STUDIES ON AMPUTATION STUMPS IN RABBITS

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Part of a large scale investigation of amputees initiated at the Orthopaedic Clinics of Uppsala University involved corollary considerations of stump healing in experimental animals. Since the amputation of an extremity is attended with a considerable alteration of the circulation, the problem was investigated first from a circulatory standpoint. In amputees with stump disturbances Leriche (1950) has described the presence of abnormal angiographic patterns, characterised by either hypervascularity of the soft tissues of the stump or tortuosity of the vessels. Abeatici and Ferrero (1953) and de Sapia and Assenso (1954) have performed angiographic studies on amputation stumps of dogs, and both have found hypervascularity of the stump ends. Another problem not previously investigated is the transformation of the bony stump and the manner of callus formation. In addition some of the mechanisms of spur origin as well as pathological hyperostoses were investigated.

MATERIAL AND METHODS

Twelve rabbits weighing between 1½ and 3 kilograms were submitted to amputation of a limb under nembutal anaesthesia. The amputation was performed at a site between the upper and middle portions of the tibia. In all cases the soft tissues were sutured with catgut around the bony stump and the vessels were tied with plain catgut. All the rabbits withstood the operation quite well and after it moved about freely in their cages. The rabbits did not stand on the stumps either at rest or when walking.

Angiography—At various intervals up to twelve weeks after amputation angiography was performed by a method previously described (Hulth and Olerud 1960). Through a cannula inserted by way of the carotid artery, 8 millilitres of 76 per cent Urografin (Schering) was injected, after which a single radiograph of the trunk and legs was taken.

Microangiography and microradiography—Immediately after angiography laparotomy was performed and the abdominal aorta was exposed and ligated. For microangiographic purposes approximately 20 per cent Micropaque solution was injected distally by an ordinary infusion apparatus with a pressure of just under 100 centimetres of water. The technique used here was basically the same as that described by Trueta and Harrison (1953). The animals usually died some minutes after the beginning of the infusion but the time of infusion was always extended to sixty minutes. After ligation of the aorta and inferior vena cava the rabbit was cut in two at mid-trunk level, the distal parts being placed in 10 per cent formaldehyde for twenty-four hours. After deep freezing (—20 degrees Centigrade at least for twenty-four hours) suitable portions were excised for further formaldehyde fixation. This material was then dehydrated and embedded in methylmethacrylate. A band saw was used to cut slices one millimetre thick. These pieces were ground to a thickness of about 200 microns and subjected to microradiography (Engström 1957). It was possible therefore to study in all samples both the mineralisation of the callus and the microangiographic patterns.

RESULTS

Arteriography in vivo with Urografin—Figures 1 and 2 show the arteriographic patterns in the lower limbs respectively two and twelve weeks after amputation. The femoral artery of the stump did not appear narrower even though it had considerably less tissue to nourish. Even after the stump had healed and the growth of the callus was complete, the artery still appeared
as wide as that of the opposite limb. In most instances the distal part of the femoral artery became tortuous. Arteriographs performed during the first three weeks after amputation (Fig. 1) showed hypervascularisation of the stump, indicative of the healing process. In

![Fig. 1](image1)

**Fig. 1**
Arteriograph two weeks after amputation showing hypervascularity of the skin and tortuosity of the branches of the femoral artery.

![Fig. 2](image2)

**Fig. 2**
Arteriograph twelve weeks after amputation. Broad vessels can be seen close to the skin where a small ulcer has formed. Signs of rapid venous return are present (arrow).

stumps several weeks old this excessive vascularity seemed to disappear, but the width of the artery was not diminished. In a few cases infection or ulceration of the stump occurred, and was followed by a widening of all vessels combined with a hypervascularity of the entire
Figure 3—Micropaque-filled stump three weeks after amputation. The marked hypervascularity and the tortuous vessels are evident. A spur can be seen on the bone stump. Figure 4—Microradiograph of the stump one week after amputation. The Micropaque-filled small vessels emerge in a fan-shaped pattern. Early callus is also present—bottom left.

Figure 5—Microradiograph of Micropaque-filled bone stump three weeks after amputation. The sealing callus has already closed the marrow canal. Figure 6—Microradiograph showing the bone structure and the vascular pattern in the periosteal callus of the stump.
stump region. In three of these stumps we noted quicker venous filling compared to the sound leg, evidence of a hyperkinetic circulation.

**Arteriography after death with Micropaque**—The radiographic appearance of a stump is shown in Figure 3. The minor vessels are clearly visible. One week after amputation pronounced hypervascularity of the stump occurred, especially in the skin area. Two to three weeks after amputation the stumps showed less cutaneous vascularity. Instead, additional vessels made their appearance in deeper parts of the stump. These were either dilated vessels or newly formed vessels within old haematomata and callus.

**Microradiograph and microangiograph**—The first change is an extension of the marrow vessels beyond the free edge of the cut bone. One week after amputation the marrow vessels have a fan-shaped appearance (Fig. 4). Later, new bone is formed along the minor end branches of the still fan-shaped marrow vessels (Fig. 5). It is noteworthy that the newly formed bone trabeculae have the same orientation as the vessel branches. This is the first appearance of the sealing callus. Two weeks after the operation the periosteal and endosteal callus also has started to appear. Callus has developed from the elevated periosteum, and the primary trabeculae show an orientation parallel to the vessel branches. The course of the vessels in the vicinity of the free stump end is vertical to the bone but more proximally the vessels have an oblique course similar to the trabeculae of the callus. The angle formed by the cortical bone and the new trabeculae decreased toward the proximal portion of the stump. The specimen examined four weeks after amputation (Fig. 6) seems to have a complete sealing callus. In this rabbit there is also a bone spur extending vertically from the tibia (Fig. 7). This spur has quite an irregular mesh-like formation as do its accompanying vessels. The old bone of the tibia undergoes resorption, the Haversian canals being widened and the vessel within the cortical bone structure becoming more prominent.
DISCUSSION

Amputation of the left hind leg below the knee in rabbits has been performed and the healing process followed by angiographs and by microradiographs in combination with microangiographs. Arteriography in the living animal has shown no narrowing of the femoral artery in any of the cases up to three months after amputation. The early hypervascularity around the stump disappears gradually but the width of the arteries remains unchanged. If infection or ulceration of the stump is present the hypervascularity persists and the diameter of all vessels in the limb increases. Venous filling occurs earlier than in the sound side, probably because of a shunt effect.

Another characteristic finding of these stump arteriographs visible both in the living and in the dead animals is a corkscrew formation of the minor arterial vessels. The reason for this phenomenon is not readily explained. Similar patterns may be seen in those collateral vessels which appear following occlusion of an artery (Allen, Barker and Hines 1946). Even proximal to an arteriovenous fistula such winding arteries may be visible. It is probable that these vessel patterns are indicative of pathological circulation. The diminished tissue mass of these stumps may sometimes cause an inadequate circulatory adaptation. Further research is necessary to determine the relationship between the deficient circulatory adaptation and disturbances of the bone of the stump.

Our microradiographic results indicate that bone healing of the stump proceeds in the same way as the healing of experimental fractures with two fragments (Nilsonne 1959). Periosteal callus develops parallel with and adjacent to the periosteal vessels. These vessels form a progressively increased angulation as they approach the distal stump (Fig. 6). Endosteal callus develops as well. The periosteal callus growth originates some distance from the free distal end of the stump. There is a tendency to the formation of exostoses with irregular trabeculae. This bone growth may possibly develop in the ingrowing connective tissue (Fig. 7). In limbs amputated in the described manner the formation of the sealing callus is initiated by the appearance of a fan-shaped proliferation of new vessels emerging from the marrow canal. The new bone is deposited around these end vessels (Fig. 4).

REFERENCES


