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BONE VASCULARIZATION AND BONE HEALING IN THE AMPUTATION STUMP

An Experimental Study

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The osseous healing process of the amputation stump was investigated in adult rabbits. Histological investigation showed that the medullary cavity was closed after 2–3 weeks, chiefly by endosteal callus. After closure of the cavity there was a gradual spongious change in the bone tip and simultaneously the cortex atrophied and the medullary cavity dilated. After amputation on the crus bone rebuilding dominated, whereas after amputation on the femur deterioration of bone was most noticeable. A combination of amputation and medullary plugging caused a change in the course of healing. The medullary cavity did not close until 7–10 weeks after operation and there was distinct periosteal callus formation.

The microangiographic investigation showed a transient hypervascularization in the cortex 3–4 weeks after amputation; whereas after simultaneous plugging of the medullary cavity the hypervascularization continued for up to 7 weeks after operation. Following amputation proximally on the crus the arterial supply of the cortex came mainly from the periost, whereas the cortex after distal amputation was vascularized from the medullary cavity. This finding can be due to an interruption of the arterial supply from the nutrient artery associated with proximal amputation, whereas this artery remains intact with amputation distally on the crus.

Key words: amputation; bone healing; bone vascularization; plugging of the medullary cavity

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Previous experimental studies of amputation (Hulth & Olerud 1962, Hansen-Leth & Reimann 1972) have shown hypervascularization in the bone in the amputation stump. Hulth & Olerud (1962) proved that the osseous healing process of the stump was the same as in an experimental fracture. Hypervascularization in fractured bone results from the interruption of bone vascularization (Rhinelander 1968). The changes in bone vascularization after interruption of the arterial supply have been investigated by Trueta (1968) and Brookes (1971), who found that vascularization of the diaphyseal cortex changed after interruption of the nutrient artery.

The aim of the present experimental study was to investigate whether the technique used for closure of the medullary cavity influences healing in the amputation stump, and to elucidate the relation between bone healing and bone vascularization especially as

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regards the effect of interruption of the nutrient artery.

MATERIAL AND METHODS

Seventy adult rabbits were used in the investigation, and apportioned according to the amputation technique performed, as shown in Table 1. The operations were performed under Nembutal anaesthesia. With proximal crus amputation the bone was sawn through at the tibio-fibular synostosis; in cases without myoplasty the muscles were cut at the same level, and in cases with myoplasty they were cut approximately 1 cm further distally. With distal crus amputation the bone was sawn through at a level distal to the course of the nutrient artery. Femur amputation was performed at the midbone level and the stump was closed with myoplasty. With knee exarticulation the gastrocnemius muscles were removed and the quadriceps sutured to the crucial ligaments. Medullary plugging was performed with cortex from the amputated bone. After amputation on the crus the animals as a rule put on weight in the amputation stump, which in eight cases resulted in minor defects on the stump tip. After amputation on the femur the stump was immobilized as a result of a flexion contracture in the hip.

The rabbits were sacrificed at intervals from 1 hour to 130 days after operation and an angiographic investigation was performed. Under Nembutal anaesthesia the peritoneum was opened and saline was perfused at 1 m pressure through a catheter into the aorta abdominalis, as simultaneously the animal, heperinized beforehand, was bled through the vena cava. Perfusion was continued with 25 per cent micropaque for approximately 30 min followed by micropaque with 10 per cent formalin and 1 per cent Berliner blue for 10 min. Finally, after removing the skin the hind part of the body was fixed in 10 per cent formalin.

For evaluation of bone healing in the stump, 1 cm of the tip was removed, fixed in 10 per cent formalin phosphate, and without previous decalcification embedded in methyl methacrylate and cut lengthwise, and sections 7 μ thick were stained according to Goldner. The vascularization of the bone was evaluated by means of microangiography of cross sections, sawn off about 1 cm proximal to the stump tip, decalcified, embedded in methyl methacrylate, ground down to a thickness of 1 mm and photographed on a spectroscope plate. As control specimens, cross sections from the contralateral tibia in 10 of the amputated animals and from the tibia of three unoperated rabbits were used.

RESULTS

Histological investigation of the bone in the amputation stump showed increased osteoblastic and osteoclastic activity corresponding to the outermost part of the endosteum 3–4 days after amputation, with formation of osteoid tissue and beak-formed spongious bone on the inside of the medullary aperture (Figure 1). Periosteally only a slight increase in osteoblastic activity was observed, located proximal to the stump tip with formation of periosteal exostoses.

After *proximal amputation on the crus with myoplasty* the stump was closed with a thin spongious layer from 18 days after surgery (Figure 2). The same was observed after *proximal amputation without myoplasty*, while after *amputation distally on the crus* the medullary cavity was seen to be

Table 1. The material apportioned according to amputation level and the technique used for closure of the medullary cavity

	cases	− myoplasty	+ myoplasty	+ medullary plugging
Amp. prox. cruris	35	11	13	11
Amp. distalis cruris	15		15	
Amputatio femoris	14		10	4
Exarticulatio genu	3			
− operation	3			
	70	11	38	15

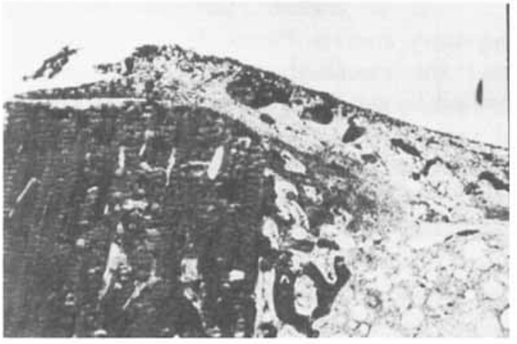

Figure 1. Six days after proximal amputation on the crus with myoplasty. The tip of the amputation stump. Cortex is seen on the left and beak-formed spongious bone on the inside of the medullary aperture.

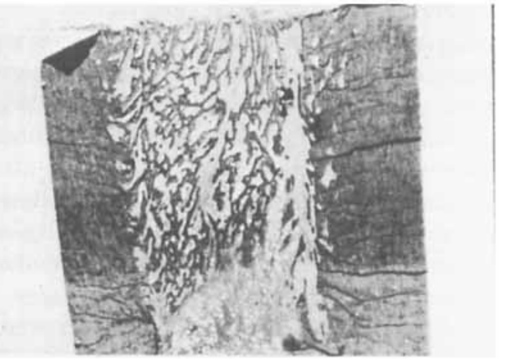

Figure 3. Twelve days after amputation distally on the crus. Spongious closure of the medullary cavity.

Figure 2. Twenty-four days after proximal amputation on the crus with myoplasty. The medullary cavity is closed with a thin spongious layer.

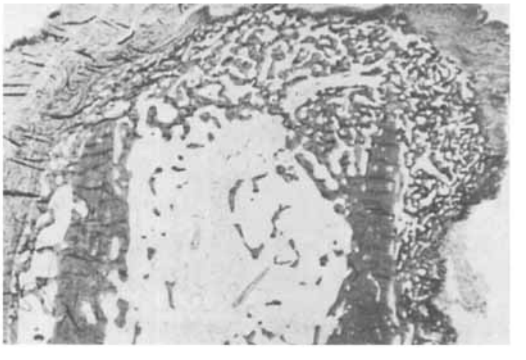

Figure 4. Forty-eight days after amputation distally on the crus. Increased spongiosity of the whole stump tip.

closed already on the 12th day. Following closure of the medullary cavity (Figure 3) increased spongiosity of the whole stump tip was observed and furthermore the cortex was atrophied and the medullary cavity dilated (Figure 4). After *amputation on the femur* the medullary cavity was closed 9 days after operation with a thin, spongious layer covered with cartilage and fibrous tissue. No further bone formation was seen and about 5 weeks later an increasing atrophy of bone with dilation of the medullary cavity was observed (Figure 5).

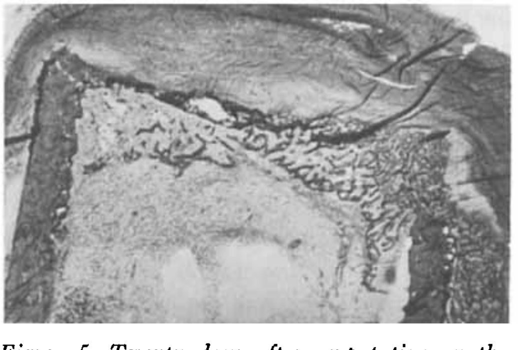

Figure 5. Twenty days after amputation on the femur. A thin spongious layer closes the medullary cavity; the cortex is atrophied and the medullary cavity dilated.

After *proximal crus amputation with plugging of the medullary cavity* there was increased periosteal reaction, as compared with amputation without plugging, and large periosteal, spongious exostoses with cartilage islands gradually developed. Forty days after amputation the stump was still not closed (Figure 6), after 50 days there was incipient closure, and after 70 days the medullary cavity was finally closed. At this stage a spongious change had occurred in the whole stump tip. The inserted plugs were mostly avital the first 3 weeks after operation. After this time an increased osteoblastic activity was seen around these plugs.

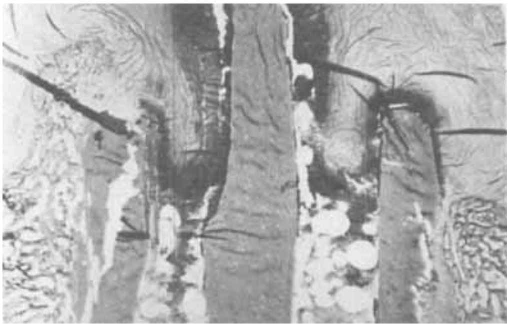

Figure 6. Forty days after amputation on the crus with plugging of the medullary cavity. Abundant periosteal spongious tissue is seen, but the medullary cavity is not closed.

Microangiographical investigation of the unoperated tibia showed only a few medullary arteries with ramifications into the central half of the cortex and a few vessels running radially from the periosteum into the superficial fifth of the cortex, and the medullary sinusoids dominated the cross section angiogram.

After amputation a hypervascularization was seen in the amputation stump, in the cortex as well as in the periosteum and the medullary cavity. In the cortex the vascularization depended on the amputation level (Table 2). After *proximal amputation on the crus* the microangiograms showed an initial reduction in cortical vascularization from medullary arteries (Figure 7). After about 10 days the vascularization in the cortex increased, mainly as a result of hyperplasticity of the periosteum (Figure 8). After 30 days vascularization in the cortex again normalized, and vascularization in the periosteum decreased, but in the medulla a hypervascularization was seen during the investigation period. *Distal amputation on the crus* caused immediate hypervascularization in the cortex and up to 40 days postoperatively there was an increased number of cortical arteries, mainly from the medulla (Figure 9). After that period vascularization normalized, in the cortex as well as in the periosteum and medulla. The vascular reaction in the bone was identical after stump closure with and

Figure 7. Microangiogram of the amputation stump. One hour after proximal amputation on the crus. The medullary sinusoids are seen below.

Table 2. Microangiographical investigation of cortical vascularization

Time	Amputation level			
	Amp. distally on crus		Amp. proximally on crus	
	PERIOSTEUM	MEDULLARY CAVITY	PERIOSTEUM	MEDULLARY CAVITY
unoperated tibia	XX	XXXX	XX	XXXX
1 hour	XXXX	XXXX	X	XX
½ week	XXX	XXXXXXX	XX	XX
—	XXXX	XXXXXX	X	XXX
1 —	XX	XXXXXXX	XX	XXX
—	XXX	XXXXXX	XXX	XXXXX
2 weeks	XXX	XXXXXXXX	XXXXX	XXX
—	XXX	XXXXXXX	XXXXXXX	XXXX
—	XXX	XXXXXXXXX	XXXXX	XXXXX
3 —	XXX	XXXXXXX	XXXX	XXXX
—	XXX	XXXXXXX	XXXX	XXXXXX
4 —	XXXXX	XXXXXXX	XXXXXXXXX	XXX
6 —	XXX	XXXXXX	XX	XXX
—			XXX	XXXXX
7 —	XX	XX		
9 —	XX	XX	XX	XX
13 —	XX	X	XXXX	XX

The cortical vascularization seen in cross sections of the tibial diaphysis of the amputation stump after amputation distally and proximally on the crus.

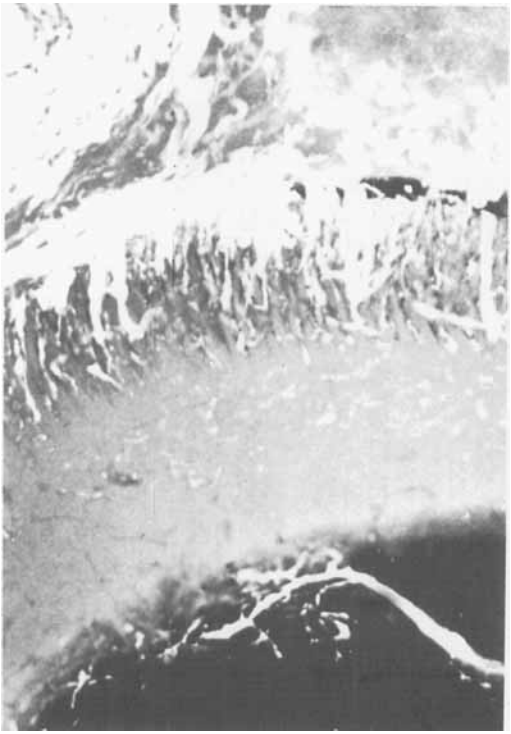

Figure 8. Microangiogram of the amputation stump. Fifteen days after proximal amputation on the crus. The hyperplastic, richly vascularized periosteum is seen above.

Figure 9. Microangiogram of the amputation stump. Three days after amputation distally on the crus. The medullary cavity is seen on the right.

without myoplasty, and after *amputation combined with plugging of the medullary cavity* there was a moderately increased vascularization from the periosteal arteries in the cortex for up to 50 days after amputation. Bone vascularization did not change after *knee disarticulation,* whereas after *femur amputation* there was a short period of slight hypervascularization of the cortex coming from the periosteum.

DISCUSSION

Several previous studies have dealt with the normal circulation in the rabbit tibia. The diaphysis of the tibia is supplied from the nutrient artery, the metaphyseal and the periosteal arteries, but there is disagreement amongst the different investigators as to the normal arterial supply of the tibial cortex. Morgan (1959), Göthman (1960), Rhinelander (1968) and Trueta (1968) found periosteal branches in the outer third of the cortex, whereas Brookes (1971) was not able to demonstrate branches from the periosteal arteries to the cortex, and he considered the subperiosteal vessels to be solely efferent veins. If one or more of the arterial systems to the tibia is obliterated the remaining vessels will, to a varying degree, take over their function (Danckwardt-Lillieström 1969). Trueta (1968) found no changes in the tibial vascularization after ligature of the nutrient artery, while Brookes (1971) proved that ligature of the artery caused an ischaemia of the diaphysis and hypervascularization in the cortex through the periosteal arteries.

In the present investigation micro-angiography of the non-operated tibia has shown that the central half of the cortex is supplied by medullary arteries, while a few radially running vessels, possibly ramifications from periosteal arteries, were seen in the superficial fifth of the cortex. After amputation on the crus vascularization of the tibial cortex depended on the level of amputation. Proximal amputation produced delayed hypervascularization in the cortex, mainly from the periosteum, whilst distal amputation caused immediate hypervascularization in the cortex through the medullary arteries. This difference may be due to the relation between the amputation level and the course of the nutrient artery (Figure 10). With proximal amputation the bone is deprived of the arterial supply from the nutrient artery, whereas this artery remains intact with distal amputation. In accordance with this, the histological investigation showed that the medullary cavity closed later after proximal amputation on the crus than after distal crus amputation and amputation on the femur where the bone has not been deprived of blood supply from the nutrient artery.

In the present investigation periosteal hypervascularization was followed by a sparse periosteal callus development, seen as exostoses slightly proximal to the stump tip; generally the periosteal callus was not involved in the closure of the medullary cavity. This observation is not in agreement with Hulth & Olerud (1962) who found that periosteal callus developed parallel with and adjacent to the periosteal vessels, while endosteal sealing callus was initiated by the appearance of fan-shaped proliferation of new

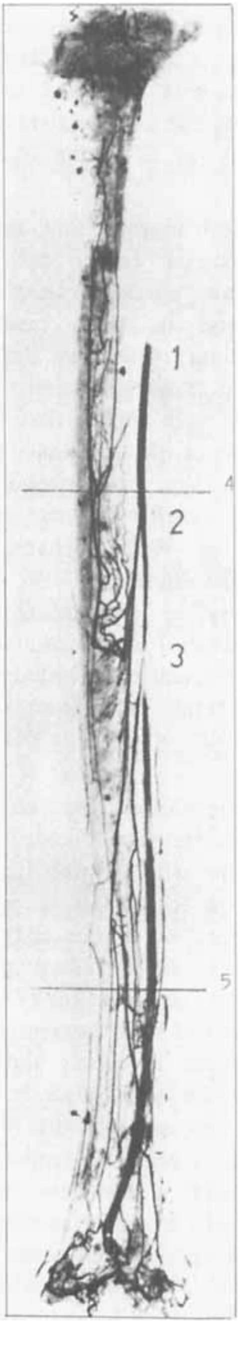

Figure 10. Microangiogram of the whole tibia of a rabbit (L. Göthman: The normal arterial pattern of the rabbit's tibia. Acta chir. scand. **120,** 201–210, 1960).
The nutrient artery (1) passes at the level 2 through the lateral cortex. In the medullary cavity it divides into ascending trunks which form a U-shaped loop, and a descending trunk. The accessory nutrient artery (3) passes distally to the lateral malleolus.
4: proximal amputation level.
5: distal amputation level.

vessels emerging from the marrow cavity. A similar fan-shaped vascular proliferation was observed after crus amputation on young rabbits in the investigation by Hansen-Leth & Reimann (1972) who also showed an extensive development of periosteal exostoses on the amputation stump. After amputation on adult rabbits, as used in the present investigation, the periosteal reaction was not so extensive as in young animals (Trueta 1968).

Histological investigation showed formation of osteoid tissue and beak-formed spongious bone on the inside of the medullary aperture, and in some cases islands of spongious bone which had developed in the peripheral part of the cavity. Previous investigations have shown that the medullary cavity reacts to intramedullary devascularization by formation of osteogenetic granulation tissue from which trabecular bone is formed (Foster et al. 1951, Richany et al. 1965, Danckwardt-Lillieström 1969). After proximal crus amputation and interruption of the nutrient artery, intramedullary vascularization is transitorily reduced and bone formation from osteogenic tissue in the peripheral part of the medullary cavity is a possibility.

After amputation the closure of the medullary cavity was followed by a gradual change in the whole stump tip; on the crus there was an increasing spongiosity of the stump, whereas on the femur bone resorption and dilation of the medullary cavity occurred. Trueta (1968) and Brookes (1971) have previously observed bone resorption and dilation of the venous sinusoids through lack of muscle function, and Geiser & Trueta (1958) found that tenotomy of the Achilles tendon on rabbits caused osteoporosis of the calcaneus and a transient hypervascularization followed by hypovascularization. They were of the opinion that these changes were due partly to abolished muscle pulling force and partly to loss of pressure strength caused by the lost weight. Brookes (1971) found likewise that insufficient muscle function resulted in increased vascularization of bone. The present investigation has shown hypervascularization in the cortex after the closure of the medullary cavity. This hypervascularization as well as the gradual changes in the bone tip and the dilation of the medullary cavity can be due to muscle inactivity. In the crus, the bone re-building dominated, but after amputation on the femur, where muscle inactivity was combined with loss of pressure strength, bone resorption was the chief feature.

The present investigation has shown that plugging of the medullary cavity changes the course of healing in the amputation stump. The medullary cavity was normally closed after 2–3 weeks; with plugging, however, the cavity was still open 5–7 weeks after amputation. At the same time, there was a strong periosteal reaction with formation of large periosteal exostoses. The inserted plugs were mostly avital in the first weeks and did not contribute to the formation of callus inside the medullary cavity. Previous investigations have shown extensive periosteal vascularization and development of subperiosteal bone after intramedullary intervention, and several explanations have been suggested for this subperiosteal bone formation. Richany et al. (1965) found that local stasis and oedema led to anoxia and prompted formation of periosteal bone, while Trueta & Cavadias (1955) were of the opinion that ischaemia caused by intramedullary procedures provoked proliferation of periosteal vessels with accompanying new bone formation, at the same time no bone formation occurred from osteogenetic tissue in the medullary cavity. This fact can explain the delayed closure of the medullary cavity after amputation combined with osseous plugging.

In a previous experimental study (Hansen-Leth & Reimann 1972) it was found that hypervascularization in the amputation stump occurred in the soft tissues as well as in the bone. In experimental investigations Zuchman (1960) and Whiteside & Lesker (1978) have shown a rich anastomotic vascular network between muscle and periosteum. Periosteum may serve as an important source of collateral circulation to the muscles. After amputation the technique used for closure of the medullary cavity influences the vascularization in the bone and furthermore the muscular vascularization in the amputation stump. Periosteal hypervascularization was seen after amputation proximally on the crus and an extensive periosteal reaction was seen after amputation combined with plugging of the medullary cavity. These findings can explain the increased muscular blood flow in the amputation stump after proximal crus amputation combined with plugging of the medullary cavity (Hansen-Leth 1976). This effect of medullary plugging may be of interest for amputation surgery in man.

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