

INTRACARDIAL ARTERIOGRAPHIC STUDY ON VASCULAR CHANGES IN AMPUTATED RABBITS

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The vascular changes in rabbits after amputation were studied *in vivo* with arteriography, after intracardial injection of contrast. In the amputated extremity an initial vasoconstriction of the arteries was found. Thereafter changes were observed in arteries and veins, both in the operated and contralateral extremities, depending on the level of amputation and the manner in which the stump was closed. After amputation distally on the extremity and after stump closure without myoplasty arteriovenous shunting was observed in the amputation stump. Arteriovenous shunts were not seen after knee disarticulation and after proximal amputation on crus with myoplasty. Inactivity of the muscles in the amputation stump is supposed to be the cause of the shunt formation. After mid-femur amputation a permanent vasoconstriction of the femoral artery was observed, whereas artery dilation was seen when amputation on the femur was combined with plugging of the medullary cavity.

Key words: amputation level; arteriovenous shunt; closure of the medullary cavity; intracardial arteriography; vascular changes in amputated rabbits

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Previous studies of vascular changes which take place after amputation have been based chiefly on arteriography. Increased vascularization in the amputation stump has been observed in both clinical and experimental investigations, but no unequivocal changes in the diameter of the related arteries have been demonstrated. Hulth & Olerud (1962) and Langhagel (1968) reported unchanged arterial diameters. Erikson & Olerud (1966) and Hansen-Leth & Reimann (1972) observed transient dilation, whilst Hasse et al. (1960) and Erikson (1965) found reduction in diameter. The reason for these changes in vessel diameter is not clearly understood; Hasse et al. suggested that reduced arteries resulted from decreased blood circulation in the amputation stump, whereas Erikson found no relationship between degree

of dilation and magnitude of blood flow. After amputation on rabbits, Hansen-Leth (1976) observed an increased muscle blood flow in the stump, which was dependent on the level of amputation and on the manner in which the marrow cavity was closed.

In this experimental study *in vivo* arteriography on rabbits has been used to determine the vascular changes after amputation and to explain the way in which the amputation level and the method of stump closure influence the vascularization in the amputation stump.

MATERIAL AND METHODS

Thirty-two adult rabbits, weighing 3-4 kg, had their left hind leg amputated under Nembutal anaesthesia. In 21 animals amputation was

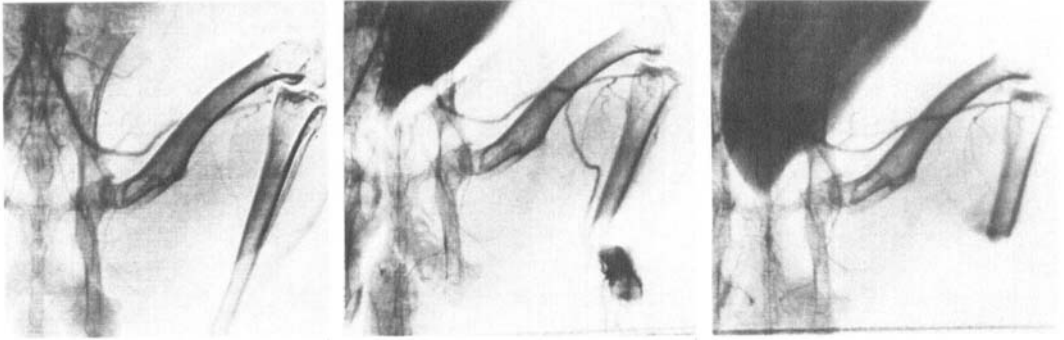


Figure 1. Arteriograms (a) Preoperatively; (b) immediately after fracture on the crus; and (c) immediately after subsequent amputation.

performed on the crus, in 5 cases distally and in 16 proximally, at the level of the tibiofibular synostosis. In the proximally amputated animals the marrow cavity was left open in four and closed with myoplasty in seven and in a further five myoplasty was combined with osseous plugging of the marrow cavity. Disarticulation of the knee joint was carried out in six animals – three of these had been subjected to amputation of the crus prior to the disarticulation. Femur amputation was performed on eight animals, three with distal amputation without myoplasty – two of these had been subjected to amputation of the crus prior to amputation on the femur – and five with mid-thigh amputation, two with myoplastic closure and three with myoplasty combined with osseous plugging of the marrow cavity. In two animals the crura were fractured prior to amputation and the immediate arteriographic changes after fracture were investigated. Two animals died, unoperated, in relation to arteriography.

Arteriography was carried out while the animals were under Nembutal anaesthesia. The animals were placed supine on the AOT – film changer. Seventy-six per cent Urographin was injected intracardially with a lumbar needle (90×0.9 mm) inserted through the skin at the tip of the xiphoid process and thrust into the heart. The needle was kept open with heparin-saline solution and a bolus of 8 cm^3 contrast was injected manually during a period of 1–1.5 seconds. Six pictures were taken with one exposure every 2 seconds. These clearly portrayed the arterial and venous circulations, and permitted estimations of the flow rate (transit-time). The degree of change in arterial diameter was measured on subtraction films at corresponding levels by the use of a magnifying glass and a scale permitting readings with a precision of 0.1 mm. The magnification factor was so small that it could be ignored (measured with a lead striped ruler placed across the pelvic area of the rabbit).

In all, 70 arteriograms were taken, seven preoperative and 63 within periods of from 1 hour to 130 days, postoperatively. One animal was arteriographed 8 times, one 6 times, two 4 times, five 3 times, ten twice and thirteen once.

RESULTS

On the preoperative arteriograms only the larger arteries were contrast-filled, the crural arteries were thread-like, none or only a few veins were seen and vein filling was late in appearance.

Arteriographs immediately after amputation disclosed a vasospasm in the amputation stump in contrast to the vasodilation immediately after a closed fracture (Figure 1). Two or four days after amputation on the crus the arteries to the stump were seen to be dilated, dependent on the closure of the stump and the amputation level.

Table 1 illustrates the contrast passage through arteries and veins (transit-time). Early appearance of veins were seen $\frac{1}{2}$ –1 week after *distal amputation with myoplasty* as well as after *proximal crus amputation without myoplasty*. This happened simultaneously with dilation of the arteries and there was a considerable formation of new vessels in the amputation stump. A similar dilation of arteries and rapid filling of veins occurred in the contralateral extremity (Figures 2, 3), but no contralateral vein filling was seen if a tourniquet was applied distally on the contralateral crus.

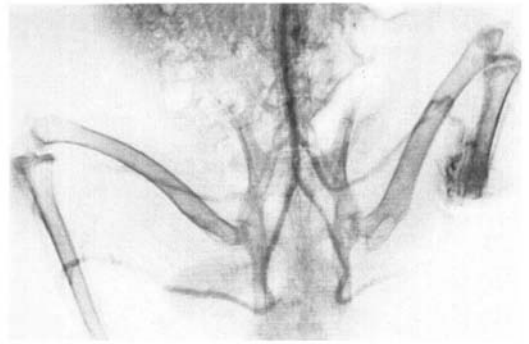
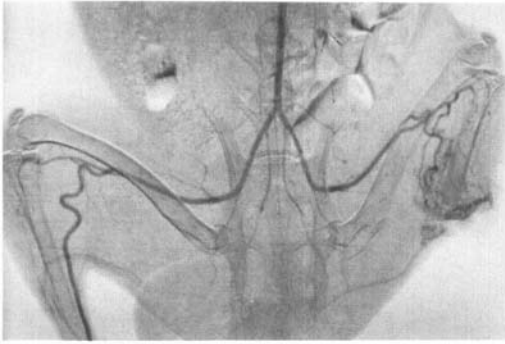


Figure 2. Arteriogram, 2 seconds after the injection, 32 days after amputation without myoplasty.

Figure 3. The same rabbit as in Figure 2. Four seconds later. Note the filling of the superficial veins.

Table 1. The rate of contrast passage (transit-time) in the arteries and veins in the amputation stump. One sign means one picture = 2 seconds.

TRANSIT-TIME

OPERATION	1 HOUR	1/2 WEEK	1 WEEK	2-3 WEEKS	4-5 WEEKS	6-7 WEEKS	>10 WEEKS
AMP. DISTALLY ON CRUS + MYOPLASTY	XXXXXX	XXXXXX XX OOO	XXXXXX XXX △△	XX OOO	XX ●●●●	XXXXXX	XXXXXX ●●●●●
AMP. PROX. ON CRUS - MYOPLASTY	XX XX	XXX XX OOOOO	XX ●●●● XX △△	XX △△△	XX ●●●●● XX ●●●●●	XX ●●●● XX ●●●●●	
AMP. PROX. ON CRUS + MYOPLASTY	XX XXX	XX XXX	X XX	XX △△△△ XX △△△	XXXXX	XXXXXX	
AMP. PROX. ON CRUS + PLUGGING	XX	XX	XXXXXX XXX △△△	XXXXX OOOO	XXX ●●● XX OO	XX OOO	XXXXXX XXXXXX ●●●
EXARTICULATIO GENU	XX	XX	XX △△△	XX △△ XX △	XXXXXX △△ XXXXXX △△		XXXXXX
AMP. DISTALLY ON FEMUR			XX OOO	XX OOO	XX OO	XX	XX OOO

- X ARTERY OCCURRENCE
- △ VEIN SLIGHT OCCURRENCE
- VEIN MODERATE OCCURRENCE
- VEIN MARKED OCCURRENCE

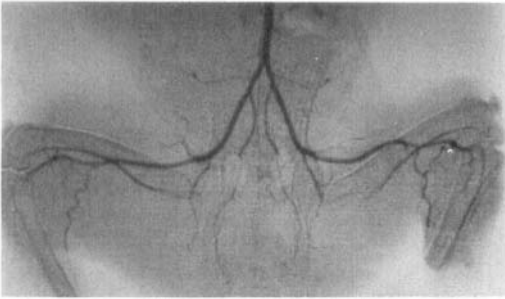


Figure 4. Twenty days after amputation with myoplasty. Six seconds after the injection.

These findings indicate that arteriovenous shunting takes place in the amputation stump and distally in the contralateral extremity. After *proximal crus amputation without myoplasty* the arterial dilation and vein filling in the amputation stump reached a maximum 4–7 weeks after amputation (Table 1).

After *proximal crus amputation with myoplasty* a moderate arterial dilation was observed on the operated side, but only a few newly formed vessels appeared in the stump and 2–3 weeks after operation only a few veins were seen on the operated side (Figure 4). Following *proximal crus amputation with myoplasty and osseous plugging of the marrow cavity* a greater number of vessels appeared in the stump and dilation of

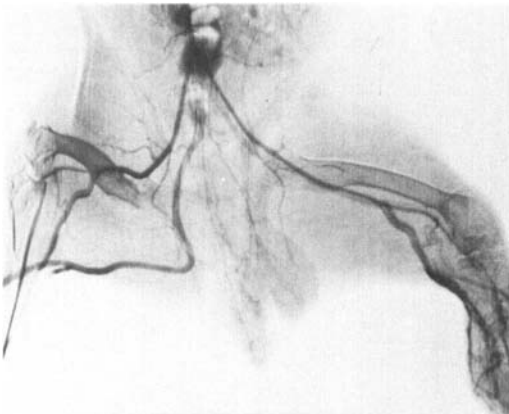


Figure 5. Eighty-five days after amputation with osseous plugging and myoplasty. Twelve seconds after the injection. Note the filling of the deep veins on the left hind-leg and superficial veins on the right.



Figure 6. Twenty-five days after knee disarticulation. The pictures were unchanged throughout the arteriography.

related arteries and appearance of veins was seen 2–3 weeks after operation. On the operated side the deep veins were observed and on the contralateral extremity the superficial veins (Figure 5).

After *knee disarticulation* there was reduced filling of the popliteal artery and of the distal part of the femoral artery on the operated side and only a few newly formed vessels appeared in the femur (Figure 6). When knee disarticulation was combined with resection of the distal femoral condyle with detachment of the muscle fixation and opening up of the marrow cavity (*distal femur amputation without myoplasty*), dilation of the femoral artery all the way to the tip of the amputation stump was observed after 1 week, and the arterial blood



Figure 7. Twenty days after distal amputation on the femur. Two seconds after the injection.

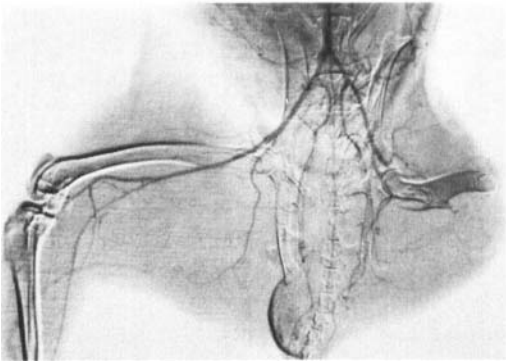


Figure 8. Eighty days after amputation on the femur. Four seconds after the injection.

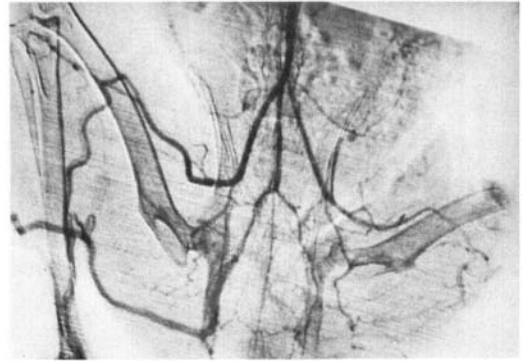


Figure 9. Thirty-five days after amputation on the femur combined with osseous plugging. Note the abundance of arteries and veins compared with Figure 8.

flow and the filling of veins were more rapid than after knee disarticulation (Figure 7).

After *amputation* at a more proximal level on the femur (Figure 8) the femoral artery on the operated side was occluded at the level of the hip joint, whilst it was dilated on the non-operated side. The arterial blood flow was slower than after distal femur amputation, vein filling was late in appearance and only a few veins were seen. However, when *femur amputation was combined with plugging of the marrow cavity* (Figure 9), a greater number of vessels appeared in the stump and dilation of related arteries and appearance of veins were seen.

DISCUSSION

After intracardial injection of contrast, serial arteriography provided a clear portrayal of the arterial and venous components of the blood circulation of amputated rabbits, and by this method the investigation could be repeated at different time intervals on any one animal.

Earlier arteriographic investigations of amputated rabbits have not employed the serial exposure method, and the experimental animals were sacrificed in relation to the arteriographic investigations (Hulth & Olerud 1962, Hansen-Leth & Reimann 1972). Erikson & Olerud (1966) performed angiographies according to an experimental method described by Ekholm et al. (1964).

The rabbits were intravenously injected with Thorotrast and a single exposure was used. This permitted the advantages of an *in vivo* study of circulatory changes, but the use of Thorotrast is prohibited nowadays. In these previous investigations the vascular changes in the amputated extremity were examined in relation to the opposite side. In the present study we found that vascular changes also occur in the contralateral extremity after amputation. This is in agreement with the findings of Hansen-Leth (1976) who, using the Xenon¹³³ clearance method, observed changes in the blood flow in the contralateral extremity, related to the amputation level and the treatment of the amputation stump.

The present investigation has shown that amputation of the crus, in contrast to closed fracture, provokes an initial constriction of the arteries to the amputation stump. This is in agreement with the findings of Erikson & Olerud (1966), who after crus amputation on rabbits with myoplasty observed an initial constriction of arteries and veins, which may be explained by an involuntarily provoked spasm. Similarly, measurement of muscle blood flow (Hansen-Leth 1976, 1977) revealed that amputation in rabbits caused an initial reduction in the flow in the amputation stump, whereas the flow in the triceps surae immediately after open fracture of the crus was unchanged.

Erikson & Olerud (1966) observed dilation of the arteries and veins in the amputated leg 3 days postoperatively. This dilation increased progressively and occasionally persisted for more than 4 months. The present investigation has shown that dilation of arteries and occurrence of veins depend on the amputation level and the method of stump closure. Dederich (1963) reported that when the marrow cavity was left open venous stasis occurred in the stump, but when closed myoplastically, venous return was improved. We found that crus amputation without myoplastic closure brought about abundant vein formation in the stump, whereas no veins appeared in the stump after amputation with myoplastic closure. However, the veins were seen to appear at the same time as the artery or immediately after, and veins appeared simultaneously with dilation of the related arteries and considerable formation of new vessels in the amputation stump. This means that the occurrence of veins must be due to arteriovenous shunts in the stump and not an expression of venous stasis. Erikson (1965) in a clinical investigation found that amputation without myoplastic closure in some cases led to formation of arteriovenous shunts. Similarly, Hulth & Olerud (1962) also proposed a shunt effect to be the reason for the more rapid vein filling in the amputated extremity observed after crus amputation in rabbits. Similar arteriographic appearances are seen after surgically produced arteriovenous fistulas (Kelly et al. 1959, Holman 1968).

It has been demonstrated in the present study that arteriovenous shunting in the amputation stump, as well as in the contralateral extremity, is pronounced after crus amputation without myoplasty. The underlying cause could be the inactivity of the muscles in the stump. Occurrence of rapid vein filling in the stump was seen $\frac{1}{2}$ –1 week after amputation. Similarly, Hulth & Olerud (1960) found arteriovenous shunting at the end of the first week after experimentally induced muscle inactivity. The proposal that the muscle inactivity is chiefly responsible for

the vascular changes is supported by a comparison of the results of knee disarticulation and femur amputation just proximal to the femoral condyle with release of the femoral muscles. After knee disarticulation the diameter of the femoral artery was reduced and only a few veins appeared, whereas after condyle resection the femoral artery was dilated all the way to the tip of the amputation stump, and there was a more rapid appearance and abundant filling of veins. However, the arteriovenous shunting after distal femur amputation is less than after amputation on the crus, and after amputation at the mid-femur level no signs of arteriovenous shunts were seen. The femoral artery was occluded at the point where it joins with the circumflex femoral arteries. Erikson (1965) also found that arteriovenous shunts appeared more frequently after crus amputation than after femur amputation and he observed a greater reduction in artery diameter after femur amputation than after amputation on the crus. Similarly, measurements of muscle blood flow (Hansen-Leth 1977) showed that femur amputation on the rabbit, in contrast to crus amputation, caused no increase in blood flow in the amputation stump.

Amputation with myoplasty combined with osseous plugging of the marrow cavity, in contrast to amputation with myoplastic closure alone, resulted in dilation of related arteries, an abundance of vessels in the stump and a greater occurrence of veins in both extremities, after crus amputation as well as after amputation on the femur. In a clinical study Langhagel (1968) found a greater number of arteries and veins in the amputation stump after osteomyoplastic closure than after myoplastic closure alone.

In previous studies the relationship between muscle blood flow and degree of dilation of related arteries in the stump after amputation is not clearly understood. Hasse et al. (1960) concluded that reduced diameters in afferent arteries resulted from decreased blood flow whereas Erikson (1965) found no relationship between degree of dilation and

magnitude of blood flow. The present investigation shows that the dilation of supplying arteries to the stump resulted from the arteriovenous shunting, and that the degree of dilation cannot be assumed to be directly related to the magnitude of muscle blood flow. The increased shunting led to a poorer nutritive circulation in capillaries and minor vessels. Similarly, measurement of muscle blood flow after amputation on rabbits (Hansen-Leth 1976) revealed that myoplastic closure of the stump, preventing muscle inactivity and arteriovenous shunting, improved the muscle blood flow in the amputation stump.

REFERENCES

- Dederich, R. (1963) Plastic treatment of the muscles and bone in amputation surgery. *J. Bone Jt Surg.* **45-B**, 60-66.
- Ekholm, J., Eriksen, U. & Skoglund, S. (1964) An improved method for experimental angiography. *Brit. J. Radiol.* **37**, 839-843.
- Erikson, Uno (1965) Circulation in traumatic amputation stumps. *Acta radiol. (Stockh.)*, Suppl. 238.
- Erikson, U. & Olerud, S. (1966) Healing of amputation stumps, with special reference to vascularity and bone. *Acta orthop. scand.* **37**, 20-28.
- Hansen-Leth, C. & Reimann, I. (1972) Amputation with and without myoplasty on rabbits with special reference to the vascularization. *Acta orthop. scand.* **43**, 68-77.
- Hansen-Leth, C. (1976) Muscle blood flow after amputation with special reference to the influence of osseous plugging of the medullary cavity. *Acta orthop. scand.* **47**, 613-618.
- Hansen-Leth, C. (1977) Muscle blood flow after amputation with special reference to the influence of the amputation level. *Acta orthop. scand.* **48**, 10-14.
- Hasse, H. M., Rau, G. & Schoop, W. (1960) Angiographische Befunde an Amputationsstümpfen. *Z. Kreis.-Forsch.* **49**, 326-330.
- Holman, E. (1968) *Abnormal arteriovenous communications; peripheral and intracardiac acquired and congenital*. Ed. 2, 245 pp. Charles C Thomas, Springfield, Illinois.
- Hulth, A. & Olerud, S. (1960) Disuse of extremities I. An arteriographic study in the rabbit. *Acta chir. scand.* **120**, 220-226.
- Hulth, A. & Olerud, S. (1962) Studies on amputation stumps in rabbits. *J. Bone Jt Surg.* **44-B**, 431-435.
- Kelly, P. J., Janes, J. M. & Peterson, L. F. A. (1959) The effect of arteriovenous fistulae on the vascular pattern of the femora of immature dogs. *J. Bone Jt Surg.* **41-A**, 1101-1108.
- Langhagel, J. (1968) *Angiographische Untersuchungen der Stumpfdurchblutung bei Beinamputierten. Arbeit und Gesundheit*. Georg Thieme Verlag, Stuttgart.

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