ACTA ORTHOPAEDICA SCANDINAVICA
SUPPLEMENTUM NO 197, VOL. 53

Investigations of the Vascular Changes following Amputation on Rabbits

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Denne afhandling er i forbindelse med omstående tidligere publicerede afhandlinger af det lægevidenskabelige fakultet ved Københavns Universitet antaget til offentligt at forsvares for den medicinske doktorgrad.

København, den 24. marts 1982.

O. Behnke, dekan.

ISBN: 87-16-09432-8
ISSN: 0300-8827
Printed in Denmark by
AiO Print as, Odense

CHAPTER I

INTRODUCTION

The experimental studies which form the basis of this survey were performed to evaluate the vascular changes that take place following amputation.

The most frequent amputation indication is vascular insufficiency, and the starting point in this investigation was the healing problems which play a decisive role in amputation surgery. The vascular reaction subsequent to amputation is of particular importance to the success or failure of the operation. This reaction is due in part to the degree of amputation trauma and in part to the ensuing healing processes in the skin, muscle and bone.

The amputation level is determined by the preoperative blood flow. However, it is not the preoperative vascularization of the amputation level but rather the postoperative blood flow in the amputation stump which is decisive during the course of healing. Radical changes in the anatomical and physiological conditions of the operated extremity occur after amputation. A reduction in tissue mass occurs whereby the supply area of the blood circulation in the extremity is altered. Muscle function in the operated extremity is changed, with inactivity of the severed muscles and with reduction of venous return from the stump. When an extremity that is supplied with collaterals is amputated, the reaction of the collaterals plays a special role in blood flow in the amputation stump.

It has been shown in previous clinical and experimental investigations using arteriography that amputation causes a number of changes in the blood circulation of the amputated extremity, but the underlying reasons for these are unknown. Arteriography in clinical investigations is most often undertaken several years after amputation, and these investigations are repeated only in a few cases; therefore it has not been possible to follow the course of the vascular changes.

By using rabbits as experimental animals it has been possible to repeat examinations on the same animal and to compare arteriographic findings with changes in the muscle blood flow. Moreover, studies can be made of the vascular reaction following amputation at different amputation levels and by different techniques, and of the relationship between the vascular changes in muscle and bone, while investigation of the vascular reaction in the skin has not been possible.

CHAPTER II

BIOLOGICAL AND BIOMECHANICAL PRINCIPLES IN AMPUTATION SURGERY

Earlier principles in the treatment of the amputation stump

Amputation is one of the oldest forms of surgical intervention, utilized for centuries as treatment for severe extremity trauma. Disarticulation, the least injurious operation, was previously the most preferred type of amputation. Velpeau performed knee disarticulations in 1830, and Syme described his method for disarticulation of the foot joint in 1843 (Harris 1956). Disarticulation produced a stable and weight-bearing amputation stump, and Pirogoff (1852) intended to obtain this by closing the stump after crus amputation with tuber calcanei. In amputations distal on the femur Gritti (1891) closed the amputation stump with the patella. Bier (1893) thought that stump pain was provoked from the bone tip, and he advised osteoplastic closure with Pirogoff's technique. Since then, different forms of osteoplastic amputation techniques have been employed. Kirchner (1920) proposed osseous plugging of the medullary cavity, and Ertl (1949) introduced the osteoperiosteal bridge between the tibia and fibula to provide greater stability in the stump of below knee amputations.

The osteoplastic amputation technique was used in these cases to obtain a weight-bearing and pain-free amputation stump, but since then it has been used in association with myoplasty (Loon 1960, Dederich 1963, Langhagel 1968) to normalize the intramedullary pressure and the venous return from the amputation stump.

Recent principles of amputation technique

Only in the last decades has an interest been aroused in muscle function and muscle blood flow in the amputation stump. In conventional amputation the muscles are retracted and atrophic changes are produced. Mondry (1952) suggested that this could be counteracted by uniting the muscles together over the bone tip and attaching them to it, whereby muscle function could be sustained. Dederich (1960) introduced myoplastic stump closure of atrophic and painful amputation stumps, often with osteoplasty, and since then myoplasty has been used in an increasing extent. At the same time the rigid stump bandage and immediate fitting was introduced. This principle was described by Villaret & Roederer in 1923, but was first utilized by Berlemont in 1958. More recently, Burgess et al. (1969), Berlemont et al. (1969), Murdoch (1969) and Vitali et al. (1969) have observed that this treatment principle achieved greater healing frequency, more rapid mobilization and provision for prostheses, and increasing frequency of amputations with preserved knees. Mooney et al. (1971) found that a rigid stump bandage produced more rapid healing than the customary stump bandage, but that ischemic skin could tolerate only minimal compression in the healing phase. This observation has resulted in beginning weight-bearing later than previously (Murdoch 1975).

Amputation in ischemic extremity disorders

The development of amputation techniques within the past decades has proceeded simultaneously with an increasing frequency of ischemic diseases. Seventy – eighty percent of all amputations are carried out on this indication (Jansen 1960, Murdoch 1967, Burgess et al. 1969). Hierton & James (1973) found an increase of 213% in the number of amputations performed on patients suffering from ischemic diseases during the period 1947–1967.

In amputations performed because of ischemia the amputation level was previously determined by the wish to obtain the highest possible fre-

quency of primary healing. Murdoch (1967) observed that that criteria in many cases led to the choice of a more proximal amputation level than necessary. In a survey of 453 patients amputated for arterial insufficiency, Warren & Kihn (1968) found primary healing after amputation of the femur in 71.1% of the cases, while it was 49.6% after crus amputation, but the technique utilized was not mentioned.

With increasing awareness that amputation is a reconstructive and function preserving operation, greater effort has been exerted to preserve the knee joint. A number of clinical observations have shown that crus amputation by conventional amputation technique could be performed despite considerable ischemic changes (Kelly & Janes 1957, Dale & Jacobs 1962, Lim et al. 1967). Burgess et al. (1971) recommended crus amputation, possibly with a modified technique. Using myoplasty with a long posterior musculocutaneous flap in amputations of the crus the frequency of reamputation of the femur was 8%. They also advised, as did Loon (1960), that muscle fascia should be preserved since it improved muscle attachment and muscle function, whereas Dederich (1960) excised the fascia because he believed it formed a barrier between muscle and skin.

The amputation level is still primarily chosen on the basis of clinical criteria. Harris et al. (1961) required a pulsation in the popliteal artery before performing a crus amputation, whereas Burgess et al. (1971) did not consider the absence of a popliteal pulsation as a contraindication to crus amputation, but they asserted that muscle bleeding and the condition of the skin were decisive factors in the choice of amputation level. Susak et al. (1978) stated that a functioning arteria profunda femoris was decisive for healing of the amputation stump of the crus. Browse (1973) claimed that no preoperative measurement method was reliable, but that both the operation technique and the bandaging were of utmost importance in the healing process. However, measurement of the cutaneous perfusion pressure and flow has been shown to be valuable in the determination of the amputation level. Holstein (1973) and Lassen & Holstein (1974) observed healing when the perfusion pressure at the amputation level was greater than 40 mm Hg, and Moore (1973) similarly found good correlation between healing and skin flow, determined by Xe^{133}.

CHAPTER III

PREVIOUS INVESTIGATIONS OF THE VASCULARIZATION OF THE AMPUTATION STUMP

CLINICAL STUDIES

Vascularization and stump pains

Earlier investigations of the vascularization of the amputation stump were conducted to ascertain the reason for stump pains. Leriche (1950) believed that pains were related to peripheral neurovascular changes, due in part to vessel spasms and vessel obliteration and in part to altered sympathetic innervation, while phantom pains were related to changes in the spinal nerves. Stump pains were accompanied by characteristic arteriographic changes, either occlusion of supplying arteries, brought about by sympathetic stimulation (hypocirculatory stump) or profuse hypervascularization, spiral-twisted vessels and signs of arteriovenous communication (hypercirculatory stump).

Similarly, Erikson & Hulth (1962) and Erikson (1965) observed hypervascularization and the presence of spiral-twisted vessels in amputation stumps with stump pains, and using plethysmography Erikson (1965) found a higher mean flow in the amputation stump with pains than in the intact leg or in the pain-free stump. By use of contrast injection in the medullary cavity Gilles (1964) detected pathological venous return from amputation stumps with pain, with sluggish flow from the medullary cavity and with sparse contrast filling in the veins. Dederich (1960, 1963) suggested that stump pains were related to deficient muscle function, which caused venous stasis and anoxia, and that normalized muscle function and circulation in the amputation stump could be achieved by myoplasty.

Vascularization after conventional amputation

In arteriographic studies after amputation without use of myoplasty, Erikson (1965) found that the diameters of the arteries in the amputation stump were smaller than those in the intact leg, and that the passage of contrast was slower. Plethysmography disclosed that the highest resting blood flow occurred in the stump, but that the maximal flow during reactive hyperemia never attained as high values in the amputation stump as in the unoperated extremity. Due to altered tissue proportions in the amputation stump, to altered perfusion in skin and bone and to a possible occurrence of arteriovenous anastomoses, Erikson did not think that an accurate determination of the magnitude of blood flow could be obtained from plethysmographic measurements. With respect to arteriographic results, Erikson found that blood flow was not determined by the degree of dilation of the larger arteries but by the physiological status of the smaller arteries, arterioles, capillaries and venules. After crus amputation Hasse et al. (1960) found reduced diameters in the superficial femoral artery, the popliteal artery and in the crus arteries, while the diameter of the profound femoral artery remained unchanged. They suggested that these changes were anatomical and not functional, and that the smaller diameters resulted from a reduced rate of blood flow and not from humoral or neural influence.

Erikson (1965) detected hypervascularization in about 50% of the amputation stumps, more pronounced after crus than after femur amputations, and that newly formed, spiral-twisted vessels appeared at the same time in the stump. Moreover, contrast filling of veins that were often twisted and dilated were also observed. Rapid venous filling, which indicates arteriovenous shunting, was seen after approximately 30% of the crus amputations, while it after femur amputation only occurred in a few cases.

Vascularization after stump closure with myoplasty and osteomyoplasty

In conventional amputation the muscles that are severed at the amputation level become retracted, resulting in atrophy and degenerative changes with the formation of fat and connective tissues. These adverse changes can be counteracted by myoplasty (Mondry 1952), whereby muscles preserve their normal tension and contractile function. Dederich (1963) demonstrated by arteriography that myoplasty produced an improved arterial supply to the amputation stump. Loon (1960) found that primary closure of the medullary cavity at amputation brought about a normalization of the venous return from the amputation stump and prevented stump edema. Immediately after amputation the pressure in the medullary cavity fell to zero, and by using intraosseous phlebography Loon observed a sluggish return flow from the medullary cavity and an absence of contrast filling of the deep veins. These conditions were normalized by closure of the medullary cavity with a bone plug. Langhagel (1968) observed with arteriography that osteomyoplasty produced a greater arterial vascularization of the amputation stump, and he found using intraosseous phlebography that the injected contrast disappeared more rapidly from this than from a non-osteoplasty treated stump.

While the skin temperature of the amputation stump after conventional amputation was lower than in the intact extremity (Loon 1960, Erikson & Hulth 1962), Weiss (1969) found an increased skin temperature in the amputation stump after myoplasty. He suggested that the temperature increase was caused by operation trauma and inflammatory reaction. In accordance with this, measurements of skin blood flow with $Xenon^{133}$ (Hansen-Leth, unpublished study) indicated that the flow in the stump closed with myoplasty was higher than the average flow at the same level in the intact extremity.

Holstein (1973) found that the cutaneous perfusion pressure in the amputation stump was around 20 mm Hg higher than the pressure measured at the same level preoperatively. In contrast, Browse (1973) showed that the cutaneous perfusion pressure in most cases was the same before and after amputation, but that in some cases where amputation was performed near to an arterial occlusion the cutaneous pressure in the amputation stump was elevated. He assumed that the reason for this was that a portion of the low pressure area in the extremity was removed with amputation.

According to a review by Reinhardt (1972), earlier arteriographic investigations after amputation have shown the following characteristic changes in the amputated extremity:

1. *Closure of the supplying artery to the amputation stump.* After femur amputation the femoral artery can be occluded at its junction with the femoral circumflex artery, and an absence in contrast filling can be seen in the posterior tibialis artery after crus amputation. Leriche (1950) suggested that vasospasm was responsible for this and that the problem could be resolved by sympathectomy. Langhagel (1968) found no evidence for the occurrence of a secondary, progressive occlusion of the supplying artery.
2. *Decreased arterial volume* as a consequence of changes in the wall of the artery has been detected in the femoral artery after amputation of the femur, but especially after crus amputation.
3. *Reduced arterial diameter* in the superficial femoral artery has been observed after both femur and crus amputations, but is less pronounced after crus amputation. This has been interpreted as a decreased need in the arterial supply area. In some cases the diameter of the profound femoral artery has been seen to increase.
4. *Increased or diminished vascularization of the amputation stump.* The diameter of minor arteries were increased and new vessels were filled with contrast. A large diameter artery emerging from the terminal artery and directed toward the end of the stump was often observed. Contrast filling of the peripheral arteries increased after intra-arterial injection of bradykinin, acetylcholine and histamine (Erikson 1965).

5. *Newly formed vessels in the amputation stump in the form of:*
   a) spiral-twisted arteries.
   b) arcade-shaped collaterals.
   The newly formed vessels were more pronounced after femur than after crus amputation. They can develop gradually after amputation, but they can also appear early and then disappear.
6. *Arteriovenous anastomoses,* which occur most frequently after crus amputation.
7. *Varicose changes in the veins of the amputation stump,* with the formation of arcade and spiral vessels.
8. *Changes in the venous flow from the bone in the amputation stump.*
9. *Restructuring of the bone in the amputation stump after femur amputation,* with atrophy, decalcification and periosteal ossification.

PREVIOUS EXPERIMENTAL INVESTIGATIONS

Vascularization of the amputation stump

Previous experimental amputation studies were based on angiographic investigations on rabbits. Hulth & Olerud (1962) found with arteriography after contrast injection into the carotid artery hypervascularization in the crus stump, with the formation of spiral-twisted vessels and signs of arterioveneous shunt formation. The diameters of the arteries remained unchanged despite the reduced tissue mass supplied by these arteries.

Erikson & Olerud (1966), using the technique described by Ekholm et al. (1964), detected an initial reduction in the diameters of the supplying arteries in the amputation stump, followed on the third day by a dilation of arteries and veins, and this condition persisted for 4 months after the operation. They proposed that the initial vessel contraction was caused by spasm or edema, while the subsequent vessel dilation resulted from increased blood flow to the traumatized region.

Itohara (1972) studied the influence of amputation technique on muscle and bone vascularization after femur amputation and found a decreased vascularization and reduced skin temperature after amputation without myoplasty. On the other hand, he observed increased vascularization in muscle and bone and normal skin temperature in amputations with myoplasty.

Osseous healing in the amputation stump

In studies using microangiography and microradiography of osseous healing in the crus stump after amputation on adult rabbits, Hulth & Olerud (1962) found that the bone healed in the same manner as experimental fractures with two fragments (Nilsonne 1959). Periosteal callus developed parallel to and in association with periosteal arteries, often at some distance from the free edge of the bone. The medullary cavity was closed similarly by a sickle-shaped callus which formed around the newly formed vessels and which expanded fan-shaped from the medullary cavity.

Erikson & Olerud (1966) also investigated bone healing after crus amputation with myoplastic closure on rabbits and found after 3–4 weeks had elapsed callus formation that resembled a spur, projecting from the periosteum and supplied by richly vascularized tissue. They proposed that the hematoma formation around the end of the stump was important for spur formation, as the hematoma was transformed into osteoid tissue and then into bone.

THE PURPOSE OF THE PRESENT INVESTIGATION

Previous experimental studies in agreement with clinical observations have shown that amputation causes a hypervascularization of the amputation stump, with the appearance of spiral-twisted vessels and the development of arteriovenous shunts. However, these vascular changes were observed by arteriography, which is a morphological research method that does not permit functional diagnosis (Erikson 1965, Murdoch 1967, Langhagel 1968). The reason for these changes and their importance to blood flow in the amputation stump has not been clarified. The purpose of the present experimental investigation has been to resolve the following questions:

1. Is the observed hypervascularization of the amputation stump tantamount to an increased muscle blood flow?
2. What causes the formation of spiral-twisted vessels and arteriovenous shunts in the amputation stump, and what role do these play in the muscle blood flow?
3. How does myoplasty change the vascularization and the muscle blood flow in the amputation stump?
4. Does the amputation level have an influence on the vascular reaction in the amputation stump?
5. Does bone healing in the amputation stump have an influence on the vascular reaction in the muscles?
6. What is the nature of the vascular reaction after amputation of an extremity supplied with collaterals?

CHAPTER IV

THE ANATOMICAL AND PHYSIOLOGICAL BASIS FOR THE INVESTIGATIONS

THE RABBIT AS AN EXPERIMENTAL ANIMAL IN AMPUTATION STUDIES

The osseous condition:

Heikel (1960) found that bone growth of one day in the rabbit corresponded with a growth of forty days in man and that the tibial epiphysis closes in rabbits at the age of 150–225 days.

The tibia in the rabbit has been used considerably in microangiographic studies of bone vascularization (Marneffe 1951, Brookes & Harrison 1957, Morgan 1959, Brånemark 1959, Göthman 1960 a, Trueta & Cavadias 1964). It has been found that the normal blood supply to the bone is conveyed through the nutrient artery, as well as through the periosteal and metaphyseal-epiphyseal arteries, while there is some disagreement on the details of supply to the cortex and on the existence of anastomoses between the 3 arterial systems. The normal arterial anatomy in the rabbit's tibia resembles principally the long tubular bones in other animal species and in man (Göthman 1960 a).

Amputation studies on the rabbit are influenced by the fact that the tibia and fibula fuse together into one bone between the proximal and medial 1/3, the diaphysis of which receives arterial blood from the nutrient artery, which emerges from the anterior tibial artery and which perforates the cortex on the fibular side about 5 mm proximal to the tibiofibular synostosis. The nutrient artery passes obliquely into the medulla where it proceeds distally while giving off branches to the medullary cavity. Thereafter, it bends proximally, giving off arterioles to the cortex and anastomoses to the vessels in the haversian system, and then continues through the medullary cavity to a proximal communication with the metaphyseal artery (fig. 1). Moreover, it gives off branches that penetrate the entire cortex, and thereby connects the nutrient artery di-

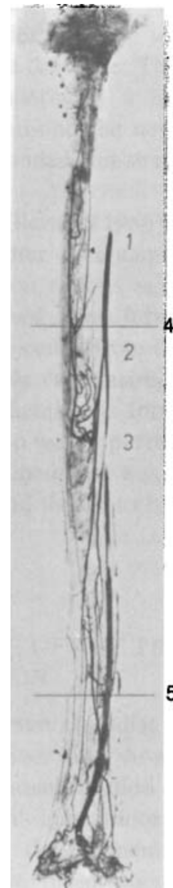

Fig. 1. Microangiogram of the whole tibia of a rabbit (L. Göthman: The normal arterial pattern of the rabbit's tibia. Acta chir. scand. 120, 201–210, 1960).

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 acessory nutrient artery (3) passes distally to the lateral malleolus. 4: proximal amputation level. 5: distal amputation level.

rectly with the periosteal system without the interposition of capillaries. The periosteal arterial system in the diaphysis consists of 6–7 longitudinally oriented arteries, from which are given off a capillary net that communicates with vessels in the haversian canals, particularly near the metaphysis.

In femur the nutrient artery begins as a branch of the lateral circumflex femoral artery, passes distally beside the iliopsoas to the foramen nutritium, which is located on the medioposterior surface of the diaphysis, immediately distal to the trochanter minor (Brookes & Harrison 1957). After giving off ascending branches to the metaphysis the artery proceeeds distally through the entire medullary cavity to the distal metaphysis. During this course it gives off arteries to the diaphyseal cortex, where arterioles can be traced to the center where they join with vessels of capillary size in the haversian and volkmannian canals (Brookes 1971).

Anastomoses between the medullary and metaphyseal vascular systems take place at a capillary level in both the tibia and femur. The epiphysis in the immature rabbit has its own separate vascularization, and until the completion of growth the epiphysic plate constitutes a vascular barrier between the epiphyseal and metaphyseal vessels. It still has not been possible to demonstrate a communication between the medullary and epiphyseal vascular systems in the adult rabbit, but it is assumed that such a communication exists (Brookes 1971).

As mentioned earlier there is disagreement on the question of how far the periosteal arteries under normal conditions participate in the arterial supply to the cortex of the diaphysis. Already in the last century Langer (1878) described numerous small vessels emerging from the periosteum and penetrating into the underlying cortex, and he suggested that these vessels supplied the peripheral portion of the cortex. Since then, Morgan (1959), Brånemark (1959), Göthman (1960a), Trueta (1968) and Rhinelander (1968) demonstrated vessels from the periosteum into the cortex. Rhinelander (1974) found in the femur that there is a communication between the intramuscular and periosteal circulation at the site of muscle attachment (linea aspera), where the fibrous periosteum is firmly bound to the bone, and that the periosteal arteries located there penetrate into the cortex where there are anastomoses with arterioles from the medullary vascular system. Brookes (1971) believed these periosteal vessels were veins and that the arterial vascularization of the cortex was performed under normal conditions exclusively from the medulla by means of a centrifugally oriented circulation toward the periosteum. Only under pathological conditions, where the original positive pressure in the medullary cavity is reduced, is the circulation reversed to a centripetal orientation toward the venous system in the medullary cavity. Brookes' point of view was shared by MacNab (1957), MacAuley (1958) and Nelson et al. (1960), while Marneffe (1951) thought the proximal part of the tibia diaphyseal cortex is primarily supplied from the medulla, while the distal part is vascularized under normal conditions from the periosteum.

A large number of venous sinusoids located in the medullary cavity are supplied by intracortical capillaries and are drained by the large central venous sinus.

The muscular condition:

The muscles which represent particular interest in these amputation studies are the triceps surae and the quadriceps. The triceps surae are composed of three components: the soleus which is attached to two joints, the talocrural and subtalar, and the gastrocnemius with two heads which, moreover, attaches to the knee joint. The function of these muscles is changed after crus amputation; the soleus no longer attaches to a joint and the gastrocnemius becomes a one-joint muscle. After femur amputation the quadriceps is changed in the same manner from a two-joint to a one-joint muscle.

A muscle contracts with maximal force when its length is slightly longer than its resting length, and when the period of contraction is short. In this case the contraction is isometric, the activity in each muscle fiber consists of a very short tetanus, whereby the energy consumption is minimal. This contraction pattern is changed after amputation, where the muscles are rapidly

fatigued and the muscle blood flow is reduced (Loon 1960).

In animal experiments it has been found that the soleus is the most powerful component and the lateral head of the gastrocnemius is the weakest component of the triceps surae. The soleus is composed of "red" muscle fibers which are distinguished from "pale" muscle fibers by their biochemical composition and their innervation. The red color is produced by myoglobin. The gastrocnemius is composed of a mixture of red and pale muscle fibers. The red fibers contract slowly (Buchthal & Schmalbruch 1970) and perform principally static functions, while pale fibers are essentially characterized by rapid, phasic movements. The capillary supply to the soleus is 2–3 times larger than the supply to the gastrocnemius.

A muscle fiber is the basic element in a muscle. It is a large cell enclosed in a cell membrane, which constitutes the innermost part of the surrounding sarcolemma. A muscle fiber contains a number of nuclei that are located peripherally in the cell. The number of nuclei is dependent on the function of the muscle fiber. A "red" muscle fiber contains three times as many nuclei as a "pale". Each muscle fiber is supplied under normal conditions with 4–5 capillaries. Fibers are separated from each other by endomysium and are collected together in fasciculi which are surrounded by perimysium, while the entire muscle is enclosed in fascia (epimysium).

The vasomotor condition:

The vascularization of the rear legs of the rabbit differs from the vascularization of the lower extremities in man. There is no profound femoral artery in the rabbit, which is of considerable importance in amputation of the femur, as well as for collateral development after ligation of the femoral artery. On the other hand, the supreme genu artery is more developed in the rabbit than in man, and it provides a well-developed vascular net in the knee region. A portion of the crus vascularization is performed by the saphenous and suralis arteries.

The muscle circulation is regulated from the vasomotor center in the medulla oblongata by sympathetic adrenergic vasoconstrictor and vasodilator nerve fibers which terminate on alpha and beta receptors in the vessel walls. Activation of the sympathetic vasoconstrictor alpha receptor elicits a constriction of resistance vessels and an increased pre-/postcapillary ratio, while stimulation of beta receptors decreases the peripheral resistance and the pre-postcapillary ratio.

As for the muscle nutritional circulation, however, the central regulation is of minor importance. In the resting muscle only a fraction of the capillaries are open, metabolism is low and the arterioles are tonically contracted. Muscle blood flow is regulated by resistance in the arterioles. When the muscle becomes active the precapillary arterioles are dilated and the number of open capillaries is increased from about 100 to 3000 per mm² (Krogh 1929). This has the effect of strongly increasing the flow rate in the supplying arteries, while the rate remains unchanged in the capillaries, as the total capillary cross-section is increased proportionally to the volume rate of flow. The regional muscle blood flow is regulated by a number of factors, such as local accumulation of metabolic products (among which is potassium, which is liberated from contracted muscle fibers), reduced oxygen tension and regional hyperosmolarity.

In contrast to the capillary circulation, the arteriovenous shunts are regulated exclusively from the vasomotor center by sympathetic fibers. These direct connections between arterioles and venules occur in large numbers only distally in the extremities, and it is not clear whether they are present in muscles under normal conditions. In the presence of shunts, which are characterized by only slight resistance (no basal tonus), a large fraction of the regional blood flow by-passes the capillaries.

The resistance in collaterals is greater than in the normally occurring vessels (Ludbrook 1966). The pressure drop in collaterals depends on length and diameter. In short, wide-bore collaterals, which return blood rapidly to the main artery, there is only a slight reduction of flow, while in long collaterals with narrow diameters the resistance is large and the flow of blood when it reaches the capillaries is greately reduced (Pois-

seuilles law: volume flow $= \frac{(P_1 - P_2)\, r^4}{L}$, where $(P_1 - P_2)$ = pressure drop, r = vessel diameter, L = length). The effect of sympathetic innervation of the collaterals is not clearly understood. While sympathectomy caused dilation of newly developed collaterals, the sympathetic effect on collaterals that have been present for a long time is less certain (Ludbrook 1966).

Whether the above described vasomotor condition present in man is also true in the rabbit is not known with certainty. It is particularly important with respect to these investigations to mention that under certain circumstances histamine causes blood vessel contraction and an increase in blood pressure in the rabbit, whereas it produces vasodilation and hypotension in man and large animals (Kruhøffer 1975).

CHAPTER V

PRESENT INVESTIGATIONS

THE MATERIAL

consisted of a total of 198 rabbits of both sexes. One hundred and ninety-one rabbits (46 immature and 145 adults) were operated on as described in table 1. In addition, the vascular conditions were studied in 7 unoperated adult rabbits.

OPERATION TECHNIQUE:

The animals were anesthetized with nembutal. The amputations on immature rabbits were performed on the right rear leg, while on adults they were performed on the left rear leg.

In proximal crus amputations the tibia was sawed through at the level of the tibio-fibular synostosis, while in distal crus amputations the amputation level was between the middle and distal 1/3 of the tibia. In the absence of myoplasty the muscles were cut through at the same level as the bone, while in myoplasty they were severed 1 cm distal to the end of the bone and then sutured over it with silk thread. In distal crus amputations the triceps surae were not severed. In knee disarticulations the gastrocnemius was severed and the quadriceps was sutured to the cruciate ligaments. In two cases, both knee disarticulation and resection of the joint surface of the femur (resectio condyli femoris) were performed, and in 3 after knee disarticulation by subsequent operations the entire femur condyle was extirpated without sev-

Table 1: The material.

Normal circulation Amputation level		Number of animals	– myoplasty	+ myoplasty	+ medullary plugging
Amp. prox. cruris	adult	51	14	20	17
	young	30	10	20	
Amp. distalis cruris	adult	16			
Exarticulatio genu	adult	5 (+2)			
	young	16			
Amputatio femoris	adult	10 (+4)		7 (+3)	3 (+1)
Amp. distalis femoris	adult	(+3)			
		128 (+9)	24	47 (+3)	20 (+1)
– operation		7			

Collateral circulation Operation	Number of animals	simultaneous lig.+amp.	Interval 1–6 days	Interval 1–5 weeks	Interval 6–18 weeks
Ligatura a. femoralis sin.	13				
Lig.+amp.proximalis cruris	49	10	17	10	12
Lig.+amp. femoris	1 (+2)				
	63 (+2)	10	17	10	12

() = previous amputation on crus.

ering the quadriceps or ligation of the femoral artery (distal femur amputation). Femur amputation was performed in the middle of the bone with ligation of the femoral artery at the same level. The quadriceps were severed about 1 cm distally, after which the muscles were sutured over the bone. Osseus plugging of the medullary cavity was implemented by hammering in a 1 cm length of cortex from the amputated bone. The femoral artery was occluded by a double ligature proximally on the artery, so that the vein and femoral nerve were spared.

Prior to amputation the arteriographic changes and blood flows following incision of skin and muscles and after fracture in the middle of the crus were studied in 8 adult rabbits.

Ulcers appeared on the amputation stumps of 25 animals at the time of examination, but there was no sign of infection and these animals were included in the investigation. Larger defects appeared in crus amputated stumps in 5 cases, after which amputation of the femur was carried out followed by healing.

INVESTIGATION METHODS:

The vascular changes in muscles were assessed by:

1. Evaluation of muscle blood flow in 73 adult rabbits.
2. Intracardial angiography of 53 adult rabbits.
3. Microangiography of 151 animals (46 immature and 105 adult rabbits).

Vascular changes in bones were assessed by:

4. Microangiography of 110 animals (46 immature and 64 adult rabbits).

Growth changes in bones were assessed by:

5. Tetracyline labelling of 46 immature rabbits.

Bone healing in amputation stumps was studied histologically by:

6. Goldner's trichromatic staining method on 61 adult rabbits.

Morphological changes in musculature were assessed histologically by:

7. Hematoxylin-eosin and with van Gieson's staining method on 30 adult rabbits.

1): Muscle blood flow in amputated and in contralateral extremities was determined by washing out Xenon 133 from intramuscular depots (Lassen et al. 1964) with addition of histamine (Lindbjerg 1965).

In preliminary examination of 12 rabbits before and after amputation of the crus, I found that the washing out curves of Xenon 133 without histamine addition were characterized by a steep initial fall during the first few minutes, followed by a more gradual fall. These curves did not permit an unequivocal estimation of muscle blood flow. Similar washing out curves were observed by Tønnesen (1969) in determinations of resting flow in isolated gastrocnemius muscles in the cat, but the curves became rectilinear after stimulation of the sciatic nerve. By nerve stimulation the number of open capillaries was increased (Kjelmer 1965), and the diffusion distance between blood and tissue was reduced. Lindbjerg (1969) suggested that the reason for the nonrectilinear washing out curves from resting muscle was due to a nonuniform exchange between blood and tissue, caused by nonuniform distribution of open capillaries in the resting muscle, and he found that addition of histamine, which opened all capillaries, produced rectilinear washing out curves.

Therefore, I added histamine to the intramuscularly injected Xenon depot and observed that the course of the washing out curves was rectilinear and that muscle blood flow (MBF) could be determined with considerable accuracy.

The examinations were carried out on nembutal anesthetized animals. An isotonic solution containing Xe^{133} (Radiochemical Center, Amersham) and histamine chloride (1 mg/ml), in the ratio 3/1, was injected into the center of the quadriceps and the triceps surae, in both the operated and the contralateral extremities. The doses varied between 0.02–0.08 ml of the mixture, injected by a Mantoux syringe and needle. Washing out of the isotope depot was recorded by a scintillation detector with a NaI crystal, shielded by a lead collimator with a window of 10 mm and coupled to a rate-meter. Immediately

after establishment of a depot the detector was placed 5–10 cm above it and the washing out curve was recorded by a semilogarithmic potentiometer pen writer during the subsequent 8–12 minutes. Muscle blood flow (MBF) was calculated as described by Lassen et al. (1964), according to the formula: $MBF = -100 \times \lambda \times Ln(10) \times d\log C/dt = 161 \times D$ ml/100 g/minute, where C is the isotope concentration in the tissue at a given time, λ is the distribution coefficient for the isotope between tissue and blood = 0.7, and D is the fraction of a decade by which the tangent to the curve falls per minute.

The washing out curves were usually rectilinear, but in some cases an adjustment phase of 1–2 minutes elapsed before the curve began to fall. In measurements on amputation stumps a third phase occurred in about $^1/_2$ of the cases, as the washing out increased for 6–8 minutes after injection, and the isotope concentration fell to zero after few minutes.

The MBF values obtained from the rectilinear part of the washing out curves after addition of histamine were approximately uniform in repeated measurements on the same animal and in measurements following the same operation on different animals, but they were lower than the values calculated from washing curves of Xenon depots without histamine added. As mentioned earlier, the histamine effect on muscle blood flow in the rabbit is equivocal, and under certain circumstances histamine induces vasoconstriction and an increase in blood pressure (Kruhøffer 1975). These measurements indicate that histamine had a vasoconstrictor effect and that the rectilinear washing out curves obtained after histamine addition resulted from a uniform distribution of functioning capillaries in the injection area.

A number of factors could play a role in the course of a washing out curve, e.g., injection trauma, the size and location of the Xenon depot and the amount of histamine added. Tønnesen (1969) found that the size of the injecting needle did not influence the washing out curves. Lindbjerg (1965) reported that both the size of the isotope depot and the amount of histamine added played a role in the blood flow. Clinically, he observed a maximal MBF after a histamine dose of between 4 and 100 μg and he found that a depot size greater than 1 ml resulted in a reduced blood flow. In the present experimental study the size of histamine dose varied between 10 and 20 μg, and the size of the depot used ranged between 0.02–0.08 ml, which is large in relation to the muscle utilized, but it was found that the depot size between 0.02–0.12 ml does not effect MBF. Variation in the course of washing out curves and difficulty in determination of MBF occurred most frequently in measurements on amputation stumps. The presence of hematoma or edema, of muscle atrophy and increased amounts of fat and connective tissues were contributing factors to these difficulties.

Normal values of blood flow in the quadriceps and in the triceps surae were determined by bilateral measurements on 20 unoperated animals. The postoperative MBF values were then compared with the preoperative average flow.

The calculated results were reviewed and statistically evaluated by *the Danish Medical Research Council's consultant service,* who performed the logarithmic calculations of MBF and who recommended the graphic presentation which was utilized.

2): In vivo angiography was conducted by a technique which has not previously been described, in which the contrast substance was injected intracardially.

Earlier experimental amputation studies employed an in vivo angiographic technique, where the contrast was injected into the carotid artery (Hulth & Olerud 1962), but that technique does not permit repeated investigations on the same animal. Angiography was performed by Erikson & Olerud (1966) by a method described by Ekholm et al. (1964) in which 25% thorotrast was injected intravenously, rendering both arteries and veins visible within $1^1/_2$ hours. That method allowed repeated investigations on the same animal, but thorotrast is no longer used because of its high radioactivity.

Intracardial angiography was performed on nembutal anesthetized animals lying on their backs on an AOT film changer. Urographin 76% was injected intracardially with a lumbar needle (90 × 0.9 mm) thrust through the skin at the tip

of the xiphoid process. The needle was kept open with heparin-saline and a bolus of 6–8 ml contrast was injected manually during a period of 1–1½ seconds. A series of 6 exposures were then taken with 2 second-intervals between each exposure, whereby both the arterial and venous phases were revealed. The rate of flow could be determined by the number of arterial pictures (transit time). The degree of arterial dilation was measured on subtraction films with the aid of a magnifying glass and scale which allowed an accuracy of 0.1 mm.

A total of 123 intracardial angiographs on 53 animals were performed, 3 animals were examined 8 times, 1 animal 6 times, 1 animal 5 times, 3 animals 4 times, 8 animals 3 times, 15 animals 2 times and 22 animals 1 time. Therefore, this angiographic technique permitted repeated examinations on the same animal in the period of investigation, i.e., preoperative and 130 days postoperative. The mortality during the investigation was about 20% in the first series (VI), but was reduced to below 10% in the second series (VII).

3): Microangiography was carried out in connection with animal sacrifice. Heparin 5000 IU was injected intravenously into a nembutal anesthetized animal, the peritoneum was opened and the body perfused with saline under a pressure of 1 m of water through a catheter inserted distally in the abdominal aorta, while at the same time the animal was bled from the inferior vena cava. The perfusion was continued with Mikropaque 25% for about 30 minutes, followed by Mikropaque 25% + formalin 10% for 10 minutes. In most cases Berliner blue 1% was also added to the final perfusion mixture. Next, the skin was removed and the hind part of the body fixed in 10% formalin and then x-ray photographed with Balleau microradiography apparatus (Machlett O.E.G. roentgen tube) at a distance of 40 cm and an exposure time of 1 minute (7 mAmp and 24 Kv).

With microangiography a static picture of the blood supply was obtained. The functional arteries were filled all the way down to the smallest capillary, while the veins normally were not filled with contrast due to rapid outflow (Rhinelander 1974). However, contrast filling of veins was observed in some cases in this investigation.

The vascular condition in the amputated and the contralateral extremities was evaluated with respect to the degree of vascularity, dilation of the supplying arteries and the appearance of newly formed, spiral-twisted vessels, described according to Erikson's classification (1965).

4): The vascular condition in the bone of the amputation stump was evaluated by microangiography. After performance of angiography as described above under 3), the bone in the amputation stump was dissected out and 1 cm of the tip sawed off. In immature animals the bone tip was examined microangiographically, whereas in adult animals it was used in a histological study of bone healing (see also point 6), and an additional 1 cm of the bone was sawed off for microangiographic examination. After decalcification in a mixture of 50% formic acid and sodium citrate, in a ratio of 1/1, the bone was embedded in methyl metacrylate and cut in ½–1 mm thick sections. A longitudinal section from the bone tip was obtained from immature animals, and a cross-section of about 1½ cm from the bone tip was obtained from adult animals. The sections were photographed on spectroscopic plates with an exposure time of 10 minutes (12 Kv and 12 mAmp).

5): Bone growth in the amputation stumps of immature rabbits was determined by labelling with tetracycline (30 mg/kg), injected intramuscularly twice with an interval of 3 days before sacrifice. After crus amputation and knee disarticulation the proximal end of the tibia and the distal end of the femur from both sides were removed and embedded in methyl metacrylate. Thereafter, 50–60 μ thick sections were examined by fluorescence microscopy and the distance between the 2 fluorescent lines on the metaphyseal side of the epiphyseal plate was measured. The growth changes were expressed in percentage of growth in the contralateral extremity.

6): Bone healing in the amputation stump was assessed by use of Goldner's trichromatic staining

method. After fixation in 10% formalin the bone tip was embedded in methyl metacrylate, cut into 6–8 μ thick sections and then stained according to Goldner's method (1938).

7): Moreover, a supplementary, unpublished histological study of the morphological changes in muscles was carried out. The triceps surae and the quadriceps were fixed in 10% formalin, cut into about 1/2 cm thich cross-sections, embedded in paraffin and cut into 6 μ thick sections and stained with hematoxylin-eosin and with van Gieson's staining method.

CHAPTER VI

MUSCLE BLOOD FLOW IN THE AMPUTATION STUMP

PRESENT INVESTIGATION

The muscle blood flow (MBF) in the amputation stump (III–IV) is shown in figure 2, where the course of MBF for each rabbit is entered. After proximal amputation on the crus there was an increase in blood flow which began after an initial fall. The initial fall persisted for several days after amputation without myoplasty, and the increased blood flow again underwent a declining tendency after about 30 days. Following amputation with myoplasty a rise in MBF appeared after 1 day, and the blood flow in the stump remained high for up to 80 days postoperatively.

In the ipsilateral quadriceps (fig. 3) decreased values in MBF were observed within the first weeks after crus amputation without myoplasty, after which the blood flow values rose. On the other hand, following closure with myoplasty a rise in blood flow occurred in the first days, after which MBF became normalized.

Distal amputation on the crus also caused an increase in MBF, but the blood flow was normalized after about 3 weeks. Following disarticulation of the knee joint (fig. 4a) an initial reduction was succeeded by an increase in MBF in the quadriceps, after which the blood flow remained high for up to 130 days postoperatively. Amputation on the femur with myoplasty (fig. 4b) caused an initial reduction followed by a transient rise in MBF in the amputation stump, but no corresponding rise as seen after crus amputation or knee disarticulation was observed.

The observed changes in muscle blood flow will in the following be described in comparison with the angiographic findings.

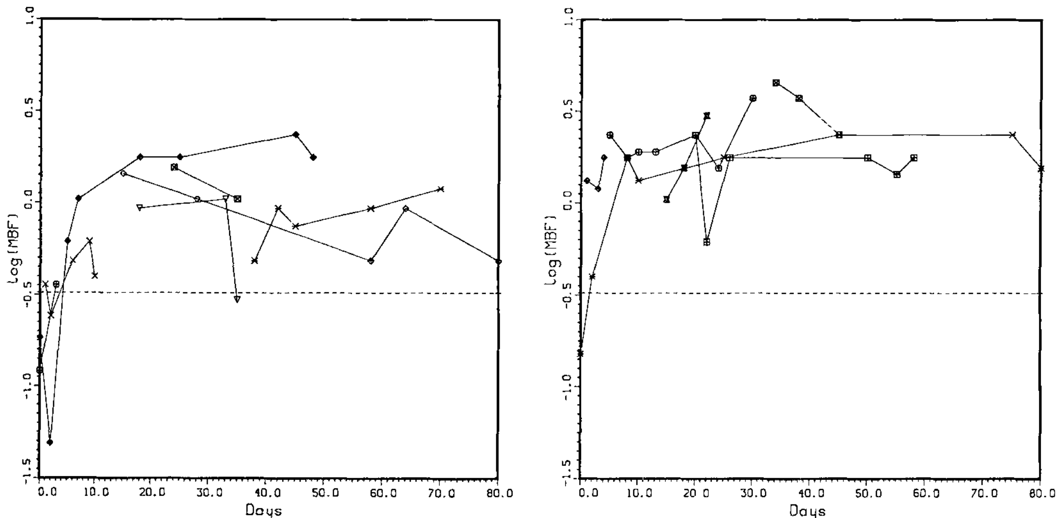

Fig. 2. MBF in the amputation stump after amputation proximally on the crus without myoplasty on 7 rabbits (a) and with myoplasty on 6 rabbits (b).

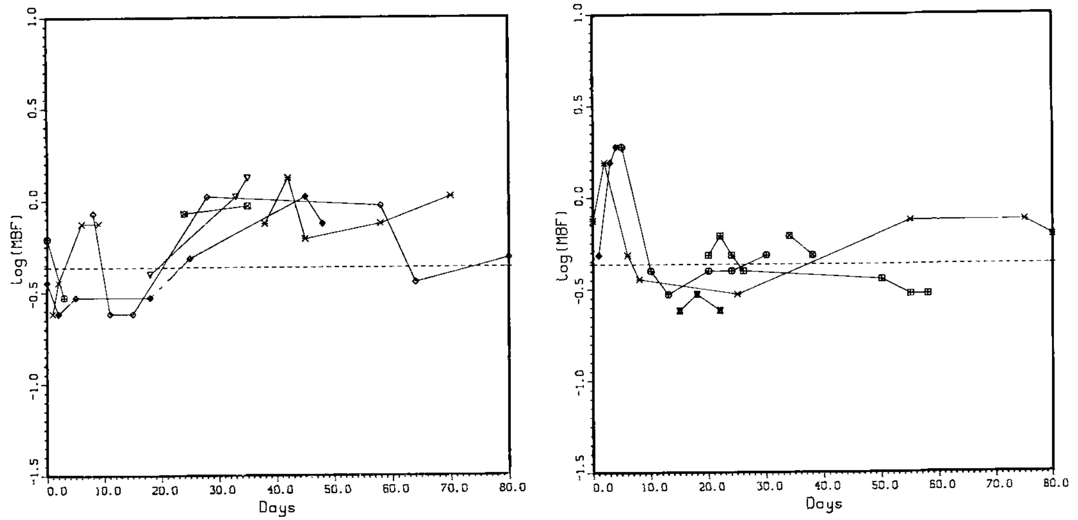

Fig. 3. MBF in the ipsilaterale quadriceps after amputation proximally on crus without myoplasty on 7 rabbits (a) and with myoplasty on 6 rabbits (b).

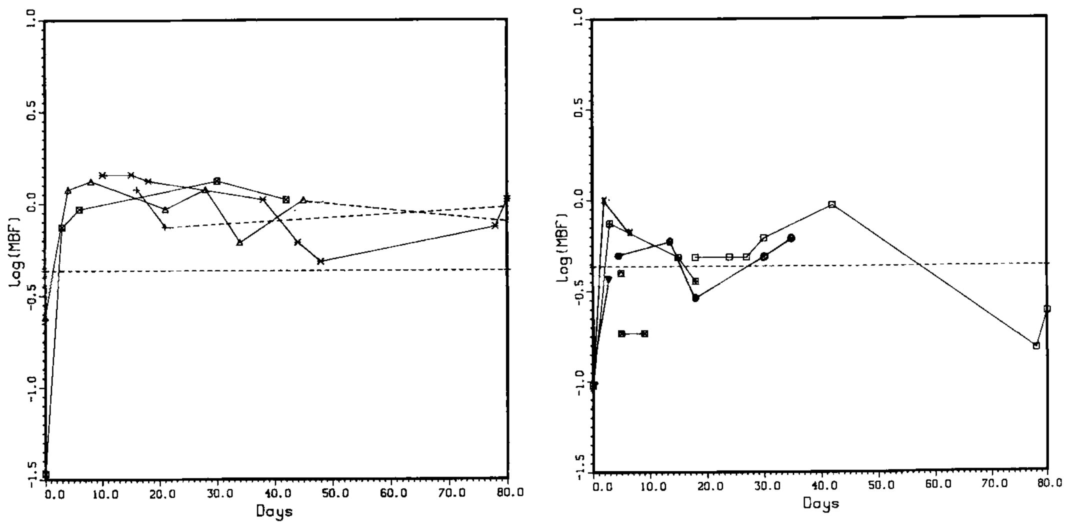

Fig. 4. MBF in the amputation stump after disarticulation in the knee on 4 rabbits (a) and after amputation of the femur with myoplasty on 7 rabbits (b).

THE IMMEDIATE VASCULAR REACTION TO AMPUTATION TRAUMA

After amputation, an interruption in vascularization as well as a traumatic effect on the soft tissue and the bone occurs. The vascularization in the amputation stump immediately after amputation was investigated by measurement of MBF and by in vivo arteriography, and these same methods were employed to ascertain the effect of arterial ligation and of soft tissue trauma in the form of incision of the skin and muscle, as well as by fracture of the crus.

It can be seen in table 2 that MBF in the amputation stump was reduced after crus amputation, knee disarticulation and femur amputation. Ligation of the femoral artery was accompanied by an immediate decrease in MBF in both the quadriceps and the triceps surae, and reduced blood flow also occurred following incision of the skin and muscle, whereas an immediate fall in MBF did not take place after fracture of the tibia.

In accordance with determinations of muscle blood flow, the *intracardial angiography* (VI) revealed that ligation of the femoral artery caused initially a reduced diameter in the arteries, both proximal and distal to the site of ligation. After amputation, an arteriogram disclosed a corresponding decrease in arterial diameter and an absence of contrast filling in the amputation stump. In contrast, a dilation in the supplying arteries was observed after fracture of the tibia. The difference between the vascularization subsequent to fracture and amputation was demonstrated by performing amputation of the crus immediately after fracture of the tibia (fig. 5).

PREVIOUS INVESTIGATIONS OF THE IMMEDIATE REACTION TO EXTREMITY TRAUMA

Immediately after crus amputation in rabbits, Erikson & Olerud (1966) found reduction in the vascularization of the amputation stump and a reduced diameter in the arteries and veins. They interpreted this as a reflex-provoked spasm, but they conceded that tissue edema and hematoma in the stump could also be contributory causes.

In 1915 Leriche demonstrated that arterial ligation elicited a vasospasm. In 1950 he proposed that the reduced arterial diameter observed in an amputated extremity was functional, related to increased sympathetic tonus, and not organic, while Pichering (1951) asserted that vasospasm could be provoked by trauma and by temporary arterial occlusion. Barnes & Trueta (1942) showed that application of a tourniquet onto an extremity of a rabbit caused a prolonged vascular spasm in both the main arteries and the

Table 2. Postoperative MBF (% of the preoperative flow).

One hour after: operation	Number of animals	Mean flow Quadriceps	Mean flow Triceps surae
Amputatio cruris	7	86%	71%
Exarticulatio genu	2	55%	
Amputatio femoris	5	54%	
Ligatura art. femoralis	4	71%	57%
Incision into skin-crus	3	134%	58%
Incision into skin-femur	2	115%	76%
Incision into muscle-crus	3	84%	81%
Incision into muscle-femur	3	77%	100%
Fractura tibiae	5	95%	110%

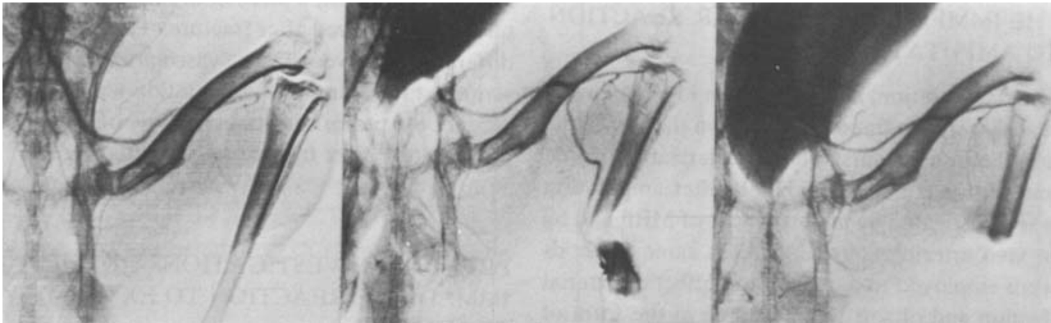

Fig. 5. Arteriograms (a) Preoperatively; (b) immediately after fracture on the crus; and (c) immediately after subsequent amputation.

collaterals in the extremity. They thought the local effect was myogenic while the collateral effect was produced by sympathetic stimulation. Jaya (1958) also observed that ligation of the femoral artery induced a widespread reflex vasospasm which included the contralateral extremity, while Kinmonth (1952) thought that traumatic related arterial spasm was evoked by local factors.

An extremity trauma can provoke vasospasm (Leriche & Fontaine 1928, Pichering 1951), but a stump extremity trauma with tissue contusion would produce vasodilation (Lewis & Lim jr. 1970, Sandegård 1974), accompanied by a transient increase in total blood flow in the traumatized extremity. Lewis & Lim jr. (1970a) showed that the same flow increase could be obtained following sympathectomy, whereas trauma subsequent to sympathectomy had no effect on flow. The flow increase must therefore be caused by a blocking of sympathetic tonus.

It has been shown in animal experiments that fracture elicits an immediate rise in blood flow in the extremity. Wray & Lynch (1959) observed increased blood volume with a maximum 9 days after closed tibia fracture in rats, and Ray et al. (1967) found that tibia fracture in rabbits was accompanied by increased blood volume for as long as 8 weeks after the fracture. Wray (1964) detected a rise in femoral arterial blood flow accompanied by arterial dilation after closed tibia fracture in dogs, which persisted for 2 hours after the trauma. The blood pressure remained unchanged, and Wray concluded that the flow increase resulted from reduced peripheral resistance as a consequence of the loss of sympathetic tonus. Moreover, Wray (1964) and Sandegård & Zachrisson (1975) found that the increased blood flow was caused by soft tissue trauma and not by isolated injury to bone or periosteum.

Baumgartl et al. (1958) found in clinical observations using arteriography that tibia fracture was accompanied by vasodilation. Kellerová et al. (1970) observed increased skin and muscle blood flow after tibia fracture, whereas Sandegård (1974) could detect no changes in blood flow after fractures with minimal soft tissue trauma, but he found slight dilation in the vessels around the fracture.

DISCUSSION OF THE PRESENT RESULTS

Experimental amputation makes possible a study of the immediate vascular reaction to amputation trauma and an analysis of the factors which are responsible for that reaction.

The results of muscle blood flow determinations and of intracardial angiography were concurrent in disclosing an initially reduced vascularization of the amputation stump. This has also been observed by Erikson & Olerud (1966).

Supplementary studies revealed that arterial ligation caused similar changes which must result from vasospasm. Proof that amputation induces vasospasm can be demonstrated when the am-

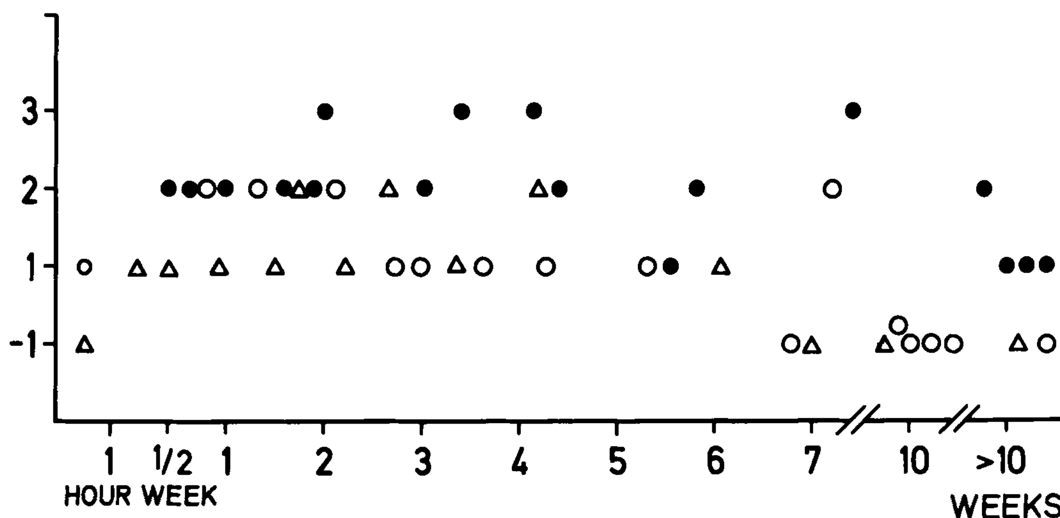

Fig. 6. Vascularization in the amputation stump after amputation proximally on crus without myoplasty = ○, proximally on crus with myoplasty = ●, and distally on crus = △. (Adult rabbits).

1: Same as the corresponding level of the contralateral extremity.
2: Moderate hypervascularization.
3: Marked hypervascularization.
– 1: Hypovascularization.

putation is preceded by a fracture causing vasodilation, and it must be assumed that both reactions are related to sympathetic influence. Incision in soft tissue also produces reduced muscle blood flow. After amputation of the crus a reduction in MBF was seen not only in the severed triceps surae but also in the ipsilateral quadriceps. Following knee disarticulation, where no muscle surgery is performed, there likewise was found a reduced blood flow in the quadriceps.

Therefore, these investigations have shown that amputation in rabbits induces an immediate vasospasm with reduction in MBF in the amputation stump. Incisions in skin or muscle are not decisive in that reaction, nor does fracture provoke vasospasm. The causative factor must therefore be the interruption in arterial supply. This conclusion is supported by the observation that ligation of the femoral artery causes a similar reduction in muscle blood flow in both the triceps surae and the quadriceps, and by the fact that simultaneous arterial ligation and amputation evoke an additional accentuation of that reduction in blood flow (Chapter XIV). In agreement with earlier observations it appears most likely that this vasospasm is related to increased sympathetic tonus.

ANGIOGRAPHIC STUDIES

It was shown in figures 2–4 that muscle blood flow in the amputation stump is increased after an initial reduction. By use of *microangiography* (IX) a corresponding hypervascularization in the amputation stump could be demonstrated (fig. 6) by comparison with the contralateral extremity. When the extremity of an unoperated rabbit was compared microangiographically with an amputated extremity, the vascularization in the amputation stump in many cases was reduced and was composed of an abnormally developed vascular network. However, microangiography is a morphological research method which cannot give information about the function of the exhibited vessels. On the other hand, in vivo angiography

permits a study of vascular function. *Intracardial angiography* (VI) has been used to show arterial and venous function in the amputated extremity and thereby clarify a number of conditions that are important in the blood flow of the amputation stump. These conditions will be discussed in the following chapters.

CHAPTER VII

MUSCLE BLOOD FLOW AND ARTERIOVENOUS SHUNT FORMATION IN THE AMPUTATION STUMP

Previous investigations have demonstrated the occurrence of arteriovenous shunts in the amputation stump (Leriche 1950, Erikson 1965, Langhagel 1968).

Using in vivo angiography of rabbits injected with contrast into the carotic artery Hulth & Olerud (1962) observed in single exposures rapid vein filling in the amputation stump following crus amputation and interpreted this as a sign of shunt formation. After crus amputation in rabbits in an investigation on the influence of immobilization on vascularization they had already observed in 1960 increased vascularization, dilation of supplying arteries and increased flow and rapid vein filling.

In clinical arteriographic studies on the amputation stump closed without myoplasty Erikson (1965) also found signs of arteriovenous shunts, more frequently after crus than after femur amputations.

Hulth & Högström (1961) measured PO_2 and pH in the venous and arterial blood of amputated patients and observed higher PO_2 values in the venous blood from the amputated than from the normal extremity, and they interpreted this as arteriovenous shunting. This difference was greatest immediately after amputation, as well as in cases of stump pains and in patients with atrophic and inactive muscles.

PRESENT INVESTIGATION

Intracardial angiography (VI) makes visible both the arterial and the venous circulation and reveals the degree of dilation in arteries and veins. The transit time can be calculated on the basis of the contrast flow in arteries and veins in 6 exposures taken at 2-second intervals (Fig. 7). The rapid vein filling began at the same time as dilation of the supplying arteries and the increased appearance of contrast filled vessels in the amputation stump (fig. 8). These findings can result from the formation of arteriovenous shunts in the amputation stump. If simultaneous contrast filling of arteries and veins is assumed to result from arteriovenous shunting, the occurrence of arteriovenous shunts as illustrated in figure 7 should be found.

It can be seen in figure 7 that a–v shunt formation was most frequent after amputation on the crus without myoplasty. On the other hand, shunt formation and vein filling occurred only rarely after proximal crus amputation with myoplasty or after knee disarticulation, and this was also true following distal amputation of the femur. After amputation in the middle of the femur no arteriovenous shunts appeared. Following amputation without myoplasty a–v shunts developed after ½ week and after distal amputation of the femur after 1 week, whereas shunt formation after the other operations was observed after the second week.

Determinations of muscle blood flow (III–IV) showed that the increase in MBF taking place in the amputation stump was larger after proximal amputation on the crus with myoplasty than after amputation at the same level without myoplasty or after distal amputation. Similarly, MBF was larger after knee disarticulation than after distal amputation on the femur. MBF after distal amputation on the crus and femur was reduced at the end of two weeks, and also after proximal crus amputation without myoplasty at the end of the fourth week, whereas MBF following proximal crus amputation with myoplasty persisted at an increased level until the twelfth week after the operation. The secondary reduction in MBF after the mentioned forms of amputation can be caused by the formation of arteriovenous shunts in the amputation stump. Measurement of MBF

OPERATION	1 HOUR	1/2 WEEK	1 WEEK	2-3 WEEKS	4-5 WEEKS	6-7 WEEKS	>10 WEEKS
AMP DISTALLY ON CRUS +MYOPLASTY	XXXXX	XXXXX XX ooo	XXXXX XXX ΔΔ	XX ooo	XX ●●●●	XXXXX	XXXXX ●●●●●
AMP. PROX. ON CRUS -MYOPLASTY	XX XX	XXX XX ooooo	XX ●●●● XX ΔΔ	XX ΔΔΔ	XX ●●●●● XX ●●●●●	XX ●●●● XX ●●●●●	
AMP. PROX. ON CRUS +MYOPLASTY	XX XXX	XX XXX	X XX	XX ΔΔΔΔ XX ΔΔΔ	XXXX	XXXXX	
AMP. PROX. ON CRUS +PLUGGING	XX	XX	XXXXX XXX ΔΔΔ	XXXX oooo	XXX ●●● XX oo	XX ooo	XXXXX XXXXX ●●●
EXARTICULATIO GENU	XX	XX	XX ΔΔΔ	XX ΔΔ XX Δ	XXXXX ΔΔ XXXXX ΔΔ		XXXXX
AMP. DISTALLY ON FEMUR			XX ooo	XX ooo	XX oo	XX	XX ooo

x ARTERY OCCURRENCE
Δ VEIN SLIGHT OCCURRENCE
o VEIN MODERATE OCCURRENCE
● VEIN MARKED OCCURRENCE

Fig. 7. The rate of contrast passage (transit-time) in the arteries and veins in the amputation stump. One sign means one picture = 2 seconds.
— = a–v-shunt.

in the ipsilateral quadriceps (fig. 3a) displayed a secondary rise in MBF after proximal crus amputation without myoplasty. This took place at the same time as the appearance of a–v shunts in the stump and can be due to increased blood flow in the proximal part of the extremity as a consequence of shunt formation.

PREVIOUS INVESTIGATIONS

Incidence of arteriovenous shunts in striated musculature

The incidence of a–v shunts in striated musculature under normal conditions has been discussed considerably. De Takats (1955) asserted that they can occur in sizeable amounts distally in the extremities. Zweifach & Metz (1955) described them as "preferential channels". Barlow et al. (1958) were not able to detect a–v anastomoses in anatomical studies of muscles in the cat and rabbit, but they assumed that the permeability of capillary beds could be altered under certain circumstances.

Effect of arteriovenous shunts

The hemodynamic changes that take place with arteriovenous shunting are best illustrated by the

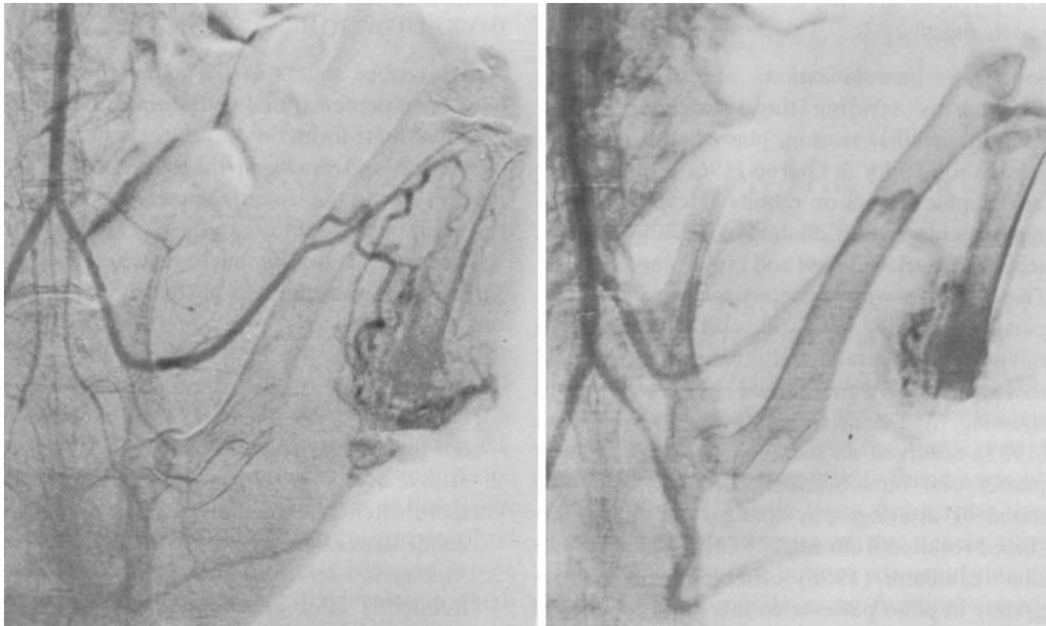

Fig. 8. Intracardial angiography. 32 days after amputation without myoplasty. a) Arteriogram 2 seconds after injection. b) 6 seconds after injection.

changes occurring with the formation of an arteriovenous fistula. Holman (1968) found that such a fistula produces a permanent reduction of the circulating blood in the normal capillary bed, which follows the lower resistance in the veins than in the capillaries. This gradually causes an increase in blood volume and dilation of the heart, arteries and veins proximal to the fistula, due to the distending effect of the increased blood volume. The reduced vascularization distally causes a compensatory collateral development. Due to its higher resistance the distal part of the extremity furnishes blood to the areas of lesser resistance. A second factor of importance in these changes following fistula formation is the venous stasis that is brought about by the increased venous pressure. Keck & Kelly (1965) believed, however, that there was an "active stasis" with more rapid flow through the developed collaterals.

Arteriovenous shunting after trauma

In 1930 Blalock & Bradburn demonstrated that soft tissue trauma was accompanied by increased oxygen saturation of the venous blood in a traumatized extremity, and they interpreted this as an expression of arteriovenous shunting. Lewis & Lim jr. (1970a) showed by intracellular labelling with radioactive Rubidium that stump trauma induced a rise in total flow in the traumatized extremity and a fall in flow in the contralateral extremity, lasting for 1–2 hours. Due to the contralateral effect a central related dilation could be excluded, and since Rb was not liberated from the blood the increased flow must be caused by a non-nutritional flow. A corresponding rise in total flow was obtained by sympathectomy. The reaction to trauma, therefore, corresponded to the reaction to sympathectomy, as stated by Barcroft & Swan (1953), and by additional investigation with I^{131}-albumin Lewis & Lim jr. (1970b) showed that the post-traumatic increase in non-nutritional flow was produced by a "physiological shunting" and not by an anatomical shunting through arteriovenous connections.

Arteriovenous shunting after immobilization and muscle inactivity

Following immobilization of an extremity achieved by severing the sciatic nerve, severing the achilles tendon, plaster casts and crus amputation Hulth & Olerud (1960) found in arteriographic studies on rabbits a development of hypervascularization, dilation of the large arteries, accelerated arterial flow and rapid venous return. These changes were detected one week after amputation and they were still substantial 10 weeks following the operation. Hulth & Olerud thought these changes were caused by arteriovenous shunting. Measuring with a flowmeter, Imig et al. (1953) observed an increased blood flow after plaster cast immobilization of dogs, and they found by arteriography changes which they assumed resulted from arteriovenous shunt formation. Braibanti (1958) observed with arteriography in polio patients an increased number of arteriovenous shunts in the paralysed extremities.

RESULTS OF THE PRESENT INVESTIGATION

Arteriovenous shunts in the amputation stump have been demonstrated with intracardial angiography. Shunt formation produces a decrease in capillary blood flow and therefore plays a significant role in the essential muscle blood flow in the amputation stump.

The present studies have shown that shunt formation in the amputation stump is dependent on:

1. *Stump closure,* as a–v shunts occur most frequently after proximal crus amputation without myoplasty. This can be due to
2. *Muscle inactivity,* as a–v shunts also develop more often after amputation distally on the femur than after knee disarticulation.
3. *Amputation level,* as a–v shunts occur more frequently after crus amputation than after amputation on femur.

CHAPTER VIII

THE INFLUENCE OF MUSCLE INACTIVITY ON THE VASCULAR CHANGES OCCURRING AFTER AMPUTATION

AMPUTATION AND MUSCLE FUNCTION

Amputation causes functional changes in the muscles of the amputation stump. After crus amputation the soleus muscle is transformed from a 2-joint muscle to a 0-joint muscle, while the gastrocnemius muscle is reduced by amputation from a 3-joint to a 1-joint muscle. After femur amputation the cessation of the quadriceps' distal fixation represents the greatest change in muscle function, in contrast to knee disarticulation where the distal muscle attachment is preserved.

Loon (1960) thought that "classical" amputation with transverse severing of muscles most often produced a complete inactivity of the retracted muscle remnant, accompanied by atrophy and partial or total fat degeneration, thus forming a barrier to venous return. In contrast, Langhagel (1968) stated that only traumatic lesions in muscles caused total muscle degeneration. The inactivation atrophy in the amputation stump was accompanied by interstitial fat deposition and diminished cell volume, but these changes are to a certain extent reversible, and the muscles that lose their normal attachment become bound by fascial connections and therefore preserve their contractile capacity. Weiss (1960) found in electromyographic studies that amputation changed the normal isometric muscle function to an isotonic contraction type, which resulted in gradual muscle atrophy. In the absence of distal attachment, he observed a reduction or cessation of electrical activity and an interruption in the centripetal signals and proprioceptivity, as well as an increased tendency toward contracture. If attachment was reestablished distally, the muscles reestablished their normal isometric function, thus counteracting muscle atrophy and interruption in muscle blood flow (Weiss: Physiological stump closure).

EARLIER INVESTIGATIONS OF MUSCLE IMMOBILIZATION AND MUSCLE INACTIVITY

In microangiographic and histological studies after immobilization of one of the rear legs of the rabbit, Hulth & Olerud (1961) found a vascular proliferation in muscle fascia and areolar tissue but no vascular changes in the muscle fibers. They concluded that both an increased shunting and a reduced muscle pump function caused a decrease in nutritional blood flow in capillaries and minor vessels, and that this brought about a stagnation in the dilated small vessels. This resulted in hypoxia and acidosis and provoked more or less extensive changes in the connective tissue (Eichelberger et al. 1956).

Eichelberger et al. (1958) found the same muscular changes after immobilization and denervation, while Ferguson & Akahoshi (1960) reported that muscle atrophy secondary to immobilization is different from the atrophy arising after inactivity. The completely relaxed muscle displays considerable atrophy, while the extended muscle undergoes modest weight loss or even hypertrophy. After immobilization of one rear leg in the rabbit they found a sizeable dilation of vessels corresponding to larger arteries, while no changes were observed in the small vessels in muscles. After nerve resection or tenotomy, on the other hand, no changes in the large vessels occurred, while changes were seen in the muscle vasculature, with formation of hair-fine vessels that were smaller than normal. In histological studies on the gastrocnemius and anterior tibialis muscles in the rabbit, Ferguson et al. (1957) found after 4 weeks of immobilization in plaster casts increased sarcolemma and fibrous degeneration but no fat degeneration or decreased muscle fiber size. Brooke & Bewick Slack (1959)

showed in rats and rabbits that growth of muscle fibers and connective tissue continued despite denervation, but that degeneration was increased. After tenotomy in rabbits Bergman & Afifi (1969) found pronounced degenerative changes after 4 weeks, with fat infiltration and atrophy of muscle fibers, while McMinn & Vrbová (1962) observed that the degenerative changes were different in the different muscle types. The "pale" muscle fibers in the peroneus were only slightly reduced after 3 months, whereas the "red" muscle fibers in the soleus were almost totally degenerated after 2–3 weeks.

After immobilization of the rear leg of the cat Cooper (1972) found that the degenerative changes in the muscles were the same as those that appeared after inactivity, but he observed that the regenerative process began rapidly when the leg with intact blood supply and innervation was freed from immobilization. The muscle fibers were more greatly reduced in size in the slow, tonic soleus muscle than in the rapid, phasic gastrocnemius muscle. An increased prominence of the nuclei was observed after 2 weeks, and after 4 weeks the muscle fibres were irregular in size and shape and the nuclei were located centrally. The number of nuclei increased from 6 to 22. Vacuoles located centrally in the fibers was interpreted as a sign of degeneration. Increased numbers of macrophages and red blood cells were observed, and connective and fat tissue increased in the interfibrillar space and the basal membrane in the vessel walls became thicker.

The muscle blood flow in immobilized muscles in the rabbit were studied by Na^{24} clearance determinations by Ferguson et al. (1957). The blood flow in the anterior tibialis and gastrocnemius was followed for as long as 10 weeks, and a rise of 39.4% with a maximum at 4 weeks was detected after immobilization in a plaster cast. Rothman & Slogoff (1967) investigated with Cr^{51}-labelled erythrocytes the capillary volume in rabbit leg muscles immobilized for 6 weeks in plaster casts, and they observed that there was no change in capillary volume compared with the contralateral extremity.

After femur amputation in the rabbit Itohara (1972) found a pronounced degeneration of muscles and degenerative vessel changes in the animals not amputated with myoplasty.

PRESENT STUDY OF MUSCULAR CHANGES IN THE AMPUTATION STUMP

An unpublished study was carried out to ascertain the morphological changes that occur in the musculature of the amputation stump. After fixation in 10% formalin, ½ cm thick cross-sections of the triceps surae were obtained from 22 animals and of the quadriceps from 18 animals, all of which had been crus amputated. After embedding in paraffin, 6 μ thick sections were cut and stained with hematoxylin-eosin and with van Gieson's staining method.

In cross-sections of the quadriceps a uniform picture was seen, comprising 5–6 edged muscle fibers of about the same size, with small, periferic located nuclei and without increased interstitial connective tissue. On the other hand, the triceps surae from the amputation stump presented a nonuniform histological picture, where normally shaped muscle fibers alternated with very abnormal fibers of variable size, containing swollen nuclei and intracellular vacuoles surrounded by abundant cell-rich connective tissue and sprinkled with adipose tissue. These signs of myogenic atrophy appeared most pronounced in amputation stumps without myoplasty (fig. 9), and they were more widely present in the soleus than in the gastrocnemius. As can be seen in table 3 these degenerative changes occurred in 6 out of 7 cases after crus amputation without myoplasty and in 4 out of 11 cases after amputation with myoplasty. These changes emerged as early as 1 week after amputation, but they could still be absent after 10 weeks in some cases.

DISCUSSION OF THE PRESENT RESULTS

There exists after amputation an immobilization of the amputation stump as well as an inactivation of the muscles within it.

Previous investigations demonstrated degenerative changes in muscles immobilized in plaster casts, or inactivated by tenotomy or nerve resection. Ferguson & Akahoshi (1960) found, however, considerable difference in muscle changes after immobilization and inactivation. Moderate weight loss or even hypertrophy as well

Table 3. Muscle degeneration in the amputation stump.

Operation	Time after amputation (weeks) 1	2	3	4	7	10	12	>12
Amp.prox.cruris – myoplasty	2	3	1 3	2 3		2		
Amp.prox.cruris + myoplasty		1	2	1	1 2	1 1 1	2	1 2
Amp.dist.cruris	1	3	3		1			

1: Normal muscle fibres and connective tissue.
2: Moderate degenerative changes.
3: Marked degenerative changes.

as dilation of the large arteries were detected after immobilization, whereas considerable muscular atrophy and development of small hair-fine vessels with no dilation of the large arteries could be seen after inactivation. Following immobilization of the gastrocnemius Ferguson et al. (1957) found increased interstitial connective tissue but no atrophy of muscle fibers and only slight weight loss, in contrast to a sizeable weight loss after tenotomy. Bergman & Afifi (1969) observed similar substantial degenerative changes after tenotomy, while McMinn & Vrbová (1962) found degeneration in the "red" soleus muscle but not in the "pale" muscles, such as the peroneus.

The morphological changes that were observed in the amputation stump in this investigation agree with those degenerative changes described

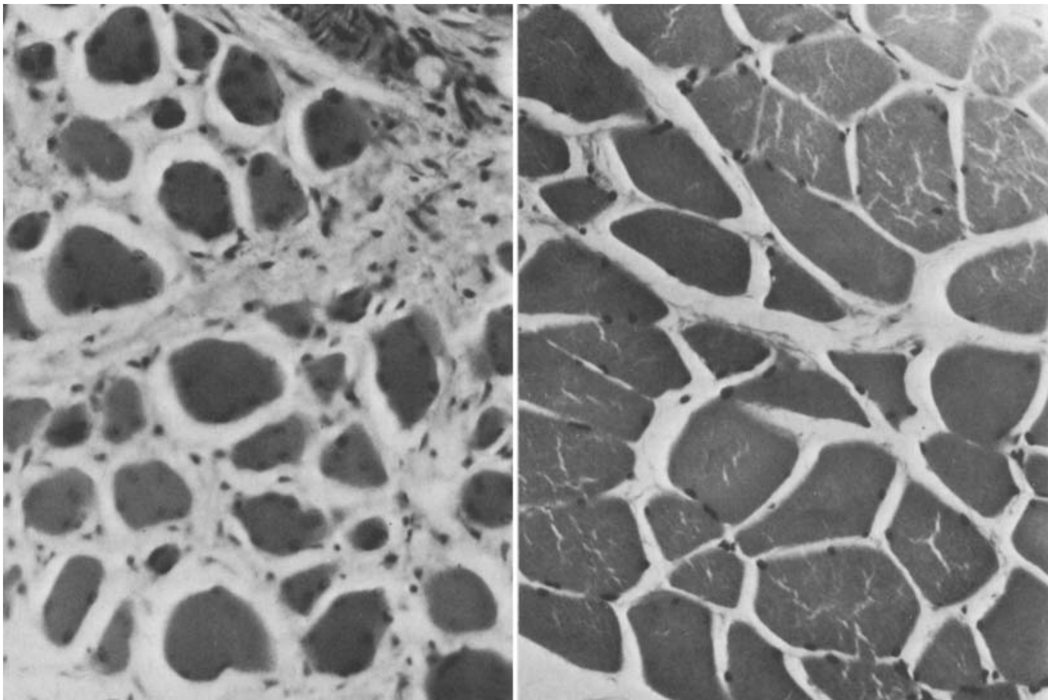

Fig. 9: Cross section of triceps surae. – a) 4 weeks after amputation proximally on crus without myoplasty. – b) 8 weeks after amputation proximally on crus with myoplasty.

by Cooper (1972). The changes were most pronounced after crus amputation without myoplasty. The resulting muscular inactivity corresponded with the inactivity following tenotomy. Moreover, the changes were found to be more extensive in the "red" soleus than in the "pale" gastrocnemius. The reason for this may be that the inactivation of the soleus, which involves the change from a 2-joint to a 0-joint muscle, is more extensive than of the gastrocnemius, which after crus amputation is still attached to the knee joint. No degenerative changes were detected in the ipsilateral quadriceps, despite the immobilization subsequent to crus amputation.

This investigation has demonstrated that stump closure with myoplasty counteracts those degenerative changes in the muscles. The effect of myoplasty on vascularization in the amputation stump will be discussed in the following chapter.

CHAPTER IX

THE INFLUENCE OF MYOPLASTY ON THE VASCULARIZATION OF THE AMPUTATION STUMP

PREVIOUS INVESTIGATIONS

The purpose of closing the amputation stump with myoplasty is, as stated by Mondry (1952) and Dederich (1960, 1963, 1967), to preserve the muscle's distal attachment, so that normal muscle tension is maintained. At the same time, an immediate closure of the medullary cavity is obtained so that venous return is normalized (Loon 1960). Dederich (1963) observed with arteriography that stump correction with myoplasty produced a greater vascularity in the amputation stump. Langhagel (1968) also found hypervascularization in the amputation stump after myoplasty, and he observed, moreover, an unchanged diameter in the supplying arteries for as long as one year, in contrast to Hasse et al. (1960) and Erikson (1965) who found reduced diameters in the main arteries in the amputation stump closed without myoplasty. Weiss (1969) reported increased temperature in the amputation stump closed with myoplasty, whereas Loon (1960), Erikson & Hulth (1962) and Erikson (1965) measured skin temperatures on the amputation stump that were lower after conventional amputation than on the intact leg.

Following experimental amputation on rabbits Hulth & Olerud (1962) and Erikson & Olerud (1966) also observed hypervascularization in the amputation stump after crus amputation with myoplasty. Moreover, Hulth & Olerud (1962) found the diameter of the femoral artery to be unchanged for up to 3 months after the operation, whereas Erikson & Olerud (1966) observed dilation of arteries and veins in the amputated extremity, maximally after 4 weeks and unchanged thereafter during the subsequent 4 months. After femur amputation on rabbits Itohara (1972) found with arteriography an increased vascularization of the stump after closure with myoplasty.

Previous investigation have shown, therefore, that myoplasty causes arteriographic changes in the amputated extremity. However, no earlier investigation has been carried out to examine the effect of stump closure with myoplasty on muscle blood flow in the amputated extremity.

PRESENT STUDIES

Muscle blood flow determinations (III) have shown that myoplasty of the amputation stump after crus amputation evokes a sudden rise in MBF in the stump (fig. 2). After amputations without myoplasty the rise in MBF took place around the fifth postoperative day and the mean flow in the first 10 days was 103% of the preoperative flow. After closure with myoplasty a rise in MBF occurred after one day, and the mean blood flow in the first 10 days was 178% of the preoperative flow. A greater blood flow also occurred in the amputation stump 1–12 weeks after the operation. In 5 animals amputated without myoplasty the mean blood flow was 145%, while in 5 animals with myoplasty the mean flow during the same period was 210% of the preoperative flow. In one animal amputated without myoplasty the MBF rose in the amputation stump to the same level as after amputation with myoplasty, the mean flow was 203% of the preoperative flow 1–7 weeks after the operation.

Microangiography of adult rabbits (IX). After proximal amputation of the crus without myoplasty the vascularization of the amputated extremity was normal for the first 2 weeks. Thereafter, there was a decreased filling of contrast in the supplying arteries and in the vascularization of the amputation stump, where small, irregular, spiral-twisted vessels were formed (fig. 10a). Following crus amputation with myoplasty, on

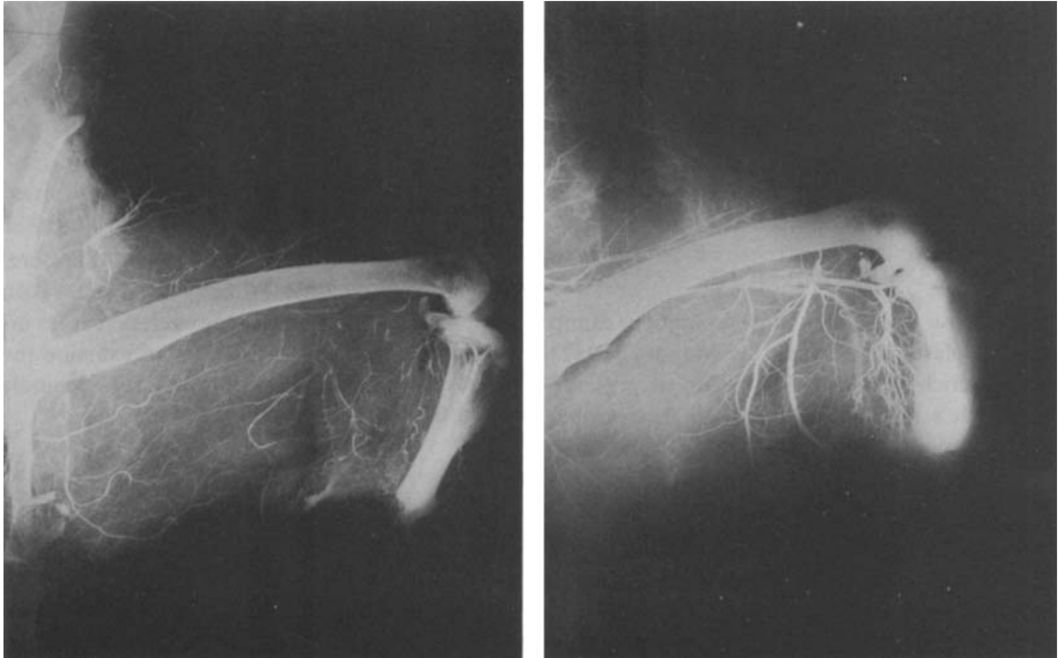

Fig. 10: Microangiography of the amputation stumps 30 days after amputation on crus a) without myoplasty and b) with myoplasty of the stump.

the other hand, hypervascularization of the amputation stump with dilation of the supplying arteries occurred 1–8 weeks after amputation (fig. 10b), after which the vascularization declined and changes in the amputation stump resembled those that take place following amputation without myoplasty. Twelve weeks after amputation, contrast filling of the veins in the amputation stump was observed.

When vascularization of the amputation stump is compared with vascularization of the triceps surae in an unoperated animal, by means of cross-sections of the musculature proximal on the crus (fig. 11a), it can be seen that the normal vessel structure is changed. Instead of observing the uniform bifurcation of the large superficial arteries in the muscle, irregular, twisted, diametervarying vessels, with a tendency to form a "ball of yarn", can be seen (fig. 11b). The abnormal changes were most pronounced in amputation stumps without myoplasty.

In a *microangiographic study of immature rabbits* (I) increased vascularization in the amputation stump after crus amputation was observed for up to 3 weeks, both after amputation with and without myoplasty. Formation of spiral-shaped arteries in the stump occurred most frequently in the animals amputated without myoplasty.

It was mentioned in chapter VII that *intracardial angiography* revealed that myoplasty counteracts formation of arteriovenous shunts in the amputation stump.

DISCUSSION OF THE PRESENT RESULTS

Myoplasty of the amputation stump causes changes in the muscle blood flow in the amputated extremity. The initial fall in MBF in the stump was short, the increase began after 1 day, and an initial increase in the blood flow in the ipsilateral quadriceps was seen at the same time (fig. 2b, 3 b). These effects may be due to a reduction by myoplasty of the initial vasospasm in

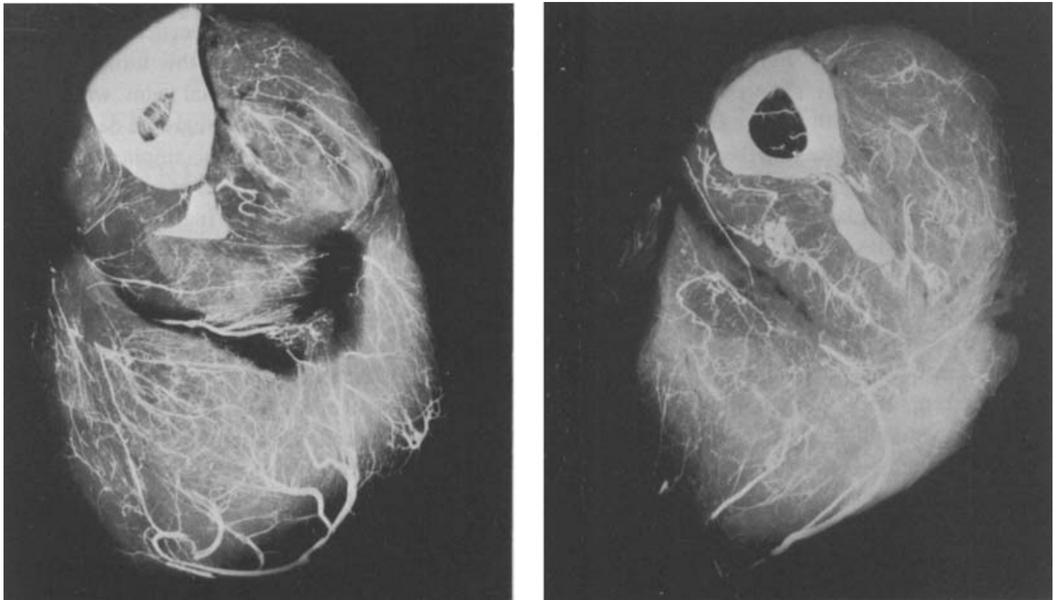

Fig. 11: Cross section of triceps surae a) in an unoperated leg, b) 6 weeks after proximal crus amputation with myoplasty.

the arteries of the extremity provoked by amputation trauma.

After proximal crus amputation with myoplasty it was found that MBF increased for as long as 80 days after the operation, whereas it decreased after 20–30 days in amputations without myoplasty. The reason for this difference may be due to preserved muscle function (Dederich 1963).

Following knee disarticulation MBF in the quadriceps increased for as long as 130 days after the operation. The distal attachment of the quadriceps remains intact in knee disarticulation, which preserves the muscle tension, and there is no opening in the medullary cavity and therefore no change in the intramedullary pressure. An attempt to obtain the same advantageous conditions for muscle function and venous return is made by stump closure with myoplasty (Loon 1960). After both knee disarticulation and proximal crus amputation with myoplasty only slight development of arteriovenous shunts occurs (fig. 7). Since the muscle blood flow after proximal crus amputation with myoplasty was somewhat greater than after knee disarticulation, the reason may be due to a better arterial supply to the triceps surae through a richly developed vascular net in the knee region than to the quadriceps through the femoral artery.

By use of microangiography (I, IX) hypervascularization of the amputation stump and dilation of the supplying arteries after proximal crus amputation with myoplasty was seen. This is in agreement with the MBF determinations. On the other hand, no hypervascularization in the stump nor dilation of the supplying arteries after crus amputation with myoplasty could be observed in *in vivo* angiography (VI) (fig. 21a). This method of investigation did reveal hypervascularization of the stump and dilation of the supplying arteries, due to formation of arteriovenous shunts (fig. 8). Therefore, arterial dilation and hypervascularization observed in *in vivo* angiography cannot be interpreted as a sign of increased muscle blood flow.

Hypervascularization detected microangiographically in the amputation stump following myoplasty can be caused by immobilization of the

amputated extremity (Ferguson & Akahoshi 1960 and Hulth & Olerud 1961). In muscles inactivated by tenotomy Ferguson & Akahoshi (1960) found vascular changes in the form of fine, hair-like vessels that were smaller than normal. In the present investigation similar changes were seen in the amputation stump after amputations without myoplasty.

Hypervascularization in the amputation stump can also be caused by trauma to soft tissue (Baumgartl et al. 1958, Wray & Lynch 1959, Ray et al. 1967 and Kellerová et al. 1970). Therefore, the reason why a corresponding microangiographic picture was not obtained from animals amputated without myoplasty may be that there was more extensive surgery on muscles and an accompanying occlusion of a large number of arteries. Hulth & Olerud (1962) assumed that the occurrence of pathological spiral-shaped vessels resulted from inadequate circulatory supply to the reduced amount of tissue in the stump. Erikson (1965) thought that tissue trauma and the subsequent arterial occlusion caused a pressure increase in – and a dilation of – the preserved arteries, and the formation of new spiral-twisted vessels. But since these spiral vessels only developed in about 50% of the cases, Erikson (1965) suggested that other factors, such as metabolic disturbances and interrupted nerve control, could play a role.

Intracardial angiography (VI) revealed the presence of veins in the amputation stump that were most pronounced in cases where the medullary cavity remained open after amputation. This could be a sign of venous stasis in the amputation stump, due to cessation of intramedullary pressure. However, contrast filling of veins requires that the return flow proceeds with a certain flow rate and positive pressure. Loon (1960) detected no contrast filling in the deep veins in the amputation stump before the medullary cavity was closed and the positive intramedullary pressure was reestablished. Vein filling, therefore, is not a sign of stasis but of a normalization of the venous return. The increasing occurrence of superficial veins in the amputation stump after closure of the medullary cavity can result from normalization of the intramedullary pressure. Langhagel (1968) showed with intraosseous phlebography that the length of the stump played a role in the distribution of the venous return between the superficial and deep veins. After crus amputation this took place essentially through the superficial veins, while after femur amputation it was through the deep veins.

The vein filling after crus amputation takes place through the superficial veins, but this is contravened by myoplasty. It must be assumed, therefore, that contrast filling of the superficial veins is conveyed cheifly through arteriovenous shunts in the amputation stump, and does not result from normalization of the intramedullary pressure. After proximal crus amputation with plugging of the medullary cavity (chapter XII), on the other hand, contrast filling of deep veins was found. This is in agreement with Loon (1960) and it can be a sign of an improved venous return from the amputation stump. Following crus amputation of an extremity supplied with collaterals (chapter XIV) and after the amputation stump was closed with myoplasty, contrast filling of deep veins also occurred. Stump closure with myoplasty, in this case, may have produced an improved venous return from the amputation stump.

The present investigation has disclosed that myoplasty has the following effects on the vascularization of the amputation stump:

1. Myoplasty counteracts the vascular changes in the amputation stump that are caused by tissue trauma and arterial occlusion. Blood flow is carried principally through normal dilated arteries, but formation of pathological, spiral-twisted vessels also occurs. The vascular changes are characterized by immobilization but not by muscle inactivity.
2. Myoplasty contravenes formation of arteriovenous shunts in the amputation stump by preservation of muscle function.
3. Myoplasty improves the venous return from the stump.
4. Myoplasty improves muscle blood flow in the amputation stump. The initial reduction of blood flow is diminished and the amputation stump reacts by increasing blood flow in the same way as an extremity normally reacts to soft tissue and bone trauma.

CHAPTER X

THE INFLUENCE OF THE AMPUTATION LEVEL ON THE VASCULARIZATION OF THE AMPUTATION STUMP

PRESENT STUDIES

Determinations of the muscle blood flow in the amputation stump revealed that the amputation level plays a role in the vascular reaction in the stump (IV). After crus amputation an increased blood flow in the amputation stump was always observed, and it was larger after proximal than after distal amputations. After knee disarticulation a persistent rise in MBF appeared in the quadriceps, while amputation of the femur only caused a transient rise in blood flow in the amputation stump (fig. 2, 4).

Angiographic investigations (VI) disclosed that after amputation in the middle of the femur no contrast filling of the femoral artery took place distal to the junction with the circumflex artery, while it was contrast filled after knee disarticulation. The reason for this is that there is no profound femoral artery in the rabbit. This is replaced by the genu suprema and saphena arteries, which branch off from the distal part of the femoral artery.

Intracardial angiography revealed additionally that development of arteriovenous shunts in the amputation stump was more pronounced after amputation of the crus than after knee disarticulation (fig. 12) and after femur amputation. No sign of shunt formation was detected after amputation in the middle of the femur, but when the amputation was performed just proximal to the knee joint, so that the femoral artery was preserved, signs of shunt formation also appeared after femur amputation.

DISCUSSION OF THE PRESENT RESULTS

It has been found in these studies on the rabbit that the vascular changes in the amputation stump are dependent on the level of amputation. When comparison is made of MBF in the amputation stump at different levels of amputation a number of the following conditions arise which are important to blood flow in the stump:

1. Anatomical condition.
2. Vascular reaction to soft tissue and bone trauma.
3. The altered function of the amputated extremity (consequences of immobilization and muscle inactivity).

1): Investigations revealed that MBF in the amputation stump is greater than the preoperative blood flow in the amputation level. An exception to this, however, is amputation on the femur where the absence of the profound femoral artery in the rabbit is expressed. On amputation, the arteries are ligated in the amputation level, which transforms them into terminal arteries that are occluded up to the junction with the first proximal side branch (Pearse 1928). This results in the amputation stump being supplied only with these side branches and with collaterals developed from them. The length and diameter of these collaterals is decisive for the perfusion pressure and thereby for the capillary circulation in the stump (Learmonth 1950). After amputation in the middle of the femur the femoral artery is occluded up to the junction with the femoral circumflex arteries, and the blood supply to the amputation stump is carried exclusively though these arteries and their collaterals. These collaterals are long, spiral-twisted and have small diameters. After knee disarticulation and distal amputation on the femur, the arterial supply though the saphena and genu suprema arteries is preserved and the amputation stump is supplied through these arteries. This anatomical condition can clarify the reason why knee disarticulation and distal femur amputation provide a greater blood flow to the amputation stump than does amputation in the middle of the femur. After amputation

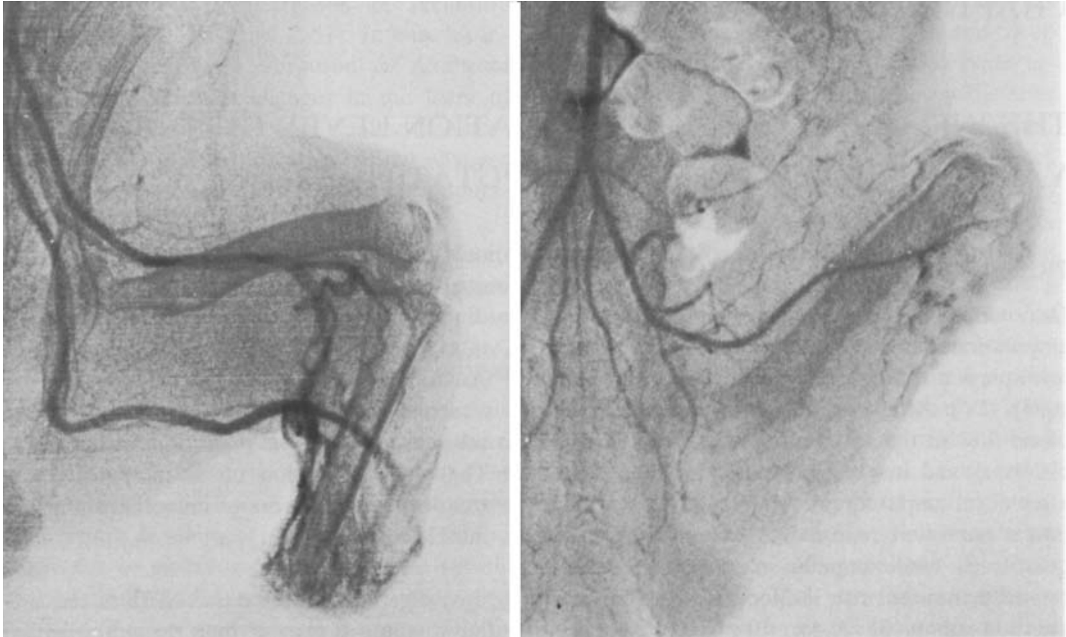

Fig. 12. Intracardial angiogram. a) 7 weeks after amputation on crus. Arteriovenous shunts are seen in the stump. – b) 3 weeks after knee-disarticulation on the same rabbit.

proximal on the crus, the amputation stump is supplied by the well developed arterial net in the knee joint region, while after amputation distal on the crus the blood supply is carried through long arteries and collaterals. This explains why blood flow in the amputation stump is smaller after distal than after proximal crus amputations.

2): Soft tissue trauma gives rise to a vascular reaction and an increased muscle blood flow in the amputation stump (Wray & Lynch 1959, Wray 1964 and Kellerová et al. 1970). This reaction is amplified by injury to the bone, which increases the "extraosseous" vascularization (chapter XI–XII). The reaction to soft tissue and bone trauma, however, cannot be mainly responsible for the increased blood flow in the amputation stump. MBF increases in the quadriceps after knee disarticulation where no surgery is performed on muscle or bone, and it increases in the triceps surae after distal crus amputation, despite the absence of surgery on the muscle. Therefore it looks like, even though there is a reduction in the muscle area of the extremity due to amputation, this is not accompanied by a proportional reduction in the blood supply to the extremity.

3): Arteriographic studies revealed that arteriovenous shunts are formed in the amputation stump. This shunt formation is dependent on the level of amputation, but is related to muscle inactivity in the amputation stump (chapter VII). Shunt development is most pronounced after amputation in the areas of the extremity where the anatomical possibilities for increased vascularization are greatest, i.e., after amputation proximal on the crus. Following amputation in the middle of the femur, which causes closure of the femoral artery, no shunt formation is detected, but if the femoral artery continues to function, e.g., after amputation distal on the femur, shunt formation does occur.

The investigations have thus shown that the am-

putation level is significant in the vascular reaction in the amputation stump.

The most ideal conditions for producing increased blood flow in the amputation stump are the following:

1. When the amputation is performed at a level where the arterial supply is conveyed through short arteries, which is the case at the level of the knee or proximal on the crus.
2. When the amputation is performed at a level where the vascular reaction in muscles and bone is greatest (see chapters XI–XII). This occurs after amputation proximal on the crus.
3. When muscle function is preserved, so that muscle inactivity and development of arteriovenous shunts is counteracted. This is obtained in amputation which preserves the knee joint.

The investigations also revealed that the most unfavourable conditions for blood flow in the amputation stump take place when the amputation is performed at a level where the arterial supply to the amputation stump is carried exclusively through a single artery, such as after amputation in the middle of the femur of the rabbit. Similar conditions will prevail after femur amputation in man, in which the profound femoral artery is occluded.

CHAPTER XI

BONE HEALING AFTER AMPUTATION AND ITS INFLUENCE ON THE VASCULARIZATION OF THE AMPUTATION STUMP

PRESENT STUDIES

The main purpose of these studies has been to ascertain the factors which have an influence on muscle blood flow in the amputation stump. One of the questions these studies has sought to answer is: how does the course of bone healing affect the blood flow in the amputation stump (VIII)?

Histological investigations of the bone in the amputation stump disclosed increased osteoblastic activity in the endosteum of the outer part of the medullary cavity, from which was developed osteoid tissue and spongy bone that sealed the cavity. (fig. 13). Following proximal amputation on the crus the cavity is sealed around 18 days after amputation, while the medullary cavity was closed 9 days after amputation in the middle of the femur and 12 days after amputation distal on the crus. No increased periosteal activity around the end of the stump was observed in association with closure of the medullary cavity, but spongy, partially cartilaginous exostosis developed on the outer side of the bone in proximity with the stump end after 2–3 weeks. When the medullary

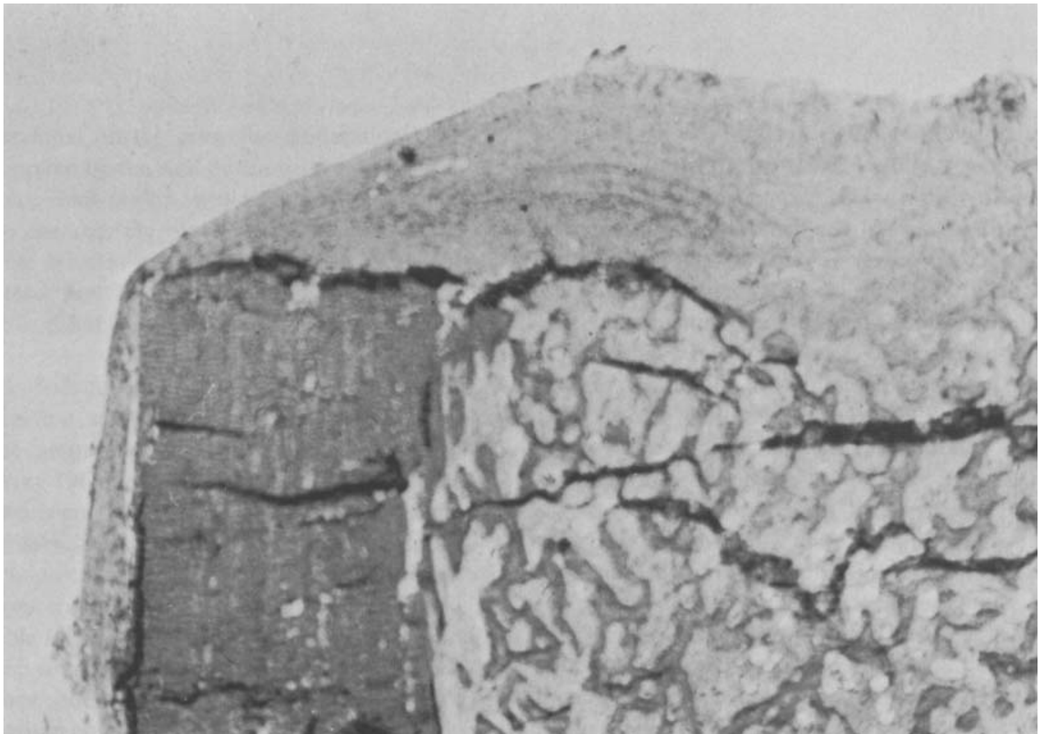

Fig. 13. The bone tip from the amputation stump. The medullary cavity is closed of spongious bone from the inside of the medullary aperture.

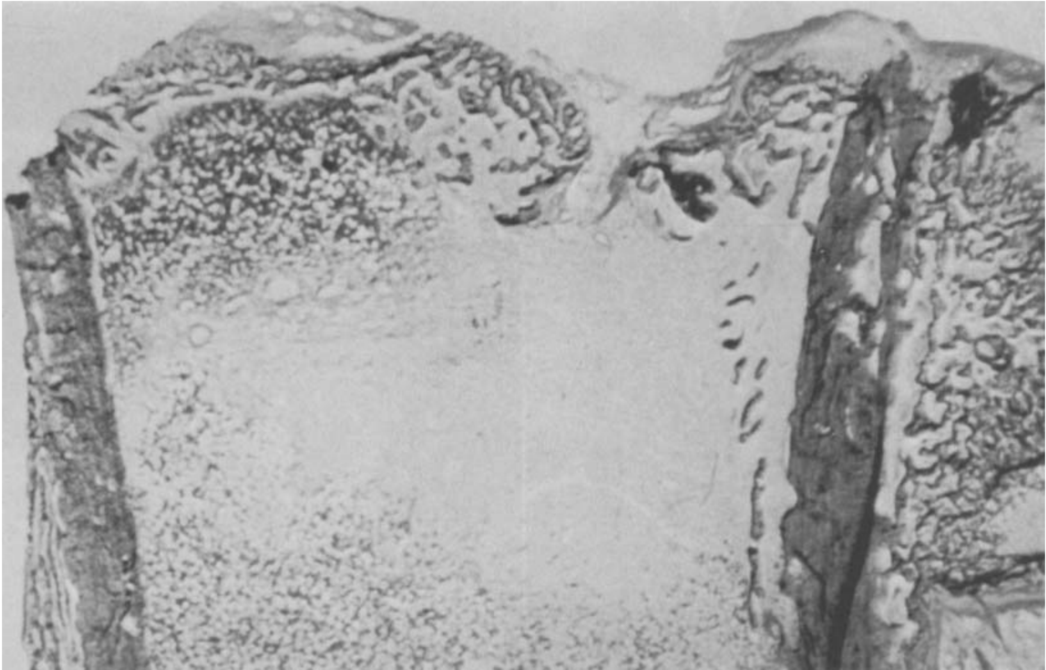

Fig. 14. The tip of the amputation stump, four weeks after amputation on femur.

cavity closed after amputation on the crus, spongy transformation of the bone end occurred, accompanied by some atrophy of the cortex and dilation of the medullary cavity. After femur amputation only sparse bone formation occurred, but pronounced cortical atrophy and medullary cavity dilation was present (fig. 14).

Microangiographic studies revealed hypervascularization of the bone in the amputation stump. Cross-sections of the bone showed that vascularization of the cortex depended on the level of amputation in crus amputations. In amputation corresponding to the tibiofibular synostosis a hypervascularization of the periosteum was seen 10–30 days after amputation, whereas hypervascularization of the cortex following distal amputation on the tibia began immediately after the operation, and was supplied essentially by medullary arteries (fig. 15). This difference may be due to disruption of the bone vascularization through the nutrient artery following amputation proximal on the crus.

In immature rabbits it was found that hypervascularization of the bone in the amputation stump was accompanied by acceleration of growth following crus amputation (fig. 16), and this occurred simultaneously with hypervascularization of the musculature. On the other hand, after knee disarticulation (II) no hypervascularization or increase in bone growth took place in the amputation stump.

PREVIOUS INVESTIGATIONS OF OSSEOUS HEALING IN THE AMPUTATION STUMP

In studies of osseous healing in the amputation stump of rabbits Hulth & Olerud (1962) found proliferation of newly formed vessels from both the periosteum and medullary cavity. Callus formed around these vessels, and the medullary cavity was closed 4 weeks after amputation. Erikson & Olerud (1966) found that the callus

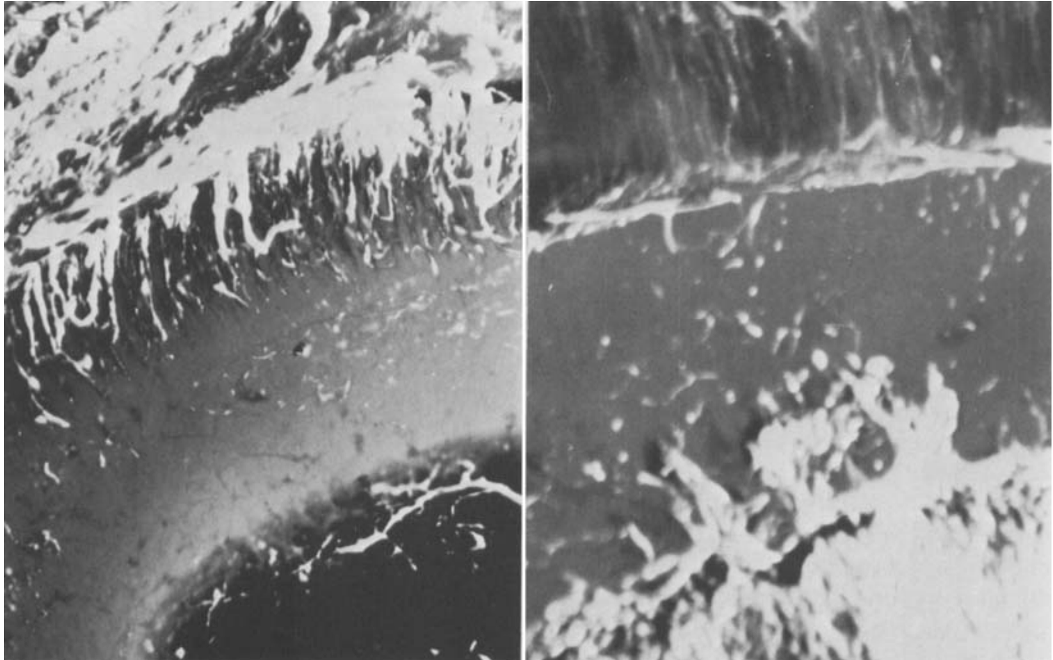

Fig. 15. Microangiogram of the amputation stump. – a) Two weeks after amputation proximal on crus. Hypervascularization of the periosteum and the superficial part of the cortex. – b) Four weeks after amputation distally on crus. Hypervascularization of the medullary cavity and the central part of the cortex.

formation often had the character of a spur, directed outwardly from the periosteum.

In clinical studies Langhagel (1968) observed that the medullary cavity was closed by a fibrous layer of connective tissue a few days after amputation. After 6 weeks he detected distinct resistance to the flow of injected contrast, there was no return flow of contrast and it accumulated in the medullary cavity. Very often the osseous closure remained eggshell thin, especially in the central region, under which loose, spongy bone structure was found. In studies on the bone end in relation to stump revision, Loon (1960) usually found no obliteration of the medullary cavity, and its opening most often was covered only by a thin layer of fibrous tissue. Reinhardt (1972) found local restructuring of the bone in the amputation stump, as well as diffuse osteoporosis and periosteal ossification.

Loon (1960) assumed, as did Erikson & Olerud (1966), that hematoma around the stump tip could be a contributing factor in spur formation, but he thought that both mechanical factors as well as release of the periosteum could also cause the formation of these spurs of spongy bone. On the femur, they were most often located at the adductor attachment, and muscle tension was ascribed particular importance to this. Dederich (1963) and Erikson (1965) with arteriography frequently observed an artery directed toward the spur, but Loon (1960) thought that such an artery was most often secondary.

Release of periosteum can cause formation of a subperiosteal cartilagenous callus (Richany et al. 1965), whereas cartilage is never formed below intact fibrous periosteum (Bast et al. 1925). Resection of the periosteum around the bone tip of the amputation stump was employed by Bunge (1899), who at the same time evacuated the extreme end of the medullary cavity. Dederich (1963) condemned this method which he though would always produce necrosis of the bone end.

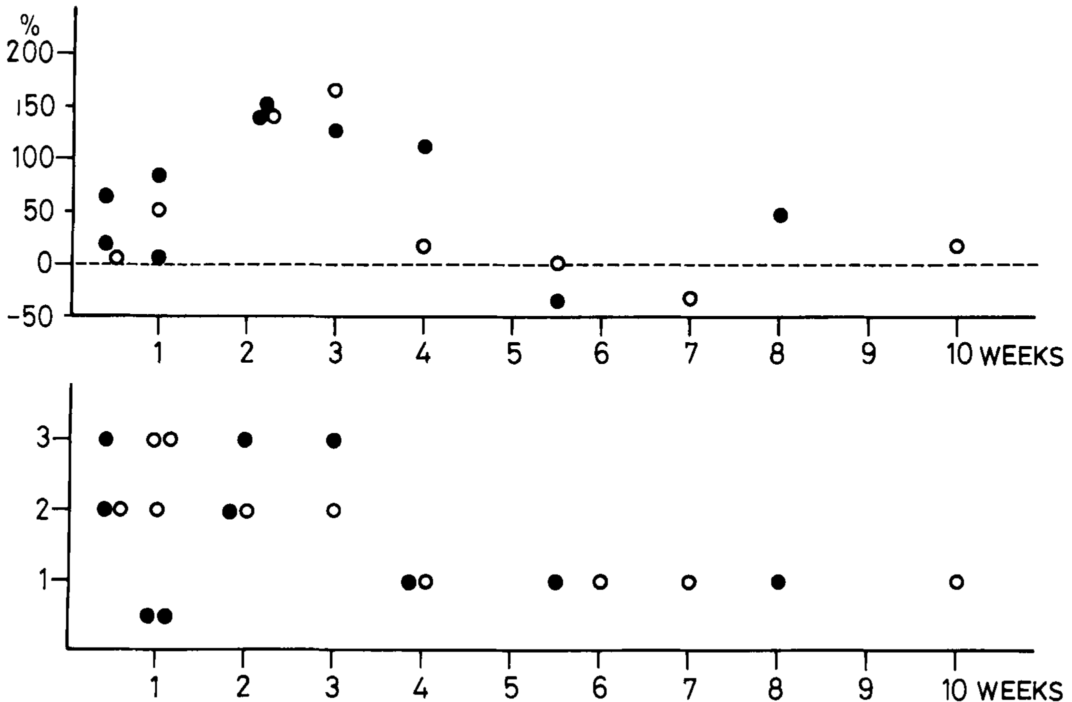

Fig. 16. Growth stimulation in bone (top) and hypervascularization in muscles (bottom) in amputation stumps after amputation on crus without myoplasty = ● and with myoplasty = ○ (young rabbits).
%: Percentage of growth in the contralateral extremity.
1–3: Explanation as fig. 6.

In contrast, Kozakiewicz (1951) found that periosteal stripping of the bone end caused fewer cases of bone necrosis and less spur formations on the stump, and Russian investigators (see Weiss 1971) have shown that periosteal stripping of the amputation stump increased the vascularization of the stump in the same manner as myoplasty.

BONE HEALING IN THE AMPUTATION STUMP AND FRACTURE HEALING

As mentioned earlier, Hulth & Olerud (1962) found that the bone end in the amputation stump healed similarly to a fracture with 2 fragments. In fracture healing the degree of dislocation and the disruption of intraosseous vascularization resulting from it are decisive in the course of healing. Rhinelander (1974) reported that callus formation after stable fractures developed as a medullary, bridge-formed callus which was vascularized exclusively from the medulla. On the other hand, a periosteal, bridge-formed callus that was supplied by the surrounding soft tissue through extraosseous arterioles developed after an unstable fracture. Periosteal callus formation can also develop after stable fractures (Rhinelander 1968). Following dislocated fractures he found that the periosteal vascularization played the most important role during the first 3 weeks of the healing process, and Göthman (1962) observed an increased arterial reaction in the periosteum for 3 weeks after dislocated tibial fractures in rabbits.

The periosteal reaction after fractures is due to disruption of the bone's blood supply through nutrient arteries. Trueta (1968) found after ligation of the nutrient artery in the rabbits tibia a hyperplasia of the periosteum, but only after simultaneous interruption of the vascularization

of the diaphysis from the metaphysis did an increased vascularization appear in the periosteum, ramifying into the cortex. In contrast, Brookes (1971) reported that ligation of the nutrient artery caused ischemia of the cortex, accompanied by cortical hypervascularization supplied from the periosteum. Shim et al. (1968) found that interruption of the nutrient artery in the femur of the rabbit produced an immediate reduction in the blood flow in the diaphysis, and Cuthbertson et al. (1964) demonstrated that ligation of the nutrient artery in the tibia and humerus of dogs caused a fall in the intramedullary pressure.

The course of the healing process in the amputation stump will depend on whether a disruption of the bone's vascularization through the nutrient artery occurs. When the nutrient artery is preserved the bone heals with a medullary callus, whereas when the nutrient artery is disrupted the periosteal reaction can be expected to be more pronounced.

Previous investigations of the relationship between bone healing and muscle blood flow

A point of particular interest in amputation is whether the described vascular changes during the course of healing in the bone were accompanied by corresponding vascular changes in the soft tissues.

Wray & Lynch (1959), Wray (1964) and Ray et al. (1967) showed in animal experiments that a fracture produced an immediate rise in the blood flow in the extremity, but Wray (1964) found that this rise was caused by trauma to soft tissue and not to the fracture.

Baumgartl et al. (1958) found with arteriography in clinical studies an expansion of the circulation and a more rapid contrast flow after fracture of the tibia. In a study of muscle and skin blood flow after tibia fracture, Kellerová et al. (1970) observed with plethysmography a rise in muscle blood flow of 216% compared with the intact leg, and this increase persisted for 9–18 months. They concluded that the increased blood flow during the first weeks was caused by trauma, in the second phase due to the healing process, and in the final phase caused by the immobilization of the extremity.

Fracture healing is dependent first and foremost on the medullary vascularization. If this is disrupted by the fracture, the "extraosseous blood supply" enters into the process (Rhinelander 1974). This is developed from torn blood vessels in the periosteum and soft tissue surrounding the fracture site and persists until the medullary blood flow is reestablished.

Zucman (1960) studied the relationship between vascularization in muscle, periosteum and bone in adult rabbits and found that revascularization of an ischemic muscle took place through anastomoses from the periosteum and bone and not from intermuscular adherences. If the muscles became separated from the periosteum no vascularization occurred, but if the periosteum was stripped from the bone a powerful dilation of the periosteal vessels and hypervascularization of the muscle took place. Similarly, Whiteside & Lesker (1978a) observed that separation of muscles from the periosteum, in contrast to subperiosteal stripping, compromised the vascularization of a traumatized muscle in the rabbit, while vascularization of normal muscles was not altered by extraperiosteal or subperiosteal dissection. They also found (1978b) that bone healing after osteotomy began more rapidly after subperiosteal stripping than after extraperiosteal dissection.

Periosteal stripping produces increased bone growth, which has been observed among others by Brodin (1955) and Solá et al. (1963). Brodin found that periosteal stripping proximal on the tibia of the rabbit elicited a growth reduction in the proximal – and growth increase in the distal – tibia epiphyses. He assumed the reason for this was an interruption of the vascular continuity between the periosteum and bone with an increased blood flow in the epiphyseal-metaphyseal region.

Previous investigations of the effect of inactivity on the bone

Loon (1960) found that reduced load and function in the amputation stump brought about osteoporosis in the bone of the stump. Sevastikoglou et al. (1969) found in clinical studies that atrophy of the bone in the amputation stump al-

ways takes place after both crus and femur amputations and, moreover, they found that a transient bone atrophy in the ipsilateral femur occurred following crus amputation.

A number of experimental studies have demonstrated the effect of immobilization on bone development (Sundén 1967). The bone and its associated musculature are considered by many to be a functional unit, where normal muscle activity regulates bone building and breakdown through the vascular system. Geiser & Trueta (1958) showed with arteriographic and histological studies that tenotomy of the achilles tendon in the rabbit caused osteoporosis of the calcaneous accompanied by a transient hypervascularization, followed by a hypovascularization. They thought these changes were due to cessation of muscle tension and to the loss of weight. Ring (1961) found a transient hyperemia and an increased longitudinal growth in the bone after nerve and tendon resection. The deficient muscle tension reduced periosteal bone formation and caused an atrophy of the cortex and a dilation of the medullary cavity. Landry & Fleisch (1964) showed that nerve resection and resection of the achilles tendon or the ligamentum patellae produced primarily reduced bone formation, followed by both increased bone formation and bone resorption. After denervation of one rear leg of the rabbit Kharmosh & Saville (1965) found that both muscle and bone atrophy appeared immediately and persisted for the following 10 weeks, after which bone resorption was replaced by bone building.

Venous stasis resulting from muscle inactivity can also influence the osseous condition. After cessation of muscle pump function, Trueta (1968) and Brookes (1971) observed resorption of bone and dilation of the venous sinusoids. Moreover, Brookes thought that venous stasis could cause an increased cartilaginous callus formation, while Pritchard & Ruzicka (1950) assumed that cartilaginous callus resulted from reduced arterial vascularization. Hutchison & Burdeaux (1954) found that venous stasis accentuated the longitudinal growth of bone, while Keck & Kelly (1965) could not demonstrate such an effect.

McPherson et al. (1961) showed that restrained venous return caused a rise in bone blood flow, as a consequence of shunting from muscle to bone. Similarly, deficient muscle function can cause an increased vascularization in the bone (Brookes 1971).

DISCUSSION OF THE PRESENT RESULTS

It has been shown in histological investigations of the amputation stump that bone healing occurs by formation of osteoid and spongy tissue from the endosteum. The closure is dependent on the amputation level, and takes place later following amputation of the tibia than of the femur.

Amputation proximal on the crus causes a transient transformation of the blood flow to the cortex, with a hypervascularization from the periosteum. The reason for this may be that the circulation through the nutrient artery is disrupted by amputation, which causes an increased vascularization through the periosteal and metaphyseal arteries. This concept is supported by an increased growth in the proximal tibia epiphysis of immature rabbits in the first weeks after amputation. A transient increase in vascularization of the cortex from the periosteum also occurred after amputation of the femur, where no disruption of nutrient arterial vascularization occurred, but no evidence was found that the periosteum played any role in the closure of the medullary cavity, as reported by Hulth & Olerud (1962). On the other hand, periosteal callus developed around the stump tip during the period after medullary cavity closure. The reason for formation of these exostoses can, as proposed by Richany et al. (1965), be a release of periosteum or, as suggested by Brookes (1971), be a consequence of venous stasis. The observation that cartilaginous exotosis occurred most frequently in cases where the amputation stump was not closed with myoplasty is in agreement with this.

It was found, as demonstrated in clinical studies by Loon (1960) and Langhagel (1968), that the osseous closure of the medullary cavity often became quite thin and spongy in nature. The medullary cavity was preserved beneath this and a spongy transformation of the original cor-

tex occurred. Thinning of the cortex and the newly formed bone that closed the medullary cavity was most pronounced after amputation of the femur. This can be a consequence of deficient muscle activity and support, as proposed by Geiser & Trueta (1958) and Ring (1961).

After amputation of the crus the effect of immobilization on the bone was less conspicuous. The extreme end of the medullary cavity was obliterated by spongy bone. Bone building dominated here over bone breakdown. This increased bone activity was accompanied by an increased hypervascularization of the surrounding muscles.

It has been shown previously that periosteal stripping elicits hypervascularization of the surrounding muscles (Zucman 1960 and Whiteside & Lesker 1978). A periosteal stripping around the amputation tip can be a contributing factor in the hypervascularization of the muscles around it, as reported by Russian investigators (Weiss 1971).

CHAPTER XII

OSSEOUS MEDULLARY PLUGGING IN AMPUTATION

PRESENT STUDIES

The reason for use of osseous plugging of the medullary cavity after amputation was based on the observation that closure of the amputation stump with myoplasty caused an improved muscle blood flow in the stump. In order to ascertain whether that effect was due to myoplasty and its favorable effect on muscle function, or to closure of the medullary cavity and the normalization of intraosseous pressure, the medullary cavity after amputation was closed with bone from the amputated portion of the extremity (III).

Determination of muscle blood flow

Figure 17 shows MBF in the amputation stump after proximal amputation of the crus combined with osseous plugging of the medullary cavity, compared with crus amputation without plugging. It can be seen that MBF in the amputation stump increased with plugging and that the initial reduction of blood flow did not appear in these cases. The rise in MBF began more rapidly and was larger after plugging than after crus amputation with myoplasty (fig. 18a). When myoplasty and plugging were combined in the amputation stump (fig. 18b) a corresponding rise in MBF occurred, but this combination did not additionally accentuate MBF. In the ipsilateral quadriceps after crus amputation combined with plugging of the medullary cavity (fig. 19), an initial increase in MBF followed by a normalization can be seen, similar to the effect following crus amputation with myoplasty (see also fig. 3b). Moreover, plugging of the medullary cavity after femur amputation caused a rise in MBF in the amputation stump which was larger than the increase after myoplasty (fig. 20).

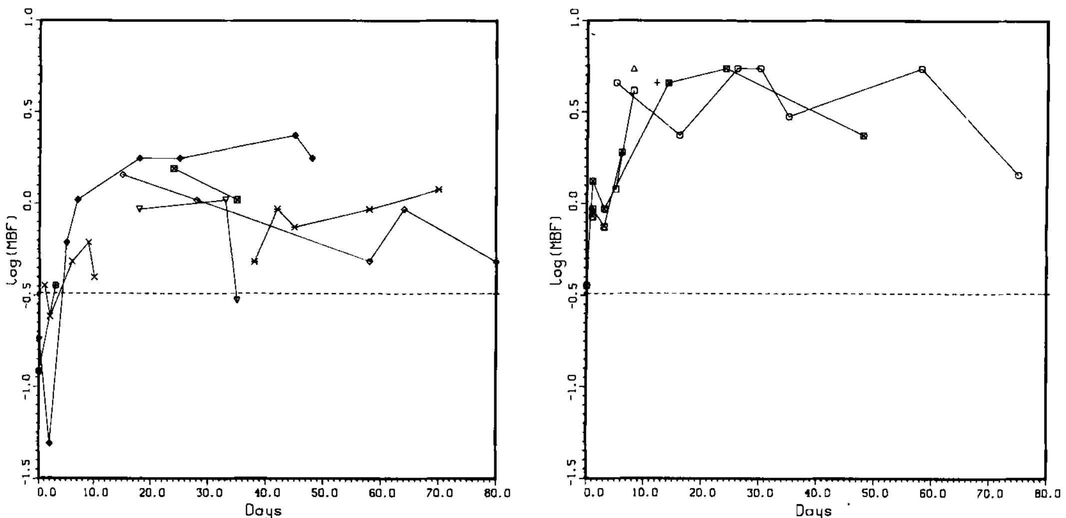

Fig. 17. MBF in the amputation stump after amputation proximally on the crus without myoplasty, 7 rabbits (a) and after osseous medullary plugging, 7 rabbits (b).

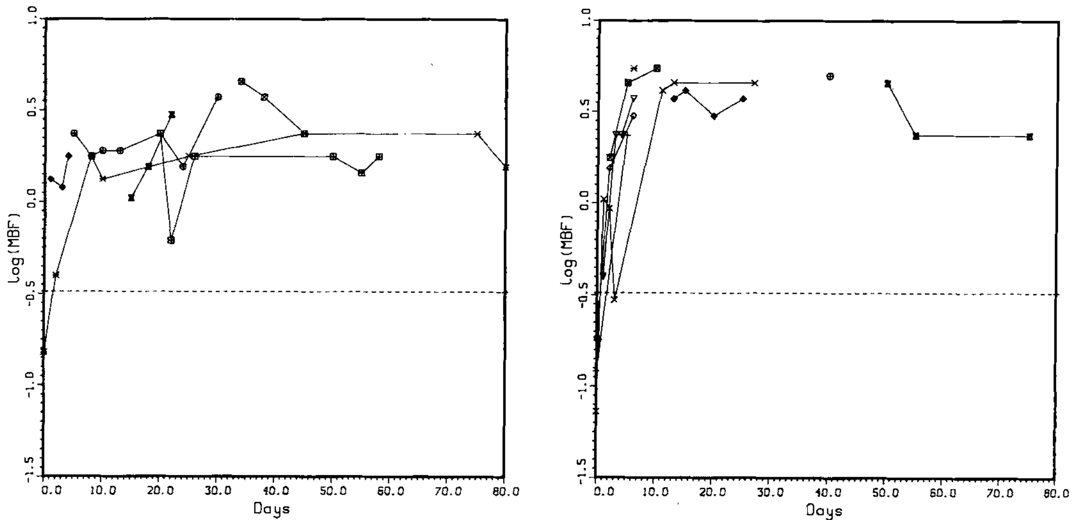

Fig. 18. MBF in the amputation stump after amputation proximally on crus with myoplasty, 6 rabbits (a) and after myoplasty combined with osseous medullary plugging, 9 rabbits (b).

Intracardial angiography

It is evident in figure 7 that medullary plugging produced an increased occurrence of arteriovenous shunts in the amputation stump, and this took place after both amputation of the crus and of the femur. In addition to filling of the superficial veins, a contrast filling of the deep veins can also be detected after plugging (fig. 21b).

A similar effect of osseous plugging was found if the crus amputation was undertaken immediately after ligation of the femoral artery (chapter XIV). Amputation immediately after arterial occlusion caused a prolonged decrease in vascularization in the amputation stump, but a more rapid appearance of arterial dilation and of rapid vein filling in the stump occurred with simultaneous plugging of the medullary cavity.

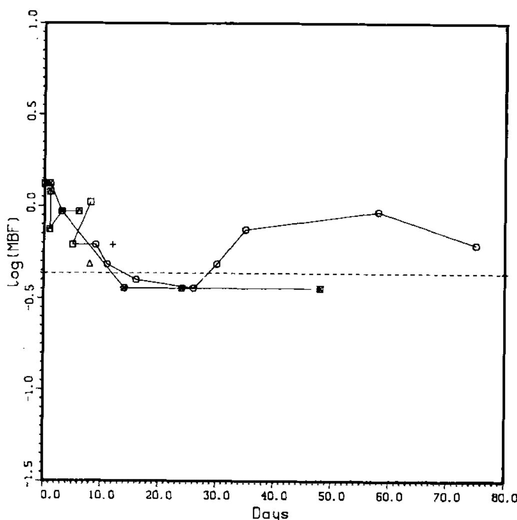

Fig. 19. MBF in the ipsilaterale quadriceps after amputation proximally on the crus combined with osseous medullary plugging, 7 rabbits.

Microangiographic and histological studies of the bone in the amputation stump (VIII) showed that plugging of the medullary cavity influenced the healing of the amputation stump. Closure of the medullary cavity began about 10 weeks after the operation, whereas it was closed at the end of about 3 weeks following myoplasty. Hypervascularization of the cortex persisted longer with than without plugging after amputation, and it came chiefly from the periosteum accompanied by periosteal callus formation, while no reaction occurred around the inserted plugs (fig. 22). Microangiography of the muscles in the amputation stump showed hypervascularization through newly formed, spiral-twisted arteries (fig. 23).

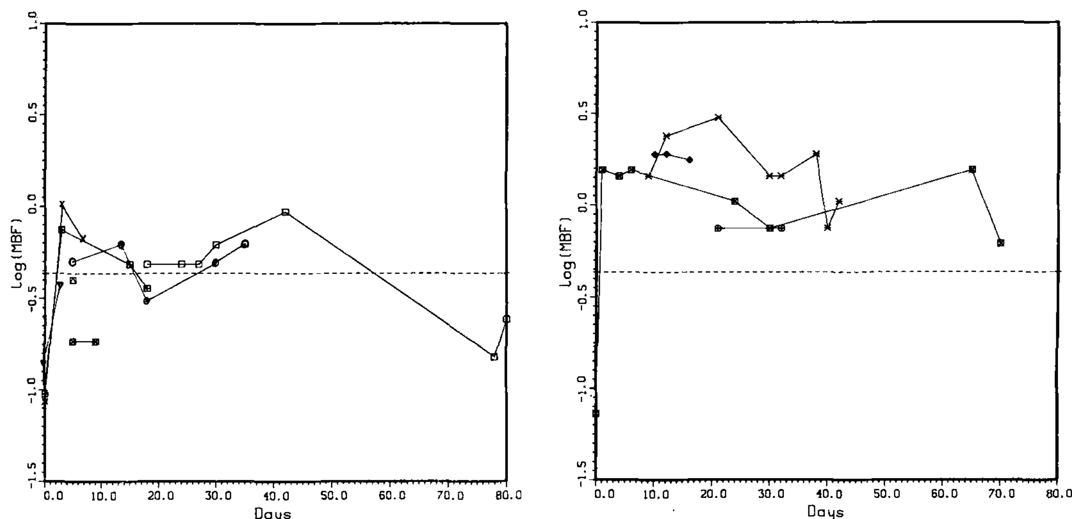

Fig. 20. MBF in the amputation stump after amputation of the femur with myoplasty, 7 rabbits (a) and after myoplasty combined with osseous medullary plugging, 4 rabbits (b).

PREVIOUS INVESTIGATIONS OF PLUGGING OF THE MEDULLARY CAVITY

As mentioned in chapter II, medullary plugging was used earlier in amputation, partly from a desire to produce a mechanically strong, weight-bearing stump (Kirchner 1920) and partly in combination with myoplasty to accelerate the closure of the medullary cavity and to normalize the intraosseous pressure (Loon 1960). However, the influence of medullary plugging on muscle blood flow in the amputation stump was not investigated previously.

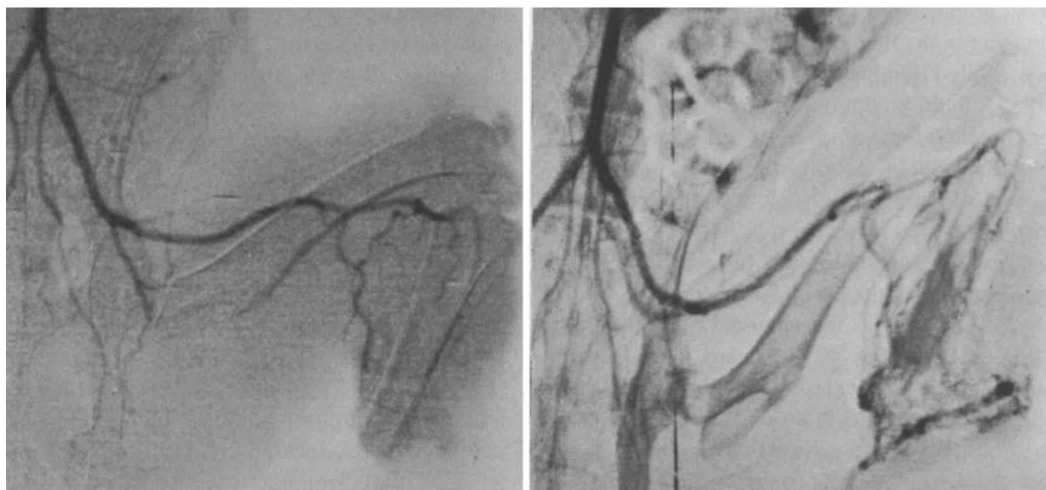

Fig. 21. Intracardial angiogram. – a) 20 days after amputation on crus with myoplasty. – b) 8 days after amputation on crus with myoplasty combined with osseous medullary plugging.

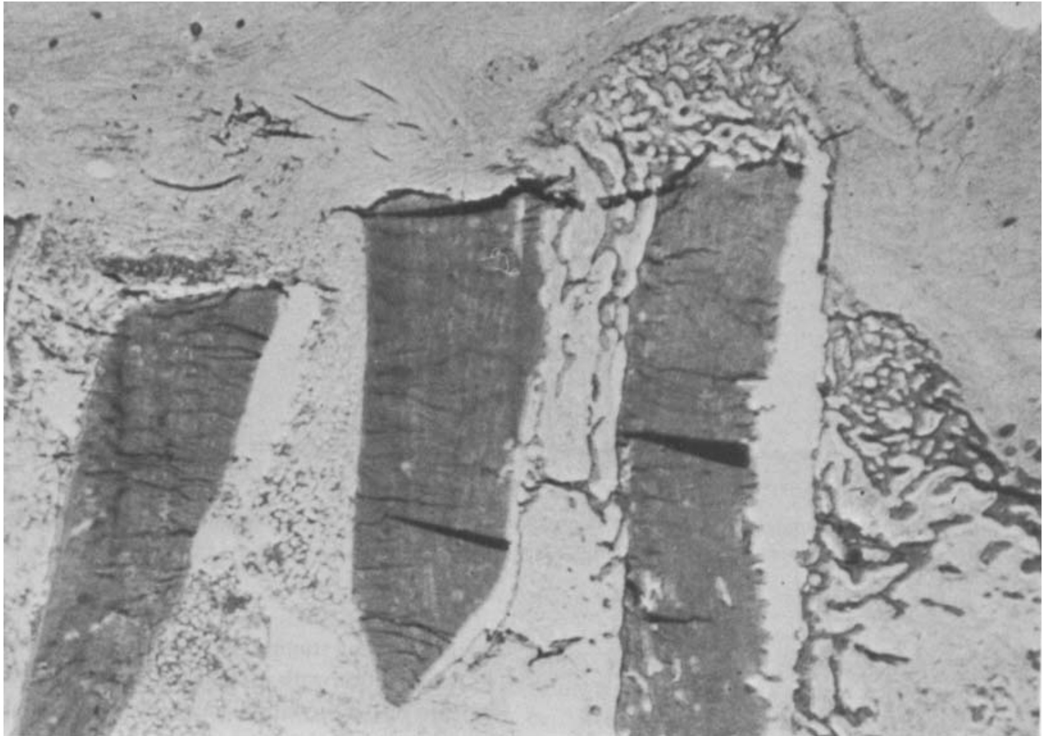

Fig. 22. The tip of the amputation stump, four weeks after amputation on crus with osseous plugging of the medullary cavity (rabbit R 43).

On the other hand, plugging was used in experimental bone growth studies. Trueta (1953) and Langenskiöld (1957) found that medullary plugging in the diaphysis caused growth acceleration, while Herndon & Spencer (1953) and Haas (1958) found growth reduction in some cases after implantation of foreigh substances in the medullary cavity. After plugging the metaphyseal portion of the medullary cavity with homologous cortical bone, Hansson (1967) observed a marked longitudinal growth. Growth stimulation, according to Ferguson (1933), resulted from a disruption of the metaphyseal blood supply, whereas Hutchison & Burdeaux (1954) proposed that it was caused by venous stasis. Trueta (1953), Carpenter & Dalton (1956) and Hansson & Wiberg (1963) suggested that an obstruction of the diaphyseal vessels produced an increased blood flow in the metaphysis and that this in turn stimulated increased longitudinal growth.

After medullary nailing of the tibia of rabbits Göthman (1960b) found an increased periosteal vascularization at the end of 10 days. Periosteal hyperplasia occurred in few cases, and no periosteal callus was formed. If medullary nailing was undertaken after fracture of the tibia (Göthman 1960c) there was no unequivocal increase in the periosteal vascularization, but a pronounced vascular reaction took place in the surrounding soft tissue. In contrast, Trueta & Cavadias (1955) found that medullary nailing of a radius fracture on immature rabbits caused a proliferation of periosteal vessels and abundant callus formation in the periosteum. Küntscher (1958) in clinical and experimental studies also found that medullary nailing stimulated the subperiosteal callus formation. Rhinelander (1974) reported that the "extraosseous blood supply" around a fractured bone persisted in the cases where the medullary arterial supply was dis-

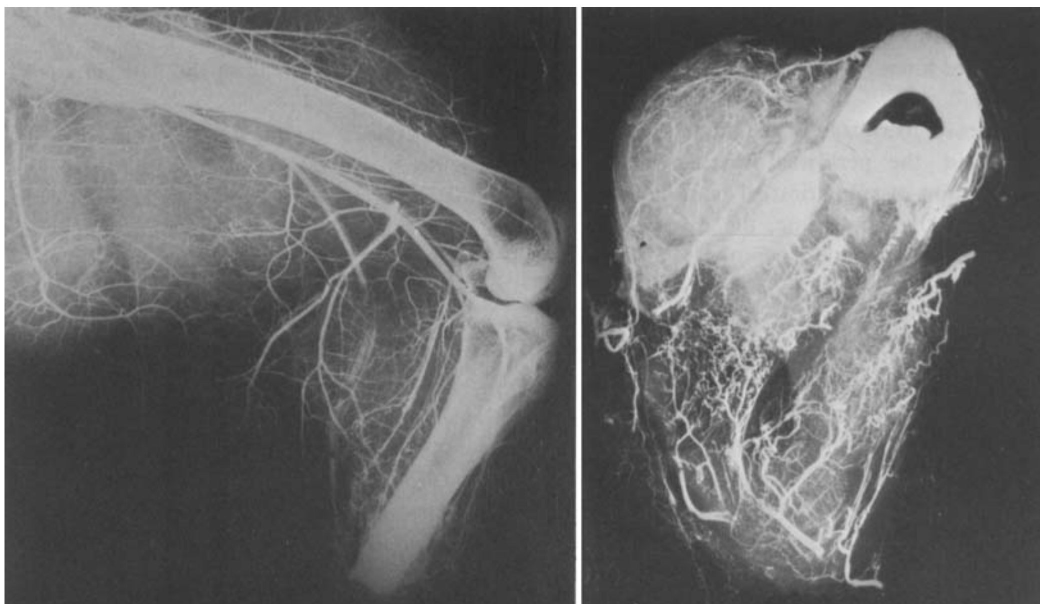

Fig. 23. a) Microangiography four weeks after amputation on crus with medullary plugging, and b) cross section of the triceps surae (R 43, the same rabbit as fig. 22).

rupted by medullary nailing or medullary plugging.

After evacuation of the medullary cavity in the femur of the cat, Richany et al. (1965) found proliferation of the fibrous periosteum and subperiosteal bone formation. When evacuation of the medullary cavity was combined with osteotomy the periosteal reaction was enhanced, healing of the osteotomy was delayed and subperiosteal cartilaginous callus formation took place. They proposed that the periosteal reaction was caused by stasis resulting from disruption of the bone's vascularization from the medulla.

DISCUSSION OF THE PRESENT INVESTIGATIONS

When amputation is combined with osseous plugging of the medullary cavity, both the healing process of the bone in the amputation stump and the muscle blood flow are changed. After proximal crus amputation it was found that plugging accentuated periosteal bone formation, which is interpreted as an interruption of the cortical arterial supply from the medulla. The periosteal reaction corresponds to the described changes following medullary nailing and evacuation of the medullary cavity. Plugging is carried out by hammering a 1 cm long piece of the cortex from the amputated bone into the extreme end of the medullary cavity. This produces no great destruction of the contents of the medullary cavity. Plugging does not produce a tight osseous closure of the medullary cavity opening. The inserted plugs during the first weeks after the operation were nonliving and caused a delay in the spontaneous closure.

Intracardial arteriography revealed contrast filling of the deep veins in the amputation stump after plugging of the medullary cavity, but before osseous closure had taken place. As demonstrated by Loon (1960), that effect must be assumed to be a sign of improved venous return from the stump.

After osseous plugging of the medullary cavity simultaneous with amputation the following conditions are found:

1. The osseous closure of the medullary cavity is delayed, the periosteal vascularization and formation of subperiosteal bone is increased and, at the same time, the osseous hèaling process in the stump is prolonged. This produces an increased vascular reaction in the surrounding muscles.
2. The venous return from the extremity is improved.
3. An increased muscle blood flow in the amputation stump takes place, as the increase in MBF begins more rapidly, reaches a higher level and lasts longer than after amputation without medullary plugging.

CHAPTER XIII

CONTRALATERAL VASCULAR REACTION FOLLOWING AMPUTATION

PREVIOUS INVESTIGATIONS OF THE CONTRALATERAL EFFECT

A contralateral effect has been described earlier in relation to the change of vascularization in the one extremity. Jaya (1958) found in studies on rats and rabbits that ligation of the femoral artery caused a constriction at the same level on the contralateral femoral artery, which lasted for from minutes to hours. If the operation was preceded by sympathectomy the constriction did not occur. Barnes & Trueta (1942) earlier described a similar effect after application of a tourniquet. Liu (1968) and Lewis & Lim (1970) observed a short-term decrease in blood flow in the contralateral extremity after blunt trauma. Following fracture in dogs Wray (1964) found a reduced blood flow and vasoconstriction in the contralateral extremity. He thought the vasodilation in the fractured extremity resulted from cessation of sympathetic tonus and that the contralateral effect was a consequence of accomodation of the vascular net in the contralateral extremity.

After femur fracture in rats Bohr (1955) detected increased bone activity in the diaphysis of the contralateral femur, and Rhinelander (1968) found with microangiography a dilation of the cortical blood vessels in the contralateral extremity after fracture in dogs.

A contralateral effect following amputation has not been described previously, but after immobilization of rabbits with plaster casts Hulth & Olerud (1960) found in arteriographic studies that arterial dilation also occurred in the contralateral extremity in some cases.

In studies on the effect of immobilization of the musculature, the changes are often compared with the contralateral extremity, but the muscle activity within it will usually be increased during the examination. This is also quite true in amputation studies. After amputation of the front legs of rats Saville & Smith (1966) observed an increased muscle mass in the rear legs, as well as an increased calcium content of their bones, and van Linge (1962) demonstrated that strenuous training of rats caused development of new muscle fibers and increased growth of these and of the interstitial connective tissue. The fact that muscle activity also has an influence on muscle vascularization was early shown by Petrén et al. (1936), and they found that the capillary density in the gastrocnemius of the guinea pig could be increased by 40% with training. Hermansen & Wachtlova (1971) could not find any difference in capillary density by means of biopsy of trained and untrained persons. Wullink (1967) observed a significant increase in open capillaries after training, but he did not think that the total number of capillaries increased, whereas Reitsma (1973) found in studies on rats a growth increase of the soleus up to 150%, increased diameter of muscle fibers and newly formed capillaries between these.

PRESENT STUDIES

In determinations of muscle blood flow (MBF) in both the operated and contralateral extremity it was found that amputation had an effect in the contralateral extremity (III–IV).

After crus amputation, MBF in the contralateral quadriceps was found to be reduced during the first weeks, after which it rose in the same manner as it did in the ipsilateral muscle (fig. 24). This increase was more substantial after ipsilateral osseous plugging of the medullary cavity. After knee disarticulation, MBF increased in the contralateral quadriceps in the same manner as it did in the amputation stump (fig. 25), whereas amputation on the femur did not produce such an effect.

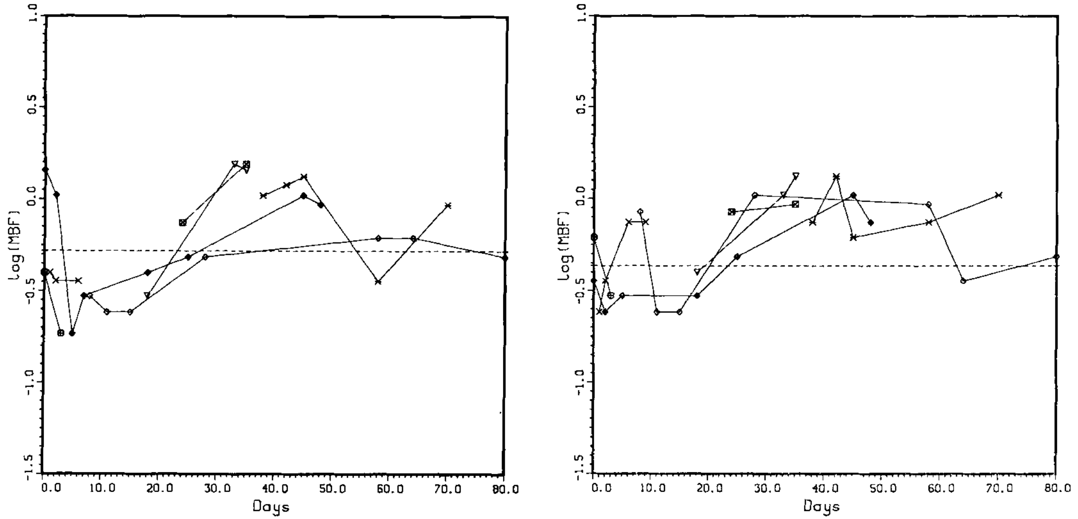

Fig. 24. MBF in the contralateral (a) and ipsilateral quadriceps (b) after amputation proximally on crus without myoplasty, 7 rabbits.

MBF in the contralateral triceps surae was observed to decrease in the first weeks after crus amputation without myoplasty (fig. 26a) and after femur amputation, whereas MBF in the contralateral triceps remained unchanged during the entire investigation period after crus amputation with myoplasty and after knee disarticulation (fig. 26 b–c).

Microangiographic studies of adult rabbits (IX) also disclosed that amputation affected vascularization in the contralateral extremity in the form of reduced contrast filling of the supplying ar-

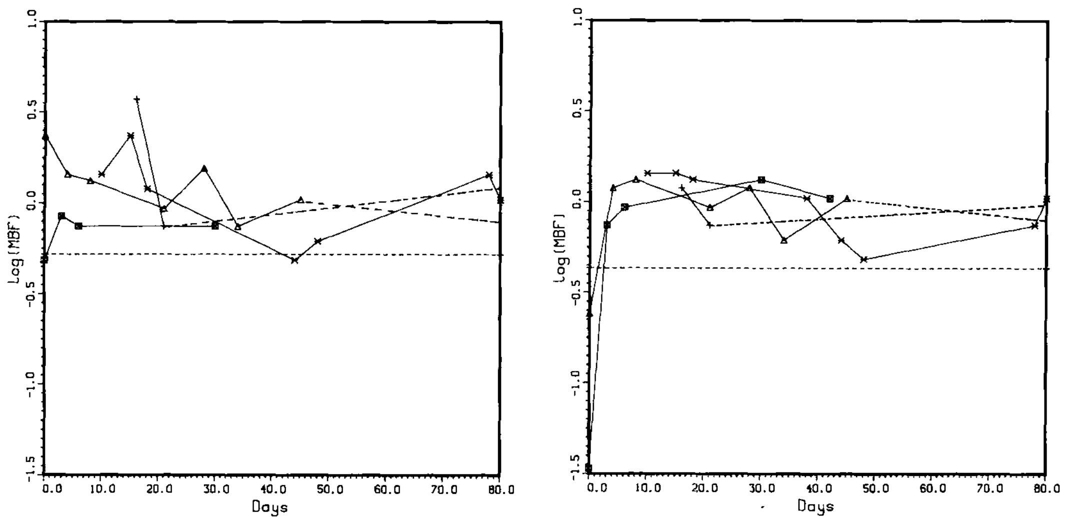

Fig. 25. MBF in the contralateral (a) and ipsilateral quadriceps (b) after disarticulation in the knee, 4 rabbits.

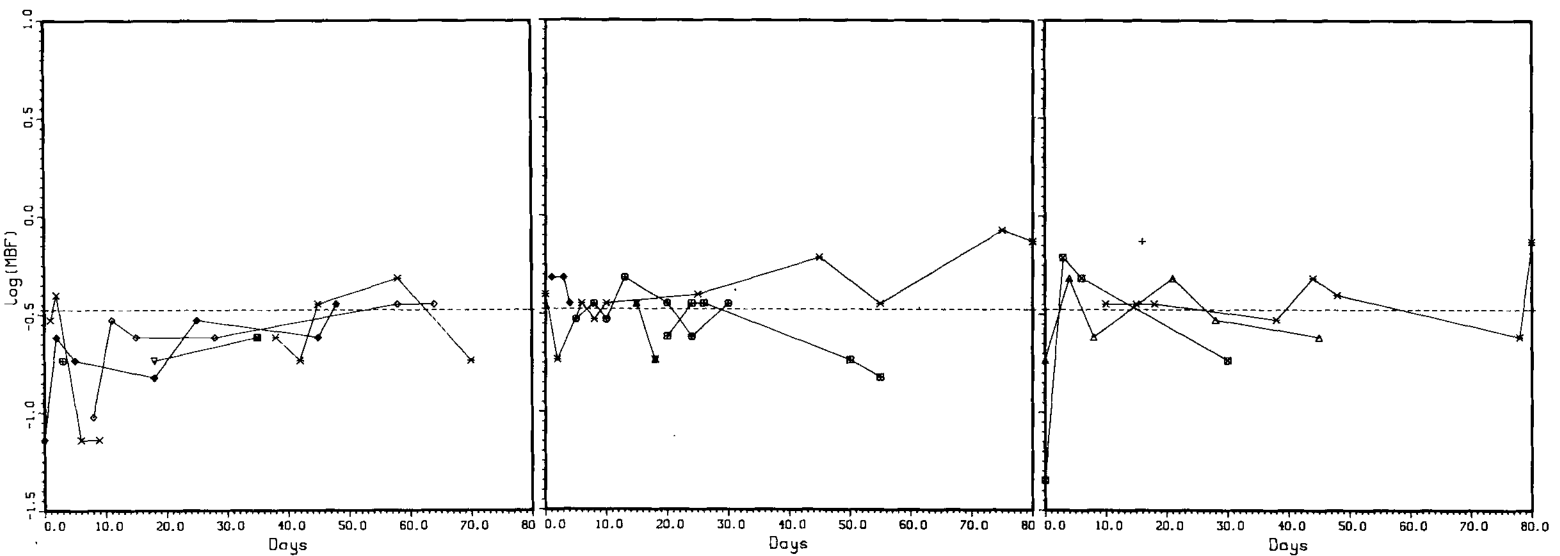

Fig. 26. MBF in the contralateral triceps surae after amputation proximally on crus without myoplasty, 7 rabbits (a), with myoplasty, 6 rabbits (b) and after disarticulation in the knee, 4 rabbits (c).

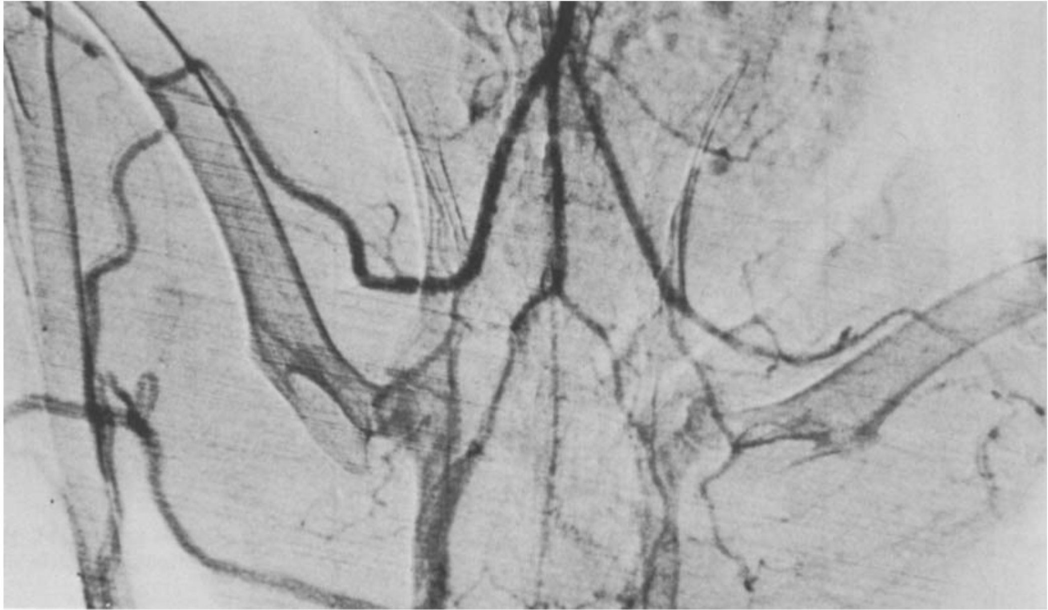

Fig. 27. A–v-shunts in the contralateral extremity after amputation of the femur combined with osseous medullary plugging.

teries. This was most pronounced after distal amputation of the crus and after femur amputation, and was least pronounced after proximal amputation of the crus with myoplasty. This type of contralateral effect did not occur after crus amputation or disarticulation of immature rabbits (I, II).

Intracardial angiography (VI) revealed signs of arteriovenous shunts in the contralateral extremity, which were most apparent if the medullary cavity was not closed with myoplasty and after distal amputation of the crus, and was least apparent after knee disarticulation. Arteriovenous shunts in the contralateral extremity also appeared after ipsilateral plugging of the medullary cavity (fig. 27).

When *muscle blood flow determinations* were carried out immediately after incision in skin and muscle (IV) decreased blood flow was found in the contralateral triceps surae, which amounted to 65% after skin incision and 76% after muscle incision. After open crus fracture, blood flow in the contralateral triceps surae was reduced to 71%. In contralateral quadriceps MBF was slightly increased after these three operations, 116%, 110% and 115%, respectively.

After ligation of the femoral artery (V) muscle blood flow in the contralateral triceps surae was immediately reduced to 66% of the preoperative flow and to 88% in the quadriceps. Blood flow in the triceps surae was decreased during the entire investigation period (mean flow: 80%), while the mean flow in the quadriceps during the interval 25–75 days was 156% of the preoperative flow.

After simultaneous ligation of the femoral artery and amputation of the crus, blood flow in the contralateral triceps surae was immediately reduced to 52% of the preoperative flow, and to 60% in the quadriceps.

Intracardial angiographic studies immediately after ligation of the femoral artery and after a combination of femoral artery ligation and amputation of the crus revealed a reduced diameter in the aorta and in the supplying arteries in both the operated as well as in the contralateral extremity (see chapter XIV).

DISCUSSION OF THE PRESENT RESULTS

Determinations of muscle blood flow have shown that ligation of the femoral artery elicits a decrease in blood flow in the contralateral triceps surae and in the quadriceps. This observation is in accordance with Barnes and Trueta (1942) and Jaya (1958). A similar reduced blood flow in the contralateral triceps surae was observed after crus fracture, which was also reported earlier by Wray (1964).

Following proximal amputation of the crus without myoplasty a corresponding reduction in blood flow in the contralateral triceps surae and quadriceps was found. This type of contralatereal effect did not occur after proximal crus amputation with myoplasty, nor after knee disarticulation.

Barnes and Trueta (1942) and Jaya (1958) thought that the contralateral vasoconstriction was caused by a sympathetic reflex, whereas Wray (1964) proposed that the contralateral vasoconstriction constituted a counterbalance to the vasodilation that arises in a fractured extremity, and thereby the blood flow into the two extremities remained constant. Kinmonth (1952) suggested that the contralateral vasoconstriction following extremity trauma resulted from a fall in blood pressure provoked by the trauma, but Jaya (1958) reported that the vasoconstriction process was independent of changes in blood pressure.

The demonstrated decrease in blood flow in the contralateral extremity after proximal crus amputation without myoplasty may result from a vasoconstriction corresponding to the reaction following arterial occlusion and after crus fracture. Myoplasty of the amputation stump is thought to contravene the contralateral vasoconstriction. The arterial occlusion brought about by muscle surgery must therefore be an essential factor in the contralateral reaction, and immediate attachment of muscles can counteract this reaction.

The initial reduction of MBF in the contralateral extremity can be interpreted as a reflex vasoconstriction, but the blood flow in the contralateral quadriceps and triceps surae remain reduced for several weeks after crus amputation without myoplasty. Following distal amputation of the crus and after femur amputation, a similar prolonged decrease in blood flow occurs in the contralateral triceps surae, whereas this does not take place after proximal crus amputation with myoplasty nor after knee disarticulation. The reason for such a prolonged reduction in blood flow cannot be vasoconstriction. However, intracardial angiographic studies have shown that arteriovenous shunts develop in both the amputation stump and in the distal portion of the contralateral extremity after crus amputation without myoplasty, as well as after distal crus amputation. These shunt formations can represent a contributing cause for the decrease in blood flow in the contralateral triceps surae.

Another cause for the changed muscle blood flow in the contralateral extremity can be the altered muscle activity in it following amputation. It has earlier been reported by Saville and Smith (1966) that amputation produces an increased muscle mass in the unoperated extremity and that increased muscle mass can evoke increased capillary density (Petrén et al. 1936). An increased muscle activity in the contralateral quadriceps can be expected after knee disarticulation and femur amputation, where the animal obtains no support from the operated extremity. After knee disarticulation the blood flow in the contralateral quadriceps was observed to be increased during the entire investigation period, in parallel with the increased blood flow in the ipsilateral quadriceps, but MBF was not increased after femur amputation in either the amputation stump or in the contralateral quadriceps. Therefore, the altered conditions of support encountered by the contralateral extremity cannot very easily be the essential cause for the increased blood flow in that extremity.

A characteristic feature observed in the amputation experiments was that the blood flow in the contralateral quadriceps changed in parallel with the blood flow in the ipsilateral quadriceps. A continuously increased blood flow in the contralateral quadriceps, taking place in parallel with the increased blood flow in the ipsilateral quadriceps, was also observed after ligation of the femoral artery. This increased blood flow

occurred at the same time as a decrease in MBF in the contralateral triceps surae, but the reason for this cannot be clarified by these studies.

Therefore, the investigations have shown that amputation has a contralateral effect in the form of a decrease in blood flow in the contralateral triceps surae, which may be due partly to vasoconstriction and partly to development of arteriovenous shunts. Moreover, amputation causes a rise in blood flow in the contralateral quadriceps, which most often occurs simultaneously with a rise in blood flow in the ipsilateral quadriceps.

CHAPTER XIV

AMPUTATION AFTER DEVELOPMENT OF A COLLATERAL CIRCULATION

The vascular reaction to amputation and the blood flow in the amputation stump have particular significance in cases where amputation is performed as a result of circulatory insufficiency. Blood flow in the amputation level is then dependent on collateral development, and blood flow in the amputation stump is dependent on the reaction of the collateral circulation to amputation trauma.

Therefore, it is interesting to investigate whether amputation has an effect on collateral development and whether the vascular reaction in the amputation stump is the same as after amputation on an extremity with a normal circulation.

PRESENT STUDIES

In these studies a model has been used in which collateral development is produced by ligation of the femoral artery, after which the vascular reaction following amputation of the crus has been investigated at increasing time intervals between arterial occlusion and amputation.

COLLATERAL DEVELOPMENT

Determinations of muscle blood flow (V) disclosed that a reduced muscle blood flow in the extremity occurred immediately after ligation of the femoral artery (table 4), after which MBF rose in both the triceps surae and in the quadriceps (fig. 28), and this increased flow continued for as long as 130 days after the operation.

Intracardial angiographic studies (VII) revealed that ligation of the femoral artery induced an initial reduction in the diameters of the aorta and the large arteries in both legs. No contrast filling occurred in the femoral artery on the ligated side, but refilling of the popliteal and crural arteries through collaterals from the circumflex femoris artery took place. Increased vascularity in the crus and increased collateral formation around the site of ligature appeared 6 days after the operation.

Microangiographic investigations (IX)

Immediately after ligation of the femoral artery a weak refilling of the distal part of the femoral

Table 4. Postoperative MBF (% af preoperative flow).

1 hour – 1 day after: Operation	Number of animals		Mean flow Quadriceps	Triceps surae
Ligatura art. femoralis	4		77%	66%
Simultaneous lig.+amp. cruris	5	1 hour	58%	
		1 day	150%	56%
Lig.+amp. – Interval 3–6 days	4	1 hour	121%	
		1 day	102%	155%
Lig.+amp. – Interval 60–130 days	6	1 hour	92%	
		1 day	180%	71%

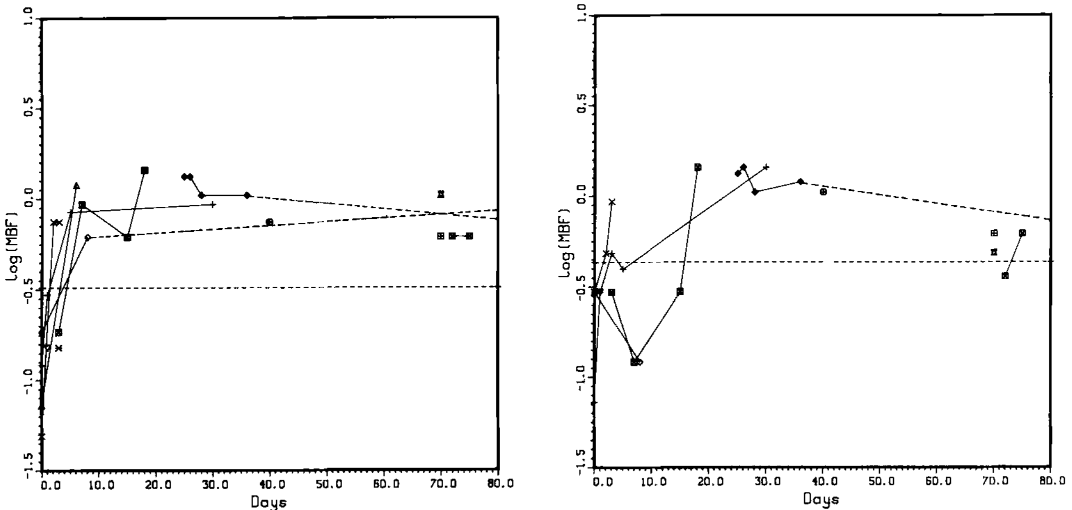

Fig. 28. MBF in triceps surae, (a) and quadriceps (b) after occlusion of the femoral artery, 12 rabbits.

artery and of the arteries on the crus was detected. The vascularization of the crus musculature was considerable reduced. Collaterals emerging from the circumflex femoris artery were first seen 6 days after occlusion. A slight hypervascularization on the crus was found simultaneously in some cases, decreasing after the fifth weeks, whereas the collaterals functioned for 16 weeks after arterial occlusion.

AMPUTATION AFTER COLLATERAL DEVELOPMENT

Determination of muscle blood flow (V)

If proximal amputation of the crus with myoplasty was undertaken immediately after ligation of the femoral artery, MBF was reduced in both the amputation stump and in the quadriceps (table 4). MBF rose in the quadriceps during the first day, while it was reduced the first 3 days in the amputation stump (mean flow: 61% of the preoperative flow), after which it rose (fig. 29a) to the same level as after amputation on an extremity with a normal circulation (fig. 2b).

After an interval of 3–6 days between ligation of the femoral artery and crus amputation an initial reduction in MBF was not seen in the amputation stump. One hour after amputation the blood flow increased (table 4) and attained the same level the following day as after amputation in a normal extremity (fig. 29b, compared with fig. 2b).

After an interval of 45–130 days an initial decrease in MBF once again appeared in the amputation stump (table 4). In 6 animals amputated 60–130 days after arterial ligation the mean flow during the first 3 days after the operation was 77% of the preoperative flow. Thereafter, MBF rose (fig. 29c) but the rise was less than after amputation performed 3–6 days after ligation of the femoral artery.

The investigation demonstrated that amputation of the crus after ligation of the femoral artery can lead to an initial decrease in MBF in the amputation stump, similar to amputation on an extremity with normal circulation. However, this reaction is dependent on the time interval between ligation and amputation. When amputation was carried out immediately after arterial ligation the reduction in blood flow was more pronounced than without ligation. If only a few days elapsed between arterial ligation and amputation, MBF increased immediately after amputation, whereas in cases where a long interval intervened between the two operations, an initial

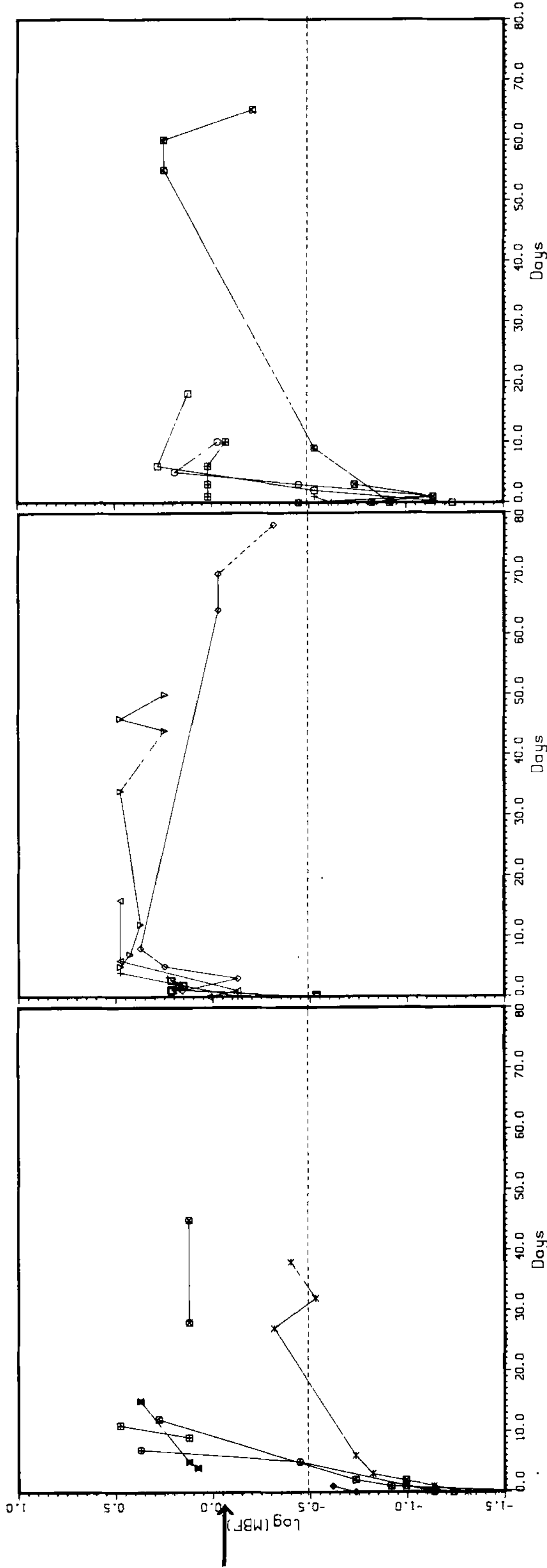

Fig. 29. MBF in the amputation stump after simultaneous occlusion of the femoral artery and amputation on crus, 7 rabbits (a), 3–6 days between artery occlusion and amputation, 5 rabbits (b) and 45–130 days between artery occlusion and amputation, 7 rabbits (c).
→ Mean flow in triceps surae 5–130 days after occlusion of the femoral artery.

OPERATION	1 HOUR	1/2 WEEK	1 WEEK	2-3 WEEKS	4-5 WEEKS	6-10 WEEKS	>10 WEEKS
LIGATURA ART. FEMORALIS	X X	X X X	X X X X X	X X X		X X	X X X
SIMULTANEOUS LIG. AND AMP. CRURIS		X X		X X X X X X	X X	X X ΔΔΔΔ X X ooo	X X ΔΔ X X ΔΔ
SIMULTANEOUS LIG. AND AMP. + PLUGGING		X X ΔΔΔ	X X ΔΔ	X X oo X X ΔΔΔ	X X X X		
INTERVAL 3-6 DAYS	X X X X X	X X X X X X	X X ΔΔ X X X X	X X ΔΔ X X ΔΔΔΔ	X X ●●● X X oooo	X X ΔΔΔ X X ooo	X X X ●●●● X X ooooo X X ΔΔΔ
INTERVAL 7-10 WEEKS	X X X X X X	X X X X		X X ΔΔΔ X X X ooo X X X ●●	X X X ●●● X X ●●●●	X X ●●●●●	X X oooo X X X ΔΔΔ
LIG. AND AMP. FEMORIS	X X X		X X	X ΔΔ	X X	X X	

X ARTERY OCCURRENCE
Δ VEIN SLIGHT OCCURRENCE
o VEIN MODERATE OCCURRENCE
● VEIN MARKED OCCURRENCE

Fig. 30. The rate of contrast passage (transit-time) in the arteries and veins in the amputation stump. One sign means one picture = 2 seconds.
— = a–v-shunt.

decrease in muscle blood flow was once again present in the amputation stump. MBF in the ipsilateral quadriceps increased during the first days after amputation (table 4), followed by normalization, corresponding to the change which occurred in MBF in the quadriceps after crus amputation with myoplasty on an extremity with normal circulation (fig. 3b).

Intracardial angiography: (VII)

If crus amputation was performed immediately after arterial occlusion the arteries were less filled with contrast than preoperatively for up to 4 weeks after the operation, and only sparse development of collaterals was seen on the femur and in the amputation stump. During the fifth and seventh week the supplying arteries were dilated, simultaneous with the appearance of contrast filling in the deep veins.

When the interval between arterial ligation and amputation was 3–6 days, the diameters of the supplying arteries were increased. As early as one hour after amputation, contrast filling of collaterals occurred on the femur and amputation stump. After 6 weeks the vascularity in the stump increased, accompanied by moderate filling of the

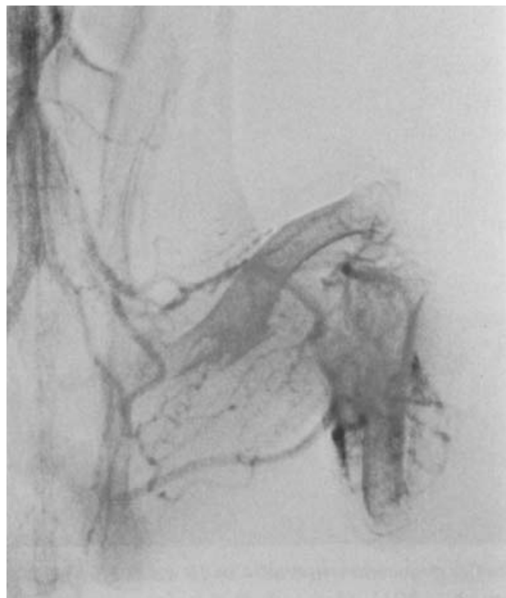

Fig. 31. Amputation 10 weeks after occlusion of the femoral artery. Arteriogram 4 weeks after amputation on crus.

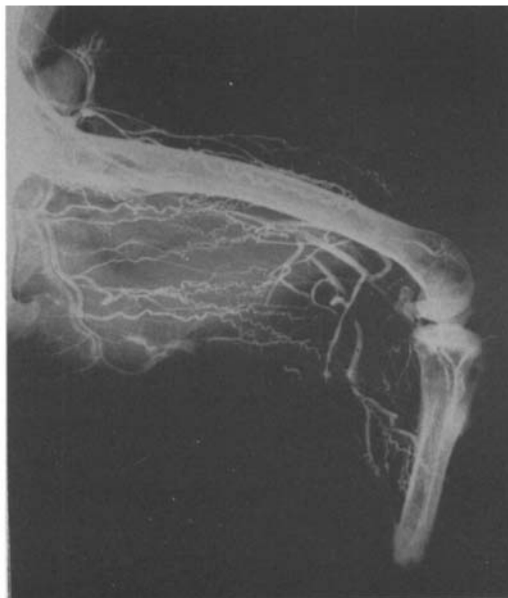

Fig. 32. Amputation 3 days after occlusion of the femoral artery. Microangiogram 10 weeks after amputation.

deep and superficial veins and by dilation of the supplying arteries. Simultaneous contrast filling of arteries and veins is interpreted as a sign of arteriovenous shunt formation (fig. 30).

Amputation 7–10 weeks after arterial ligation caused no primary change in the diameters of the supplying arteries, but dilation of the crus arteries and filling of the deep and superficial veins occurred 2 weeks after amputation. The vascularity in the amputation stump was greater than in amputation after a short time interval (fig. 31).

Microangiographic investigations (IX)

In cases of crus amputation immediately after ligation of the femoral artery a weak contrast filling of the femoral artery and of the popliteal artery was detected after 1 day, but no visible collateral formation was seen, and the vascularization of the amputation stump was reduced.

If amputation was undertaken ½–4 weeks after ligation of the femoral artery, an immediate dilation of collaterals branching off from the circumflex femoris artery and a normal vascularization of the amputation stump was seen, followed during the next 3 weeks by moderate hypervascularization. Ten weeks after amputation, pronounced development of spiral-twisted, dilated arteries and veins on the femur were observed, accompanied by only slight contrast filling in the amputations stump (fig. 32).

When the time interval between arterial occlusion and amputation was increased, minor contrast filling of collaterals in the amputation stump occurred in the first day after amputation. After an interval of 18 weeks a significant decrease in vascularization in the stump was seen, despite pronounced dilation of the collaterals (fig. 33).

PREVIOUS INVESTIGATIONS OF COLLATERAL DEVELOPMENT

Collaterals are arterio-arterial anastomoses which develop after arterial occlusion from the existing vascular network (Longland 1953, Krahl et al. 1654, Bellman et al. 1959, Schoop & Jahn

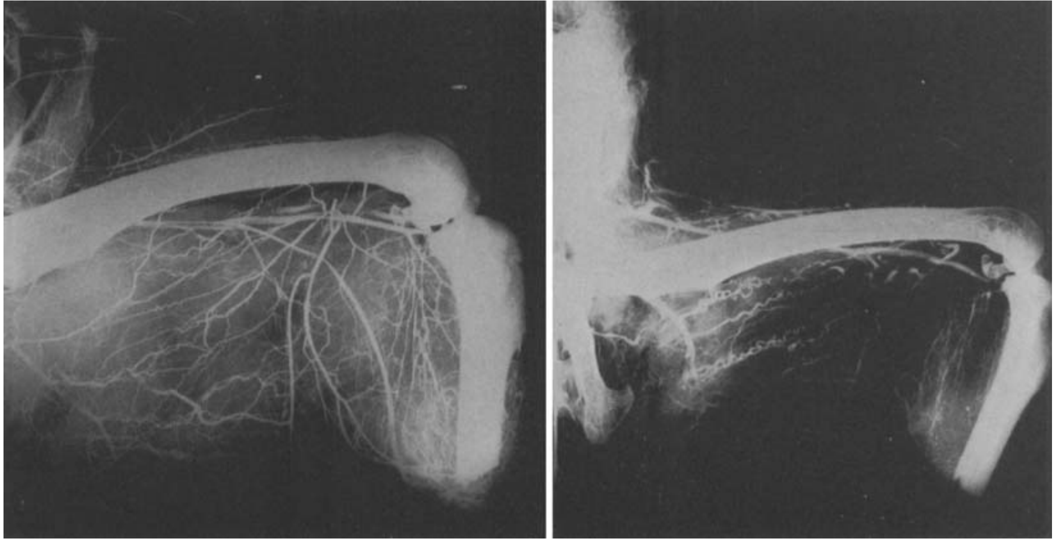

Fig. 33. a) 2 weeks after femoral artery occlusion and 1 week after amputation on crus. – b) 18 weeks after femoral artery occlusion and 2 days after amputation.

1961, Lambert et al. 1961 and Virkkula et al. 1969). Schoop & Jahn (1961) distinguished between "primary" collaterals (nutrient collaterals), which are arteries that are normally nonfunctional but immediatly spring into function when the pressure falls distal to the occlusion, and "secondary" collaterals (functional collaterals), which are developed during weeks or months depending on the increased rate of blood flow. Immediately after ligation of the femoral artery Schoop & Jahn (1961) observed with arteriography contrast filling of the primary collaterals, followed 2 weeks later by dilated, spiral-twisted secondary collaterals, which underwent additional development during the ensuing weeks. Krahl et al. (1954) distinguished between preformed main collaterals and newly formed accessory collaterals, developed from muscle blood vessels. They reported that collateral development depended partly on the location of arterial occlusion and partly on whether occlusion provoked arterial spasm, which could include a greater part of the vascular domain distal to the occlusion. These spasms were most pronounced after rapidly imposed occlusions, and they thereafter impeded the function of the collaterals.

In determinations of blood flow immediately after ligation of the femoral artery in the dog, Robertson et al. (1950) found a reduced flow, which then rose toward the normal value during the next 20 minutes. Thulesius (1962) observed similar conditions in studies on the cat, and so also did Shepherd (1950) and Dornhorst & Sharpey-Schafer (1951) in clinical investigations.

The vasospasm provoked by arterial occlusion or arterial ligation is counteracted or abolished by sympathectomy (Barnes & Trueta 1942, Jaya 1958). Leriche thought that impulses from the occluded vascular area stimulated the sympathetic system which thereby increased the tonus in collaterals, and he found that resection of the occluded arterial area produced a more rapid and more extensive collateral development (Leriche & Fontaine 1928). Virkkula et al. (1969) observed with arteriography in the dog a more rapid contrast passage after resection than after arterial ligation. They concluded that arterial resection yielded a better collateral development than arterial occlusion because the free ends of the severed arteries provided better possibility for collateral formation. In contrast, Pearse (1928) found after ligation of the femoral artery

that vasa vasorum in the arterial wall participated in collateral development.

The effect of amputation on collateral development was investigated by Holman (1949) who showed in animal experiments that amputation distal to an arteriovenous fistula with a well-developed collateral circulation caused no changes in the collaterals. This is opposite to the effect of vasospasm occurring after amputation of an extremity with normal circulation (Erikson & Olerud 1966), which is thought to be dependent on an increased sympathetic tonus.

While there is general agreement that sympathectomy produces dilation in newly developed collaterals, the concept of the effect of the sympathetic system on collaterals, which has persisted for a long time, is now less certain (Beaconsfield 1954, Ludbrook 1966). Collaterals normally possess a sizeable resistance in contrast to normal arteries. Edholm et al. (1951) found that the resistance was high at low pressure and flow, but low at normal or increased flow. Ludbrook (1966) concluded that the resting tonus of collaterals was minimal, while Thulesius et al. (1973) found that collaterals possess vasoconstrictor tonus and that sympathectomy reduced the collateral resistance transiently. Clinical studies (Folse et al. 1965, Nielsen et al. 1973) revealed that sympathetic blockade produced only very slight effect on collateral blood flow. Nielsen et al. (1973) explained the lack of effect by sympathetic blockade on the peripheral vascularization of circulation insufficiency by proposing that sympathetic tonus was weaker in collaterals than in the peripheral resistance vessels.

DISCUSSION OF THE PRESENT RESULTS

Intracardial arteriography has shown (VII) that ligation of the femoral artery elicits a reduced diameter in the arteries proximal and distal to the ligation site, and that a contrast filling of the collaterals occurs simultaneously around it. The ligation provokes vasospasm, but the collaterals rapidly become functional and the blood flow in the triceps surae rises above the preoperative level during the course of a few days.

In cases of crus amputation immediately after ligation of the femoral artery, a prolonged reduction of MBF takes place in the amputation stump, and a corresponding decrease in contrast filling of collaterals and in the amputation stump is observed with arteriography. In vivo arteriography disclosed that the decreased contrast filling is due to functional and not organic changes in the supplying arteries. Therefore, arterial ligation must provoke a vasospasm which is further accentuated by the subsequent crus amputation. The vasospasm brings about a reduced flow in the supplying arteries and counteracts the development of collaterals (Krahl et al. 1954). At low blood flow the resistance in collaterals is large (Edholm et al. 1951). These conditions can explain the observed decrease in blood flow in the amputation stump.

Three days after ligation of the femoral artery the collaterals were functional. If amputation was performed after that time no vasospasm was detected with arteriography, the collaterals were contrast filled and an immediate rise in MBF occurred in the amputation stump. The collaterals resemble those described by Schoop & Jahn (1961) as "primary collaterals". On the other hand, if amputation was carried out 45–130 days after arterial ligation, at the time when the "secondary" collaterals were developed, no initial rise in muscle blood flow in the amputation stump and only sparse contrast filling could be detected. This may result from the greater resistance in these "secondary" collaterals.

While amputation immediately after arterial ligation provoked vasospasm in the collaterals, this was not observed after amputation on an extremity with well-developed collaterals, which can be explained as a reduced sympathetic tonus in these collaterals (Ludbrook 1966, Folse et al. 1965 and Nielsen et al. 1973). This agrees with Holman's observations (1949) that amputation does not produce vasospasm in developed collaterals.

Intracardial angiography revealed a greater occurrence of a–v shunts in the amputation stump after crus amputation with myoplasty of an extremity supplied with collaterals than after the same operation on an extremity with normal circulation. Contrast filling of venous collaterals on the femur was detected by microangiography (fig.

32). This can be caused by an increased occurrence of a–v shunts on the femur.

Determinations of MBF in the amputation stump showed a rise of the same magnitude following crus amputation after collateral development as following amputation on a normal extremity, but since MBF in the triceps surae was increased by collateral development before amputation, the rise in MBF was correspondingly less (fig. 29). This may be due to increased resistance in the collaterals as well as to the presence of an increased number of arteriovenous shunts in the amputation stump.

Therefore, the investigation has shown that amputation of an extremity supplied by collaterals causes vascular changes which differ from the changes found after amputation on an extremity with a normal circulation:

1. The amputation can inhibit development of collaterals and thereby reduce the muscle blood flow in the amputation stump.

2. The initial vasospasm that normally occurs after amputation is amplified if the amputation is undertaken immediately after arterial occlusion, but does not appear if the extremity is supplied by developed collaterals.

3. The muscle blood flow in an amputation stump supplied with collaterals increases less than after amputation on an extremity with normal circulation. This can be caused partly by the greater resistance in the collaterals and partly by the increased tendency for development of arteriovenous shunts in the amputation stump and on the femur.

CHAPTER XV

CONCLUSIONS

These amputation studies were undertaken on rabbits, and the vascular changes observed after amputation have been qualified by the special anatomical and physiological conditions in this animal. The studies have had the character of a model experiment where a number of hemodynamic conditions that play a role in amputation in humans were not taken into consideration. Among others, this has been true in studies of amputation of an extremity supplied with collaterals.

Bearing this reservation in mind, it is felt that the following conclusions can be drawn from the investigation:

Amputation causes the following changes in the vascularization of an amputated extremity:

1. After amputation of an extremity with normal circulation a vasoconstriction is provoked which causes an initial fall in muscle blood flow in the amputation stump. It is assumed that the vasospasm is related to an increased sympathetic tonus, and that it is due to disruption of the supplying arteries and not to trauma to muscle or bone.

   After amputation of an extremity supplied with collaterals the initial reaction depends on the extent of collateral development. Amputation immediately after arterial occlusion will produce a more prolonged vasospasm, which impedes the function of collaterals. If the extremity is supplied with newly developed, "primary" collaterals, the amputation will not provoke a vasospasm in these collaterals and no initial reduction of blood flow in the amputation stump will be seen. On the other hand, if collateral development has progressed for a longer time so that the amputation stump is supplied by "secondary" collaterals, a decrease in blood flow is detected in the stump immediately after amputation.
2. After the initial vasoconstriction the blood flow in the amputated extremity is increased. The reason for this rise may be due to the healing process in muscle and bone. The reaction is dependent on the technique used for closure of the amputation stump. According to conventional amputation the muscles are severed at the amputation level, after which they retract. This brings about occlusion of muscle blood vessels and development of pathological, spiral-twisted vessels. When the stump is closed with myoplasty, muscle continuity is reestablished in the amputation stump, a hypervascularization through normally invisible vessels can be seen and blood flow in the stump is increased more rapidly and rises to a higher level than after conventional amputation.
3. The healing process in the bone is also thought to have an effect on blood flow in the amputation stump. Closure of the medullary cavity takes place after a few weeks, but the osseous activity in the stump persists in the form of continued construction and breakdown of bone tissue. This can be a contributing cause for the increased vascular activity and for the increased muscle blood flow in the stump occurring after closure of the medullary cavity. This assumption is supported by investigations where amputation was combined with osseous medullary plugging. This results in a delayed closure of the medullary cavity, a greater periosteal reaction and an increased muscle blood flow in the amputation stump.
4. After conventional amputation the severed muscles in the amputation stump are rendered inactive. This induces the development of arteriovenous shunts in the stump, and the blood flow in muscle capillaries is diminished after some weeks. Myoplasty contravenes muscle inactivity and thereby the formation of arteriovenous shunts, and muscle blood flow in the amputation stump is not reduced as after conventional amputation.

5. This investigation has shown that muscle blood flow in the amputation stump is greater than in the preoperative amputation level, which is also true after conventional amputation where the supplying arteries in the stump are occluded and replaced by newly formed, spiral-twisted vessels, and after knee disarticulation where no healing process in muscles and bone takes place. After knee disarticulation the distal muscle fixation is preserved, there is no inactivity changes in the form of arteriovenous shunts and there is no secondary reduction in muscle blood flow. This continuous increase in blood flow must be a sign that hypervascularization in the amputation stump is not only a factor in the healing process in muscle and bone, but must be due to the fact that the blood supplied to the amputated extremity is not reduced to the same degree as the amount of tissue is reduced.
6. The studies have revealed that blood flow in the amputation stump depends on the amputation level. The greatest increase in blood flow was obtained after proximal amputation of the crus. Since the blood flow in this case is greater than after knee disarticulation, the reason may be that the richly vascularized vascular network around the knee joint is intact, in addition to the above-mentioned increased activity which accompanies the healing process in the muscles and bone in the amputation stump. After distal amputation of the crus the blood flow in the stump decreased after a few weeks. This can be a consequence of the greater tendency for development of arteriovenous shunts distally on the extremity. The exceptional condition in the rabbit, i.e., there is no profound femoral artery, means that amputation of the femur does not induce an increased blood flow in the amputation stump. The superficial femoral artery is occluded at the junction with the circumflex femoris artery, and vascularization of the amputation stump is carried by collaterals with high resistance. After amputation of the femur an immobilization and inactivation of the muscles occurs in the amputation stump. Despite this, the development of arteriovenous shunts is less pronounced after femur amputation than after crus amputation.
7. In studies of an amputation on an extremity supplied with collaterals, it was found that amputation influences collateral development. A simplified model was used where collateral development was provoked by ligation of the femoral artery. Determination of muscle blood flow prior to amputation revealed that the developed collaterals convey an increased blood supply to the extremity. The muscle blood flow in the amputation stump after crus amputation rose less than after amputation of an extremity with normal circulation. This may result from the higher resistance in the collaterals and from the greater tendency for development of arteriovenous shunts in the amputated extremity.

The studies have shown, moreover, that amputation can affect blood flow in the contralateral extremity. This contralateral effect appears to result partly from an initial vasoconstriction in the contralateral crus and partly from an ensuing development of arteriovenous shunts distally. These conditions produce a reduction of blood flow in the distal portion of the contralateral extremity. This contralateral effect is most pronounced after proximal amputation of the crus, but can be counteracted by myoplasty of the amputation stump.

CLINICAL ASPECTS

The results of the investigation do not permit direct conclusions to be made on how vascularization is changed by amputation in man, but they constitute a basis for new clinical studies from the following observations:

a) The vascular bases upon which the amputation indication is made and upon which the amputation level is decided are altered by amputation.

   Amputation initially induces a vasospasm in the supplying arteries, but thereafter the muscle blood flow in the amputation stump rises above the preoperative value. If amputation is performed at a level where there is only one supplying artery, blood must be conveyed in the amputation stump by collaterals.

b) After amputation of an extremity supplied by collaterals, the extent of the development of collaterals at the time of amputation plays a role in the vascular reaction in the amputation stump.

c) Preservation of muscle function is decisive for normal blood flow in the amputation stump, as development of pathological vessels and arteriovenous shunts is thereby prevented.

d) The vascular reaction in the bone of the amputation stump can play a role in the muscle blood flow. After osseous plugging of the medullary cavity the vascular reaction in the bone and the surrounding musculature is increased, and the venous return from the amputation stump is improved.

e) Amputation produces a vascular reaction in the contralateral extremity, partly as an initial vasospasm and partly as a subsequent development distally of arteriovenous shunts.

SUMMARY

CHAPTER I

In the introduction a review is made of the foundation of the investigation. The most essential problem after amputation is to obtain healing of the amputation stump. The amputation level is determined generally on the basis of the preoperative blood flow in the extremity. However, it is not the preoperative blood flow in the amputation level but the postoperative blood flow in the amputation stump which is decisive for healing. The purpose of the investigation has been to study blood flow in the amputation stump in relation to blood flow in the amputation level, and to ascertain the factors that are important for vascularization in the amputation stump, i.e., the postoperative reaction in soft tissue and bone, the altered muscle function and the consequences of the reduced vascular supply area in the extremity after amputation.

CHAPTER II

A short review is made of the principles which earlier formed the basis for amputation surgery. The various types of disarticulation were employed to provide a painless, weight-bearing amputation stump. The increasing understanding of the importance of preservation of muscle function and of an intact knee joint for mobility has characterized amputation surgery in the past decades. The rising occurrence of amputation due to ischemic diseases of the extremities has generated an increased interest in the healing problems in the amputation stump, and has led to the development of new techniques of amputation and of new bandaging and types of prostheses.

CHAPTER III

Previous investigations of vascularization of the amputation stump are described. The earliest studies attempted to clarify the reason for stump and phantom pains. By use of arteriography, changes in the diameters of the supplying arteries and the occurrence of pathological vessels and arteriovenous shunts in the amputation stump could be observed. Arteriography revealed an improved arterial supply to the amputation stump following stump closure with myoplasty.

Hypervascularization in the amputation stump was also found in earlier experimental amputation studies which utilized arteriography.

Previous investigations have, therefore, demonstrated a number of arteriographic changes. An attempt to clarify the reason for these changes is made in the present experimental studies on rabbits.

CHAPTER IV

A review is made of the anatomical and physiological conditions that are important in the studies. The special conditions of the rabbit's tibia and arterial supply in the rear legs are described.

CHAPTER V

The material, the technique of operation and the investigation methods in the present studies are described. The vascular changes in muscles are assessed by determination of the muscle blood flow, as well as by in vivo angiography and microangiography studies, carried out in connection with sacrifice of the animals. The bone in the amputation stump was investigated with tetracyline labelling to detect any growth changes. The course of healing in the bone stump was examined with Goldner's staining method, and vascular changes were studied microangiographically.

Determination of muscle blood flow with Xe^{133} and histamine is discussed. A description is made of an in vivo arteriographic method not previ-

ously used before this investigation, in which the contrast material is injected intracardially. This method allows repeated studies on the same animal and permits the opportunity to evaluate the rate of blood flow, vein filling and the occurrence of arteriovenous shunts.

CHAPTER VI

The results of the determinations of muscle blood flow are summarized in this chapter. It was found immediately after amputation that muscle blood flow in the amputation stump was always less than the preoperative blood flow in the amputation level. Intracardial angiographic studies revealed that the initial reduction in muscle blood flow was caused by vasospasm. This was related to the disruption of the arterial blood supply and not to surgery on muscles or bone.

After the initial reduction, the blood flow rose in the amputation stump above the preoperative blood flow in the amputation level. This rise in muscle blood flow was dependent on the technique utilized in closure of the amputation stump and on the amputation level.

In chapters VII–X the results of the studies are analysed with regard to an explanation of the reasons for the dependence of these conditions on the blood flow in the amputation stump.

CHAPTER VII

Signs of arteriovenous shunts in the amputation stump were detected with intracardial angiography. Earlier investigations of arteriovenous shunt formation after amputation are presented. The possible reason for the development of these shunts is discussed. It was found in the present investigation that shunt formation occurred most frequently after amputation of the crus without myoplasty. Shunt formation in these cases can be caused by muscle inactivity.

CHAPTER VIII

Changes in muscle function in the amputated extremity are discussed in this chapter. Amputation causes both an immobilization of the extremity and muscle inactivity of the severed muscles. Previously observed morphological and vascular changes in these conditions are discussed. The results of the present investigation on morphological changes in muscle tissues after crus amputation are presented. Only degenerative changes in the triceps surae could be detected. These changes were least pronounced in cases treated with myoplasty.

CHAPTER IX

Previous studies of the effect of myoplasty on vascularization of the amputation stump are discussed. Earlier studies using arteriography demonstrated an increased vascularization of the amputation stump, as well as unchanged diameters in the supplying arteries. It was found in the present investigation that myoplasty increases muscle blood flow in the amputation stump, and that the initial reduction of blood flow was less. The reason for this may be a faster establishment of vascularization through normal, dilated arteries. Less occurrence of pathological, spiral-twisted vessels was found. Formation of arteriovenous shunts was prevented when the amputation stump was closed with myoplasty.

CHAPTER X

Muscle blood flow determinations showed that blood flow in the amputation stump depended on the amputation level, which was found with angiography to be due to the realtionship between the amputation level and the supplying arteries.

If the supplying artery was a terminal artery, as is the femoral artery after amputation in the middle of the femur, it was occluded and the blood flow in the stump was carried by long collaterals with high resistance. In this case no increase in blood flow took place in the amputation stump. In contrast, after distal amputation of the femur and knee disarticulation, where the femoral artery is preserved, an increased muscle blood flow occurred. The most ideal conditions for increased blood flow in the amputation stump were obtained after proximal amputation of the crus. The reason for this is due partly to anatomical conditions, which provide possibilities for the best arterial supply, and partly to the large vas-

cular reaction to muscle and bone trauma. Moreover, preservation of the knee joint produced less muscle inactivity and thereby less development of arteriovenous shunts in the amputation stump.

CHAPTER XI

Bone healing in the amputation stump and its relation to vascularization of the bone is described in this chapter. The medullary cavity was closed with osteoid tissue and spongy bone developed from endosteum. Thereafter, following amputation of the crus, a spongy transformation of the bone tip occurred, whereas only sparse bone formation took place in the femur stump, with pronounced cortex atrophy and dilation of the medullary cavity. The changes can be due to altered muscle activity and to altered conditions of support in the extremity.

Vascularization of the bone was found to be increased. After crus amputation, vascularization of the cortex depended on the relationship between the level of amputation and the course of the nutrient arteries. Therefore, a transient hypervascularization from the periosteum occurred after proximal crus amputation. The reason for this may be disruption of the bone's vascularization by the nutrient artery. Previous investigations of the importance of the nutrient artery for vascularization of the tibia are discussed.

The relationship between bone healing and vascularization in the surrounding musculature has been demonstrated in earlier studies. Prolonged increase in blood flow in the surrounding muscles has been observed after fracture. Previous studies have also reported that periosteal stripping can induce hypervascularization of the surrounding musculature. The importance of these conditions for increased blood flow in the amputation stump is discussed.

CHAPTER XII

Osseous plugging of the medullary cavity was employed in connection with amputation to ascertain how "primary closure" of the medullary cavity affected muscle blood flow in the amputation stump. It was found that this produced a greater blood flow than did closure with myoplasty. Angiographic studies revealed a greater vascular reaction accompanied by contrast filling of the deep veins and an increased occurrence of arteriovenous shunts. Studies of the bone disclosed that the healing process was changed, closure of the medullary cavity was delayed and a powerful periosteal reaction with increased formation of subperiosteal callus occurred.

Earlier studies of medullary plugging have demonstrated osseous hyperemia and acceleration of growth. After more extensive destruction of the medullary cavity, previous studies found a powerful periosteal reaction with vascular proliferation and abundant subperiosteal callus formation.

The effect of osseous plugging of the medullary cavity following amputation must be caused by the increased healing process in the bone, which is accompanied by a larger blood flow in the musculature and by an improved venous return from the amputation stump.

CHAPTER XIII

Muscle blood flow determinations after amputation were conducted on the quadriceps and triceps surae in both the amputated and in the contralateral extremity. It was found that the muscle blood flow in the contralateral extremity was changed after amputation. This reaction was dependent on the amputation level and on stump closure. In the contralateral quadriceps and triceps surae the blood flow was found to be less during the first weeks after crus amputation without myoplasty. A similar response occurred in the triceps surae after ligation of the femoral artery, but not after crus amputation with myoplasty nor after knee disarticulation. In the contralateral quadriceps after crus amputation and knee disarticulation the blood flow rose to the same level as in the quadriceps on the amputated side.

It was found with angiography that the contralateral effect was due partly to vasoconstriction and partly to development of arteriovenous shunts.

This type of contralateral effect had not previously been observed after amputation, but earlier studies of a contralateral effect after arterial ligation and fracture are discussed.

CHAPTER XIV

In order to study the blood flow in the amputation stump on an extremity supplied with collaterals, amputation of the crus was performed after ligation of the femoral artery. Muscle blood flow determinations under these conditions also disclosed increased blood flow in the amputation stump, but the rise was less than after amputation on an extremity with a normal circulation. Moreover, the magnitude of the blood flow was dependent on the time interval between arterial occlusion and amputation. The initial reduction that normally appeared after amputation was increased if the amputation was carried out immediately after arterial occlusion. The reason for this, as determined by intracardial angiography, was an increased vasospasm. If amputation was performed after 3 days the blood flow in the amputation stump rose immediately above the preoperative level, and was accompanied by immediate contrast filling of "primary" collaterals and of the amputation stump, as determined by in vivo angiography and microangiography. After a long interval of time between arterial ligation and amputation, an initial reduction in blood flow appeared once again in the amputation stump. In accordance with this finding, microangiographic studies revealed a reduced contrast filling of the amputation stump through twisted, dilated "secondary" collaterals. Development of arteriovenous shunts in the amputation stump was observed by means of in vivo angiography. The increased resistance in the collaterals and the development of arteriovenous shunts can contribute to the reduced elevation in muscle blood flow in an amputation stump supplied with collaterals.

CHAPTER XV

The results of the investigation are summarized in this chapter, where it is pointed out that they are qualified by the special anatomical and physiological conditions of the rabbit. Amputation provokes a vasospasm which causes an initial decrease in muscle blood flow in the amputation stump. Following amputation on an extremity supplied by collaterals, this response depends on the extent of development of the collaterals at the time of amputation. Thereafter, blood flow in the amputation stump rises above the blood flow in the preoperative amputation level. This rise can be due partly to the healing process in muscle and bone, as the muscle blood flow can be increased additionally by osseous plugging of the medullary cavity, but it can also be due to the fact that the blood supply to the amputated extremity is not reduced proportional to the reduction in amount of tissue. A secondary reduction in blood flow in the amputation stump results from development of arteriovenous shunts, which are related to muscle inactivity. This can be contravened if the amputation stump is closed with myoplasty.

RESUMÉ

KAPITEL I

I indledningen redegøres for begrundelsen for undersøgelserne. Ved amputation er det væsentligste problem at opnå heling af amputationsstumpen. Amputationsniveauet bestemmes almindeligvis på grundlag af den præoperative gennemblødning i ekstremiteten. Imidlertid er det ikke den præoperative gennemblødning i amputationsniveauet, men den postoperative gennemblødning i amputationsstumpen, som er afgørende for helingsforløbet. Formålet med undersøgelserne har været at undersøge gennemblødningen af amputationsstumpen i relation til gennemblødningen i amputationsniveauet og at belyse de faktorer, som kan have betydning for vaskulariseringen i amputationsstumpen, således den postoperative reaktion i bløddele og knogle, den ændrede muskelfunktion og det forhold, at ekstremitetsarteriernes forsyningsområde reduceres ved amputationen.

KAPITEL II

Der gives en kort gennemgang af de principper, som tidligere har dannet grundlag for amputationskirurgien. De forskellige former for eksartikulation blev anvendt for at skabe en smertefri, vægtbærende amputationsstump. Den stigende forståelse for bevarelse af muskelfunktionen og knæleddets betydning for gangfunktionen har præget amputationskirurgien gennem de sidste årtier. Den øgede forekomst af amputation på grund af iskæmiske ekstremitetslidelser har skabt en stigende interesse for helingsproblemerne i amputationsstumpen og medført udvikling af ny amputationsteknik og nye bandagerings- og protesetyper.

KAPITEL III

Der redegøres for de tidligere undersøgelser af vaskulariseringen i amputationsstumpen. De tidligste undersøgelser søgte at klarlægge årsagen til stump- og fantomsmerter. Ved arteriografi kunne man påvise ændring af diameteren i de tilførende arterier samt forekomsten af patologiske kar og arteriovenøse shunts i amputationsstumpen. Efter lukning med myoplastik viste arteriografi en forbedret arteriel forsyning til amputationsstumpen.

Ved tidligere eksperimentelle amputationsstudier fandt man ligeledes ved arteriografi hypervaskularisering i amputationsstumpen.

Tidligere undersøgelser har således vist en række arteriografiske ændringer. I disse eksperimentelle undersøgelser på kaniner har man søgt at klarlægge årsagen til disse ændringer.

KAPITEL IV

I kapitlet gennemgås de anatomiske og fysiologiske forhold, som kan have betydning for undersøgelserne. De specielle forhold i kaninens tibia og *i arterieforsyningen af kaninens bagben* beskrives.

KAPITEL V

I kapitlet beskrives materialet, operationsteknik og undersøgelsesmetoder ved egne undersøgelser. De vaskulære ændringer i musklerne er bedømt ved bestemmelse af muskelgennemblødningen, samt ved in vivo angiografi og ved mikroangiografi, som blev foretaget i tilslutning til dyrenes aflivning. Knoglen i amputationsstumpen blev undersøgt med tetracyclinmærkning med henblik på vækstændringer; helingsforløbet i knoglestumpen blev belyst ved hjælp af Goldners farvemetode, og de vaskulære ændringer her blev påvist med mikroangiografi.

Blandt undersøgelsesmetoderne diskuteres den anvendte teknik ved bestemmelse af muskelgennemblødningen med Xe 133 tilsat histamin. Ved in vivo arteriografi har man anvendt en ikke tidli-

gere beskrevet metode, hvor kontraststoffet injiceres intrakardielt. Denne metode tillader gentagende undersøgelser på samme dyr og giver mulighed for at bedømme gennemblødningshastighed, venefyldning og forekomsten af arteriovenøse shunts.

KAPITEL VI

I kapitlet opgøres resultaterne af bestemmelserne af muskelgennemblødningen. Man fandt, at gennemblødningen i amputationsstumpen umiddelbart efter amputation altid var mindre end den præoperative gennemblødning i amputationsniveauet. Ved intrakardiel angiografi kunne man vise, at den initiale nedsættelse af muskelgennemblødningen skyldtes en vasospasme. Denne var betinget af afbrydelsen af den arterielle blodforsyning og ikke af indgreb på muskler eller knogle.

Efter den initiale nedsættelse steg gennemblødningen i amputationsstumpen over den præoperative gennemblødning i amputationsniveauet. Denne stigning i muskelgennemblødningen var afhængig af den anvendte teknik ved lukning af amputationsstumpen og af amputationsniveauet.

I kapitlerne VII–X analyseres undersøgelsesresultaterne med henblik på at klarlægge årsagerne til, at gennemblødningen i amputationsstumpen er afhængig af disse forhold.

KAPITEL VII

Ved intrakardiel angiografi fandt man tegn på arteriovenøse shunts i amputationsstumpen. Tidligere undersøgelser af arteriovenøs shuntdannelse ved amputation omtales. De mulige årsager til, at der udvikles disse shunts diskuteres. Ved egne undersøgelser fandt man, at shuntdannelsen var hyppigst ved amputation på crus uden myoplastik. Shuntdannelsen kan i disse tilfælde være en følge af muskelinaktivitet.

KAPITEL VIII

I kapitlet omtales den ændrede muskelfunktion i den amputerede ekstremitet. Amputation medfører dels en immobilisering af ekstremiteten, dels en inaktivitet af de overskårne muskler. De tidligere påviste morfologiske ændringer ved disse tilstande omtales. Resultaterne af egne undersøgelser af de morfologiske ændringer i muskelvævet efter crusamputation fremlægges. Der kunne påvises degenerative forandringer i triceps surae. Disse forandringer var mindst udtalte i de tilfælde, hvor der blev foretaget myoplastik.

KAPITEL IX

Tidligere undersøgelser af myoplastikkens indflydelse på vaskulariseringen af amputationsstumpen omtales. Ved arteriografi er der tidligere påvist en øget vaskularisering i amputationsstumpen og uændret diameter af de tilførende arterier. Ved egne undersøgelser fandt man, at myoplastik forøgede muskelgennmblødningen i amputationsstumpen. Den initiale reduktion i gennemblødningen var mindre. Dette kan skyldes en hurtigere etablering af vaskulariseringen gennem normale, dilaterede arterier. Man fandt mindre forekomst af patologiske, spiralsnoede kar. Dannelsen af arteriovenøse shunts modvirkedes, når amputationsstumpen blev lukket med myoplastik.

KAPITEL X

Bestemmelse af muskelgennemblødningen viste, at gennemblødningen af amputationsstumpen afhang af amputationsniveauet. Ved angiografi fandt man, at dette skyldes amputationsniveauets relation til de tilførende arterier.

Hvis den tilførende arterie er en endearterie, som arteria femoralis ved amputation midt på femur, vil denne okkluderes, og gennemblødningen af amputationsstumpen sker gennem lange kollateraler med stor modstand. Der ses ingen øget gennemblødning i amputationsstumpen. I modsætning hertil vil amputation distalt på femur og knæeksartikulation, hvor arteria femoralis er bevaret, medføre en øget muskelgennemblødning. De bedste betingelser for øget gennemblødning af amputationsstumpen opnås ved amputation proksimalt på crus. Det skyldes dels de anatomiske forhold, som giver mulighed for bedst arterieforsyning, dels den større vaskulære reaktion på muskel- og knog-

letraumet. Desuden medfører bevarelsen af knæleddet en mindre muskelinaktivitet og dermed en mindre udvikling af arteriovenøse shunts i amputationsstumpen.

KAPITEL XI

I kapitlet beskrives knoglehelingen i amputationsstumpen og dens relation til vaskulariseringen af knoglen. Marvhulen lukkedes med osteoid væv og spongiøs knogle, som udvikledes fra endosteum. Derefter indtrådte der ved amputation på crus en spongiøs omdannelse af knoglespidsen, medens der i femurstumpen kun forekom sparsom knoglenydannelse med udtalt cortexatrofi og dilation af marvhulen. Disse forandringer kan skyldes den ændrede muskelaktivitet og de ændrede belastningsforhold i ekstremiteten.

Vaskulariseringen af knoglen fandtes forøget. Ved crusamputation afhang vaskulariseringen af cortex af amputationsniveauets relation til forløbet af arteria nutritia. Ved proksimal crusamputation fandt man således en forbigående hypervaskularisering fra periosteum. Dette kan skyldes afbrydelse af knoglens vaskularisering gennem arteria nutritia. Tidligere undersøgelser af arteria nutritias betydning for tibias vaskularisering omtales.

Tidligere undersøgelser har vist relation mellem knoglehelingen og vaskulariseringen i omgivende muskulatur. Efter fraktur er der fundet en langvarig forøget gennemblødning i de omgivende muskler. Det er ligeledes tidligere vist, at periostal løsning kan medføre hypervaskularisering i den omgivende muskulatur. Disse forholds betydning for den øgede gennemblødning af amputationsstumpen diskuteres.

KAPITEL XII

Ossøs plugging af marvhulen blev anvendt i forbindelse med amputation for at undersøge, hvorledes »primær lukning« af marvhulen påvirkede muskelgennemblødningen i amputationsstumpen. Man fandt, at gennemblødningen her var større end efter lukning med myoplastik, såvel ved amputation på crus som på femur. Ved angiografi påvistes en større vaskulær reaktion med kontrastfyldning af de dybe vener og øget forekomst af arteriovenøse shunts. Ved undersøgelse af knoglen fandt man, at helingsforløbet ændredes, marvhulens lukning forsinkedes, og der var kraftig periostal reaktion med forøget dannelse af subperiostal callus.

Tidligere undersøgelser af medullær plugging har vist ossøs hyperæmi og vækstacceleration. Ved mere omfattende destruktion af marvhulen har man tidligere fundet kraftig periostal reaktion med vaskulær proliferation og rigelig subperiostal callusdannelse.

Effekten af ossøs plugging af marvhulen ved amputation må skyldes den forøgede helingsproces i knoglen, som ledsages af en større gennemblødning i muskulaturen, samt det forbedrede venøse tilbageløb fra amputationsstumpen.

KAPITEL XIII

Bestemmelse af muskelgennemblødningen efter amputation blev foretaget i quadriceps og triceps surae, såvel i den amputerede, som i den kontralaterale ekstremitet. Herved fandt man, at gennemblødningen i modsidige ekstremitet ændredes efter amputation. Denne reaktion var afhængig af amputationsniveau og stumplukning. I modsidige quadriceps og triceps surae fandtes gennemblødningen nedsat de første uger efter crusamputation uden myoplastik. En lignende reaktion i triceps surae forekom efter ligatur af arteria femoralis, men ikke efter crusamputation med myoplastik eller efter knæeksartikulation. I modsidige quadriceps steg gennemblødningen til samme niveau som i quadriceps på den amputerede side, såvel efter amputation på crus som efter knæeksartikulation.

Ved angiografi kunne man vise, at den kontralaterale effekt dels skyldtes vasokonstriktion, dels udvikling af arteriovenøse shunts.

En sådan kontralateral effekt er ikke tidligere vist ved amputation, men tidligere undersøgelser af kontralateral effekt ved arterieligatur og fraktur omtales.

KAPITEL XIV

For at undersøge gennemblødningen i amputationsstumpen i en ekstremitet, som forsynes gennem kollateraler, blev der foretaget amputation på crus efter ligatur af arteria femoralis. Bestemmelse af muskelgennemblødningen viste også herefter en forøget gennemblødning i amputationsstumpen, men stigningen var mindre end efter amputation på ekstremitet med normalt kredsløb. Desuden var gennemblødningen afhængig af tidsintervallet mellem arterieokklusion og amputation. Den initiale reduktion, som normalt forekom efter amputation, var forøget, hvis amputationen blev foretaget umiddelbart efter arterieokklusionen. Ved intrakardiel angiografi kunne man vise, at dette skyldtes en forøget vasospasme. Hvis amputation blev foretaget efter 3 dages forløb, steg gennemblødningen i amputationsstumpen straks over det præoperative niveau, og ved in vivo angiografi og mikroangiografi fandt man umiddelbar kontrastfyldning af »primære« kollateraler og af amputationsstumpen. Ved langt tidsinterval fandt man atter en initial nedsættelse af gennemblødningen af amputationsstumpen. Mikroangiografi viste i overensstemmelse hermed en mindre kontrastfyldning af amputationsstumpen gennem snoede, dilaterede »sekundære« kollateraler. Ved in vivo angiografi fandtes udvikling af arteriovenøse shunts i amputationsstumpen. Den øgede modstand i kollateralerne og udviklingen af arteriovenøse shunts kan være årsagen til den mindre stigning i muskelgennemblødningen i amputationsstumpen, som forsynes gennem kollateraler.

KAPITEL XV

I kapitlet sammenfattes undersøgelsesresultaterne, idet det pointeres, at disse er betinget af de specielle anatomiske og fysiologiske forhold hos kaninen. Ved amputationen udløses en vasospasme, som medfører en initial nedsættelse af muskelgenneblødningen i amputationsstumpen. Ved amputation på ekstremitet, som forsynes gennem kollateraler, er denne reaktion afhængig af kollateraludviklingen på amputationstidspunktet. Derefter stiger gennemblødningen i amputationsstumpen over gennemblødningen i det præoperative amputationsniveau. Denne stigning kan dels skyldes helingsprocesser i muskel og knogle, muskelgennemblødningen kan således forøges yderligere ved ossøs plugging af marvhulen, men den må også skyldes, at blodforsyningen til den amputerede ekstremitet ikke reduceres i samme omfang som vævsmængden. En sekundær nedsættelse af gennemblødningen i amputationsstumpen skyldes udvikling af arteriovenøse shunts, som er betinget af muskelinaktivitet. Denne kan modvirkes, hvis amputationsstumpen lukkes med myoplastik.

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