over a large extent with regenerated immature bone or fibro-bone tissue against the background of disturbed microcirculation, which hindered the completion of reparative processes even in the long term.

The variability of bone stump forms (thickening of the diameter due to pronounced periosteal bone formation, curvature of the axis with asymmetry, narrowing of its end section with the acquisition of a conical shape or flask-like expansion, shortening of the formed stump due to significant resorption of bone material at the end of the filing) is due to the absence of a closed bone marrow channel and uneven muscle tension. In such stumps, normalization of the intraosseous circulation did not occur even in the long term, and the completion of reparative processes was not observed, which led to a pathological rearrangement of bone tissue. On mascara-dyed preparations, many deviations were revealed both in the microcirculation system and in the condition of the intraosseous main vessels. Due to the imperfection of the overlapping of the medullary canal, the main vessels did not reduce, their network expanded, occupying a significant part of its lumen and going into the connective tissue bordering the end surface of the stump, and therefore there was no possibility of forming a perfect closure bone plate. In the intraosseous microcirculatory network, sinusoids unusual for the diaphysis of the normal bone and juxtacapillary circulation paths appeared in the form of tissue cysts. In these cases, resorption of the cortical diaphyseal plate passed along its inner surface, in response to this, bone formation processes proceeded in the medullary canal. Resorption on the periosteal surface of the cortical diaphyseal plate and the formation of periosteal regenerates were due to improper tension on the stitched muscles of the stump. In this case, the uneven tension and the formation of periosteal regenerates associated with it were also accompanied by significant disturbances in the intraosseous circulation, which led to massive resorption of the cortical diaphyseal plate and a sharp deformation of the bone stump. In all cases of stump formation without exception, the bone formation activity of the endosteum was fundamental in reparative regeneration; the perioste participated in bone formation only under strictly defined conditions associated with increased muscle tone. It should also be noted that in none of the observations the endost was the source of the formation of periostally located bone and bone-cartilage regenerates.

The formation of the cone-shaped end of an irrational bone stump was not the result of the growth of the endoste from the bone marrow canal outside the bone experiment; it occurred due to significant resorption of the edges of the cortical diaphyseal plate and bone formation in response to this when the absorbed bone is defectively replaced.

In creeping fractures in the medullary canal at the fracture level, loose fibrous connective tissue with many large tissue cysts filled with mascara, thin-walled vessels of large diameter, as well as dilated branches of the feeding artery was always revealed. In the resorption zone in the loose fibrous connective tissue, diffusely located macrophages, lymphoid and plasma cells were found. A large number of ink-filled sinusoids were detected. These changes with a slowdown in blood flow, apparently, led to local tissue disorders in the form of hypoxia and local ischemia with the capillaries of compact bone tissue turned off, which caused first resorption and then the appearance of a creeping fracture.

6. CONCLUSIONS

(1) The rapid completion of the reparative process and the normalization of the intraosseous circulation with the formation by the end of the 1st month after amputation of the closure bone plate covering the medullary canal while maintaining the usual structure and normal bone marrow tissue in it is possible only with tight closure of filing.
(2) In a cult with significant changes in the structural organization of the bone, the shape always differs from the shape of the normal diaphysis, the closure of the bone marrow occurs by regeneration of immature bone or fibro-bone tissue against the background of impaired microcirculation, which inhibits the completion of reparative processes.
(3) Changes in the structural organization of the bone stump are manifested by a thickening of their diameter due to periosteal bone formation, axis curvature, conical shape, creeping fractures.
(4) Along with the closure density of the medullary canal, the main pathogenetic mechanism of disturbances in the structural organization of the bone stump is the state of tension of truncated muscles during plastic surgery.

Conflict of interest

None declared.

Funding

None declared.

Ethics

All studies were carried out in compliance with international bioethical rules in accordance with the requirements of the Helsinki Declaration and the rules of humane treatment of laboratory animals. Ethics committee approval for this study was obtained (approval No 5/2020).
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