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Reaming of the Medullary Cavity and Its Effect on Diaphyseal Bone

A Fluorochromic, Microangiographic and Histologic Study on the Rabbit Tibia and Dog Femur

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Introduction

Intramedullary nailing with solid bars of bone, ivory or metal has been used sporadically for the fixation of diaphyseal fractures in long bones since the end of the 19th century. For literature review, see Küntscher, 1962.

The Rush brothers introduced in 1927 curved, flexible solid steel pins, for intramedullary osteosynthesis. In the fixation of diaphyseal fractures with these pins, an elastic three-point fixation is produced, which, however, often allows relatively large movements in the fracture line. The mechanical stability of the osteosynthesis is often inadequate. As a rule, therefore, further complementary external fixation is required.

In 1940 Küntscher introduced a new principle of osteosynthesis with a steel nail, which was initially V-shaped but was later given the shape of a clover-leaf. The nail could be compressed elastically from the sides. Küntscher intended that it should be squeezed against the cortex inside the medullary cavity. In order to obtain stable fixation the nail has to be pressed firmly in the medullary cavity in both the proximal and distal fracture fragment. With hour-glass-shaped medullary cavities this means that the nail gives adequate fixation only in relatively transverse fractures within the narrowest area of the diaphysis.

Rigid fixation, preferably stable enough for weight-bearing, gives important advantages by allowing earlier mobilization of the patient and thus more rapid restoration of the general state of health, with a decreased risk of thrombosis and renal calculi as a result. As regards local conditions of the affected limb, there will be reduced muscular atrophy, reduced periarticular fibrosis and oedema and more rapid removal of breakdown products from the fracture region.

In order to increase the possibilities of osteosynthesis of diaphyseal fractures with an intramedullary nail, sufficiently stable for weight-bearing, Küntscher developed in 1950 hand-operated reamers for widening the medullary cavity, and in 1954 electrically driven reamers on a flexible axis, which can be threaded over a guide wire which is inserted into the medullary cavity. By enlarging the medullary cavity, space is created for a nail of such a diameter that it can take up the mechanical forces when weight is placed on the limb. In this way the risk is also avoided of the nail becoming wedged in the medullary cavity and splitting the cortex, or separating

the fracture fragments by wedging in the distal fragment. By enlarging the medullary cavity in the middle part of the bone the nail can be held fast along a greater proportion of its length than is otherwise possible. Less force is required for driving home the nail, which probably means a reduced risk of fat embolism.

The pressure conditions in the distal metaphysis of the tibia were studied by Wehner (1968) with closed reaming and nailing of fractures of the tibial diaphysis in man. He found that 5–8 days after the fracture the medullary cavity pressure was 9–10 mm Hg. When the medullary cavity was opened with an awl, its pressure rose to 20–25 mm Hg within a short time. Closed reduction resulted in the same degree of pressure increase. When the guide wire was first inserted in the medullary cavity a pressure increase of about 40 mm Hg was obtained. On subsequent reaming, there was a maximal pressure increase of 120 mm Hg. The pressure increase was greatest when the reaming began in the distal fragment and was greater the wider the calibre of the reamer. When the reamer was withdrawn from the medullary cavity, a negative pressure of about 100 mm Hg was recorded. On driving in the nail, practically no measurable pressure increase was obtained. With an open operation on the fracture a smaller pressure increase was obtained than with a closed technique. Operations on the femur gave similar results to those on the tibia.

Since 1964 we have employed Küntscher's instrument for reaming of the medullary cavity and have now used it on about 30 fresh fractures of the femur and in a few pseudarthroses. After about 1 week of preoperative extension therapy, the operation was performed on an extension table, under radiographic control with a television monitor. For two reasons the fracture was reduced by the open technique, through an incision lateral to vastus lateralis; firstly to provide a possibility of more exact reduction, and secondly to counteract the pressure increase in the medullary cavity and in the fracture haematoma associated with the reaming of the medullary cavity. The medullary cavity was reamed from the tip of the greater trochanter, using short reaming stages and with the least possible pressure against the reamer. The calibre of the reamer was increased step-wise by $^1/_2$–1 mm each time, from 10 mm. The reaming was discontinued when both fracture fragments had been adequately reamed. A nail of greater diameter than 11.5 mm and $^1/_2$–1 mm narrower than the widest reamer was chosen. The length of the nail was determined by measurement, on the television screen, of the guide wire which had been inserted in the medullary cavity on reaming. In fractures on the distal part of the middle third of the bone, the nail was inserted to about 1 cm above the intercondyloid fossa, to be secured there in the cancellous bone. Proximally, the nail should extend no higher than to

about $^1/_2$ cm above the tip of the trochanter. Large intermediate fragments were secured to the bone by cerclage, and the cerclage wires were removed within 3–4 months after the operation. The periosteal blood supply of the intermediate fragment was maintained as far as possible. The clinical results of the operations have been promising. The main advantage of the method, namely that the patient can usually put full weight on the limb within 2 weeks postoperatively, has been fully utilized. A more detailed report of the clinical results will be published elsewhere.

The periosteal cuff of callus was always several cm wide and the callus mass was assessed as considerable. Periosteal callus was visualized as a thin, cloud-like shadow 3–4 weeks after the operation. The callus tissue showed its maximal extent on the radiograph about 6 weeks after the operation. After this time there was usually no increase in the amount of callus.

Within the area in the vicinity of the fracture where, by reaming, the cortex had become greatly thinned—in some cases to half of its previous thickness—the periosteal callus tissue does not at first become attached to the thinned bone, but bridges this area. An area of low density is then seen on the radiograph between the callus tissue and the underlying bone, which is probably totally avascular and necrotic. After some months this bone becomes clearly eroded from the periosteal side and seems to be largely replaced by periosteal callus.

An intermediate fragment in which the periosteal circulation is retained and which is fixed into the osteosynthesis by cerclage, is covered by a large amount of periosteal callus, which seems to become attached to the fragment from the beginning except within a narrow zone nearest to the fracture gaps. This finding is considered by Charnley (1968) to be a sign of ischaemia of the fracture ends.

In some younger patients the periosteal callus formation was not only localized to the fracture area but was also found as a thin cuff of bone around the greater part of the diaphysis.

The fracture gap was often seen radiographically to be still open 6 months after the operation.

A large amount of periosteal callus in a fracture is usually due to instability of the fracture (Karlinger and Sas, 1961; Küntscher, 1962; Müller *et al.* 1965). Müller *et al.* in the AO group consider every radiographically visible periosteal callus formation occurring during the healing of a fracture fixed with a compression plate or compression screws as a sign of instability of the fracture. According to Böhler (1948) and Geiser (1967), in intramedullary nailing of diaphyseal fractures considerable periosteal callus formation may be due to the fact that the limb bears weight to a more normal extent than with other methods of osteosynthesis.

The following factors oppose the view that in this material the development of periosteal callus was due essentially to instability:

1. The callus tissue was already of the same extent when it first became visible radiographically after 3–4 weeks. No gradual increase, of the type seen in the development of a hypertrophic pseudarthrosis, was observed.

2. No line of low density passing through the entire periosteal callus cuff was seen, as can be observed when there is considerable instability in a fracture.

3. No bone resorption around the nail occurred until the fracture had healed.

4. The patients were already free from pain in the fracture area about 1–2 days after the osteosynthesis.

Reports have been published of several experimental studies on the reaction of the long bone after Küntscher nailing and after evacuation of the medullary cavity. On the other hand, no studies appear to have been made of the reaction of the long bone to the removal of bone from the endosteal surface by reaming of the medullary cavity.

The aim of the present investigation was to study, by means of Indian ink angiography, bone labelling with tetracycline, and histological methods, the effect of reaming of the medullary cavity on the formation of callus and on the microcirculation, in order to obtain an idea of the conditions of healing with this method of treatment. To investigate the effect *per se,* the study was made of reaming of the medullary cavity of non-fractured long bones.

CHAPTER 1

Review of Literature

THE NORMAL VASCULAR SUPPLY OF THE TIBIAL DIAPHYSIS IN THE RABBIT

The investigations of Langer in 1876 and the angiographic studies of Lexer *et al.* in 1904 provided the basis for our understanding of the arterial vascular supply in the bone. The anatomy of the larger vessels is now well known, while the microcirculation of the cortex is still obscure in many respects.

The diaphysis of the long bone obtains its arterial blood supply from three sources, namely the nutrient artery, the metaphyseal arteries and the periosteal arteries. The nutrient artery of the tibia in the rabbit arises from the anterior tibial artery and perforates the tibia on the fibular side about 5 mm above the tibiofibular synostosis (Brookes & Harrison, 1957; Göthman, 1961). The artery passes obliquely through the cortex of the tibia in the distal-tibial direction without giving off any branches to the cortex during its transit (de Marneffe, 1953; Brookes, 1964). In the medullary cavity the nutrient artery divides into ascending and descending branches, which anastomose with the metaphyseal vessels (Göthman, 1961). Branches from the nutrient artery supply the bone marrow. To the cortex pass both relatively wide arterioles, which penetrate half the cortex and anastomose with vessels in the Haversian systems (Brookes, 1964), and branches which penetrate the innermost layer of the cortex, then to return to the medullary cavity and empty into venous sinusoids (Röhlich, 1941; Brånemark, 1959). In addition to these vessels there are branches which penetrate the entire cortex and directly connect the periosteal arterial system with the nutrient artery without interceding capillaries (Brookes & Harrison, 1957; Göthman, 1961). In practically all cases there is a secondary nutrient artery, even though rudimentary (Göthman, 1961). This perforates the tibia ventro-distally to the tibiofibular synostosis and supplies the fibular-distal part of the tibial medullary cavity. Sometimes the descending branch of the primary nutrient artery is lacking. In these cases branches from the secondary nutrient artery supply the whole of the medullary cavity distal to the synostosis.

The metaphyseal arteries arise from the periosteal vascular plexus around the metaphysis and perforate the cortex with fairly wide branches in several places. The arteries send off branches within the metaphyseal area and

anastomose with branches of the nutrient artery, resulting in the formation of a medullary arterial system (Brookes & Harrison, 1957; Göthman, 1961; Trueta & Caladias, 1964). The growing epiphysis has a separate vascular supply. After closure of the epiphysis, anastomoses occur between the metaphyseal and the epiphyseal arteries (Trueta & Caladias, 1964). According to Göthman (1961), the anastomoses with the vessels in the distal epiphysis are wide but narrow with the vessels in the proximal epiphysis.

The periosteal arterial system of the diaphysis consists of six or seven longitudinal artieries, which run along the entire length of the diaphysis and unite the arterial, periosteal plexus at the metaphyses (Morgan, 1959). In growing bones, branches pass from the periosteal arteries to a capillary network located in the osteogenic layer of the periosteum immediately adjacent to the bone surface (Brookes, 1964). This periosteal capillary network is, in addition, supplied with a large number of small vessel branches from musculature attached to the bone. The periosteal capillary network communicates with the vessels in the Haversian canals via several narrow vessels, which arise from the network almost at right angles. These vessels, which penetrate the outer cortex, are most profuse nearest to the metaphyses (Morgan, 1959; Trueta 1963; Göthman, 1961).

The main venous vessel in the rabbit tibia consists of a thin-walled canal located in the centre of the medullary cavity. This canal is about 10 times wider than the nutrient artery (Brookes, 1964). According to Morgan (1959) there are two principal veins in the distal part of the tibia, which unite just below the middle of the tibia. From this central vein radiate, like spokes of a wheel, a large number of venous sinusoids into which intracortical veins and medullary vessels empty. The central vein is emptied partly through an emissary foramen situated inferio-anteriorly to the entrance of the secondary nutrient artery, and partly through several small veins, especially in the metaphyseal areas, to veins outside the bone.

The vessels in the diaphyseal cortex lie in bone canals, the diameters of which are 10–20 μ (Brånemark, 1959). Brookes (1964) states that within the periosteally formed bone in young adult rabbits, the bone canals converge towards the primary ossification centre of the shaft. In the endochondrally formed bone the bone canals show a mainly longitudinal course. Numerous transverse anastomoses are found between the bone canals. The intracortical canals tend to be wider towards the endosteal than towards the periosteal surface (Cohen & Harris, 1958). The bone canals usually contain one vessel, which consists of a single endothelial tube of constant diameter even after repeated divisions and anastomoses (Brookes, 1964). According to Brånemark (1959) the diameter of this capillary is 7–8 μ. On vital microscopy of the endosteal part of the cortex in the rabbit fibula,

Brånemark (1959) found in some intracortical canals two vessels with different flow rates and opposite flow directions. Trueta (1963) found, on ultraviolet microscopy of subperiosteal bone from the rabbit after injection of tetracycline, intracortical canals containing two vessels, one of which he considered to be a precapillary and the other a vein. Brookes (1964) observed further, that in the endosteal part of the cortex there was a relatively small number of wide canals, which contained precapillaries.

There is disagreement between different investigators as to which parts of the diaphyseal cortex in the rabbit are normally supplied by the different arterial systems. The various observations have been made by histological and angiographic studies (de Marneffe, 1951, 1953; Brookes & Harrison, 1957; Morgan, 1959; Göthman, 1961; Trueta, 1963; Trueta & Caladias, 1964) and by vital-microscopic investigations (Brånemark, 1959). The circulation in the cortex is described by Brookes (1964) as mainly centrifugal. According to Brookes the cortex is supplied with blood from branches of the nutrient artery in the medullary cavity, which penetrate half the cortex and anastomose there with the capillary system in the Haversian canals. Depending upon the pressure conditions in the medullary cavity and in the veins outside the bone, the blood flows from the central parts of the cortex in a centrifugal direction to the periosteal vessels, or in a central direction to the venous system of the medullary cavity. According to Röhlich (1941), the majority of the veins of the compact bone empty into the sinusoids of the medullary cavity.

The vessels in the subperiosteal bone are considered by Brookes (1964) to be solely efferent veins. This opinion is shared by Brookes & Harrison (1957), McAuley (1958), Macnab (1958), Gustilo *et al.* (1964) and Harrison (1966), and is based principally on angiographic studies using a suspension of fine-grained barium sulphate (Mikropack) as contrast medium. According to Brookes & Harrison (1957), however, Mikropack does not fill the vessels distal to the arterioles. This means that the capillaries leading from the periosteum into the cortex "often remain empty of the perfusion medium" (Trueta, 1963). Morgan (1959) and Trueta (1963) consider that the outer one-third of the cortex is supplied by periosteal vessels and the inner two-thirds by branches of the nutrient artery. De Marneffe (1953) claims that the cortex in the upper part of the tibial diaphysis in the rabbit is supplied mainly from medullary vessels, and that in the middle part from both periosteal and medullary vessels, while in the distal part of the diaphysis the vascular supply takes place almost exclusively from periosteal vessels. Communications between the periosteal and endosteal vascular systems via vessels which are wider than capillaries and which pass through the entire cortex, have been shown by Brookes & Harrison (1957), Morgan (1959),

Göthman (1961), Trueta & Caladias (1964), Brookes (1964) and Rhinelander *et al.* (1968), among others.

From angiographic studies in the rabbit, Koekenberg (1963) summarizes the intracortical circulation as follows: "... the cortical network can be considered the capillary system of both the periosteal and the marrow vessels. The cortical network itself forms an anastomosis between periost and marrow vessels". He considered the most important source of blood supply to be the nutrient artery, however. This opinion is shared by Rhinelander *et al.* (1968), Kelly (1968) and others.

THE EFFECT OF SELECTIVE OCCLUSION OF THE MAIN BLOOD VESSELS OF THE LONG BONE ON THE INTRACORTICAL AND MEDULLARY CIRCULATION

The long bone obtains its arterial blood supply from three vascular systems: the nutrient artery, the metaphyseo–epiphyseal arteries and the periosteal vessels. For understanding of the nutrition of bone after operations on the diaphyseal cortex, it is essential to know to what extent the different vascular systems can take over each others' normal supply areas. Attempts have been made to clarify this question by selectively occluding, in animals, one or more of the vascular systems and then studying by histological and angiographic methods the extent of the necrosis which has occurred and the deficiency of vascular visualization in the tissue. Pressure measurements in the medullary cavity have also been made in an attempt to elucidate this problem.

Division of the Nutrient Artery

The nutrient artery has been divided outside the bone. For the blood supply to the bone, the periosteal and the metaphyseo-epiphyseal vessels then remain. Such studies were performed on the rabbit by Bragdon *et al.* (1949) and by Trueta & Caladias (1964). On histological examination, Bragdon *et al.* found in the femur of young rabbits small areas of infarction in the medulla and also in the inner parts of the cortex. In some cases large areas of cortical necrosis were found. Trueta & Caladias studied the radius in young and adult rabbits by histological methods and by angiography with Mikropack. They reported normal visualization of the vessels in the medulla and cortex, and histologically no necrosis was seen either in young or adult animals.

Ligation of the nutrient artery outside the bone was performed in the dog

tibia by Drinker *et al.* (1922). On perfusion of the remaining vessels with Indian ink, normal visualization of the intracortical and medullary vessels was obtained.

Shaw (1964) ligated the nutrient artery in the femur of young cats with open epiphysis and found a pronounced fall in the intramedullary pressure and blood flow, while in adult animals the operation resulted in only a slight reduction of the intramedullary pressure and blood flow. Cuthbertson *et al.* (1964) reported that ligation of the nutrient artery of the tibia and humerus in dogs caused an immediate and profound fall in the intramedullary pressure, and it remained low for 1–3 hours in 9 out of 19 bones and for 4–22 days or more in the 10 other bones.

Division of the Nutrient Artery Combined with Blockade of the Periosteo-Cortical Circulation

After division of the nutrient artery and occlusion of the periosteal and cortical vascular communications, only the metaphyseo-epiphyseal vessels remain for providing the arterial blood supply. The function of the periosteal vessels will have been temporarily discontinued by the fact that the periosteum is lifted up from the cortex and then replaced against it. Revascularization of the cortex from the periosteum is then not prevented. Permanent occlusion of the periosteo-cortical communication has been brought about by placing teflon foil between the periosteum and bone after raising the periosteum from the cortex, the teflon foil then preventing revascularization of the cortex from the periosteum. Such studies have been performed on the rabbit by Foster *et al.* (1951) and Trueta & Caladias (1964). After temporary occlusion of the periosteal circulation and division of the nutrient artery, Foster *et al.* found in the femur of young rabbits, on histological examination, total necrosis of both the bone marrow and the cortex. In adult animals they obtained very varying results. Thus in many animals both the bone marrow and the cortex remained normal. Trueta & Caladias found in young rabbits after permanent occlusion of the communications between periosteum and cortex, with division of the nutrient artery, that the nutrient artery in the medullary cavity did not fill with Mikropack on angiography and that histologically there was total necrosis of both the bone marrow and the cortex. In adult rabbits the nutrient artery filled with contrast medium inside the medullary cavity. The bone marrow remained normal histologically but the outer and middle parts of the cortex became devitalized.

Corresponding studies were performed on the dog by Johnson (1927), Brunschwig (1929), Larson *et al.* (1961) and Silberman *et al.* (1967). After

temporary occlusion of the periosteo-cortical vascular communications and division of the nutrient artery in young dogs, Brunschwig found necrosis of the bone marrow and inner parts of the cortex on histological examination, Larson *et al.* found almost complete filling of the intramedullary vessels with Mikropack on angiography and a fully viable cortex histologically, and Silberman *et al.* observed almost total avascularity of the cortex on angiography with Mikropack seven days after the operation, but a viable cortex on histological studies. This might be due to factors discussed on page 74.

After permanent occlusion of the communications between periosteum and cortex and simultaneous division of the nutrient artery in young dogs, Larson *et al.* found that in some cases the bone marrow lacked Mikropack-filled vessels, while in other cases the vascular visualization was increased. The cortex remained viable in some cases, and was necrotic or partially necrotic in others. Silberman *et al.* observed total necrosis of the bone marrow and cortex.

On temporary occlusion of the periosteo-cortical communications and division of the nutrient artery in adult dogs, Brunschwig and Larson *et al.* found no damage to the bone marrow or cortex. On permanent occlusion of the periosteo-cortical communications and division of the nutrient artery, also in adult dogs, Larson *et al.* observed some necrosis of the bone marrow, while Silberman *et al.* and Johnson found the marrow to be normal. Larson *et al.* and Silberman *et al.* both found a normal cortical circulation, while Johnson observed necrosis of the outer two-thirds of the cortex.

Blockade of the Periosteo-Cortical Circulation

After blockade of the periosteo-cortical circulation, the nutrient artery and the metaphyseal vessels remain for vascular supply of the cortex. Trueta & Caladias (1964) found partial necrosis of the outer parts of the cortex after permanent blockade of the periosteo-cortical circulation in the rabbit tibia. The bone marrow and remaining parts of the cortex remained viable.

Blockade of the Periosteo-Cortical Circulation and of the Metaphyseal Vessels

After blockade of the periosteo-cortical vascular communications and of the metaphyseal vessels, the nutrient artery is solely responsible for supplying the diaphysis. The metaphyseo—epiphyseal vessels have been blocked by opening the medullary cavity in the metaphyses and scraping out the bone marrow. In order to obtain permanent blockade of the metaphyseal vessels the medullary cavity has been plugged with, for example wax, in the metaphyseal area.

After permanent blockade of the metaphyseal vessels in this way, and permanent occlusion of the periosteo-cortical vessels with interposition of teflon, Trueta & Caladias (1964) found normal visualization of the vessels of the bone marrow and of the inner $^{2}/_{3}$–$^{3}/_{4}$ of the cortex on angiography with Mikropack in tibiae of both young and adult rabbits. The vessels in the outer $^{1}/_{3}$–$^{1}/_{4}$ of the cortex did not fill with Mikropack and these parts of the cortex showed necrosis. Johnson (1927), after similar operations on the dog, observed necrosis of the outer two-thirds of the cortex but otherwise normal cortex and medullary contents.

The function of the nutrient artery can also be studied by perfusion of the vessel. Perfusion with Indian ink of the nutrient artery alone, without interference with the remaining vascular systems, was performed by Drinker *et al.* in 1922. After this procedure they obtained visualization of the entire cortical and medullary vascular systems and noted that Indian ink left the bone through periosteal vessels. Shim *et al.* (1968) found on studying the arterial supply of the femur in young rabbits with strontium^{85} that the nutrient artery was responsible for 71% of the blood supply to the diaphysis, including the bone marrow.

Division of the Nutrient Artery and Occlusion of the Metaphyseal Vessels

The possibility of the periosteal arteries to compensate for loss of the nutrient artery and metaphyseal vessels is of especial interest with regard to operations in the medullary cavity. Different investigators have obtained very divergent results in such studies, which has probably been largely due to the different methods used. Trueta & Caladias (1964) divided the nutrient artery outside the tibia in young and adult rabbits and at the same time made a permanent break in the communication between the metaphyseal and diaphyseal vessels within the medullary cavity by scraping out the bone marrow in the metaphysis and plugging the cavity with wax. They found that on angiography with Mikropack the intracortical vessels were not visualized until about one week after the operation, and also that the bone marrow and inner two-thirds of the cortex had become necrotic. Macnab (1958), McAuley (1958) and Gustilo *et al.* (1964) considered after perfusion studies with Mikropack that the periosteal vessels were unable to supply any part of the cortex. Johnson (1927) found after obliteration of the medullary circulation in the tibial diaphysis of the dog that total necrosis of the bone marrow had occurred and necrosis of the inner half of the cortex. He also found that on Indian ink angiography the cortex was "very little injected". De Marneffe (1951) claims that the periosteum in the distal part

of the rabbit tibia can supply the entire cortex. Rhinelander & Baragry (1962) consider that the periosteal circulation of long bones can take over when the medullary circulation has been interrupted.

Summary

The diaphysis of the long bone is supplied from the nutrient artery, the metaphyseal and the periosteal arteries. If one or more of these arteries is obliterated the remaining vessels will to varying extents take over their function.

The periosteal and the metaphyseo-epiphyseal vessels are able together to supply both the bone marrow and the cortex when the nutrient artery has been divided. In growing animals the periosteal arteries are then of the greatest importance, while in adult animals, in which the epiphysis is closed, the most important vessels are the metaphyseal and epiphyseal arteries (Trueta & Caladias, 1964).

In young rabbits the metaphyseal vessels cannot alone supply the contents of the medullary cavity and the cortex even if revascularization of the cortex from the periosteum is not prevented. In young dogs the metaphyseal vessels protect the cortex from necrosis if revascularization from the periosteum is not rendered impossible, but if revascularization is prevented, varying degrees of cortical necrosis occur. In both adult rabbits and adult dogs the metaphyseo-epiphyseal vessels alone can essentially supply the contents of the medullary cavity and at least the inner parts of the cortex.

If only the nutrient artery remains and the periosteo-cortical circulation is permanently interrupted, necrosis occurs in the outer third of the cortex, but when teflon foil is interposed between the periosteum and cortex, the venous outflow from the cortex is probably also affected, and this can contribute to the cortical damage. Rhinelander *et al.* (1968) has pointed out that a blocked cortical outflow can cause the occurrence of bone necrosis under a compression plate lying closely against the bone.

In cases where the periosteal vessels alone remain, several investigators have found that the intracortical vessels are not visualized primarily on angiography with Mikropack. This has been considered to mean that no functioning vessels are present. Trueta (1963) pointed out, however, that a lack of visualization of the vascular system with Mikropack does not preclude the presence of a nutritive flow. When the medullary cavity of the metaphyses were plugged with wax in order to close off the metaphyseal circulation, a pressure increase may have been produced in the medullary cavity, which might have affected the circulation in the cortex. Thus there

is no definite evidence that the periosteal vessels cannot take over the nutrition in part of the diaphyseal cortex.

There seems thus to be no marked difference between the rabbit and dog with regard to the potentialities of the three different vascular systems for taking over each others' circulatory regions.

THE EFFECT OF SURGERY ON THE MEDULLARY CAVITY OF THE DIAPHYSIS OF THE LONG BONE

Partial Destruction of the Medullary Circulation

Surgery to the medullary cavity can disturb the medullary and cortical circulation to different degrees. On intramedullary nailing of the rabbit tibia *without fracture,* Göthman (1961) found that remnants of the nutrient artery were often visualized in the medullary cavity on arteriography. During the first days after the operation he observed slight filling of the intracortical vessels with Mikropack contrast medium. Not until after 10 days were the intracortical vessels better visualized. The osteocytes in the cortex remained viable.

Trueta & Cavadias (1955) studied fractured radii in rabbits treated with intramedullary nailing and found on angiography with Mikropack that the medullary vessels were practically always completely destroyed. Even far from the fracture line, necrosis of the inner third to two-thirds of the cortex was observed histologically, in both young and adult animals.

On intramedullary nailing of osteotomized rabbit tibiae, Göthman (1961) often found remnants of the medullary arteries in the medullary cavity. The vascular filling in the cortex away from the fracture line was similar to that in tibiae subjected to intramedullary nailing but not fractured. In two out of 35 rabbits a long, irregular area of periosteal callus was seen along both fracture fragments. On angiography of osteotomized tibiae in rabbits treated with intramedullary nailing, using Mikropack + Berlin blue as contrast medium, Koekenberg (1963) obtained a similar degree of visualization of the medullary vessels as Göthman (1961).

Rhinelander *et al.* (1967) performed osteotomy and intramedullary nailing of the ulna in dogs and found on angiography with Mikropack that the cortex in contact with the medullary nail showed reduced vascularity of the inner half to two-thirds, while that which had no contact with the nail was vascularized.

Gustilo *et al.* (1964) found on Mikropack angiography of osteotomized dog femurs treated with intramedullary nailing that the periosteal vessels did not penetrate the cortex to supply it.

Göthman (1961) performed intramedullary nailing of osteotomized tibiae in monkeys. He found on angiography with Mikropack that the medullary arteries were less extensively damaged than in the rabbit. In monkeys, also, he observed almost no filling of the intracortical vessels with contrast medium during the first week postoperatively.

Intramedullary nailing of osteotomized long bones in animals without angiographic examination has been performed by several investigators, including Küntscher (1961) and Anderson (1965).

Total Destruction of the Medullary Circulation

If the medullary contents are removed, the cortex is completely dependent upon the periosteal circulation for its nutrition. Such operations have been carried out on rabbits by Röhlich (1941), Brånemark (1964) and Zucman *et al.* (1968), on cats by Richany *et al.* (1965) and on rats by Mital & Cohen (1966). In connection with this operation, angiography has only been performed by Mital & Cohen. Röhlich removed the bone marrow of the diaphysis with gauze after dividing the bone through the metaphyses. Brånemark removed the marrow through a groove sawn out on the anterior side of the bone. Richany *et al.* washed out the marrow with salt solution and, after having put down a rotating stainless steel pin in the cavity, brushed the medullary cavity with a pipe cleaner. He obtained massive necrosis of the cortex except within the very outermost layer. Mital & Cohen removed the medulla by several different methods, including suction, brushing with a baby-bottle brush and filling of the medullary cavity with agar, and found necrosis in the cortex on maximal injury.

After operations in the medullary cavity with partial or total destruction of the bone marrow, typical reactions from the periosteum, cortex and medullary cavity are obtained.

Periosteal Reaction

On disturbance of the endosteal circulation, the periosteum reacts with increased vascular filling and proliferation of the vessels (Trueta & Cavadias, 1955; Göthman, 1961; Zucman *et al.*, 1968). Trueta & Cavadias consider that proliferation of the periosteal vessels is always accompanied by the formation of new bone. Despite considerable vascular proliferation in the periosteum after intramedullary nailing of non-fractured tibiae in rabbits, Göthman found no new bone formation in the periosteum, however, except in a very few cases.

Increased periosteal new bone formation in the diaphysis of the long bone has been demonstrated by several investigators after destruction of the me-

dullary vessels. The bone formation was most extensive over the areas where the medullary and intracortical circulation was most severely damaged (Axhausen & Bergmann, 1937; Trueta & Cavadias, 1955; de Marneffe, 1951; Gustilo *et al.*, 1964; Richany *et al.*, 1965; Andersson, 1965; Mital & Cohen 1966). Richany *et al.* found that the amount of periosteal bone formation corresponded exactly with the extent of medullary removal. The increased subperiosteal bone formation has been considered by Küntscher (1957) to be due to local acidosis, and by Trueta & Cavadias to periosteal hyperaemia over necrotic cortex. Richany *et al.* and Johnson (1966) considered that local stasis with oedema and periosteal anoxia was the cause of the periosteal bone formation, while Zucman *et al.* (1968) showed that medullary fragments were squeezed out through the cortex to the subperiosteal space and there gave rise to extensive formation of bone.

The subsequent development of the periosteal callus tissue has been studied by Trueta & Caladias (1964), Richany *et al.* (1965) and Zucman *et al.* (1968). Trueta & Caladias found in young rabbits that the subperiosteal cells proliferated after 2 days, that new bone was present after 4 days and that after 6 days there were new trabeculae subperiosteally. After 12 days the new bone covers the whole ulnar surface of the radius. In adult rabbits a thin smooth layer of lamellar bone was found subperiosteally after 6 weeks. Richany *et al.* observed, in young adult cats, early marked cellular proliferation with circumferential subperiosteal bone deposition involving the diaphyses. A maximal amount of subperiosteal bone was seen after an observation time of 20 days, and subsequently the bone mass gradually decreased. Periosteal bone was transformed to mature Haversian compact bone.

Zuchman *et al.* (1968) studied growing rabbits after reaming of the medullary cavity without removing bone from the endosteal surface. They found on radiographic examination that callus tissue was observed 15 days postoperatively, and this tissue reached a maximal size on the 15th to 21st day. In the centre of the callus, cavities developed, and in the periphery of the callus corticalization was observed. Cavities in callus tissue were described by Falkenberg (1961) in osteotomies with intramedullary nailing in rabbits. He observed the first cavity 30 days after the operation. According to his photographic illustrations it would seem that the cavities were localized more and more centrally in the bone with increasing time postoperatively.

Cortical Reaction

When a necrosis of the inner part of the cortex has occurred, e.g. after operations in the medullary cavity, the necrotic area is revascularized from

the viable outer part of the cortex (Phemister, 1930; Röhlich, 1941; Trueta & Cavadias, 1955; Koekenberg, 1963; Ricany *et al.,* 1965; Rhinelander *et al.,* 1968). The channels in the viable part of the cortex become wider and contain more blood vessels (Axhausen & Bergmann, 1937; Brookes, 1960). Vessels with preceding osteoclasts, so-called cutting heads, bore channels through the necrotic bone in the direction towards the medullary cavity. The bone channels then become gradually narrower behind the "cutter head" by the formation of bone on their walls (Schenk & Willenegger, 1963; Johnson, 1966). In young animals, large, irregular resorption cavities can be formed in the necrotic bone (Axhausen & Bergmann, 1937; Richany *et al.,* 1965), but in adult animals small, round cavities are formed (Johnson, 1966). This rebuilding process also affects viable bone but the resorption cavities are smaller in viable than in necrotic bone (Axhausen & Bergmann, 1937).

In young adult cats the maximal reconstruction of the cortex takes place 40 days after disturbance of the endosteal circulation, when the periosteal reaction is already declining (Richany *et al.,* 1965). Trueta & Cavadias (1955) found that in growing rabbits cortex, two–thirds of which had primarily shown necrosis, was almost completely rebuilt after 7 weeks, while in adult animals necrotic areas were still present after 8 months. Resorption in the inner part of the cortex was established radiographically after an observation time of 4 weeks (Trueta & Cavadias, 1955; Richany *et al.,* 1965). According to Phemister (1948), large necrotic areas in the cortex are never completely rebuilt. Especially in older individuals, the reparative stimulus seems to become exhausted. Axhausen & Bergmann (1937) consider that the cells from which new bone formation occurs in the necrotic bone areas arise from the periosteum or from the medullary cavity, while in the opinion of Trueta (1963) they arise from the vascular endothelium. Other authors believe that the source of origin is the perivascular mesenchymal tissue (Brookes, 1960). The amount of new bone that is formed to replace the degenerated necrotic bone is highly dependent upon the functional stimulus to which the limb is subjected (Phemister, 1930). As a rule, more bone is broken down within the zone than is reformed (Axhausen & Bergmann, 1937).

Medullary Reaction

The healing processes in the medullary cavity after the evacuation of its contents have been studied by Röhlich (1941), Trueta & Caladias (1964), Brånemark (1964) and Richany *et al.* (1965), among others. All these investigators give similar descriptions for these processes, but the time course varies considerably. After 3 days Brånemark observed widening of the Haversian canals nearest to the medullary cavity and at the same time the

haematoma in the medullary cavity began to be organized. Röhlich, using histological methods, observed bone formation in the medullary cavity after 14 days. Trueta & Caladias noted some new vessels in the medullary cavity 9 days after operation. Röhlich found that the bone bridges began to be resorbed within its peripheral areas 19 days after operation. Brånemark observed this 3 weeks after operation. According to Richany *et al.* the normal medullary structures were rebuilt after 160 days. Röhlich observed that the vessels in the medullary cavity nearest to the residual avascular region were narrow, while those around the bone bridges in the more peripheral area of the medullary cavity were wide and thin-walled. Trueta & Caladias found that in adult rabbits blood vessels did not invade the medullary cavity from the cortex until after 6 weeks, and infarctions still persisted in the medullary cavity 6 months postoperatively.

HISTOLOGICAL CHANGES ON NECROSIS OF CORTICAL BONE

When the blood circulation to bone tissue is occluded the bone dies in the course of a few days (Phemister, 1948; Bonfiglio, 1954). Woodhouse (1962), after occlusion of the circulation to the femoral head for 6 hours in the dog, found total necrosis of the femoral head in 3 cases out of 6. After occlusion of the circulation for 12 hours he found total necrosis of the femoral head in all cases.

Axhausen & Bergmann (1937) studied the development of bone necrosis and found that during the first week after the circulation to the cortical bone had been occluded no histologically visible changes occurred. Later there was pyknosis of the osteocytic nuclei, which became fragmented, decreased in size and stained darker. Axhausen & Bergmann considered that the recognition of pyknosis was rather difficult and required high magnification and very careful observation. They also considered that the most reliable sign that the bone was necrotic was the presence of nucleus-free osteocytic lacunae. According to Axhausen & Bergmann, the degeneration and removal of the pyknotic nucleus demands circulating tissue fluid. The time it takes for the osteocytic nuclei in cortical bone to dissolve is dependent upon the thickness of the bone tissue and the penetration capacity of the vessels which force their way in and by which means the dissolution takes place. According to Schenk (1969) osteocytic nuclei in cortical bone disappear about 2 weeks after bone necrosis. In dead bone the nuclei disappear first from the surface, and the layer beneath contains pyknotic osteocytic nuclei (Axhausen & Bergmann, 1937). In ischaemia of cortical bone without total occlusion of the circulation, the cells in the interstitial lamellae to which

the nutrition is normally poorest die first, while the cells in the Haversian systems are still intact (Brookes, 1960). On further deterioration of the blood supply the osteocytes in the remaining parts of the bone die, while the cells in the bone marrow and in the periosteal soft tissues can remain viable. The osteocytes are thus very sensitive to anoxia (Halshofer 1937).

Bonfiglio (1954) found stainable osteocytic nuclei in the femoral head of the dog 2–3 weeks after total permanent occlusion of the circulation to the femoral head. Catto (1965) obtained similar results in studies of the femoral head in man after medial femoral neck fractures, and Rokkanen *et al.* showed in 1965 that histologically visible changes in the rabbit femoral head did not occur until 2–3 weeks after total occlusion of the circulation to the head.

Other workers have found considerably more rapid removal of the osteocytic nucleus from necrotic cortical bone than the investigators referred to above. Ham & Harris thus found in 1956 in fractures of cortical bone that the osteocytic lacunae at the fracture ends were already empty 2–3 days after the fracture. Foster *et al.* (1951), after ligation of the nutrient artery and stripping of the periosteum in the rabbit, observed that the osteocytes in the cortex had faded already within the first 24 hours. Within 3 days the osteocytic nuclei had disappeared except in some areas.

CHAPTER 2

Reaming, Brushing and Suction of the Medullary Cavity of the Rabbit Tibia

Material and Methods

Adult and growing non-pregnant rabbits of different breeds and of both sexes were used for the experiments. The rabbit was chosen as the main experimental animal both because its tibia is sufficiently large to allow reaming of the medullary cavity and because a relatively large number of animals was required.

The information given by the breeders on the ages of the animals was not always reliable. The material was therefore divided into two groups, of adult and growing animals, according to whether the fibular epiphysis was found to be closed or not on radiographic examination, which was performed at the end of the experiment. According to Heikel (1960) the epiphysis of the rabbit fibula closes at the age of 5–7 $^1/_2$ months, and according to Geiser (1963) this takes place at 5 months.

Of 178 rabbits used, 4 were used for determining the heat development and pressure conditions in the medullary cavity on reaming and brushing, 22 for a study of intracortical fat and 24 for correlation of the amount of newly formed bone to the degree of traumatization of the cortex. From the remaining 128 animals, which were used for general morphological studies, after reaming 25 were excluded after fracture had occurred on the treated leg, and 17 died for various reasons during the experimental period. The remaining 86 animals are presented in Table I.

Preliminary studies showed that the morphological changes which occurred could most suitably be recorded at observation times of 3 days and 1, 2, 4, 8 and 12 weeks. Large groups of animals were therefore studied at these observation times after operation, and these were called the main experimental groups. In 61 of the 86 animals (28 adults and 33 growing animals), the medullary cavity of the left tibia was reamed, while the right tibia served as a control. In order to obtain a larger amount of material for morphological studies, both the left and right tibia were reamed in 25 animals (7 adults and 18 growing animals). In these cases the left tibia was used for fluorescence microscopic studies and the right tibia for angiographic and histological studies.

Table 1. *Rabbits for morphological studies after reaming of the medullary cavity.*
* indicates that the animal is reamed in both tibias.

Obs. time	Growing animals	Adult animals
2 hours	27*	
4 h	28*	—
8 h	32* 33*	—
1 day	40* 61	9*
2 d	38*	10*
3 d	3 81 114 123 124 125	54 55 63 109 110
4 d	37* 41*	—
7 d	64 66 95 104 105 106	112 121 126
10 d	4	—
2 weeks	6 42* 45* 59 101 108 211 220 281 285	57 58 86 87 119
3 w	5 46* 47*	—
4 w	7 15* 51 52 96 97 216	75 88 89 100 113
5 w	13* 31*	73
6 w	30* 36*	—
7 w	—	11* 23*
8 w	14* 50 65 92 111	21* 67 77 83 84 85
10 w	—	39*
12 w	—	43* 56 69 70 76

The rabbits were kept separately in ordinary rabbit cages with floors of wire netting. They were given free access to water and to food pellets with a standardized content of calories, minerals and vitamins.

Reamer Equipment for Widening of the Medullary Cavity

For reaming of the medullary cavity plain excavating burrs for dental use, manufactured by Hager and Meisinger, Düsseldorf, were used. Eleven different burr numbers were used with burr head diameters of 2.52–4.72 mm. The increase of the burr head diameter between each burr number was 0.1–0.3 mm. A 15–20 cm long shaft in the form of a wire spiral was constructed of piano wire. The external diameter of the wire spiral was 2.5 mm for the narrowest burrs and 3.5 mm for the widest.

The burr was divided 10 mm below the head, and the neck of the burr was ground down to a diameter of 1 mm. The ground burr neck was inserted into the central canal of the wire spiral to about 2 mm from the head, and soldered into the canal (Fig. 1). The reamers so produced were kept standing in spirit when not in use. For reaming of the medullary cavity the reamer shaft was fixed in a sterilized chuck, which was driven by a flexible drive axle covered by a sterile linen bag, and this axle was coupled to an electric motor with a rotation rate of 300 r.p.m. The motor was controlled by a foot switch.

Operation

The rabbits were anaesthetized with Nembutal® Abbot for veterinary use, in a dose of about 30 mg/kg body weight, which was given as an intravenous injection through an aural vein. Complementary injections of Nembutal® were given until the Achilles reflex was no longer present. The animal was strapped in the supine position on to a special sled-formed operation table with the hip and knee joints flexed at 60°. The hind limbs were supported under the thigh, the knee joint and the heel, while the lower leg was free. The leg was fixed to the operation table by straps over the thigh and foot. In order to avoid traumatization of the lower leg, no pressure was exerted against it during the operation. After washing of the skin with spirit, a 2 cm long arciform skin incision was made lateral to the knee joint. A skin flap with its base on the medial side was dissected free and folded over medially. The patellar ligament was exposed and incised longitudinally. An awl which had been ground so as to produce four sides was then inserted into the medullary cavity under the patellar ligament so as to produce a hole about 4.5 mm in diameter, and slowly and carefully rotated about 1 cm into the cavity.

The contents of the medullary cavity were removed by one of the following three methods:

1. *Reaming.* The narrowest reamer, with a diameter of 2.52 mm, was first inserted into the medullary cavity slowly and under rotation. The reaming was then repeated with successively wider reamers until a layer of cortex of an estimated thickness of $^1/_4$-$^1/_2$ mm had been removed from the middle part of the bone. The reaming was performed intermittently, about a half-minute of reaming being followed by a half-minute interval.

2. *Suction.* The contents of the cavity were sucked out through a polyethylene catheter with an inner diameter of 1.5 mm. The suction was repeated three times and between each suction an elastic metal spiral about 1 mm thick and with a curved end was inserted into the medullary cavity under rotation in order to mobilize bone marrow attached to the endosteum.

3. *Brushing.* The content of the medullary cavity was mobilized by means of a bottle-brush 7 mm thick and 2 cm long attached to a wire shaft. The brush was inserted into the medullary cavity as far as the distal metaphysis and then withdrawn. The brushing was repeated twice.

The skin incision was closed with silk sutures and the wound covered with Nobecutan®.

Bone Labelling

In order to label the newly formed bone an intravenous or intraperitoneal injection of tetracycline (Dumocyclin®, Dumex[1]) was given in a dose of

[1] Dumocyclin was kindly supplied by Dumex Ltd., Denmark.

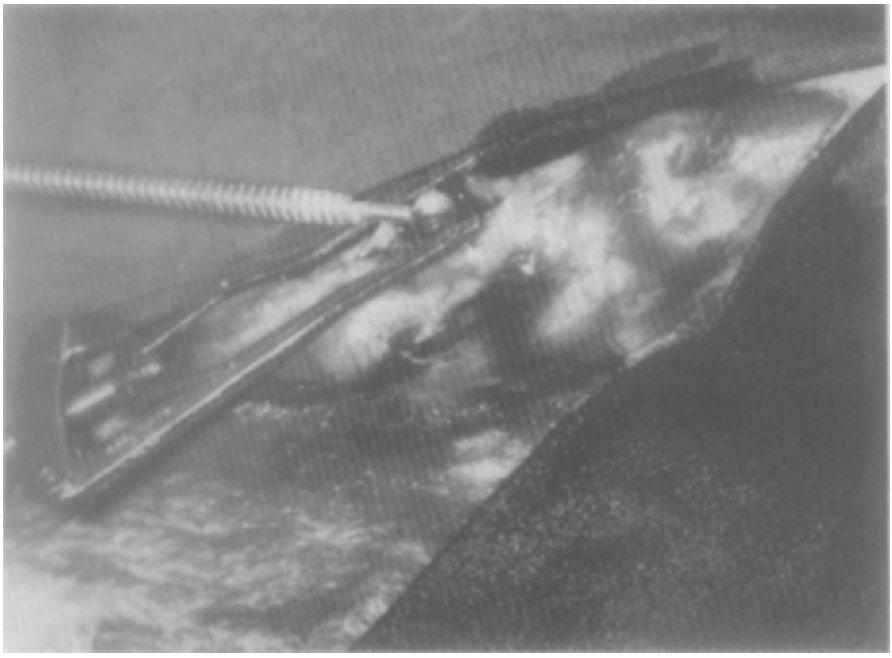


Fig. 1. The reamer with its flexible axis is inserted through the patellar ligament which was incised longitudinally.

50 mg/kg body weight. Animals studied at shorter observation times than 3 days did not undergo bone labelling. All other animals were given the labelling substance intraperitoneally 2 days before the angiographic examination. This labelling was called labelling 2. In the main experimental groups all animals were given one further labelling, called labelling 1, intravenously or intraperitoneally 0–7 days after the reaming of the medullary cavity. As a rule, for labelling 1, the growing animals were given tetracycline 0–1 day after the operation and the adult animals 1–4 days after the operation. The remaining animals not belonging to the main experimental groups with an observation time of >3 days underwent labelling 1 at varying times after the operation.

Microangiographic Procedure

200 ml of Pelikan Indian ink suspended in 800 ml of physiological saline was used as the infusion medium. At the end of the observation time the animal was anaesthetized with Nembutal® and 2 ml of heparin (5000 U/ml) was given intravenously. The animal was placed in the supine position and

the abdomen was opened by a midline incision. The small blood vessels in the abdominal wall which had been severed at the laparotomy were left open. The aorta was exposed below the renal arteries and ligated proximally. Through an incision in the aorta a polyethylene catheter with an inner diameter of 1.1 mm was inserted distally until its tip lay immediately above the aortic bifurcation. A strong blood flow was obtained through the catheter. The catheter was connected to a drip infusion apparatus for infusion of the Indian ink solution. The infusion pressure was about 130 cm H_2O. As a rule the animals died when 200–300 ml of the solution had been infused. Towards the end of the infusion the infusion rate decreased spontaneously and gradually. After infusion of 800–1000 ml the infusion was discontinued. Both legs were then exarticulated at the hip joint. The skin was removed from the thigh and lower leg, after which the limb was fixed in 10% neutralized formalin solution contained in a large vessel. After 2 days the tibia was dissected free from the skin and outer soft tissue. Fixation in formalin was then continued for a further 5 days, after which the preparations were frozen to −20°C.

Macroradiography

Macroradiographs were taken of both tibias in the frontal projection.

Preparation

In the general morphological series the tibia in the deep-frozen state was divided with a saw 2.5 and 7.5 cm from the tibiotalar joint. The intermediate 5 cm of the diaphysis was used for the preparation of sections for fluorescence microscopy, microangiography and conventional histology. From each end of the diaphyseal preparation $^1/_2$ cm was sawn off and decalcified. A further $^1/_2$ cm was sawn off from each end and embedded in methyl metacrylate. The remaining 3 cm long part of the diaphysis was sawn longitudinally into one fibular and one tibial half. The fibular half was decalcified and embedded in paraffin. The tibial half was embedded in methyl metacrylate. In the other series of experiments the tibia was divided according to some different scheme, as described in the relevant chapters.

The preparations intended for plastic embedding were dehydrated in absolute alcohol which was changed daily for 5 days. They were then transferred to non-polymerized methyl metacrylate in which they were kept for 4 days, after which they were embedded, in glass tubes, in partially polymerized methyl metacrylate which was finally polymerized in a heat chamber at 30°C during a period of 3–4 days. Slices $^1/_2$ mm thick were then sawn

from the methyl metacrylate-embedded specimens, parallel with the cut surface of the bone. For this purpose a bandsaw was used with a 100 μ set blade, whereby a distance of 300 μ was obtained between the preparations. The slices were ground and polished by hand with sandpaper to a thickness of 50–100 μ and then mounted in Permount under a cover glass. Fluorescence microscopy was performed with a conventional binocular microscope with Zeiss large fluorescence equipment. Selected fluorescence preparations and Spalteholz preparations were photographed on Echtachrome High Speed 23 din for daylight, Kodachrome II or Addox kb 14.

The bone preparations intended for preparation of Spalteholz specimens and conventional histological specimens were decalcified with 5% nitric acid and embedded in paraffin. From the paraffin-embedded preparations $^1/_2$ mm thick sections were sawn parallel with the cut surface of the bone. The paraffin was removed with xylol, the sections treated according to the Spalteholz technique, and studied in a binocular microscope.

In order to assess the amount of newly formed bone, the amount of bone removed on reaming and the degree of intracortical vascular damage the following methods were used.

A. Division of Preparations

Colour diapositives of transverse plastic-embedded cross sections were projected in a magnification apparatus and the images drawn on paper, so that the total longitudinal magnification was 30.1 times. The images from the treated side and from the control side were always projected in such a way that equivalent images were obtained (Fig. 2). On the profile drawing the outer boundary of the outer fluorescence line, the inner boundary of the inner fluorescence line and the borderline between the medullary cavity and the cortex were drawn in. On the profile drawing from the treated side the points at which the reamer had cut into the cortex were marked on the latter borderline. The central borderline for the bone which had been removed during the reaming was determined by comparison with the corresponding area from the control side, as follows. The diapositive from the control side was projected on to the profile drawing from the treated side, so that the corresponding endosteal line of the control side passed through the points in the drawing of the treated side at which the reamer had cut into the cortex. The intermediate endosteal borderline of the control side was then drawn on the profile drawing of the treated side.

The profile drawing was divided into four sectors: (1) tibial, (2) dorsal, (3) fibular, and (4) ventral. The boundaries between the sectors were determined by drawing a line through the tibial boundary of the medullary cavity, so that the intersection between this line and the outer fluorescence line

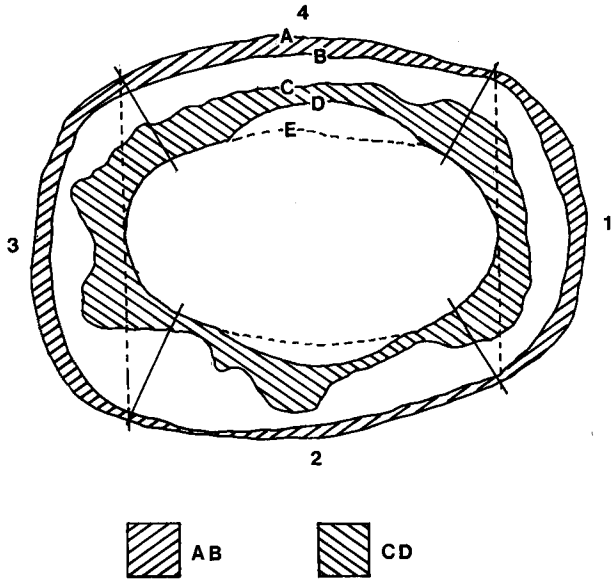


Fig. 2. Drawn image of the distal fluorescence section with the vascular front drawn in. The drawing is divided into 4 sectors: 1, tibial; 2, dorsal; 3, fibular; 4, ventral.
Line *A:* The outer boundary of the outer fluorescent line, resulting from labelling 2.
Line *B:* The inner boundary of the inner fluorescent line, resulting from labelling 1.
Line *C:* The vascular front obtained from the Spalteholz preparation.
Line *D:* The peripheral boundary of the bone removed on reaming.
Line *E:* The inner borderline of the orginal cortex against the medullary cavity.
When no bone was removed on reaming, lines *D* and *E* coincide.
Each sector is divided into the following areas:
AB: Bone formed between and during labellings 1 and 2.
BE: Original cortex.
BC: Vascularized part of original cortex.
CD: Avascular part of original cortex.
DE: Part of original cortex removed on reaming.

(line *A*, see below) lay on the fibular side of both tibial corners of the profile drawing. A line was drawn parallel with this line, through the fibular boundary of the medullary cavity. The fibular corners then lay within the fibular sector. From the respective intersection points with the outer fluorescence line, lines were then drawn straight through the cortex to the medullary cavity. These lines coincided essentially with the principal direction of the intracortical vessels.

B. Assessment of Vascularization

On the plastic-embedded, non-decalcified cross sections which had been photographed, the Indian ink filling of the intracortical vessels could only be assessed incompletely. For this purpose thicker preparations treated according to the Spalteholz technique were required. From the plastic-embedded preparations a further $^1/_2$ mm thick section was therefore cut

adjacent to the section which was used for preparing the photographed fluorescence section. The methyl metacrylate was removed with chloroform, after which the section was decalcified and treated according to the Spalteholz technique. By means of a microscope and a table which could be raised or lowered, the image of this Spalteholz preparation was given the same size as and projected onto the drawn image of the corresponding fluorescence preparation. The vascular pattern in the projected image of the Spalteholz preparation comprised a summation image of the vessels in the $^1/_2$ mm thick section. The borderline between the intracortical vessels which had filled with Indian ink and those which lacked Indian ink was called the vascular front. This line was drawn between that part of every Indian ink-filled vessel which lay nearest to the medullary cavity. In cases where no Indian ink-filled vessels were found within a distance of 3–4 mm, the borderline was drawn towards the periphery until an Indian ink-filled vessel was seen within this distance. No Indian ink-filled vessels were then found in the cortex central to the vascular front, but peripheral to this borderline some Haversian canals without Indian ink-filled vessels could be seen.

The distance between the plastic-embedded preparation and the corresponding Spalteholz preparation was about $^1/_2$ mm, and the profile drawing obtained thus comprised a summation image of one fluorescence preparation and one Spalteholz preparation taken about $^1/_2$ mm from one another.

When the vascular front had been drawn, the following lines were then present on the profile drawing, counting from the periphery (see Fig. 2): *A*, the outer boundary of the outer fluorescence line. This fluorescence line resulted from tetracycline labelling 2, which was given 2 days before the angiographic examination. *B*, the inner boundary of the inner fluorescence line. This fluorescence line resulted from labelling 1. *C*, the vascular front obtained from the Spalteholz preparation. *D*, the peripheral boundary for the bone removed on reaming. *E*, the inner borderline of the cortex against the medullary cavity, and the inner boundary of the bone removed on reaming. In the unreamed areas of the cortex, lines *D* and *E* coincided. See fig. 2.

The profile drawing of the cortex was thus divided into the following areas within each sector:

1. *Area AB*. The part of the cortical cross-section area which was labelled by the two tetracycline injections, and the part between these labelling lines.

2. *Area BC*. The area of original cortex, in which the vessels were filled with Indian ink on angiography (vascularized part of original cortex).

3. *Area CD*. The part where the vessels were not filled with Indian ink (avascular part of original cortex).

4. *Area DE*. The part wich had been removed on reaming of the medullary cavity (part of original cortex removed on reaming).

The size of the different areas was usually determined planimetrically and expressed in per cent of the whole cortical area at the start of the experiment, the original cortex, i.e. of the area *BE* (see below).

C. Special Methods of Measurement

In the following cases the position of line *A* could not be determined exactly on profile drawing of the colour diapositive of the fluorescence preparation:

1. When there was no periosteal new bone formation at the second tetracycline labelling. In these cases the borderline between periosteum and underlying bone could sometimes not be identified.

2. When the bone formation was very rapid at the second labelling, especially on the formation of trabecular bone. The borderline between fluorescence-labelled bone and later formed non-labelled bone was then often diffuse and could not be determined exactly.

The position of line *B* on the projected image was uncertain in the following cases:

1. When labelling 1 gave weak fluorescence. The fluorescence line which then occurred could then be impossible to identify on the projected image.

2. When at the first labelling the bone grew rapidly with the formation of primary osteones. In these cases the fluorescence from the maturing osteones central to the labelling line B could completely obscure this line.

The technical error in drawing of the projected image could also be of great importance for the reliability of the recording if the distance between lines *A* and *B* on the profile drawing was very small and only amounted to about one mm.

If the preparation was sawn obliquely the fluorescence lines would appear broader than they were in reality. If, for example, the preparation was sawn at an angle of 10° and was 50 μ thick, the distance *AB* would be lengthened by 8.8 μ. This would mean that the value obtained for the bone area *AB* would be approximately 0.1 % too large calculated in per cent of the area *BE*. If, for example, area *AB* comprised 0.5 % of area *BE*, the value obtained for area *AB* would be 0.6 %.

In order to avoid the above difficulties, direct measurements were also made on fluorescence preparations in the microscope for recording of the

positions of lines *A* and *B*. The measurements were performed at a magnification of 320 times. The microscope could then be focused exactly to the upper layer of the preparation, which eliminated essentially the increase in breadth which occurred if the preparation was sawn obliquely. The exact positions of lines *A* and *B* could also be more easily established even if the labellings were weak or if irrelevant fluorescence disturbed the image.

Approximation of area *AB* was performed as follows: On the profile drawing line *A* was divided in each sector into six equal parts by means of dividers. The division points were denoted points 0, 1, 2, 3, 4, 5 and 6. The length of each sixth was recorded on the profile and denoted *a*. On the preparation the distance between lines *A* and *B*, included the width of the two fluorescent bands, was measured with an ocular micrometer at points 1, 3 and 5 and the mean value of these measurements was given as the thickness (*T*) of the newly formed bone. With the magnification used one ocular micrometer unit, U, corresponded to 3.5 μ. Area *AB* was expressed in the same planimeter unit as was used in the planimetric determination of the other areas on the profile drawing of the fluorescence preparation. The values for area *AB* so obtained were expressed in per cent of area *BE*.

For approximation of area *AB* the following formula was used for each sector:

$$\frac{T \cdot 3.5}{1000} \cdot 30.1 \cdot 6a \cdot \frac{10.82}{10\,000} \text{ units}$$

where

$\frac{T \cdot 3.5}{1000}$ = the mean value of the three thicknesses of *AB* measured in the microscope at points 1, 3 and 5, expressed i mm

a = $\frac{1}{6}$ of the length of line *A* within the sector, expressed in mm

30.1 = the longitudinal enlargement of the fluorescence profile drawing obtained as the mean value of 10 control measurements with S.E. = ± 0.05.

$\frac{10.82}{10\,000}$ = the number of planimeter units corresponding to 1 mm^2.

Discussion of the Methods

The flexible shaft of the reamer made it possible for the reamer to follow the S-shaped tendency of the medullary cavity. In the tibial diaphysis, especially in the distal part, the medullary cavity is oval. The reamer thus removed bone practically solely from the ventral and dorsal sectors, while only small amounts of bone were removed in the tibial and fibular sectors.

From the area of the tibia from which the distal fluorescence section was taken, 3.0–3.5 cm above the tibio-talar joint, a mean cortical cross-

sectional area of 7.4% (S.E. ±0.64) was removed on reaming. (In growing animals 6.6% and in adult animals 8.4%).

Tetracycline Labelling of Newly Formed Bone

Tetracycline was used for labelling of the newly formed bone, since this is a simple method which in several investigations has given almost perfect correlation with microroentgen and autoradiography (Harris *et al.*, 1962) and also with histological studies of osteoid bands (Kelly *et al.*, 1965; Vanderhoeft *et al.*, 1962). Tetracycline forms chelates with calcium ions. Whenever calcium ions are interchanged or set free, i.e. during mineralization and demineralization, an equilibrium reaction with tetracycline takes place (Eger *et al.*, 1967). Injected tetracycline becomes bound to all cartilage and bone which is undergoing mineralization (Frost *et al.*, 1961; Vanderhoeft *et al.*, 1962). The level of tetracycline in the blood is adequate to label the bone for approximately 8 hours following an intraperitonal injection (Tapp, 1966). Tetracycline given by this route can be expected to give the same good labelling as that injected intravenously (Ahlgren, 1968). The diffuse tetracycline fluorescence in the tissues disappears within 2 days after the injection (Harris *et al.*, 1962; Frost *et al.*, 1961), and in order to avoid this source of error labelling 2 was given 2 days before the angiographic examination.

Labelling of Howship's lacunae, which was recorded by Hulth & Olerud (1962), takes place, according to Eger *et al.* (1967), during the resorption phase of the bone. This labelling occurred often in the preparations of the present study. The labelling band on bone resorption can be distinguished from that on bone formation by its thinness and by the fact that in the former case the border of the bone surface is uneven. The binding of tetracycline in the calcification zone—if the animal has not been given tetracycline during the last day before death—has been described by Frost *et al.* (1961) and Harris *et al.* (1962) as a form of surface stain. According to Hansson (1967) this tetracycline deposition occurs mainly postvitally, *inter alia* in the fixation bath. This labelling was sometimes observed in the present study as an extremely thin fluorescent band on the surface of the bone outside the fluorescent band from labelling 1 or 2, if the latter band lay at a short distance from the bone surface. Since the labelling band in the calcification zone is always very thin it can usually be identified easily.

The Bone-suppressing Effect of Tetracycline

According to Eger *et al.* (1967), tetracycline causes hypoplasia of dental enamel by (1) suppressing the formation of crystal nuclei; (2) preventing

the growth of crystallites; and (3) blocking the conversion by hydrolysis of primary octacalcium-orthophosphate into apatite.

Saxén (1966) found in *in vitro* studies that tetracycline in a concentration of 500 μg/ml completely inhibited the development of the calcified zone in bone rudiment. The effect was irreversible after it had been present for 5 days. Hansson (1967) found that tetracycline in a dose of 25 mg/kg body weight disturbed both the endochondral calcification process and the resorption of the cells of the degenerated cartilage.

Tetracycline in the dose used here, approximately 50 mg/kg, can thus probably have a negative influence on the mineralization and growth of bone tissue. Whether tetracycline also interferes with resorption of bone tissue is unknown. The effect is greater at higher tissue concentrations (Hansson, 1967).

In this material, on evaluation of the amount of newly formed bone the treated side was compared with the control side. The rate of periosteal bone formation was, at least at first, higher on the treated than on the control side. A negative effect of tetracycline on bone mineralization and bone formation should therefore tend to reduce the difference between the treated tibia and the control tibia, and thus not result in overestimation of the differences obtained.

Absence of Bone Labelling

Six of the animals, nos. 46, 50, 92, 85, 77 and 56, showed a complete absence of fluorescent bone after labelling 2. All of these animals were given tetracycline in an intraperitoneal injection at the second labelling. The reason for the lack of fluorescence is not clear but it might be that the tetracycline was injected into the large intestine and was therefore not absorbed. These animals could not be evaluated completely on fluorescence microscopy. Labelling 1 resulted in fluorescence of bone in all cases.

Angiographic Technique

At the angiographic procedure the cut vessels in the abdominal wall were left open in order to counteract an increase of the central venous pressure and thereby reduce the risk of extravasation of Indian ink, which easily occurs especially in the medullary cavity. In order to obtain an idea of what pressure occurred in the venous system, the central venous pressure was recorded in seven animals on a graded tube during infusion of Indian ink. A polyethylene cather was introduced into the vena cava via the renal vein. At the start of the infusion practically no measurable venous pressure was recorded. When the vessels in the abdominal wall were left open after the laparotomy the venous pressure rose slowly to 15–20 cm

water, while about 200 ml of Indian ink was infused. The animal usually died after infusion of this amount of Indian ink. The flow through the small abdominal wall vessels was then profuse. In two animals the bleeding vessels in the abdominal wall were ligated. The venous pressure then rose during the infusion to about 40 cm of water. Subsequently bleeding occurred from the omentum, among other places, after which the venous pressure gradually decreased. No venous pressure exceeding 23 cm of water was recorded during the infusion when the abdominal wall vessels were left open.

The profuse blood flow obtained from the catheter which had been inserted into the distal part of the aorta showed that the lower extremities are supplied with blood even if the aorta is ligated. During the infusion the Indian ink was therefore mixed in successively higher concentrations with the blood which perfused the legs right up to the time when the animal died. This method of perfusion has been found to give good visualization of the entire vascular system of the diaphysis, including intracortical capillaries and sinusoids in the medullary cavity. The vessels leading from the periosteum into the cortex are very narrow throughout, however, and there is a risk that these vessels may be obliterated by the Indian ink, with the result that the filling of vessels in the underlying cortex will be incomplete. In order to counteract the deposition of fibrin on the ink particles the animals were heparinized. Studies of longitudinal sections of Spalteholz preparations have also shown that within a height difference of a few mm, large differences in Indian ink filling of the intracortical vessels seldom occur. The vascular pattern in the Spalteholz preparation taken about $^1/_2$ mm from the fluorescence preparation can therefore be considered to reflect satisfactorily the Indian ink filling in the cortex of the fluorescence preparation.

In the Spalteholz preparations which were prepared from paraffin-embedded sections, the soft tissue structures and cortex were well preserved. These preparations make possible detailed studies of soft tissue vessels and intracortical vessels. The Spalteholz preparations prepared from the primary plastic-embedded bone swelled when the methyl metacrylate was dissolved with chloroform, as a result of which the soft tissues around the bone and in the medullary cavity were destroyed. Bone preparations often buckle, but as a rule the cortex remains otherwise intact. These preparations are therefore only suitable for evaluation of the intracortical vascular filling and not for evaluation of the vessels of soft tissues.

Errors of Calculation

The error on calculating the area *CD*, i.e. that area of the cortical cross section in which the vessels did not fill with Indian ink at angiography,

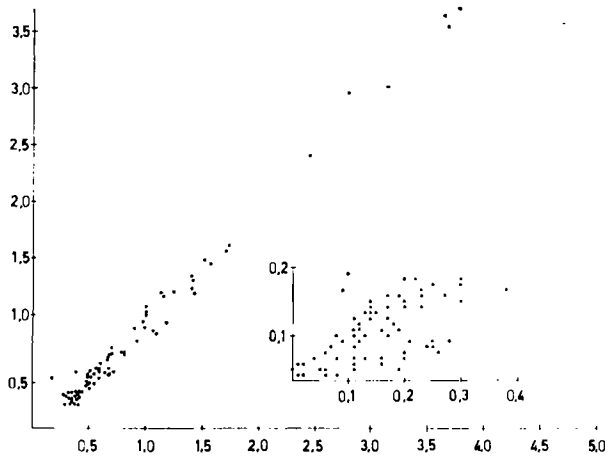


Fig. 3. Relationship between the size of the area *AB* within each sector measured planimetrically on a drawn image (se p. 29) (abscissa) and approximated according to the formula given on p. 32 (ordinate). All sectors with approximated *AB* values greater than 0.2 U are included. Of sectors with *AB* values below 0.2 U, a representative number are included. The figure shows that there is good correlation between the two methods of recording at approximated *AB* values greater than 0.2 U, but a low correlation at lower *AB* values.

was determined by drawing the same preparation, from animal 125, on five different occasions. The mean value for area *CD* amounted to 50.5 %, with S.E. = ± 0.32. The reliability of the recording was considered to be adequate.

On measurement of area *AB* the primary aim was to use planimetry as was used in the measurement of the other areas on the profile drawing. It was found, however, that on measurement of small areas the error in the drawing could be very large, often several hundred per cent. Area *AB* was therefore always approximated with the previously given formula (p. 32). The error in this approximation was studied by correlating the results obtained on approximation of the area with the formula and the results obtained on planimetry (see Fig. 3). With large *AB* values the correlation between the two methods was very high, but with small *AB* values, where approximation should give the most reliable value, the correlation was low. Approximation with the formula should thus be a better method in these cases than planimetry.

Measurement of the thickness of *AB* in the microscope was performed at three points within each sector. The change of the thickness within one sector usually takes place continuously, and three measurements within each sector were considered to be representative of the sector.

In order to determine the size of the error of measurement for the thick-

Table 2. *Mean value and standard deviation for 10 measurements of the thickness of AB in each of 5 sectors with trabecular bone, primary osteones and circumferential lamellae*

	Trabecular bone					Primary osteones					Circumferential lamellae				
Animal no....	125	45	45	45	281	125	281	244	240	237	51	58	123	220	96
Mean	32.5	117.4	72.6	123.4	95.4	7.8	32.6	7.1	22.8	38.6	23.1	8.8	3.2	11.1	24.6
S.D.	1.52	5.40	2.70	5.04	9.04	1.14	1.54	1.05	0.95	3.33	0.55	0.47	0.47	1.10	1.14

ness *AB*, 10 separate measurements of the thickness were performed within each of five sectors with trabecular bone (*t*), primary osteones (*o*) and circumferential lamellae (*l*) (Table 2). For each series of 10 observations the mean value and standard deviation were calculated. The size of the error of measurement for each individual bone structure was calculated with the aid of the standard deviations of the five individual series according to the formula:

$$\sqrt{\frac{\sum \text{S.D.}^2}{5}} = \text{S.D.}_{t,o,l}$$

The error of measurement ($\text{S.D.}_{t,o,l}$) of the individual observation was ± 5.4 U for trabecular bone, ± 1.83 U for osteones and ± 0.75 U for circumferential lamellae. These errors comprise a reasonable estimate of the error to which the individual observation value for the respective bone structure is subject. On measurement of the thickness *AB*, the mean value of several determinations within one sector or within several different sectors was calculated. In these cases the error of the mean will be smaller than that of the individual observation.

The variation caused by the systematic error of measurement can be correlated to the biological variation by forming the quotient

$$\frac{\sum \text{S.D.}^2}{5} / \text{S.D.}^2 m_{t,o,l}$$

$\text{S.D.}^2 m$ is the variance for the mean value of the five observation series for trabecular bone, primary osteones and circumferential lamellae, respectively, and in this material is mainly an expression of the biological variation. With the above formula, the quotient for trabecular bone was 0.021, for osteones 0.017 and for circumferential lamellae 0.008. This is a rough measure of the relative importance of the error of measurement, and the quotient is so low that the error of measurement can be regarded as of secondary importance in relation to the biological variations.

The area of the bone removed on reaming was approximated from the profile drawing for the control side. The error arising in this approximation was assessed by recording the natural difference between the right and left sides within the ventral and dorsal sectors in five control animals. The endosteal line within the middle $^4/_6$ of the sectors from the profile image from the left tibia was drawn over the corresponding line on the profile image from the right tibia, and the area between these lines was calculated and expressed in per cent of area *BE*. The mean difference in the ventral sector was -0.4%, S.D. ± 6.6 and in the dorsal sector 0.5%, S.D. ± 2.7.

Pressure Variations in the Medullary Cavity on Reaming and Brushing out of the Cavity in the Rabbit.

Wehner (1968) has shown that on closed reaming and nailing of tibial fractures in man, pressure variations from -100 mm Hg to $+120$ mm Hg can occur in the medullary cavity (See p. 6.) Larsen (1938) considers that an intramedullary pressure increase can give rise to necrosis of the cortex, and Nick *et al.* (1965) claim that the rise in pressure to about 75 mm Hg which can be observed in a fracture haematoma may be an important factor in fracture healing.

Recording of the intramedullary pressure variations which occur in the medullary cavity on reaming and brushing by the method used in the present study, was considered to be motivated here as an aid to establishing the cause of the intracortical vascular damage.

Material

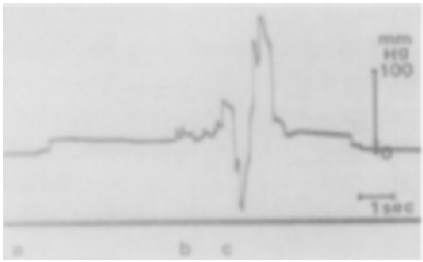
Two adult rabbits were used.

Method

The anaesthesia and operative technique were as described on pages 25.

The intramedullary pressure was recorded in the distal tibial metaphysis which was exposed ventrally about $1\,^1/_2$ cm above the tibiotalar joint. A hole 3.3 mm in diameter was drilled here through the ventral cortex. The hole was plugged with a plastic plug through which a polyethylene catheter with an outer diameter of 1.5 mm and with a wall thickness of 0.2 mm was inserted. The catheter was filled with heparin solution and was connected to a pressure transducer (Elema-Schönander EMT 490A), the nominal range of recording of which is 0–300 mm Hg. The pressure was recorded by means of a direct-writing ink recorder (Mingograf 42B, Elema-Schönander, Solna, Sweden). The apparatus was calibrated between each recording and correction for drift was made.

Pressure recording was performed during the following stages of the operative procedure:



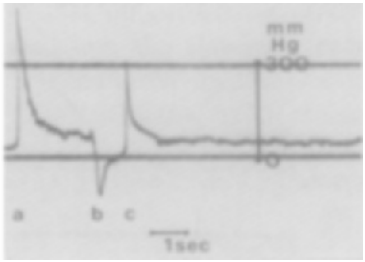


Fig. 4 Fig. 5

Fig. 4. Recording of intramedullary pressure when the medullary cavity is opened with an awl under the ligamentum patellae. At (*a*) the medullary cavity is opened carefully with the awl, which is then held still in the hole. At (*b*) the hole is widened carefully, and at (*c*) the awl is forced down into the medullary cavity and rotated at the same time. Pressure variations considerably exceeding 100 mm Hg can be seen.

Fig. 5. Recording of intramedullary pressure on insertion of a reamer into the medullary cavity. At (*a*) the rapidly inserted, rotating reamer comes into contact with endosteal bone. At (*b*) the reamer is withdrawn a few cm and is then again forced into the medullary cavity (*c*). When the reamer is moved up and down in narrow parts of the medullary cavity considerable pressure variations can occur.

1. When the medullary cavity was opened with an awl below the patellar ligament.
2. When the reamer was inserted in the medullary cavity.
3. On reaming of the endosteal cortex in the medullary cavity.
4. When the reamer was removed from the medullary cavity.
5. When a bottle brush was inserted into and withdrawn from the medullary cavity.

Results

The spontaneous pressure in the medullary cavity was found to be about 30 mm Hg.

Recording at stage 1: When the medullary cavity was opened with an awl a fall in pressure was first noted. When the awl was held still the pressure lay at a level of about 30 mm Hg. On moving the awl again a further rise in pressure of the order of 25 mm Hg was obtained. When the awl was forced down into the medullary cavity and rotated at the same time a pressure increase to considerably over 100 mm Hg occurred. On movement of the awl considerable negative pressure was also observed (Fig. 4).

Recording at stage 2: When the reamer was introduced under rotation into the medullary cavity a pressure rise was recorded. When it was inserted slowly very small pressure increases occurred, but when it was inserted quickly pressure increases to above 300 mm Hg were noted. Large reamer heads appeared to cause greater pressure increases than smaller reamer heads, under otherwise identical conditions (Fig. 5).

Recording at stage 3: During the reaming a moderate and varying pressure rise of fairly long duration can be seen, but rapid pressure variations of the

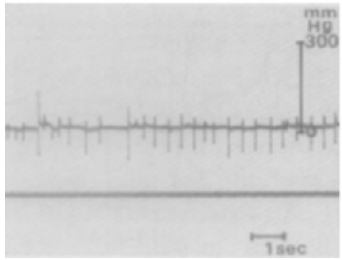


Fig. 6

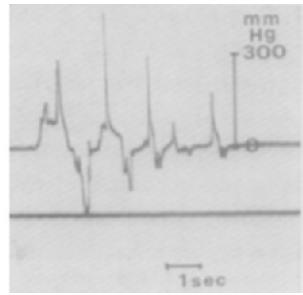


Fig. 7

Fig. 6. Recording of intramedullary pressure on reaming of the medullary cavity. A moderate pressure increase of long duration and rapid pressure variations can be seen.

Fig. 7. Recording of intramedullary pressure on brushing in the medullary cavity. Very large pressure variations can be seen.

order of 200 mm Hg also occurred. The rapid pressure variations were introduced by a negative phase and followed by a positive phase, the negative phase being the greater (Fig. 6).

Recording at stage 4: When the reamer was withdrawn from the medullary cavity the pressure in the cavity decreased to below zero. The order of magnitude of the decrease was dependent upon the rate at which the reamer was withdrawn. Considerable negative pressures were recorded when the reamer was withdrawn rapidly (Fig. 5).

Recording at stage 5: When a bottle-brush was inserted into the medullary cavity a pressure increase to considerably over 300 mm Hg, more than twice the range of the transducer, was recorded. When the brush was withdrawn there was a considerable decrease in pressure (Fig. 7).

Discussion

When the medullary cavity is opened carefully with a rotating awl, which is only inserted about 1 cm into the medullary cavity, and the reamer is then introduced slowly and under rotation, the pressure in the distal part of the medullary cavity does not increase to more than about 60 mm Hg, which is twice the normal medullary cavity pressure. It seems that under these circumstances the reamer head is able, by a pump action, to gradually transport backwards parts of the contents of the medullary cavity past the reamer head and thereby help to prevent the occurrence of a large pressure increase. On reaming in endosteal bone, large pressure variations can occur, and variations up to 200 mm Hg were recorded. These pressures are of the same order as those reported by Wehner (1968). The pressure variations give rise to varying pressures and a suction action in the Haver-

sian canals of the diaphysis. It is probable that on occurrence of these pressure variations material is easily squeezed into the Haversian canals from the medullary cavity. The pressure variations in the medullary cavity can probably also cause the vessels in the Haversian canals, which are very fragile, to tear.

Brushing of the medullary canal can give rise to even greater intramedullary pressure variations than reaming.

Conclusions

Intramedullary pressure variations are probably an important cause of intracortical vascular damage on reaming of the medullary cavity. The pressure variations can be reduced if the medullary cavity is opened carefully and if the reamer is moved up and down slowly in the medullary cavity.

Determination of Temperature Increase on Reaming of the Medullary Cavity in the Rabbit

One possible reason for the occurrence of vascular trauma in the cortex on reaming of the medullary cavity may be that the reamer, by friction heat, can produce thermal damage. Baar (1968) found that the function of the enzyme systems in human red blood cells was not affected negatively on heating if the temperature was kept below 42°C. Lieber (1946) claimed that pain was induced from dentine if the temperature exceeded 55°C. According to Hudoch *et al.* (1939) irreversible changes occur in cell protoplasm at temperatures of 43°–44°C.

In order to determine whether friction heat could give rise to thermal damage in the present material, the temperature increase was recorded on reaming of the medullary cavity in the rabbit tibia.

Material

Two rabbit tibias were used immediately after death of the animals.

Method

The temperature was measured with a Thermocouple Applicator Type H 1 coupled to a Lab. Thermocouple thermometer Type TE 3 with a measurement range of 0–50°C (Electrolab, Copenhagen). The Applicater Type H 1 measures with a thin measuring wire and has a setting time of 1–2 sec. On recording, a constant value was first obtained, followed by a slow reduction. The constant value has been given as the measurement value obtained.

A water bath was heated to 38°C. The thermoelement was placed in a stand 15 cm from the water bath and shielded from the bath. In order to record the fall

in temperature on the reamer head when this was transferred from the water bath to the thermoelement the head and about 3 mm of the shaft of reamer no. 16 were held in the water bath for 1 min. The reamer was then moved at a constant rate into contact with the measuring electrode. This was done by inserting the measuring wire into one of the grooves of the reamer, so that as large a contact surface as possible was created between the reamer and the measuring electrode. The recording was repeated five times. The same experiment was repeated six times with the water bath heated to 45–48°C.

Freshly removed rabbit tibias were divided transversally in the middle of the diaphysis. The distal part of the bone was fixed in the water bath, which had been heated to 38°C, so that the osteotomy surface lay a few mm above the surface of the water. The head of reamer no. 16, which is 3.9 mm in diameter, was heated in the water bath for 1 min. The medullary cavity of the tibia was then reamed for $^1/_2$ min, using slight pressure against the bone and with a rotation rate of 300 r.p.m., after which the reamer was moved towards the thermoelement at the same rate as in the preparatory experiments, and the temperature on the reamer head was recorded. Five reaming experiments were performed. The same reamer equipment was used as in the experimental series.

Results

The mean reduction in temperature when the reamer was heated in the water bath and then transferred to the measuring electrode was 5°C (range 4–8°C) when the water bath temperature was 38°C. At a water bath temperature of 45–48°C the mean temperature reduction was 6°C with a range of 5–6°C.

On reaming of the medullary cavity after preheating of the reamer, the maximal increase in temperature was 3°C with a range from −2 to +3°C when correction was made for the temperature fall of 5°C noted when the reamer was transferred from the water bath to the thermoelement, and 4°C when correction was made for a temperature fall of 6°C.

Discussion

In these experiments the reamer lost heat via radiation, fluid evaporation and conduction through the neck of the reamer to its shaft. An attempt was made to keep these factors as constant and comparable as possible in the preparatory and main experiments.

In odontology the development of heat on the drilling of teeth has attracted great interest. Peyton (1952) found that the heat development on drilling is dependent upon (1) The material in which the drilling is performed. Drilling in enamel causes heat development three times as great as drilling in dentine. (2) The type of instrument used. Small drill heads give less heat development than large. (3) The number of revolutions of the drill. A low rate of rotation gives less heat development than higher rates.

(4) The pressure applied to the instrument. Stronger pressures against the instrument give greater heat development.

Brånemark (1958) observed a temperature increase of 10–12°C on grinding of the cortex in rabbit fibulas at 300 r.p.m. when no cooling fluids were used. Vaughn & Peyton (1951), on drilling in dental enamel with drills 1.4–1.7 mm in diameter, at 1300 r.p.m., found a temperature increase of about 30°C. Since the heat development in enamel has been estimated to be three times as great as in dentine (Peyton, 1952), the heat development in dentine under these experimental conditions would have been about 10°C. According to Brånemark (1958), cortical bone is most closely comparable with dentine.

In the present series of experiments on living rabbits reamers of relatively wide calibre were used. The diameters of the reamer heads ranged from 3.02–4.92 mm, the diameter increasing by up to 0.3 mm for each reamer number. The reaming of the medullary cavity was performed successively, the cavity being widened by a maximum of 0.3 mm with each reamer used, and reaming of bone took place only by the most peripheral part of the reamer head. Under these circumstances the heat development should be considerably less than if reamers of corresponding size were used in the reaming of solid bone, in which case the whole of the anterior surface of the reamer would be in contact with the bone. The rate of rotation used —300 r.p.m.—is also relatively low. The temperature increase observed in these erperiments thus corresponds well with previous experimnetal results.

During operations in the medullary cavity in living animals, the reamer is cooled by blood circulating in the cortex and by the blood which flows into the medullary cavity from the cortex. With the method used for assessing the temperature increase on reaming, the rise in temperature on reaming in living bone is probably not underestimated.

Conclusions

Although the experimental conditions differ somewhat from those in reaming of the medullary cavity in living animals, it is reasonable to assume that about the same temperature increases, max. 3–4°C, are produced locally in the medullary cavity in both cases.

On intermittent reaming with no appreciable pressure against the reamer, the temperature increase occurring should not damage the bone tissue.

CHAPTER 3

Effect of Reaming of the Medullary Cavity in the Rabbit Tibia

GENERAL REACTION OF THE ANIMALS

The animals bore weight on the treated leg on the day after the operation. In no case was there any clinically manifest infection in the operation area, and neither did any wound-rupture occur.

MACRORADIOGRAPHIC STUDIES

On specimens observed at 0–2 weeks, thinning of the cortex on the treated side was often observed in the middle part of the tibia. At an observation time of 2 weeks and later some animals showed a periosteal deposition of relatively low contrast density, which was sometimes thicker than the original cortex. The periosteal deposition occurred in some cases along the greater part of the cortex, but in others it was localized to a limited area, often located in the proximal fibular part of the diaphysis dorsal to the insertion of the fibula on the tibia. In one preparation observed at 5 weeks (13) (Fig. 8) a zone of low density was observed at that location between the original cortex and the periosteal callus tissue. At the same time, in some animals a patchy area of increased contrast density was seen in the medullary cavity. In some animals studied at an observation time of more than 2 weeks there was considerably increased density in the medullary cavity and at the same time the borderline between the cortex and medullary cavity was indistinct.

FLUORESCENCE MICROSCOPIC STUDIES.

Normal Transverse Growth in the Diaphysis of the Long Bone in the Rabbit

The normal transverse growth of the long bone takes place in the diaphysis by the formation of lamellar bone on the periosteal surface, at the same time as bone is resorbed from the endosteal surface. The lamellar bone can consist of primary osteones or of circumferential lamellae. When the rabbit is 3–4 months old, its periosteum in the distal part of the diaphysis in the ventral sector often forms primary osteones so rapidly that several layers of osteones mature simultaneously. In the dorsal sector the formation of la-

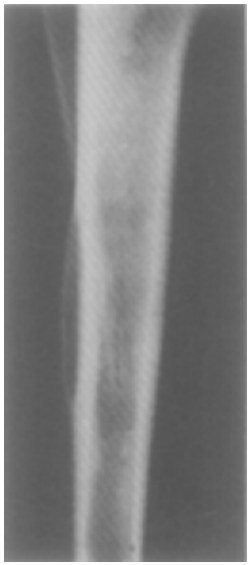


Fig. 8. X-ray picture of rabbit tibia 5 weeks after reaming of the medullary cavity. A zone of low density between the old cortex and a newly formed, thin cortex on the dorsal side of the bone indicates a cavity in the callus. Increased contrast density is seen in some areas in the medullary cavity. (The same animal as in Fig. 20.)

mellar bone is, as a rule, alternated with bone resorption during this time. In the tibial and fibular sectors lamellar bone is usually formed. No woven bone was observed in this material during normal periosteal bone growth. The rate of periosteal bone formation decreases during growth. The previously formed primary osteones are superimposed during growth by circumferential lamellae.

On the endosteal surface resorption alternates with the formation of lamellar bone.

Parts of the cortex are reconstructed successively. According to Owen *et al.* (1955) this takes place especially in those areas of the cortex where, in their opinion, there are residual fragments of calcified epiphyseal cartilage. On tetracycline labelling these areas of reconstruction appear on cross section as fluorescent ring-shaped structures and on longitudinal section so-called cutter heads (Schenk, 1963) can be observed during the formation of secondary osteones (Color pl. 1 *a, b,* 3 *a).*

Fluorescence Microscopic Observations after Reaming of the Medullary Cavity

The material for this study comprised animals in Table 1. In the subperiosteal layer the morphology and reconstruction of the newly formed

bone were especially studied, and in the cortex the initial absence of bone formation and the rebuilding of vascularized and avascular cortex. In the medullary cavity a study was made of the primary absence of fluorescence on endosteal bone, new bone formation, bone resorption and restoration of the normal medullary structures.

For the evaluations, each section was compared with the corresponding section from the control side in cases where only one tibia was treated (61/86 animals). The animal numbers are referred to table 1. D indicates the distal transverse section and P the proximal transverse section.

Observation time: 0–2 days

These animals were not given tetracycline. Two of the animals (27 and 28) showed subperiosteal bleeding at the insertion of the fibula on the tibia.

Observation time: 3 days

Labelling 1 was given 1 day postoperatively.

Periosteum. The periosteum on the treated side had usually formed bone and osteoid tissue of the same structure as on the control side. In some cases, however, woven bone had formed on the treated side, while on the control side lamellar bone was observed.

In the formation of woven bone, beams or spicules of osteoid were first formed, which later become calcified between two periosteal blood vessels. Two closely adjacent beams were then united by bone, which formed a bridge over vessels lying nearby.

The development of the periosteal bone formation could be followed by giving tetracycline at different time points after the operation and studying the location in the bone tissue at which the tetracyline was deposited. When tetracycline was given at the time of the operation the fluorescent band always had the same appearance and thickness on the treated side as on the control side. The structure of the bone and osteoid outside the fluorescent band showed, however, that woven bone could have been formed later than 8 hours postoperatively.

When tetracycline in growing animals was given on the day after the operation, homogeneously fluorescent beams or spicules of woven bone, up to 100 μ high, were found between two blood vessels during the formation of trabecular bone (Fig. 9) (125 D). In some preparations (125 P, 124 P) incomplete fluorescent bridges between adjacent beams could be seen peripheral to the blood vessels. When primary osteones were formed within an area where such osteones had been formed before the operation, the postoperatively formed primary osteones were often built of lamellar bone alone. Beam-shaped structures of woven bone sometimes occurred instead between

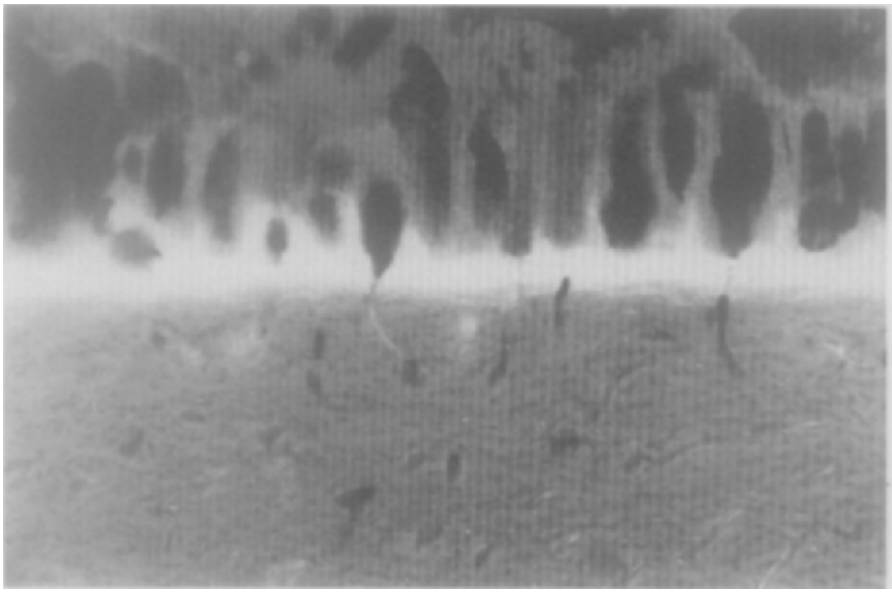


Fig. 9. Distal fluorescence cross section of ventral cortex, observation time 3 days. Growing animal 125, labelled on day 1. Fluorescent spicules of newly formed subperiosteal bone are seen, and between these, radiating blood vessels. The visible part of the cortex is well vascularized from periosteal vessels.

blood vessels, in the same way as in the formation of trabecular bone. This latter type of primary osteone developed even when circumferential lamellae had been formed before the operation or when preoperatively the periosteum had been inactive.

In the adult animals many sectors showed no active bone formation, but when bone formation did occur, almost only circumferential lamellae were observed at 3 days.

In a preparation from the growing animal 125 P, a "haemorrhage" about 1 mm thick, which had not taken up Indian ink, was seen on the fibular side, cranial to the insertion of the fibula on the tibia. In the angle between the raised periosteum over this haemorrhage and the cortex, newly formed trabecular bone was observed (Fig. 10). The smooth bone surface under the centre of the haemorrhage completely lacked flourescence. Minor haemorrhages in the corresponding region were seen in a further three growing animals (3, 114, 124) and two adults (54, 110).

Cortex. In the cortex, signs of absence of bone formation activity were observed on the treated side. Thus fluorescence was lacking in many areas adjacent to the medullary cavity and on the endosteal surface, where fluorescent bone was present on the control side (Color pl. 1). This absence of

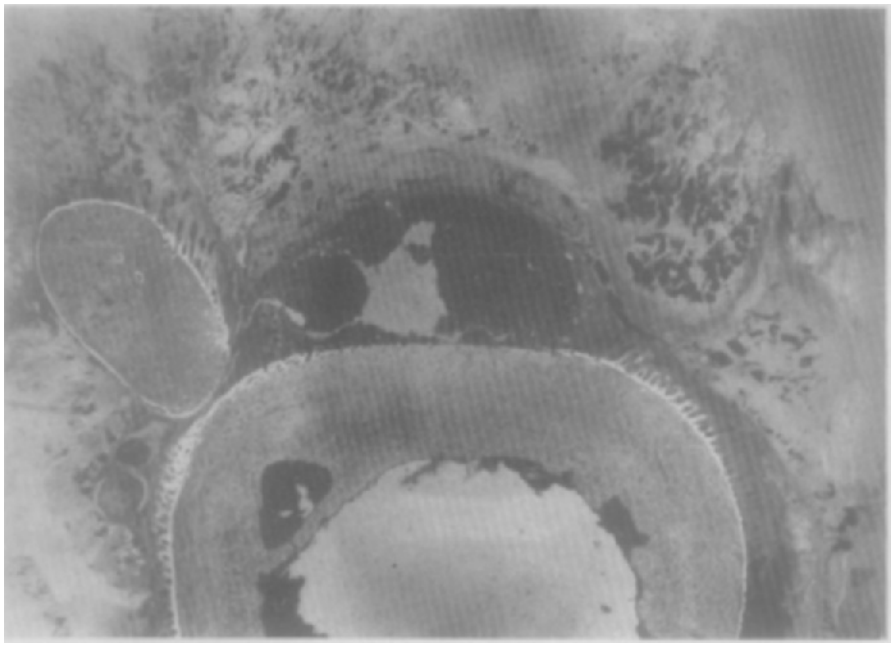


Fig. 10. Proximal fluorescence cross section of fibular cortex, observation time 3 days. Growing animal 125, labelling on day 1 postoperatively. There is a subperiosteal haematoma dorsally and between the tibia and fibula. The bone surface is inactive in the central part under the haematoma, but around this part, even on the fibula, trabecular bone is formed.

bone formation was most clearly observed in the young animals in which normally there is active reconstruction of the inner part of the cortex. In the cortical areas in which no bone formation activity was observed, Indian ink filling of the intracortical blood vessels was also lacking.

Medullary cavity. In one preparation (124 P), an almost sector-shaped area in the medullary cavity containing fluorescent bone fragments was found (Fig. 11). These bone fragments had been detached from the endosteal cortex on reaming. Passing to this area from the periosteum through the cortex was an Indian ink-filled blood vessel, and extravasation of Indian ink was observed in the medullary cavity blood clot between the fluorescent bone fragments and the endosteal surface. In the remaining parts of the preparation the medullary cavity was filled with a clot containing non-fluorescent bone fragments.

Observation time: 1 week

Labelling 1 was given 1 day and labelling 2, 5 days postoperatively.

Periosteum. When lamellar bone had formed, the fluorescent bands from

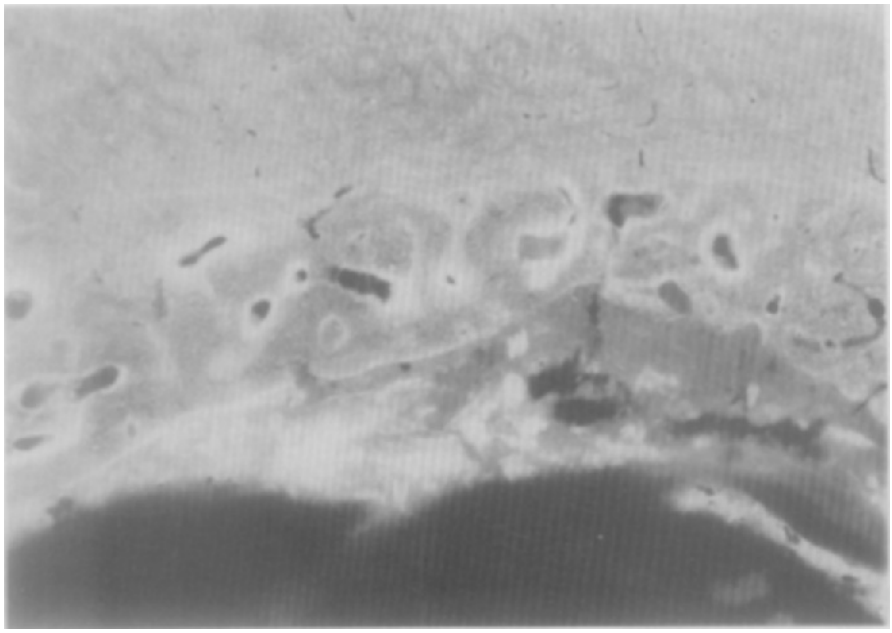


Fig. 11. Proximal fluorescence cross section of fibular cortex, observation time 3 days. Growing animal 124, labelled on day 1. Indian ink filled blood vessels are traversing the cortex to the medullary cavity. Extravasation of Indian ink is seen in the medullary cavity close to the endosteum. Bone fragments in the medullary cavity adjacent to the Indian ink extravasation are fluorescent, which is a sign that tetracycline has diffused out into the medullary cavity blood clot here 1 day after the operation.

the first and second labellings were often confluent. In some of the youngest animals, however, two separate fluorescent bands were seen. In these cases the outer band was broader than the inner (e.g. 64). This indicates that the rate of bone formation was higher 5 days than 1 day after the operation, assuming that the absorption conditions of the tetracycline were the same on the two labelling occasions. Since tetracycline was given as an intraperitoneal injection both at labelling 1 and 2, these conditions should have been identical.

Primary osteones with woven and trabecular bone which had been labelled 2 days postoperatively showed in growing animals broader fluorescent beams between the blood vessels than those which were labelled on the day after the operation (Fig. 12). Complete bridges were often observed over the blood vessels (65, 97). From the labelling given 3 days after the operation (83), both broad beams between the blood vessels and bridges over them were fluorescent in both growing and adult animals. A suggestion of fluorescence of beams leading to osteones of the next generation was

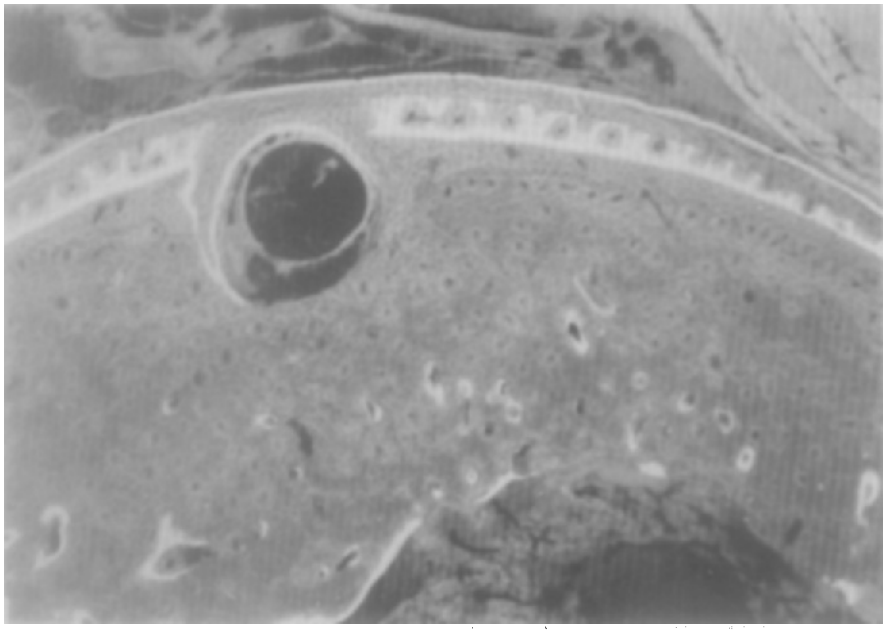


Fig. 12. Proximal fluorescence cross section of the fibular part of the cortex, observation time 8 weeks. Growing animal 65, labelled 2 and 54 days postoperatively. There are trabeculae of woven bone and bridges over vessels fluorescence-labelled 2 days postoperatively. Labelling 54 days postoperatively shows that the osteones were closed at that time. The osteones are covered with circumferential lamellae after partial superficial resorption. Reconstruction is taking place in the inner half of the cortex. The bone trabeculae in the visible part of the medullary cavity are resorbed and replaced by almost normal marrow.

also frequently observed. When tetracycline had been given 4 days postoperatively, bridges to osteones of the second generation and beams to third generation osteones were sometimes fluorescent. When tetracycline had been given 7 days after the operation, the beams of woven bone between blood vessels to primary osteones of the first generation were no longer fluorescent (Fig. 13). In these preparations fluorescence was observed instead in a ring-shaped layer of lamellar bone around the blood vessels (7).

The woven bone which had been labelled 1 and 5 days after the operation showed, however, massive, almost homogeneous fluorescence of all the newly formed bone (105).

In the adult animals the formation of circumferential lamellae predominated. In two animals (121, 112), the formation of primary osteones was also seen. These showed a somewhat lower degree of development than the primary osteones in the young animals.

In several growing animals, (e.g. 104, 105), very active formation of tra-

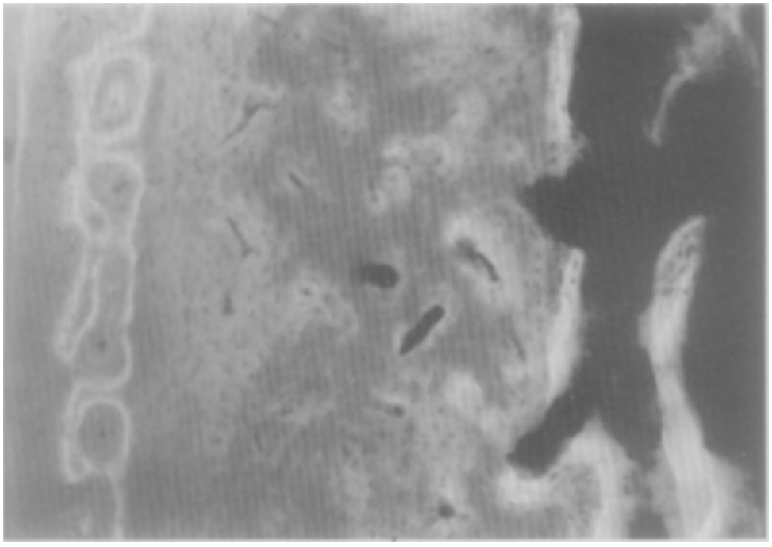


Fig. 13. Distal fluorescence cross section of ventral cortex, observation time 4 weeks. Growing animal 7, labelled on days 7 and 26 postoperatively. In the subperiosteally formed primary osteones ring-shaped structures of lamellar bone show fluorescence after labelling 1. The osteones are almost closed at labelling 2, and are covered by circumferential lamellae. In the inner part of the cortex reconstruction is taking place. The bone trabeculae in the medullary cavity, surrounded by wide Indian ink filled vessels, are undergoing extensive resorption, but deposition of bone is also observed.

becular bone and primary osteones was observed at the insertion of the fibula on the tibia, without any signs of resorption or reconstruction in this callus tissue. In no animal was any major subperiosteal bleeding observed.

Cortex. In one animal (95) there was a tendency to widening of some bone canals in the subperiosteal part of the original cortex. In the inner parts of the cortex an absence of fluorescence was observed in the same way as described for an observation time of 3 days.

Medullary cavity. The medullary cavity contained a blood clot containing bone fragments. In several preparations newly formed blood vessels had invaded the medullary cavity. Around these vessels a wreath of small fluorescent old bone fragments was seen, but no newly formed bone was observed in the medullary cavity.

Observation time: 2 weeks

Labelling 1 was given 1 to 7 days and labelling 2, 12 days postoperatively.

Periosteum. In one growing animal, in which lamellar bone was formed during labelling on days 1 and 12 after the operation, the fluorescent band

after labelling 1 was narrower than that after labelling 2. In another growing animal which was labelled on days 7 and 12, the fluorescent band after labelling 1 was broader than that after labelling 2. The rate of bone formation thus appeared in the one case to be higher 12 days than 1 day postoperatively, and in the other case higher 7 days than 12 days postoperatively (59, 6). The tetracycline was given in the same way at both labellings in the respective animals.

The newly formed, primary osteones and the trabecular bone had matured further. From labelling 2, in first generation osteones ring-shaped lamellar bone was fluorescent around blood vessels (Fig. 14), and in second and third generation primary osteones and in trabecular bone, beams of woven bone fluoresced between blood vessels. The perivascular soft tissue had decreased correspondingly. In many sectors resorption was in progress from the surface of the newly formed bone. In the ventral and sometimes also the dorsal sector, the bone formation often seemed to be greatest over that part of the cortex which had been most thinned by the reaming (Color pl. 1 *b;* Fig. 18). Often in these cases primary osteones were formed in the area which was most thinned, and circumferential lamellae at the sides of this area.

In the region around the insertion of the fibula on the tibia, no subperiosteal haemorrhage was observed in any of the preparations. In many growing animals a large area of callus consisting of trabecular bone was found instead in this area. Also in many adult animals trabecular bone was observed at this location, but to a smaller extent than in the growing animals. In the central parts of this callus an extensive resorption of the newly formed bone beams was in progress (Fig. 16). In the peripheral parts of the callus new, often lamellar bone was deposited on the bone bridges. In this way the bone tissue became condensed peripherally and transformed successively to cortical bone (6). A similar reconstruction of callus was also observed in other areas of the diaphysis, but only seemed to take place where a large amount of woven bone had been formed primarily and not when lamellar bone had been formed.

Cortex. In the outer parts of the cortex signs of reconstruction of the bone were observed in both growing and adult animals. The bone canals for many of the blood vessels which passed from the periosteum into the cortex were thus wider on the treated side than on the control side in all growing animals and in two of the five adults. The walls of the bone canals were often uneven due to the presence of Howship's lacunae, which indicated bone resorption (Fig. 14). In growing animals large resorption cavities occurred on the surface of the original cortex, but only in areas over which trabecular bone had been deposited (45) (Color pl. 2 *a*). In revascularized parts of the

Fig. 14. Proximal fluorescence cross section of fibular cortex, observation time 2 weeks. Adult animal 86 labelled on days 2 and 12 postoperatively. Active periosteal callus formation with no appreciable resorption. A canal in the subperiosteal original cortex is filled with an Indian ink filled vessel and is increasing in calibre, which is evident from its width and rough surface.

cortex nearer to the medullary cavity, many of the bone canals were considerably wider than in the outer area of the cortex. These canals ran through the cortex in the direction of the medullary cavity. Their foremost part was often widened into a pear shape and comprised so called cutter heads (Fig. 15). The bone surface was rough and partially labelled with a thin fluorescent layer of the type which can be seen on bone undergoing resorption. Further peripherally on the walls of the bone canals new bone had been laid down, whereby a secondary osteone had been formed. In growing animals large resorption cavities occurred, not infrequently, in the inner part of the cortex. In these cavities some preparations showed new formation of bone on the surface facing the medullary cavity. In adult animals similar changes were seen to those in the growing animals. The resorption canals were narrower throughout, however, and no large resorption cavities were found in the cortex.

Medullary cavity. Blood vessels had invaded the medullary cavity in many of the growing animals and in some of the adults. In no preparation was newly formed bone observed in the medullary cavity.

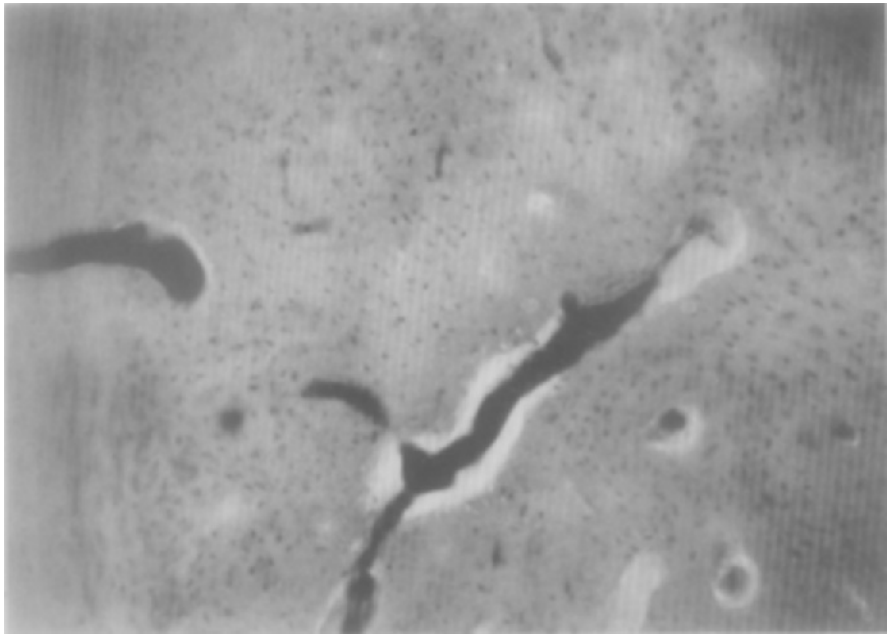


Fig. 15. Distal fluorescence cross section of tibial part of cortex, observation time 2 weeks. Growing animal 108, labelled on days 1 and 12 postoperatively. Revascularization of the cortex is taking place by means of "cutter heads" in the direction towards the medullary cavity (to the right). The cortex is being resorbed around the leading narrow vessel loop, and new bone is being deposited on the wall of the eroded bone canal further peripherally close to fairly wide blood vessels massively filled with Indian ink.

Observation time: 3 weeks

Periosteum. The blood vessel canals in the primary osteones formed postoperatively had decreased further in width. Active formation of lamellar bone was in progress on the walls of the bone canals around the blood vessels. Extensive resorption was observed on the surface of the newly formed bone.

Cortex. In the cortex many of the bone canals had widened further and on the walls in many of the resorption cavities in the inner parts of the cortex fluorescent new bone was observed.

Medullary cavity. In several preparations from growing animals newly formed blood vessels and also a sparse amount of newly formed fluorescent woven bone was seen in the medullary cavity adjacent to the endosteum.

Observation time: 4 weeks

Labelling 1 was given 0–3 days and labelling 2, 26 days postoperatively.

Periosteum. In the first generation primary osteones formed after the

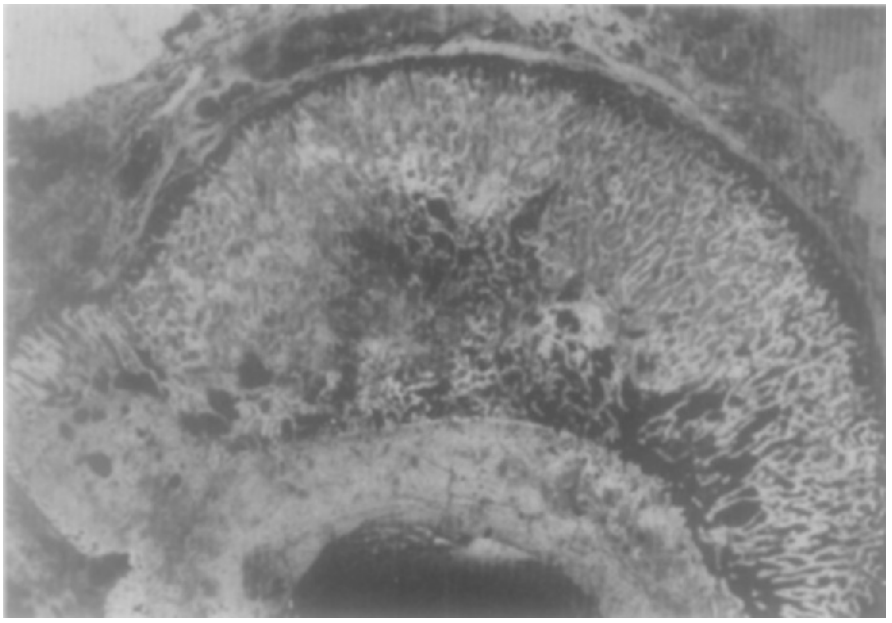


Fig. 16. Proximal fluorescence cross section of fibular cortex, observation time 2 weeks. Growing animal 6, labelled on days 7 and 12 postoperatively. A large amount of trabecular bone has been formed in the dorsal angle between the fibula and tibia. The central part of the newly formed bone is undergoing resorption, while the peripheral part is being condensed to cortical bone.

operation, the bone canals showed practically the same width as in the original cortex (Fig. 13). The bone formation on the walls of these bone canals had almost ceased (7, 97). The first-formed, primary osteones were in many cases superimposed by circumferential lamellae.

In the area around the insertion of the fibula on the tibia, a large amount of trabecular bone appeared to have formed primarily after the operation in two young animals. At this observation time a hood of newly formed, condensed periosteal bone was found in this area (Fig. 17). Under this hood there was a cavity in the callus tissue filled with a tissue which on the fluorescence preparation resembled normal medullary tissue. The cavity lay on the section right inside the callus tissue. It was separated from the original cortex by a thin layer of bone, which was partially undergoing resorption from inside the cavity. On the largest part of the peripheral wall of the cavity active bone formation was observed (51, 52). Only in preparations from one animal (97) was formation of woven bone under the periosteum in progress; for the rest, only lamellar bone.

Cortex. In the young animals several bone canals in the outer part of the cortex were widened and filled with wide, Indian ink-filled blood vessels.

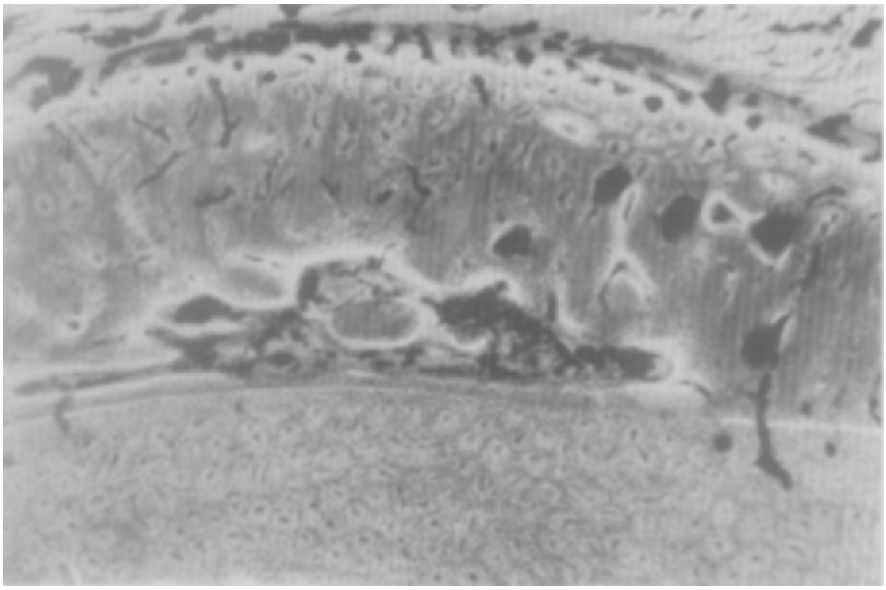


Fig. 17. Proximal fluorescence cross section of fibular cortex, observation time 4 weeks. Growing animal 52, labelled on days 1 and 26 postoperatively. There is a cavity in the periosteal callus tissue of trabecular bone. The cavity is separated from the surface of the original cortex by a thin layer of bone which is largely undergoing resorption from the cavity, on the peripheral surface of which bone formation is taking place.

On the walls of these canals active bone reconstruction, with both resorption and bone formation, was in progress. Many bone canals in the outer part of the cortex did not seem to be undergoing any reconstruction, however, but showed the same appearance as on the control side. In the inner parts of the cortex some of the growing animals showed large resorption cavities (Fig. 19), which were partly lined with new fluorescent bone on the surface facing the medullary cavity, while the opposite surface usually exhibited resorption. In other parts of the inner cortex small cavities were seen, often with bone formation around the entire circumference (Fig. 13).

In the adult animals, qualitatively the same reconstruction process was observed in the cortex as in the growing animals, but the widened vascular canals were regularly considerably narrower and no large resorption cavities were found in the cortex.

Medullary cavity. In five of the seven growing animals and two of the five adults, fluorescent bone trabeculae built of woven bone were found in the medullary cavity. In some of the young animals and in one of the adults the central parts of the trabeculae were not fluorescent (Figs. 13, 18). This was observed especially in the trabeculae lying nearest to the endosteum.

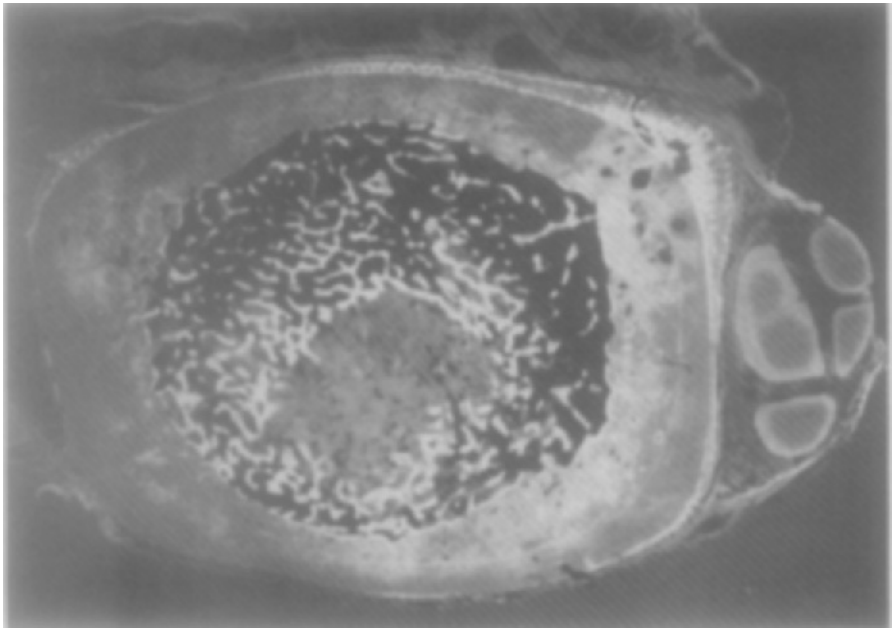


Fig. 18. Distal fluorescence cross section, observation time 4 weeks. Growing animal 7, labelled on days 7 and 26. Almost completed periosteal new bone formation. Extensive superficial resorption in the dorsal sector (below). Ventrally primary osteones were first formed, and ventro-fibularly (above right) trabecular bone, on which circumferential lamellae were deposited. Reconstruction of the inner cortex is taking place, especially in the fibular sector (right). In the centre of the medullary cavity there is a sparsely vascularized area with no newly formed bone trabeculae, and peripheral to this area newly formed bone trabeculae with active bone formation are seen. In the surrounding areas there are relatively few Indian ink filled vessels. Adjacent to the endosteal cortex, especially in the fibular and tibial areas, bone trabeculae which are surrounded by wide vessels massively filled with Indian ink have been largely resorbed.

The centre of these trabeculae had thus become mineralized earlier than 2 days before labelling 2 was given. The trabeculae in the middle of the medullary cavity were often fluorescent on all surfaces, but in some cases some surfaces lacked fluorescence, often surfaces facing the periphery. The nonfluorescent surface was then often uneven due to the presence of Howship's lacunae. In three of the growing and three of the adult animals newly formed blood vessels of wide calibre, usually with thin walls, were observed in the medullary cavity adjacent to the endosteum.

Observation time: 5–6 weeks

Periosteum. In one animal a large cavity was found in callus tissue in the area around the insertion of the fibula on the tibia (13) (Fig. 20). From this cavity resorption of the underlying cortex was in progress.

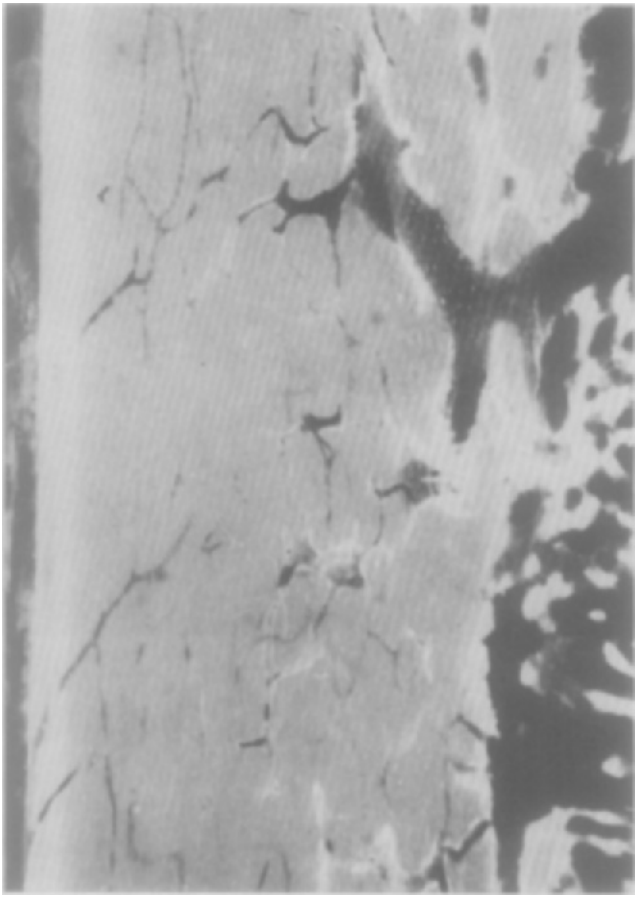


Fig. 19. Longitudinal fluorescence section, observation time 4 weeks. Growing animal 97, labelled on days 2 and 26. Some of the bone canals in the outer part of the cortex are widened and filled with rather wide vessels. In the inner parts of the cortex large resorption cavities which are partly lined with new fluorescent bone can be seen.

Observation time: 8 weeks

Labelling 1 was given 0–4 days and labelling 2, 54 days postoperatively.

Periosteum. The periosteum was inactive or had formed circumferential lamellae when labelling 2 was given. The primary osteones which had formed previously in the postoperative course had usually become covered with a layer of circumferential lamellae (Fig. 12). In some cases this had taken place after resorption of the peripheral parts of the woven bone which were fluorescent after the first labelling (65).

In the area of the insertion of the fibula on the tibia, one animal showed

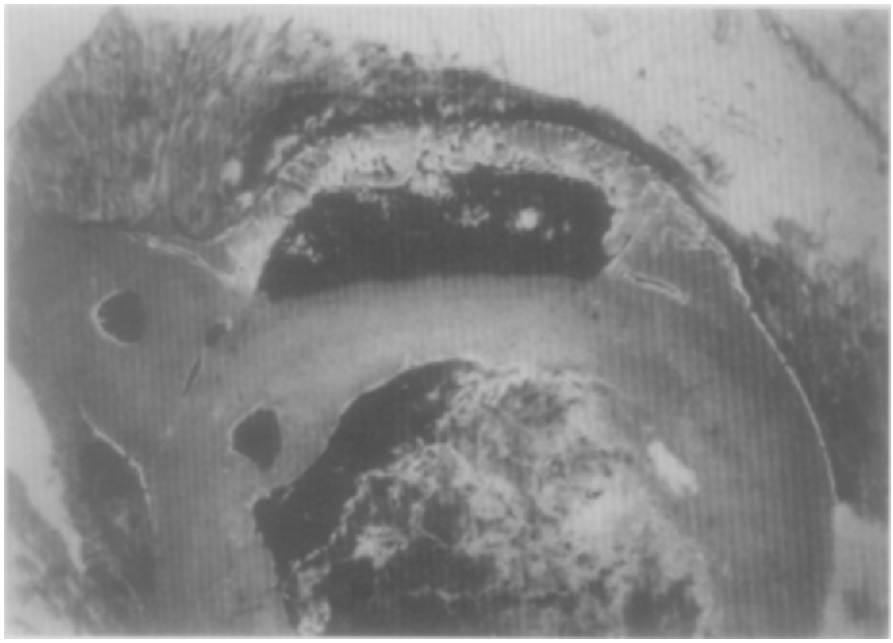


Fig. 20. Proximal fluorescence cross section of fibular cortex, observation time 5 weeks. Growing animal 13, labelled on days 26 and 33. There is a cavity in the periosteal callus tissue of trabecular bone. From the cavity, which is filled with almost normal marrow tissue, erosion is taking place of the underlying original cortex, which in the centre under the cavity is avascular. The same animal as in Fig. 8.

a fairly large cavity in the callus in the dorsal region (92). This cavity had a deeper location than in animals with shorter observation times (Fig. 21). More than half of the cavity lay within the original cortex. The surface of the cavity facing the medullary cavity showed a suggestion of fluorescence, and on the other surface resorption of original cortex was in progress.

Cortex. In the growing animals the resorption processes in the cortex seemed to be less pronounced than at an observation time of 4 weeks, and new bone formation predominated. On the walls of most of the resorption canals and resorption cavities bone formation was observed. In the inner parts of the cortex in growing animals large cavities were seen with only sporadic bone formation on the wall facing the medullary cavity. In adult animals both resorption and new formation of bone were in progress on the walls of the resorption canals.

Medullary cavity. In all animals, both growing and adult, newly formed bone was found at some place in the medullary cavity. In adult animals, however, the bone formation was often very sparse. In the medullary cavity

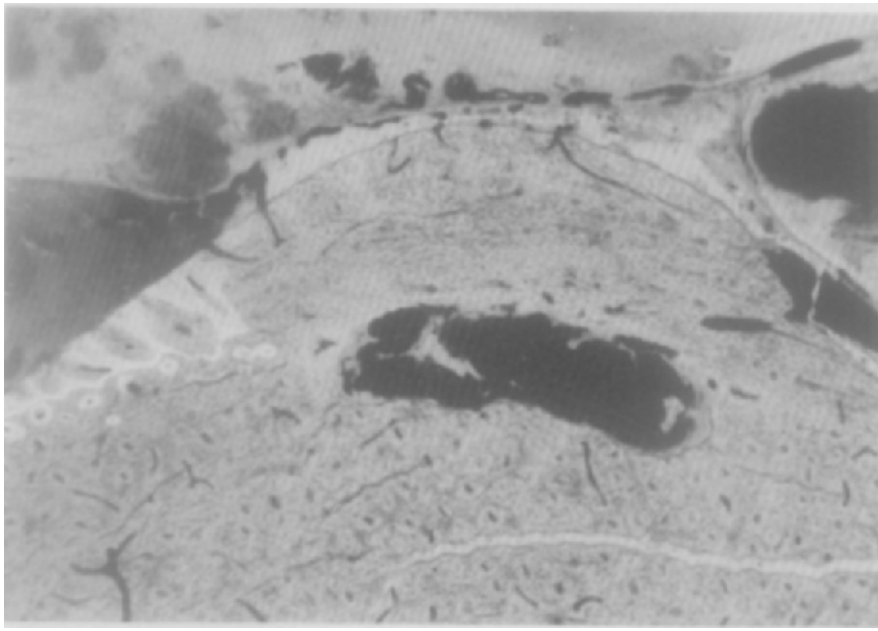


Fig. 21. Proximal fluorescence cross section of fibular cortex, observation time 8 weeks. Growing animal 92, labelled on days 1 and 54. Condensed periosteal callus tissue, which is undergoing resorption from the surface. Between the callus tissue and the original cortex, partly located in the original cortex, there is a cavity whose central wall is undergoing resorption.

in these animals large areas which had not become revascularized were seen. In other areas there was revascularization by sparsely situated blood vessels without formation of new bone around the vessels. In the growing animals resorption of bone predominated over new bone formation. Many of the trabeculae observed at the border between the richly vascularized and the central sparsely vascularized areas lacked fluorescence even on the surface facing the centre. The peripheral parts of the medullary cavity completely lacked trabeculae in many preparations. In these areas the trabeculae had been resorbed and replaced by bone marrow which seemed on the fluorescence section to have a normal structure. This bone marrow contained wide blood vessels, usually veins. On the endosteal surface, especially within the reamed area, a layer of newly formed lamellar bone was often found.

Observation time: 12 weeks.

Labelling 1 was given 1–4 days and labelling 2, 92 days postoperatively. The group comprised only adult animals.

Periosteum. The fluorescence microscopic picture resembled that at an observation time of 8 weeks.

Cortex. In the outer parts of the cortex occasional blood vessel canals which were considerably widened were found. On their walls bone formation was observed in some cases but as a rule there was no bone formation activity. In the inner parts of the cortex resorption cavities were found, the walls of which showed inactivity or bone formation, but in fairly large areas of the cortex there were still no signs of reconstruction.

Medullary cavity. All the adult animals showed active bone formation in the medullary cavity, but resorption of the trabeculae predominated in the peripheral areas of the medullary cavity. Vessels of wide calibre, usually veins but in some preparations also arteries, were found in the medullary cavity. The medullary cavity also contained areas with restored fat marrow of normal appearance in four of the five animals.

Discussion

On reaming of the medullary cavity the medullary blood vessels in the diaphysis of the bone are completely destroyed at the same time as bone is removed from the endosteal surface of the cortex. Reactive changes occur in the periosteum, cortex and medullary cavity.

Reaction of Periosteum

As in many previous investigations, the periosteum in this study reacted with increased bone formation. The newly formed bone was of two morphological types, lamellar bone and woven bone.

Lamellar bone, which can comprise circumferential lamellae or primary osteones, is formed on normal periosteal growth in rabbits of the ages studied here, and the formation of this bone could therefore be regarded as resumed or accelerated normal bone formation. Frost (1963) pointed out that this bone, when completely developed, has maximum resistance to the physical loads on the skeleton. This agrees with the present findings, which showed that the central parts of the newly formed lamellar bone were not reconstructed during the period of maturation but appeared to have acquired their final properties at the initial stage.

The causal mechanism for the formation of lamellar bone under the periosteum could not be elucidated by this material. According to Frost (1963) the formation of lamellar bone is influenced by growth hormones, thyroid hormones and space polarizing factors. In the present study it was observed that the subperiosteal blood vessels were dilated during the time in which the periosteal bone formation was increased and during the subsequent phase when the bone resorption was potentiated. The primary vascular dilatation may have been due to stasis in the blood vessels. This can

lead to local anoxia and acidosis, which according to Richany *et al.* (1965) and Johnson (1966) can induce subperiosteal bone formation. According to Trueta (1963), dying or hypoxic osteocytes and endothelial cells influence the blood vessels, either by their action or by their inhibition, via vascular stimulating factors. He considers that the bone formation can occur as a result of the vascular reaction.

The formation of woven bone, which can consist either of primary osteones or trabecular bone, results initially in a much more rapid increase in the thickness of the bone than the formation of lamellar bone, and when trabecular bone is formed the cortical thickness can inrease twofold within the course of about 2 weeks. In the normal growth of rabbits of the ages studied here, woven bone appears never to develop subperiosteally, and its presence can be considered to be pathological, so-called callus formation. The formation of trabecular bone means that large amounts of woven bone have developed. In this series trabecular bone was formed especially frequently within the area around the insertion of the fibula on the tibia. In this area preparations studied at short observation times often showed subperiosteal haemorrhages, which seemed to become resorbed within the course of about 1 week. In preparations studied at longer observation times an abundance of woven bone, which had probably been induced by such a haemorrhage, was often observed at this site (see also p. 118). The central parts of the trabecular bone were often reconstructed by resorption of the bone trabeculae and their replacement by normal medullary tissue, whereby a cavity occurred in the callus tissue. The callus tissue outside the cavity was reconstructed to cortical bone by the deposition of lamellar bone on the trabeculae around the blood vessels. The callus cavity appeared to be formed, at least partly, inside the callus tissue and subsequently seemed to move in the direction of the medullary cavity by the deposition of bone on its peripheral wall simultaneous with resorption of its central wall. The original cortex under the cavity was probably necrotic, which may have been the reason that the callus cavity moved centrally. Zucman *et al.* (1968) demonstrated that a subperiosteal "haemorrhage" occurring after reaming of the medullary cavity consisted partly of squeezed out marrow fat, which they found gave rise to abundant periosteal bone formation in which cavities could occur. The woven bone thus seems to be a pathological bone formation which is often rebuilt during the process of maturation in order to adapt to the physiological demands placed on the extremity. Frost (1963) considers that the formation of woven bone is governed by local stimulating factors and not by growth hormones, thyroid hormones or space polarizing factors. Neither is the structure of the collagen fibres adapted to the physical load on the skeleton, according to Frost (1963). In the present material woven

bone was formed during the first 2–4 weeks after the operation, but subsequently only lamellar bone was formed subperiosteally. Charnley (1968) has pointed out that woven bone is a provisional bone, which is resorbed when it becomes covered by lamellar bone. In the woven bone the calcium crystals are less closely packed. The bone is therefore permeable to tetracycline, which then stains the entire bone tissue (Eger & Kämmerer, 1967).

On the surface of the newly formed periosteal bone, alternating resorption and deposition of bone takes place after an observation time of about 2 weeks. The newly formed woven bone is then covered with lamellar bone, often when the surface of the woven bone has first been resorbed. This alternation between superficial resorption and deposition of bone is, according to Schenk (1967), the natural process of adaptation of the periosteum to different functional demands on the bone.

Cartilage formation under the periosteum was not observed in any of the preparations of the present study. This is in agreement with the findings of Bast *et al.* (1925) and Richany *et al.* (1965). They found cartilage formation from the periosteum only when the latter had been opened, and never under intact periosteum.

Reaction of Cortex

After reaming of the medullary cavity different areas of the cortex reacted in different ways, depending, among other things, upon whether or not the blood circulation in the area remained undisturbed. Growing and adult animals also reacted somewhat differently.

Reconstruction of vascularized cortex

In those parts of the cortex where the circulation remained after the intramedullary reaming, many of the intracortical bone canals began to increase in width during the first 1–2 weeks after the operation. At an observation time of 4 weeks several of the widened canals had begun to decrease again in width by the deposition of bone on their walls. Other canals continued to become wider, on the other hand. The increased circulation through the outer part of the cortex to its inner part and to the medullary cavity appeared in this way to be concentrated to a smaller and smaller number of blood vessels, while the remaining vessels gradually became of decreased importance.

Reconstruction of avascular cortex

The avascular areas of the cortex were revascularized from areas supplied with blood, partly by means of cutter heads. Schenk & Willenegger (1963)

described these cutter heads in fracture healing in the dog, while Geiser (1963) claimed that they do not occur in the rabbit. In the present study cutter heads were found to drill relatively narrow canals in the bone in an oblique direction towards the medullary cavity. In addition to this form of revascularization of the cortex, resorption cavities were formed in some cases in association with broom-like vascular reactions. In some growing animals very large cavities were formed. There was a tendency for new bone to be formed on the peripheral surface of the cavity, while at the same time resorption took place from its central surface. In this way the cavity moved in the direction of the medullary cavity with increasing observation times. An invasion of blood vessels and reconstruction of avascular bone was evident at an observation time of 2 weeks. In growing animals the bone resorption in avascular cortex appeared to be most pronounced at an observation time of about 4 weeks, while the new bone formation seemed to be most active at about 8 weeks. The rebuilding in the cortex thus took place when the periosteal bone formation activity had decreased. This time course corresponds well with the findings of Andersson (1965) in the dog and of Richany *et al.* (1965) in adult cats. The small cavities in growing and adult animals appeared, as a rule, to close up completely. This process took a long time, however, and at an observation time of 12 weeks many small cavities with active bone formation on the walls were observed. Large cavities in the inner part of the cortex seem to have a tendency not to close completely. In the present material at an observation time of 8 weeks, cavities were found in the inner part of the cortex with no signs of either resorption or new bone formation on their walls. Richany *et al.* found large resorption cavities filled with normal bone marrow at an observation time of 4 weeks.

Richany *et al.* studied the development in adult cats over a long period and found that reconstruction in the cortex was essentially completed after $5\ ^1/_2$ months. Trueta & Cavadias (1955) found that in adult rabbits necrotic areas were still to be seen in the cortex 8 months after disturbance of the endosteal circulation.

Reaction of Medullary Cavity

Regeneration of the structures in the medullary cavity after scraping away of the bone marrow has been studied in detail histologically by Brånemark (1964) and Richany *et al.* (1965). The results obtained for the growing animals in the present study agree essentially with the findings of these authors. Tetracycline labelling makes it possible to follow the formation and resorption of the bone trabeculae in the medullary cavity with greater reliability than do histological methods, however.

In the peripheral part of the medullary cavity in the growing animals of the present study at an observation time of 4 weeks, resorption of the bone trabeculae facing the periphery of the bone often took place, while bone formation still proceeded on the surface facing the parts of the medullary cavity which had not yet become revascularized. At the same time new trabeculae were formed more centrally in the medullary cavity. At an observation time of 8–12 weeks the trabeculae in the peripheral parts of the medullary cavity in the growing animals had often become completely resorbed and had been replaced by richly vascular medullary tissue. The bone formation had then often partially ceased, even on the trabeculae which lay in the centre of the medullary cavity on the border of areas which had not yet become vascularized or which were sparsely revascularized. Brånemark (1964) observed incipient resorption of the bone trabeculae after an observation time of 3 weeks, and restored marrow tissue after 4 weeks. Richany *et al.* (1965) found that the marrow regeneration was largely accomplished 25 weeks after medullary removal.

In the present material the adult animals showed considerably less bone formation in the medullary cavity than the growing animals. Often at an observation time of 12 weeks large areas of the medullary cavity were still not revascularized. In no preparation had the bone formation in the medullary cavity ceased 12 weeks after the operation.

MICROANGIOGRAPHIC STUDIES

Normal Angiographic Pattern on Control Side

On Indian ink angiography with the method used here, the entire vascular bed including intracortical capillaries and medullary cavity sinusoids was filled with the dye. In the different vascular systems the vessels showed different degrees of Indian ink filling. As a rule the periosteal vessels were massively, homogeneously filled with the dye, but the Indian ink columns in the vessels were sometimes interrupted by small unfilled or partially filled areas. In the diaphyseal cortex parts of the capillaries were massively filled or lined with Indian ink. Between these well filled parts, short stretches of capillary which completely lacked contrast medium were seen. Arterioles from the medullary cavity were, as a rule, massively filled and could often be followed to the middle of the diaphyseal cortex. The cortex adjacent to the medullary cavity also showed, however, wide blood vessels in which the Indian ink lined the walls without filling the lumen. These vessels were often found to open in funnel-shaped fashion into the intramedullary sinusoids. The blood vessels in the subperiosteal bone were usually wider in the growing animals than in the adults.

In the medullary cavity both homogeneously filled, relatively narrow blood vessels, namely those which were seen to pass through half of the cortex and which were considered to be arterioles, and sinusoids, the walls of which were lined with grains of Indian ink, were found. Small areas of medullary cavity sinusoids were completely empty of the dye. The central vein of the medullary cavity was, as a rule, lined with grains of Indian ink.

The calibre of the periosteal blood vessels and their number were highly dependent upon the bone formation activity of the periosteum. On formation of primary osteones, relatively wide, somewhat tortuous blood vessels were seen to cross each other in several layers. On formation of circumferential lamellar bone, the blood vessels were narrow and straight throughout, and the vascular layer thinner. On completion of bone formation only occasional narrow vessels were seen in the subperiosteal layer.

In the growing animals the blood vessels in the subperiosteal bone were usually relatively wide in calibre and in some cases two vessels were found in the same bone canal. The bone canals in the middle part of the cortex usually contained only one blood vessel. At this location two vessels in the same canal were only observed following so-called "cutter heads", in the formation of secondary osteones (Color pl. 3 *a*). In the growing animals the bone canals around the blood vessels were often considerably wider than the enclosed vessel,but in the adults the bone canal was only very slightly wider than the blood vessel. In the subendosteal bone two blood vessels were sometimes found in the same bone canal. In these cases one of the vessels was often wide and its walls lined with grains of Indian ink, while the other was narrow and massively filled with the dye. The former vessel was considered to be a venule, and the latter an arteriole.

Microangiographic Pattern after Reaming of the Medullary Cavity

The material for this study comprised the animals in Table 1, with the exception of 81, on which angiography was not performed.

The periosteal vascular pattern varied with the local growth situation of the periosteum. Corresponding areas on the treated and control sides always had to be compared, therefore, before the effect of intra-medullary reaming on the periosteal blood vessels could be evaluated.

Observation time: 0–2 days

Periosteum. In all preparations the periosteal blood vessels were massively filled with Indian ink. Two of the nine animals (27, 28) showed subperiosteal haemorrhages in the area of the insertion of the fibula on the tibia. In the subperiosteal haemorrhages a small leakage of Indian ink was

seen in some places. In preparations observed at 24 hours (61), the periosteal blood vessels were wider on the treated than on the control side.

Cortex. At an observation time of less than 24 hours the filling of the intracortical capillaries was often difficult to evaluate, since the Indian ink-filled parts of one capillary were surrounded by long, unfilled areas, especially in the middle and inner part of the cortex. At an observation time of 24 hours these unfilled areas had decreased greatly in number and length. The vascular front (see p. 30) could then be determined with more reliability.

Under the subperiosteal haemorrhages the cortex usually showed no Indian ink filling of the blood vessels.

Medullary cavity. In all animals occasional blood vessels from the periosteum were seen to penetrate the entire cortex to the medullary cavity. In the medullary cavity in front of the Indian ink-filled vessel, a minor extravasation of the dye was usually observed.

Observation time: 3 days

Periosteum. It was a regular finding that the periosteal vessels were massively filled with Indian ink. In four of the five young animals (3, 114, 124, 125) and two of the five adults (54, 110) there were subperiosteal "haemorrhages" localized to the area of the insertion of the fibula on the tibia (Fig. 10). In no case was the haematoma infused with Indian ink. In all young animals the periosteal blood vessels were wider and often more tortuous on the treated side than on the control side. The same change was observed, but much less pronounced, in adult animals.

Cortex. From the periosteum, Indian ink-filled blood vessels penetrated into the cortex. A tendency to a funnel-shaped widening of these vessels nearest to the periosteum was noted on the treated side in all growing animals but was not observed in adult animals. The dilatation of the blood vessels in the bone canals appeared to have taken place without the bone canals becoming wider at this time. Under the subperiosteal haemorrhages the cortex showed a considerable lack of Indian ink filling of the blood-vessels. The filling of the intracortical vessels varied. In the subperiosteal bone the blood vessels were often massively filled, while those further inside the cortex often showed only grains of Indian ink along the walls. In two of the five young animals (3, 124) and one of the five adults (55), blood vessels filled with Indian ink were seen to run from the periosteum through the cortex to the medullary cavity.

Medullary cavity. In no preparation were any blood vessels filled with Indian ink found in the medullary cavity. A minor extravasation of Indian ink was often observed just in front of the Indian ink-filled blood vessels

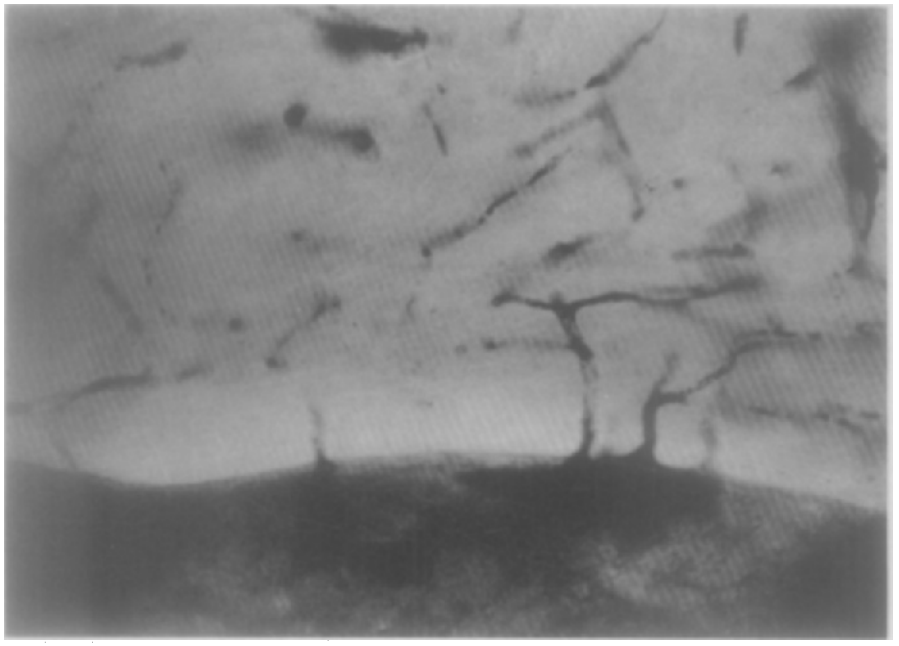


Fig. 22. Distal Spalteholz preparation from ventro-tibial part of cortex, observation time 3 days. Growing animal 124. Practically all blood vessels in the cortex nearest to the medullary cavity are filled with Indian ink. From several vessels in the non-reamed part of the cortex, extravasation of Indian ink into the medullary cavity blood clot is seen.

which opened into the medullary cavity (Figs. 11, 22), as in preparations at shorter observation times.

Observation time: 1 week

Periosteum. In all animals with the exception of one adult (126), periosteal blood vessels were observed which showed changes compared with corresponding areas on the control side. The vascular reaction varied. In the least pronounced reaction the blood vessels were widened and often more tortuous than on the control side. This was observed in the formation of circumferential lamellae. In stronger reactions, which occurred by formation of primary osteones, several layers of dilated and tortuous vessels were seen. In the strongest reaction long, slightly tortuous vessels were observed with pronounced calibre variation, running practically perpendicular to the bone surface. The blood vessels anastomosed with each other in a U-shaped fashion at varying heights, often immediately outside the original cortex. These blood vessels were present where trabecular bone was being formed. (See color pl. 2 *a.*)

Cortex. Blood vessels passing from the periosteum into the cortex widened in funnel-shape fashion in three of the six young animals (64, 66, 95) and in one of the three adult animals (121). A tendency to the same reaction was observed in one further young animal (105). The intracortical blood vessels were more massively filled with Indian ink than those observed at 3 days, but otherwise had the same appearance.

Medullary cavity. Newly formed blood vessels had invaded the medullary cavity in all young animals and in two of the three adults (121, 44). In all cases the vessels had only invaded from endosteal surface which had not been damaged by the reamer. The vessels which first invaded the medullary cavity comprised a three-dimensional, irregularly shaped network of mutually anastomosing vessels with pronounced calibre variation. In cross-section the blood vessels were often polygonal, with a diameter of between 2 and 30 μ.

Observation time: 2 weeks

Periosteum. The vascular reactions in the periosteum were similar to those at an observation time of 1 week, but as a rule more pronounced (Color pl. 2 *a*). Apart from the previously described vascular reactions, wide, round blood vessels of equal calibre lined with grains of Indian ink were now seen in the deeper parts of the periosteal callus tissue, in the areas where the long periosteal vessels perpendicular to the bone surface had formed previously. The course of these vessels was essentially parallel to the bone surface. This vascular reaction occurred on bone resorption when cavities were formed in trabecular bone. In all of the young animals and in three of the five adults the blood vessels passing from the periosteum into the cortex were dilated.

Cortex. In the outer part of the cortex many, but not all, of the intracortical vessels were dilated. In deeper parts of the cortex there were two forms of vascular reaction, which could occur within the same section. The most common reaction, which was observed in all growing and adult animals, comprised loops of blood vessels forming a ball with a diameter of about 20–35 μ. In some cases several vessels could be seen leading to these loops. This accumulation of blood vessels appeared to occur at the border between cortical areas in which the vessels had filled with Indian ink on angiography and areas with no Indian ink filling. It comprised the vascular component of a "cutter head", which revascularized the cortex (Color pl. 3 *a*). The other vascular reaction consisted of large broom-shaped bundles of blood vessels inside the cortex or between the original cortex and periosteal newly formed bone (Color pl. 2 *a*). At the base of the vascular "broom" a narrow, massively filled blood vessel and several wider vessels

Fig. 23. Distal Spalteholz section, observation time 2 weeks. Growing animal 281. Newly formed blood vessels are seen in the medullary cavity. In some areas the endosteal part of the cortex is not yet revascularized.

lined with Indian ink were often observed. When this vascular reaction occurred from the surface of the original cortex, a pronounced vascular reaction was usually observed at the same time in the overlying periosteum.

Medullary cavity. The vascular reaction in the medullary cavity was similar to that observed at 1 week. Newly formed blood vessels were seen in the medullary cavity in all animals except one young animal (45). In only one case (281) (Fig. 23) were blood vessels seen to emerge from the cortex at a location where the endosteal bone had been directly damaged by the rotating reamer. In the other cases the blood vessels emerged from the cortex in the non-reamed areas.

Observation time: 4 weeks

Periosteum. The reaction in the periosteal blood vessels had diminished considerably and was only observed in one of the six young animals (97) and one of the five adults (89). The vascular density in the subperiosteal bone which had developed after the reaming had decreased greatly. The blood vessels had also decreased in diameter, had become straighter and had assumed essentially the same appearance as the deeper located intracortical

vessels. In two young animals (51, 52) cavities were seen in the callus at the insertion of the fibula on the tibia. The blood vessels in the cavity showed essentially the same appearance as in normal medullary tissue.

Cortex. In the outer cortex many of the blood vessels were wider on the treated side than on the control side. Several wide vessels, often massively filled with Indian ink, were seen to run from the periosteum through the cortex to the medullary cavity. The Indian ink columns in these vessels were about 40 μ in diameter. The cortex lying immediately under the callus cavities was sparsely revascularized by fairly thin blood vessels. In the deeper parts of the cortex here there was a complete absence of Indian ink-filled vessels.

Medullary cavity. In all animals blood vessels were seen to emerge from almost all parts of the cortex. Wide vessels, as a rule veins, were observed in three of the seven growing and two of the five adult animals. In the centre of the medullary cavity in the growing animals occasional immature blood vessels were seen, of the type described at an observation time of 1 week, viz. polygonal, of varying calibre and massively filled with Indian ink. Peripheral to this area there was a large number of mutually anastomosing blood vessels of circular cross-section and with a diameter of 30–40 μ. These vessels formed a network with meshes measuring about 70 μ. Woven bone was seen between the vessels. In other areas of the medullary cavity there were sparsely located, narrow, somewhat tortuous vessels of equal calibre, which in the form of a brush ran in one direction. The tissue structure between these blood vessels appeared on the angiography preparations to be homogeneous. Adjacent to the endosteum there were long blood vessels about 100 μ wide, with a circular cross-section and lined with grains of Indian ink. These wide vessels lay in direct contact with intermediately located bone trabeculae (Fig. 24). Alternating with these wide vessels adjacent to the endosteum, straight vessels about 50 μ thick and massively filled with Indian ink were seen.

Blood vessels from the medullary cavity returned to avascular parts of the cortex and revascularized the inner part of the cortex.

Observation time: 8 weeks

Periosteum. The periosteal vascular reaction appeared to have disappeared completely. No difference in the width or tortuosity of the blood vessels or of the depth of the vascular layer was found on comparison between the treated and control sides. The blood vessels in the newly formed periosteal bone had assumed a completely mature appearance.

Cortex. The difference between the angiographic pattern in the outer and inner parts of the cortex was pronounced. In the outer cortical regions most

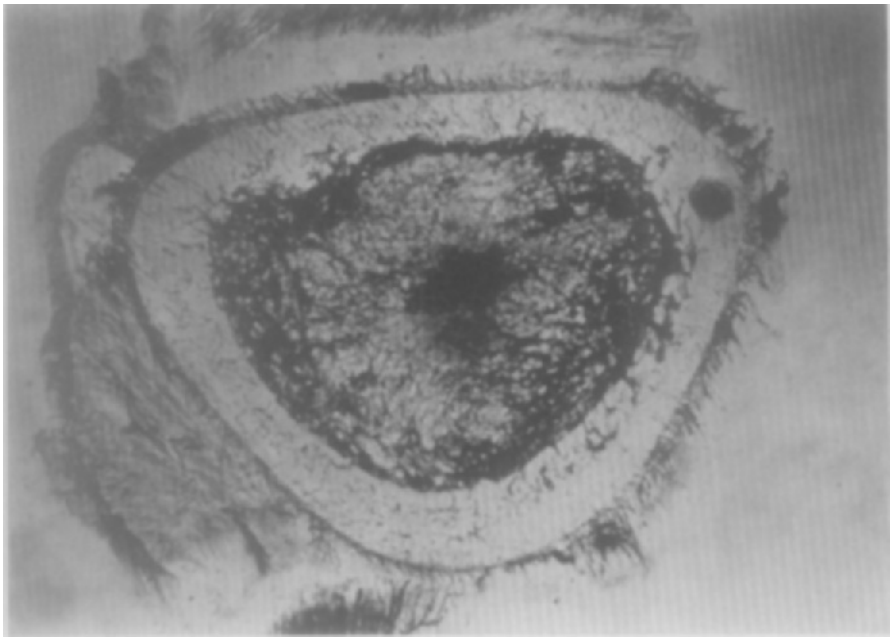


Fig. 24. Proximal Spalteholz cross section, observation time 4 weeks. Growing animal 15. Three different vascular areas can be seen in the medullary cavity. Nearest to the central extravasation of Indian ink there are small areas penetrated by narrow vessels (fibrotic areas). Peripheral to these areas there is a broad zone of wider vessels between lighter zones (bone trabeculae). Nearest to the endosteum a small number of light zones (bone trabeculae) surrounded by wide, Indian ink filled vessels are seen. In the innermost part of the cortex resorption cavities are observed, especially fibularly.

vessels showed a normal appearance, but some wide vessels connecting the vessels of the periosteum and those of the medullary cavity were observed. In the inner part of the cortex a large number of wide vessels were found, often located in resorption cavities in the bone. These blood vessels communicated with the medullary cavity by a large number of vessels of fairly wide calibre. Blood vessels were found in almost the entire cortex in all growing and in two of the six adult animals, but between the vessels in the inner part of the cortex there were small areas of bone tissue which were not revascularized. In this area several bone canals contained two blood vessels, one of which was narrow and massively filled with Indian ink, while the other was wider, with Indian ink only lining its walls.

Medullary cavity. Blood vessels were found in the medullary cavity in all animals. In all animals except one (83) blood vessels were seen to have invaded the medullary cavity even from endosteal bone which had been damaged by the reamer. Vessels of wide calibre, in some cases arteries

with thick walls, were observed in all young animals and in three of the six adults. In addition to the types of blood vessels described for the 4-week preparations, normal sinusoids were also seen in all animals.

Observation time: 12 weeks

Periosteum. The periosteal blood vessels had the same appearance on the treated side as on the control side.

Cortex. Three of the five animals showed revascularized cortex. Even in these animals, however, small areas of central cortex which had not been invaded by blood vessels were seen.

Medullary cavity. In the medullary cavity in all animals, areas with normal sinusoids were found. In many animals, however, there were areas which had not been revascularized. The types of blood vessels described for the 4-week and 8-week preparations were also found at this observation time. The immature vessels observed at 1 week were not seen in the 12-week preparations, however.

Discussion

Reaction in Periosteum

Three days after reaming of the medullary cavity a distinct reaction was observed in the periosteal blood vessels, especially in the young animals. These blood vessels had become wider and more tortuous and were filled more massively with Indian ink than on the control side. Koekenberg (1963) observed this vascular reaction one week after fracture in the rabbit tibia. He called these vessels type 1 and considered that they represented hyperaemia in existing blood vessels.

The blood vessel type which Koekenberg named type 2 consisted of mutually parallel vessels perpendicular to the bone surface, which initially showed varying calibres but which after about 2 weeks were found to be of equal width. Koekenberg found this vascular reaction in his preparations 2 and 4 weeks after fracture in the rabbit tibia. In the present material such blood vessels were observed between periosteum and cortex in those cases where fluorescence preparations showed that trabecular bone had been formed. The underlying cortex was then, as a rule, avascular. Göthman (1961) observed this vascular reaction subperiosteally 2–6 weeks after nailing of rabbit tibias without fracture, "in only a few cases". The typical pattern with blood vessels of type 2 is eliminated when the bone is reconstructed. A similar vascular reaction can sometimes be seen in the medullary cavity on the formation of a homogeneous, fibrous tissue. In the present

study the vascular reaction in the periosteum was most pronounced at an observation time of 2 weeks, it had decreased at 4 weeks and was completely absent at 8 weeks.

At observation times of less than 1 week, subperiosteal haemorrhages were often seen within the area of insertion of the fibula on the tibia. As a rule, no Indian ink had leaked into these haemorrhages, indicating that they had occurred before the Indian ink angiography. Subperiosteal haemorrhages were sometimes also found in other areas of the diaphysis. The reason for these haemorrhages will be discussed in chapter 4. On preparations observed at 1 week or later, no subperiosteal haemorrhages were seen, and it is probable that they had been resorbed. Within the area in which they were usually found, trabecular bone was often observed instead.

On the resorption of bone trabeculae in the central parts of newly formed trabecular bone under the periosteum, wide, round blood vessels of equal thickness and parallel with the peripheral surface of the original cortex were found. These vessels were of the same type as occurred in the medullary cavity on resorption of the bone beams there. In both cases this type of blood vessel was often later replaced by normal sinusoids.

Reaction in Cortex

The first vascular reaction in the cortex was widening of the blood vessels in that part of the outer cortex in which the blood circulation was still present after reaming of the medullary cavity. A tendency towards this reaction was found in young animals at an observation time of 3 days, and the reaction was pronounced at 1 week. The blood vessels appeared first to become wider in the bone canals without the latter being changed, but later the bone canals also increased in calibre. Rhinelander & Baragry (1962) considered that parts of the intracortical capillary network were normally "resting", but that on increased functional demands, e. g., following a fracture of the contralateral bone, the blood vessels opened, and thus could be filled with contrast medium. Göthman (1961) and Brookes (1964) found that Micropack suspension did not pass into the cortex from periosteal vessels until about 1 week after a disturbance of the endosteal circulation in the rabbit. Brookes considered that the intracortical vessels increased in calibre due to "reversal of the normal blood flow". The present experiments indicated that immediately after reaming of the medullary cavity the blood vessels passing from the periosteum into the cortex were able to lead blood into the cortex, and that these vessels then became successively wider. The deficiency in vascular filling with contrast medium observed by Rhinelander & Baragry and other workers was probably due to the fact that Micropack

has difficulty in passing through these blood vessels before they have increased in calibre, which does not take place to a sufficient degree until about one week after the operation. Some of the blood vessels in the outer cortex continued to increase in width during the 12 weeks of the present experiments. Other vessels, which had increased in size during the first weeks after the intramedullary reaming, appeared to decrease again in calibre after about 4 weeks, at the same time as their bone canals became narrower. The circulation from the periosteum into the cortex thereby became more and more concentrated to a smaller number of increasingly wide vessels, while the majority of blood vessels in the outer part of the cortex got the same appearance as on the control side.

The reaction in the inner part of the cortex depended upon whether or not the area had retained its circulation after the reaming. In those areas which had retained their circulation, the blood vessels appeared to react in the same way as in the outer vascularized part of the cortex. From the borderline area between those parts of the cortex in which the blood vessels still functioned after reaming of the medullary cavity, and those which no longer functioned, special vascular reactions were observed after about 2 weeks. These appeared to be of two types; either a ball of blood vessel loops was formed, which comprised the vacsular component in a "cutter head", or a fairly large broom-shaped bundle of vessels. Around the bundle of blood vessels so much bone was sometimes resorbed in young animals that a very large cavity was formed in the cortex. Arciform blood vessels could then be seen passing to the underlying bone, in which resorption was taking place. The "cutter heads" and bundles of blood vessels widened canals or cavities in the direction towards the medullary cavity and in this way revascularized the cortex. At this stage their vascular communication was only with the periosteal blood vessels.

Reaction in Medullary Cavity

On the first few days after the operation, most preparations showed occasional blood vessels which passed from the periosteum through the entire cortex as far as the medullary cavity. These vessels were seen in areas which had not been damaged directly by the rotating reamer. That these blood vessels really were functioning for the transport of fluid was shown by fluorescence preparations from animal 124 at an observation time of 3 days. This animal had been given tetracycline 24 hours after the operation. The tetracycline had diffused out into the medullary cavity around a blood vessel which had opened on the endosteal surface, and had become bound to bone fragments immediately outside the vessel. The tetracycline

had then not become reabsorbed into the circulation but was visible on the fluorescence preparation. The binding of tetracycline to the bone fragments was probably due to the fact that they were becoming halisteretic by lying in an acid environment. In the development of halisteresis, there is a release of calcium ions, and if these are not removed owing to a poor circulation, tetracycline can become bound to them (Eger & Kämmerer, 1967). See p. 48, Fig. 11.

The first blood vessels to develop in the medullary cavity were observed at an observation time of 1 week. These vessels were irregular in shape and varied in calibre, and formed a three-dimensional network. They closely resembled the type referred to by Koekenberg as type 3. Koekenberg found type 3 blood vessels during fracture healing in the rabbit at an observation time of 2–7 weeks, and considered that they were associated with chondral ossification. In the present material they were always the first blood vessels to form on revascularization of subperiosteal haematomas or of the medullary cavity. In no preparation was cartilage formation observed. Often in places where blood vessels had invaded the medullary cavity from the cortex, a narrow blood vessel massively filled with contrast medium which was considered to be an artery, was observed, as well as several round blood vessels lined with grains of Indian ink, which were considered to be veins. The bone formation in the medullary cavity did not appear to take place in direct association with the types of blood vessel referred to by Koekenberg as type 3, but later, when round vessels of medium calibre and anastomosing in network form had developed. Within the meshes of the network, between these blood vessels, woven bone was formed. Resorption of bone trabeculae in the medullary cavity appeared to take place near to 100 μ wide, anastomosing round blood vessels, which bordered closely on the surface of these beams. After resorption of the bone trabeculae the blood vessels underwent a change to apparently normal sinusoids.

On Indian ink filling of intracortical vessels by the method described here, the entire vascular bed, including the capillaries, became filled. The method is therefore suitable when the nutritive flow in the cortex is to be studied, but does not allow determination of the direction of the blood flow on a material not subjected to operation. This has also been pointed out by Brookes (1960).

STUDY OF THE EXTENT OF INTRACORTICAL VASCULAR DAMAGE

Previous investigations concerning the capacity of the periosteal vessels to take over the intracortical circulation when the contents of the medullary

cavity have been destroyed have given varying results (see chapter 1). It was therefore considered of interest to study by means of Indian ink angiography and the Spalteholz technique the extent of the intracortical vascular damage that occurs after reaming of the medullary cavity, and to follow the revascularization of the cortex. Indian ink angiography gives good visualization of the intracortical vessels, even one day after the operation and with the Spalteholz technique it is possible to study the Indian ink filling by stereomicroscopy and to record the vascular front (see p. 30).

Material and Methods

The material comprised the 67 animals—36 growing and 31 adults— shown in Table 1 which were studied at observation times of 1–3 days, 1 week, 2 weeks, 4 weeks, 8 weeks and 12 weeks. Animal 81, in which angiography was not performed, was not included. From animals with observation times of 1–3 days, two angiography sections from the diaphysis were examined—one about 2.8 cm and one about 7.3 cm from the tibiotalar joint. From the remaining animals sections from the distal part of the diaphysis were examined. The percentual part of the original cortex (area *BE*) which lacked Indian ink-filled vessels (area *CD*) was recorded.

The areas of the section in which the vessels were filled with Indian ink were called vascularized cortex and those which lacked Indian ink-filled vessels avascular cortex. The borderline between Indian ink-filled and non-Indian ink-filled areas was called the vascular front.

At observation times of 8 and 12 weeks many vessels were observed which first penetrated through the cortex into the medullary cavity and then invaded the cortex within another area. The cortex was thus revascularized also from the medullary cavity. Vessels were then found in the cortex both close to the medullary cavity and in the outer part, but interlying areas of the cortex could lack blood vessels. In these cases the assessment of the surface of avascular bone on the basis of the vascular front was not reliable, and the percentual part of the cortical surface which was avascular had to be estimated instead.

Results

The mean values of the percentage of avascular cortex within each experimental group are given in Table 3 *a* for growing animals and in Table 3 *b* for adult animals. The tables also show the ranges of variation within the different groups.

From the table for growing animals can be seen that in the group observed at 1–3 days 26% of the cross-section area of the cortex was avascu-

Table 3 *a, b. Mean value and variation range of the percentage of avascular cortex (CD·100/BE) in individual sectors and whole sections in experimental groups studied at observation times of 1 day to 8–12 weeks*

P indicates the proximal section and *D* the distal section.

Obs. time	No. of animals	Section	1 (Tibial) (%)	2 (Dorsal) (%)	3 (Fibular) (%)	4 (Ventral) (%)	5 (Total) (%)
a. *Growing animals*							
1–3 d	8	P	20 (7–38)	24 (2–40)	32 (7–56)	35 (16–50)	26 (12–37)
1–3 d	8	D	32 (0–80)	22 (12–43)	24 (4–64)	28 (13–57)	26 (9–61)
1 w	6	D	19 (3–41)	15 (5–29)	13 (3–28)	16 (5–25)	16 (8–25)
2 w	10	D	32 (5–84)	29 (8–60)	26 (5–71)	25 (8–60)	28 (8–67)
4 w	7	D	14 (0–37)	10 (0–38)	7 (0–27)	10 (0–25)	11 (0–26)
8 w	5	D	0	0	0	0	0
b. *Adult animals*							
1–3 d	7	P	39 (5–66)	29 (15–52)	41 (3–66)	51 (4–78)	40 (8–58)
1–3 d	7	D	34 (8–67)	28 (0–67)	33 (7–58)	25 (12–43)	31 (10–50)
1 w	3	D	34 (11–67)	28 (15–40)	23 (2–58)	23 (13–38)	26 (11–52)
2 w	5	D	26 (2–80)	18 (5–46)	14 (0–32)	17 (9–30)	19 (4–44)
4 w	5	D	27 (1–48)	22 (12–38)	14 (5–26)	30 (2–72)	24 (5–41)
8 w	6	D	6 (0–30)	1 (0–5)	0	7 (0–15)	4 (0–20)
12 w	5	D	0	6 (0–30)	4 (0–20)	10 (0–40)	7 (0–15)

lar. The avascularity was fairly evenly distributed within the four sectors. The values for the proximal and the distal sections corresponded well. The range of variation was very wide, in the distal section between 9 % and 61 %. The variation was smaller in the proximal sections. In one animal, (125) the whole of the tibial sector of the distal section was vascularized. At observation times of longer than 2 weeks there was a tendency for a smaller part of the cortex to be avascular. Thus at 4 weeks there were two animals in which the whole of the cortical cross section area was revascularized (7, 216), and at 8 weeks all growing animals showed complete revascularization of the cortex.

The adult animals had a somewhat higher mean percentage of avascular cortex at an observation time of 1–3 days, both in the distal and proximal section, than the growing animals. Also in the adult group a very large variation was found between individual animals. There was some tendency for the lowest percentage of avascular cortex in any animal in an experimental group to decrease with increasing observation times. At an observation time of 8 weeks two animals showed completely revascularized cortex (67, 77), while in the others between 5 % and 20 % of the cortex was estimated to be still avascular. At 12 weeks two animals (70, 76), showed revascularized cortex, and in the other three about 10 % of the cortex was avascular.

Discussion

During the first hours after reaming of the medullary cavity incomplete filling of the intracortical vessels is obtained on angiography with Indian ink (see p. 67), but after about 24 hours the visualization is so good that the vascular front can be recorded. Revascularization of the cortex takes place from residual intracortical vessels, which are supplied from periosteal vessels. According to Axhausen & Bergmann (1937), the first budding of new vessels on revascularization of a fracture area begins at the end of the second day. The percentage of avascular cortex which is recorded on sections at observation times of 1–3 days should therefore give a good reflection of the vascular damage caused by reaming of the medullary cavity. In this material about 30% of the cortical cross-section was avascular both within the distal and proximal part of the diaphysis. If this corresponds to the area of the cortex which cannot be supplied by the periosteal vessels when the medullary cavity is destroyed, these findings are in disagreement with several previous results. Thus de Marneffe (1953) claims that within the distal part of the tibial diaphysis in the rabbit the cortex is supplied to the largest part from periosteal vessels, while in the proximal part of the diaphysis only the outer part of the cortex is supplied from these vessels. Trueta & Cavadias (1955) found in rabbits that only the outer third of the cortex was supplied from periosteal vessels and the rest from endosteal vessels. In the experimental studies of these latter authors, however, considerable pressure may have occurred in the medullary cavity when the metaphyses were plugged, which may have caused damage to the intracortical vessels (see p. 110). As a perfusion medium they used mainly Micropack which probably gives poorer filling of the cortical vessels than Indian ink. These factors may explain their different results.

The vascular damage in the cortex was possibly somewhat greater in the adults than in the growing animals. Since the vessels in the subperiosteal bone are narrower in the adults than in the growing animals, this difference may be due partly to the fact that the Indian ink suspension did not fill the intracortical vessels so completely in the adult animals despite the fact that these had functioned intravitally.

The variations in that part of the cortex which became avascular, between the different animals and between different sectors in the same animal, was so great that the avascularity could not have been due solely to occlusion of the blood supply from the medullary cavity, but must also have had other causes. This will be discussed in chapter 4.

At an observation time of 8 weeks the cortex in the growing animals was completely revascularized. This finding was made by Trueta & Cavadias

(1955) after an observation time of 7 weeks in growing rabbits. In adult rabbits avascular parts of the cortex were still seen in many cases after observation times of both 8 and 12 weeks.

STUDY OF THE AMOUNT OF NEWLY FORMED PERIOSTEAL BONE

Material

The material consisted of 39 rabbits, 22 of which were growing animals and 17 adults. The material comprised those animals in Table 1 in which *one* tibia was operated on, the other tibia serving as a control. The observation times, i.e. the lengths of time after the operation to the angiography, were 1, 2, 4 and 8 weeks. Growing animals in which labelling 1 (see p. 25) was performed at time points 0–2 days after reaming of the medullary cavity and adult animals in which labelling 1 was performed 1–4 days after the operation were included. The two growing animals, 6 and 7, and the adult animal, 75, in which labelling 1 was not performed until 7 days after the reaming were thus not included.

Method

The area of the newly formed periosteal bone (area *AB*) was calculated on the distal transverse fluorescence section from approximately 3.3 cm above the tibiotalar joint (see p. 36). Area *AB* is expressed as a percentage of the area of the original cortex (area *BE*). The calculation was made separately for each sector and also for the whole cross-section on the treated side and on the control side. In the calculation of the whole cross section, the sum of *AB* from the four sectors is expressed in per cent of the sum of *BE* from the four sectors. A sector from the treated side and the corresponding sector from the control side are called a pair of sectors. The section from the treated side and the section from the control side are called a pair of sections. The percentage value obtained for the periosteally formed new bone is called, within each sector, the bone formation value (BF). The mean bone formation value for all animals in an experimental group is denoted the mean bone formation (MBF). The term Z is added when the whole cross-section has been calculated, the term C when the calculation refers to the control side, and O when it refers to the reamed side. For example: MBFZC = mean bone formation for the whole sections on the control side in one experimental group.

The difference between the bone formation values on the treated and control sides within each pair of sectors is called the bone formation difference (BFD). The mean bone formation difference for all animals in an experimental group is denoted the mean bone formation difference (MBFD). The term Z is used in the same way as in the calculation of the bone formation values.

Significance testing of the difference in periosteal bone formation between the main experimental groups was performed by means of the Wilcoxon test (see *Documenta Geigy*, 6th ed., p. 124).

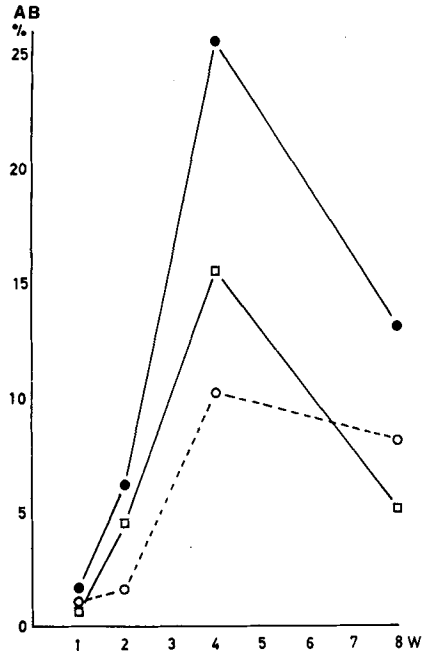


Fig. 25. Growing animals. Mean periosteal bone formation (area *AB*) in per cent of the area of the original cortex (area *BE*) in the whole section in the experimental groups with observation times of 1 to 8 weeks. ●—●, treated bone (MBFZO); ○--○, control bone (MBFZC); □—□, difference between treated and control bone (MBFDZ).

Results

Growing Animals (Fig. 25)

Observation time: 1 week. MBFZC was 1.1%. BFZC varied between the individual animals from 0.4–1.6%.

MBFDZ was 0.6%. BFDZ varied between the individual animals from 0.1 to 1.3%. MBFD was greatest in the ventral sector (0.9%) and smallest in the fibular sector (0.3%). BFD was greatest (1.8%) in the tibial sector in animal 95 and smallest (−0.2%) in the dorsal and tibial sectors in animal 106.

Observation time: 2 weeks. MBFZC was 1.6%. BFZC varied between the individual animals from 0.4 to 3.0%.

MBFDZ was 4.5%. BFDZ varied between the individual animals from 0.3 to 10.5%. MBFD was greatest in the fibular sector (8.2%) and smallest in the ventral sector (2.8%). BFD was greatest (31.1%) in the fibular sector in animal 281 and smallest (−0.1%) in the ventral sector in animal 101.

Observation time: 4 weeks. MBFZC was 10.2%. BFZC varied between the individual animals from 3.2% to 33.5%.

MBFDZ was 15.5%. BFDZ varied between the individual animals from 0.4 to 60.8%. MBFD was greatest in the fibular sector (20.5%) and smallest in the tibial sector (8.5%). BFD was greatest (82.0%) in the fibular sector in animal 52 and smallest (−1.4%) in the ventral sector in animal 216.

Observation time: 8 weeks. MBFZC was 8.1%. BFZC varied between the individual animals from 2.6 to 19.7%.

MBFDZ was 5.0%. BFDZ varied between the individual animals from 3.0 to 8.0%. MBFD was greatest in the fibular sector (8.2%) and smallest in the tibial sector (2.7%). BFD was greatest (15.8%) in the dorsal sector in animal 111 and smallest (−0.2%) in the tibial sector in animal 65.

In the group observed at 1 week only small amounts of bone had been formed subperiosteally, both on the treated and the control side.

On comparison between the groups observed at 1 and 2 weeks, an increase in the area of newly formed bone was found on the treated side, which was significant at the 5% level, while the bone area on the control side had increased only very slightly. A significant difference at the 10% level was found between the treated and the control side in the group observed at 2 weeks.

On comparison between the groups observed at 2 and 4 weeks, a further increase in the mean bone area on the treated side was found. This increase was due largely to the considerable bone formation in one animal (52) and is not significant. On the control side, the bone area had increased significantly at the 1% level, on comparison between the same groups of animals. No significant difference was found between the treated and control side in the group observed at 4 weeks.

Comparison between the groups of animals observed at 4 and 8 weeks showed no significant difference in the area of newly formed bone either on the treated or on the control side.

Adult Animals (Fig. 26)

Observation time: 1 week. MBFZC = 0.2%. BFZC varied between the individual animals from 0 to 0.7%.

MBFDZ was 0.4%. BFDZ varied between the individual animals from 0.1 to 0.9%. MBFD was greatest in the ventral sector (0.7%) and smallest in the fibular sector (0.0%). BFD was greatest (2.1%) in the ventral sector in animal 121 and smallest (−0.1%) in the fibular sector in animal 121.

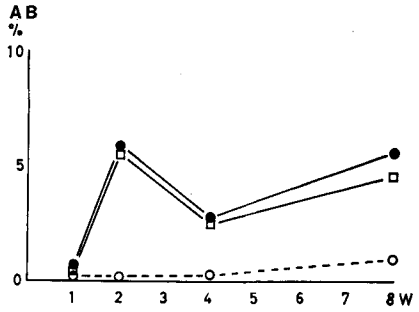


Fig. 26. Adult animals. Mean periosteal bone formation (area *AB*) in per cent of the area of original cortex (area *BE*) in the whole section in the experimental groups with observation times of 1 to 8 weeks. (For notation see Table 25.)

Observation time: 2 weeks. MBFZC was 0.2%. BFZC varied between the individual animals from 0.0 to 0.8%.

MBFDZ was 5.5%. BFDZ varied between the individual animals from 0.3 to 21.8%. MBFD was greatest in the dorsal sector (7.1%) and smallest in the ventral sector (2.9%). BFD was greatest (24.3%) in the dorsal sector in animal 86 and smallest (−0.2%) in the fibular sector in animal 58.

Observation time: 4 weeks. MBFZC was 0.3%. BFZC varied between the individual animals from 0 to 1.0%.

MBFDZ was 2.5%. BFDZ varied between the individual animals from 0.3 to 6.9%. MBFD was greatest in the tibial sector (4.7%) and smallest in the fibular sector (1.3%). BFD was greatest (16.7%) in the tibial sector in animal 89 and smallest (0.0%) in the dorsal sector in animal 88.

Observation time: 8 weeks. MBFZC was 1.0%. BFZC varied between the individual animals from 0.7 to 1.6%.

MBFDZ was 4.6%. BFDZ varied between the individual animals from 1.2 to 7.6%. MBFD was greatest in the ventral sector (7.2%) and smallest in the fibular sector (2.9%). BFD was greatest (13.3%) in the dorsal sector in animal 85 and smallest (−0.1%) in the dorsal sector in animal 77.

On comparison between the groups observed at 1 and 2 weeks and between those observed at 2 and 4 weeks, no significant difference in the area of newly formed bone was found, either on the treated or the control side. The high mean value for the bone formation on the treated side at an observation time of 2 weeks was mainly due to the large amount of new bone formed in one animal, 86.

Comparison of the bone formation in the combined groups observed at 1, 2 and 4 weeks with that in the group observed at 8 weeks showed a significant increase at the 5% level on the treated side and a significant in-

crease at the 2% level on the control side. The difference in area of newly formed bone between the treated side and control side on comparison between these groups was significant at the 10% level.

Discussion

With the method of recording used, measurements were made of the cross-sectional area of the periosteal tissue whose bone component was mineralized within 8 hours after labelling 2 (see p. 33). This area comprised bone tissue with varying percentages of vessels and perivascular tissue (vascular tissue). If circumferential lamellae has been formed there is approximately the same percentage of vascular tissue as in normal, cortical bone. If primary osteones have been formed, there is a high percentage of vascular tissue when the osteones are newly formed. The percentage of vascular tissue decreases continuously when the osteones mature, and after about 4 weeks the bone tissue has the same percentage of vascular tissue as normal, cortical bone. If trabecular bone has been formed the development of the bone tissue can vary. In one case the trabecular bone can be condensed to compact bone and then has a normal percentage of vascular tissue. In another case the central parts of the trabecular bone can, instead, be broken down and replaced by a richly vascularized cavity (see p. 62).

That part of the area of the newly formed bone which consists of actual bone tissue will therefore be dependent upon the length of the observation time and the morphology of the bone.

In this series of experiments the variations between the individual animals in the experimental groups and between the different sectors in the same animal were considerable. In all growing and adult animals the periosteal bone formation in the whole section was greater on the treated than on the control side. In individual pairs of sectors the bone formation was greater on the treated side than on the control side in 82 of the 88 pairs of sectors in growing animals and in 65 of the 68 pairs of sectors in adult animals. Evaluation of the significance of the difference between the different experimental groups was therefore not made on the basis of the mean values and distribution of the mean values of the groups, but according to the Wilcoxon test using the order of rank of the measurement values obtained.

After causing a disturbance of the endosteal circulation of the long bone, many authors have observed increased periosteal bone formation (Axhausen & Bergmann, 1937; Röhlich, 1941; Trueta & Cavadias, 1955; Küntscher, 1957; Richany *et al.*, 1965; Mital & Cohen, 1966; Flatmark, 1967; Zucman *et al.*, 1968). These investigators have assessed the increased bone

mass histologically or by repeated radiographic studies during the course of the experiment.

With histological techniques the boundary between the original cortex and the newly formed periosteal bone can only be established with certainty if the newly formed bone has a different morphology from that of the original cortex. As a rule, therefore, the exact borderline cannot be determined if the newly formed bone comprises circumferential lamellae or primary osteones; only with certainty if it is trabecular. Acceleration or restimulation of the normal bone formation can then often not be demonstrated with certainty, but only the real callus formation.

Repeated radiographic examinations during the course of the experiment cannot, as a rule, reveal minor changes in the bone mass.

In this investigation two tetracycline injections were given—one at the start of the experiment and the other at its end. In this way the bone formed during the experimental period was delimited. By this method even small amounts of bone could be measured. Similar techniques for the assessmet of newly formed bone or dentine have been used by Frost (1963), Tapp (1966), Hansson (1967) and Ahlgren (1968). There appear to have been no previous reports in the literature on the use of bone labelling techniques in studies of the amount of periosteal bone formation after disturbances of the endosteal circulation of the long bone.

During the first week after reaming of the medullary cavity in growing animals, only very small amounts of new bone were formed subperiosteally both on the treated and the control side. This first week comprises the adrenergic corticoid postoperative phase for the animal, during which an increased quantity of adrenocortical steroids are released, which on experimental administration have been found to result in depression of the bone formation (Hulth & Olerud, 1964; Tapp, 1966). The main bone formation on the treated side in growing animals takes place during the second week of the experiment, which should comprise the anabolic postoperative phase. On the control side the main bone formation takes place during the third and fourth weeks postoperatively, when the bone formation on the treated side is already declining. At this time the compensation for the previously retarded formation of bone on the control side can begin.

In adult animals the development is more difficult to evaluate. No definite increase in the bone formation is evident between subsequent animal groups, either on the treated or the control side.

The results obtained here for growing animals are in agreement with the findings of Richany *et al.* in 1965. By histological methods they observed in young adult cats that after cleaning of the medullary cavity in the femur the bone mass newly formed periosteally was maximal after an observation

time of 3 weeks and was reduced after a further 3 weeks. In the present material there was no markedly greater bone formation in any particular sector, as was found by Richany *et al.* in the region around the linea aspera.

The difference in bone formation within the different pairs of sectors in an individual animal was sometimes greatest within that pair of sectors where, on the treated side, the vascular damage in the underlying cortex was greatest, or where the largest amount of bone had been removed from the endosteal surface. The possible relationship between the amount of newly formed bone and the degree of damage to the underlying cortex will be discussed further in chapter 5.

STUDY OF THE BONE FORMATION ACTIVITY OF THE PERIOSTEUM

After reaming of the medullary cavity the periosteum of the bone is, as a rule, activated to increased bone formation. The bone formation activity was assessed in the present study by (*a*) recording in each sector whether the outer fluorescent band which occurred from labelling 2 was present or not, and (*b*) measuring in each sector the thickness of this fluorescent band together with bone and osteoid located peripherally to it.

The outer fluorescent band comprises essentially that bone which has been mineralized within 8 hours after the last tetracycline injection (Tapp, 1966). The bone and osteoid tissue lying peripheral to the outer fluorescent band was formed during the last 40 hours before the angiographic examination, since this examination was performed 2 days after labelling 2. The observed thickness was thus an expression of the bone formation wich had taken place during the days immediately preceding the angiographic examination, in contrast to the total bone formation which referred to the whole observation time. The measurements were performed with a Zeiss ocular micrometer on the distal transversal fluorescence section at a magnification of 320 times.

The periosteum can be activated at different time points after the operation in different animals and in different sectors in the same animal, and the activity can be of varying strengths. Each sector must therefore be evaluated separately. A sector on the treated side and the corresponding sector on the control side have been called a pair of sectors. To obtain a better idea of the bone formation activity of the periosteum this was studied with the aid of several variables, which are defined below. Thus for each sector and pair of sectors, respectively, recordings were made of the activity value (A), activity difference (AD), resorption value (R), resorption difference (RD), absolute growth value (G), absolute growth difference (GD) and stan-

dardized growth difference (SGD). The mean value for these variables was calculated for all sectors or pairs of sectors in each individual animal and for all sectors or pairs of sectors in all animals in each experimental group.

In this series of experiments the aim was to determine, in relation to the time of operation, (1) when the periosteal bone formation started to increase, (2) when the periosteal bone formation was maximal, (3) when the difference in bone formation activity between the treated and the control side was maximal, and (4) how long the increased bone formation activity persisted.

Material

The material consisted of 49 rabbits, 28 of which were growing animals and 21 adults. These animals comprised those shown in Table 1 in which one tibia was subjected to operation while the other served as a control, and for which the observation times were 3 days and 1, 2, 4 and 8 weeks. The five animals, 50, 56, 85, 77 and 92, in which no fluorescent bone was observed after labelling 2, were excluded from the material (see p. 34).

Methods

Determination of Activity Values and Activity Differences

When the outer fluorescent band occurred in any part of the middle $^4/_6$ of a sector this sector was called active, and when it did not, this sector was called inactive. An active sector was given the value 1 and an inactive sector the value 0. The value thus given to a sector was called the activity value (A) of the sector. A could thus have the value 0 or 1. The mean activity on the treated side or the control side for all four sectors in the same animal was denoted MA. MA could have any of the values 0, $^1/_4$, $^1/_2$, $^3/_4$ or 1. The mean activity for all sectors in all animals in an experimental group was denoted MAT (= mean activity total). MAT could have values between 0 and 1.

The difference within a pair of sectors between the activity value on the treated side and that on the control side was denoted the activity difference (AD). AD was given the value + 1 if the treated side was active and the control side inactive, the value −1 if the treated side was inactive and the control side active and the value 0 if both sides were inactive or both active. The activity difference for a pair of sectors (AD) could have any of the values −1, 0 or +1. The mean activity difference for all four pairs of sectors in one animal was denoted MAD. MAD could have any of the values −1, $-^1/_2$, 0, $+^1/_2$ or +1. The mean activity difference for all pairs of sectors in all animals within an experimental group was denoted MADT. MADT could have values between −1 and +1.

A regression analysis was performed of the relation of the activity values and activity differences to the observation times, these values or differences being used as dependent or y-variables, and the length of the observation time and the square of the length of the observation time as independent or x-variables. In the regression ana-

lysis an observation time of 3 days was given the value 1; 1 week the value 2; 2 weeks the value 3; 4 weeks the value 4, and 8 weeks the value 5. The calculations were performed by means of a computer (Uppsala Computer Centre).

Determination of Absolute Growth Values and Absolute Growth Differences

In each sector the maximal thickness of the outer fluorescent band together with bone and osteoid lying peripheral to it was measured within the middle $^4/_6$ of the sector. For each sector the value obtained on measurement was called the absolute growth value (G). The mean of the absolute growth values for all four sectors in the same animal was called the mean absolute growth value (MG). The mean of the absolute growth values for all sectors in all animals in one experimental group was denoted MGT. The difference between the absolute growth values on the treated and control sides in each pair of sectors was called the absolute growth difference (GD). The mean of the absolute growth differences for all four sectors in the same animal was called the mean absolute growth difference (MGD). The mean of the absolute growth differences for all four sectors in all animals in an experimental group was denoted MGDT.

Determination of Standardized Growth Difference

In a simple calculation of the mean value for the differences in growth values between the treated and control sides, high values within individual sectors, e.g. animal 125—dorsal sector, will dominate. For this reason quantitation of the growth difference to $+1$, -1 and 0 was performed. This was called for each pair of sectors the standardized growth difference (SGD). This was given the value $+1$ when the thickness on the treated side was significantly greater than on the control side, -1 when the thickness on the control side was significantly greater than on the treated side and 0 when the thicknesses on the two sides were the same (see below). SGD could thus be given any of the values 1, 0 or -1. The mean value of the standardized growth difference for all pairs of sectors in one animal was denoted MSGD. MSGD could have any of the values ± 0, $\pm^1/_4$, $\pm^1/_2$, $\pm^3/_4$ or ± 1. The mean standardized growth difference for all four pairs of sectors in all animals in one experimental group was denoted MSGDT and could have values between -1 and $+1$.

Determination of Resorption Values and Resorption Differences

When resorption was found within more than half of the periphery of a sector, this sector was called a sector undergoing resorption and was given the value 1, while a sector not undergoing resorption was given the value 0. The value given was called the resorption value of the sector (R). The mean resorption values (MR and MRT), the resorption difference (RD) and the mean resorption differences (MRD and MRDT) were defined and calculated analogously to the corresponding variables used in assessment of the activity.

The resorption could only be evaluated in the growing animals, since signs of superficial resorption were very unreliable in the adults.

Discussion of the Method

In order to assess when the measured growth difference between the right and left side was greater than the expected spontaneous growth difference

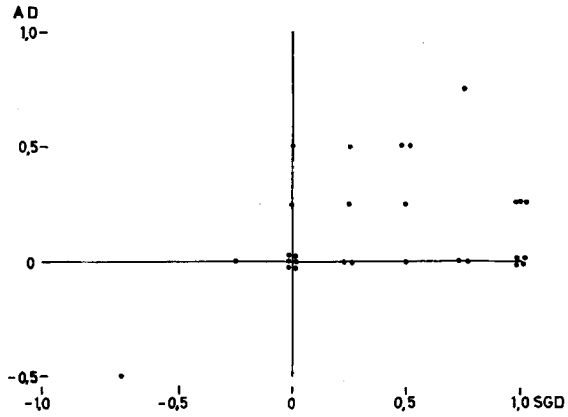


Fig. 27. Growing animals. Correlation between values of activity difference (AD) and standardized growth difference (SGD) in individual animals. Coefficient of correlation (R) = 0.21.

between the sides, the growth difference was studied in five growing rabbits which did not undergo operation. The animals were given tetracycline 2 days before the angiographic examination. The thickness of the newly formed bone was measured in the same way as in the experimental series. The maximal difference of the thicknesses between the sectors, within the pairs of sectors, was 1 unit (U) = 3.5 μ, see p. 32. Only if one of the sectors in a pair was inactive during simultaneous resorption and the other was active did the maximal difference amount to 2 units. In this series of experiments it was therefore required that in comparisons between sectors in a pair of sectors the difference in the measured thicknesses should be at least 1 unit for an estimated standardized growth difference to be said to exist. If one of the sectors was inactive during simultaneous resorption, while the other was inactive, the difference should be at least 2 units.

In animals observed at 1 week the fluorescent bands from labellings 1 and 2 were usually confluent. Also the width of the inner fluorescent band from labelling 1 was therefore taken for measurement of the thickness. Since all young animals had a positive activity value in all sectors at an observation time of 3 days, it was required in these cases that the thickness should be more than 1 unit for the sector to be considered to have a positive activity value at an observation time of 1 week. The criterion for a positive standardized growth difference was that the difference in thicknesses should be at least 2 units. On evaluation of the absolute growth, the whole value obtained for the thickness was used in the calculation. The group of animals observed at 1 week was attributed little importance in the study.

An analysis was performed of the correlations (*a*) between the activity

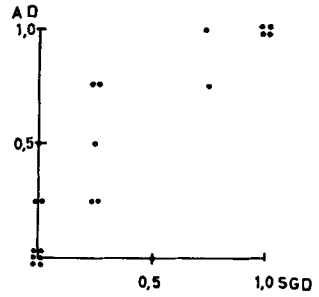


Fig. 28. Adult animals. Correlation between values of activity difference (AD) and standardized growth difference (SGD) in individual animals. Coefficient of correlation (R) = 0.41.

difference (AD) and the standardized growth difference (SGD), and (*b*) between the absolute growth difference (GD) and the standardized growth difference (SGD).

In growing animals the correlation coefficient (r) between AD and SGD was 0.21 (Fig. 27). There was thus a correlation between these methods of recording, but it did not reach the significance level. The weakness of the correlation is due to the fact that activity was also present on the control side in many cases with a positive standardized growth difference, especially in animals with a short observation time. AD would then be equal to 0, but SGD would be greater than 0.

In adult animals the correlation coefficient (r) between AD and SGD was 0.41 (Fig. 28). The correlation was thus stronger than in the growing animals but did not reach the significance level. The relatively low r value is due to the fact that an activity difference was found in many sectors, especially in animals with long observation times, where the thickness measured was so small that only small standardized growth differences occurred.

The ratio of the mean value of AD : SGD was 0.33 for growing animals and 1.25 for adult animals, which also illustrates that the reason for the low r value differed partly in growing and adult animals.

The correlation between GD and SGD reached a significant level in both growing and adult animals (Figs. 29, 30). This is a consequence of the definition of SGD. The diagram shows, however, that when SGD = 1 there was considerable variability of GD, expecially in the growing animals.

Results

The values obtained on measurement are given in Tables 4 and 5.

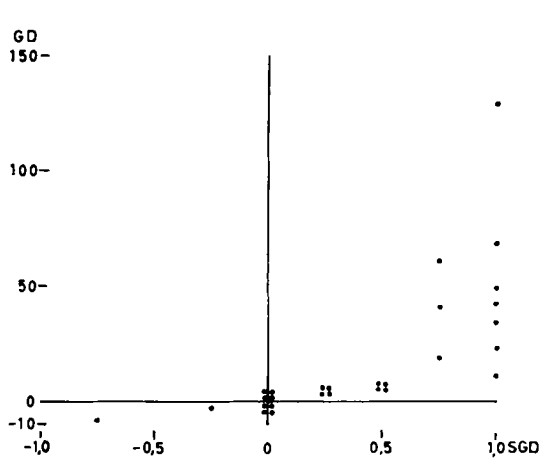


Fig. 29

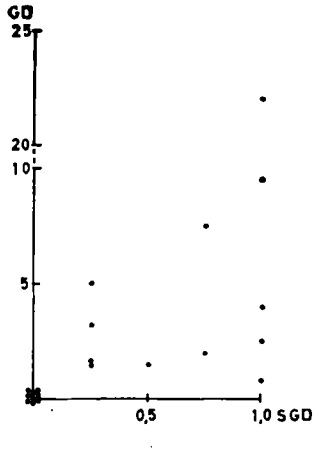


Fig. 30

Fig. 29. Growing animals. Correlation between values of growth difference (GD) and standardized growth difference (SGD) in individual animals. Coefficient of correlation (R) = 0.64.***

Fig. 30. Adult animals. Correlation between values of growth difference (GD) and standardized growth difference (SGD) in individual animals. Coefficient of correlation (R) = 0.69.***

Studies of the Activity Values

Growing animals

For the control side the regression curve for the activity values (A) of the individual sectors was a downward convex parabola which was found to have different strengths in different sectors. For the dorsal and tibial sectors the parabolic form is almost significant but for the ventral and fibular sectors there was only a suggestion of this parabolic form. On the treated side there was a tendency to the same parabolic form in the ventral and dorsal sectors but not in the tibial and fibular sectors. In the regression curve for AD (see Table 6) the tendency to a more curved form on the control side was strongly evident. For the ventral and tibial pairs of sectors there was an upward convex almost significant parabola and in the dorsal and fibular pairs of sectors, a suggestion of a parabola of the same appearance. At time point 2.8 (an observation time of 12.6 days) and around this point, the AD values were almost significantly increased throughout, which is evident from the coefficient of intercept. At time point 2.8, AD was 0.34 for the ventral sector, 0.19 for the dorsal sector, 0.31 for the tibial sector and 0.29 for the fibular sector.

The time point for the parabolic maximum was calculated from the formula $t_{max} = 2.8 - B/2$, which was obtained by derivation of the equation

Tables 4, 5. *The measured thickness, in each sector on the distal fluorescent section, of the fluorescent band from the tetracycline labelling given 2 days before sacrifice, together with bone and osteoid located peripherally to it*

Observation time 3 days to 8 weeks after reaming of the medullary cavity. *R* indicates that the sector is undergoing resorption; *t* = treated side; *c* = control side.

Obs. time	Animal no.	Sectors							
		Tibial		Dorsal		Fibular		Ventral	
		t	*c*	*t*	*c*	*t*	*c*	*t*	*c*
Growing animals									
3 days	3	3	1	20	3	5	4	24	3
	81	10	6	25	7	8	6	35	10
	114	5	5	5	3	5	4	4	4
	123	1	2	3	3	3	3	2	2
	124	3	3	4	3	4	3	4	3
	125	30	3	70	3	15	3	26	3
1 week	64	8	5	8	5	6	4	15	4
	66	6	5	10	5	6	6	7	6
	95	6 *R*	1 *R*	6	3	9	3	27	7
	104	2	2	3	2	3	4	4	3
	105	7	3	15	4	6	3	25	1
	106	1	1	2	1	2	2	3	1
2 weeks	6	3	0 *R*	0 *R*	0 *R*	3	0 *R*	2 *R*	2
	59	3	0 *R*	0 *R*	0 *R*	3	0 *R*	3 *R*	3 *R*
	101	4	3	5	1	5	1	0	0
	108	0 *R*	0 *R*	0 *R*	0 *R*	0 *R*	0 *R*	1 *R*	0 *R*
	211	7	1	6 *R*	0 *R*	5	1	9	2
	220	2	1 *R*	4	0 *R*	2	1 *R*	3	1
	281	5	0 *R*	0 *R*	0 *R*	50	0 *R*	6	0 *R*
	285	5	2	5	3	5	2	7	4
4 weeks	7	0 *R*	0 *R*	0 *R*	0 *R*	2	0	1	0 *R*
	51	5	5	3	3	4	0 *R*	4	4
	52	2 *R*	3	4	4	4	4	2 *R*	4
	96	2	2	2	2	3	3	2	2
	97	26	3	29	3	15	3	11	3
	216	0 *R*	0	0	0 *R*	0 *R*	0 *R*	0	0
8 weeks	65	0	2	2	5	3	4	0	2
	111	4	3	4	4	4	4	5	4
Adult animals									
3 days	54	3	1	4	2	3	2	3	2
	55	1	1	2	2	1	1	2	1
	63	0	0	1	0	0	0	2	0
	109	0	0	0	0	0	0	0	0
	110	0	0	0	0	0	0	0	0
1 week	112	0	0	12	0	0	0	1	0
	121	6	1	6	2	2	2	25	4
	126	2	0	0	0	0	0	0	0
2 weeks	57	2	0	3	0	2	0	3	0
	58	4	0	1	0	0	0	1	0
	86	35	0	19	0	20	0	15	1
	87	4	0	25	0	3	0	6	0
	119	3	0	0	0	3	0	2	0

Tables 4, 5 (*cont.*)

Obs. time	Animal no.	Sectors							
		Tibial		Dorsal		Fibular		Ventral	
		t	*c*	*t*	*c*	*t*	*c*	*t*	*c*
4 weeks	75	0	0	0	0	0	0	0	0
	88	0	0	0	0	0	0	0	0
	89	4	0	4	0	4	0	4	0
	100	0	0	0	0	0	0	1	0
	113	0	0	1	0	0	0	1	0
8 weeks	67	1	1	0	0	0	0	0	0
	83	0	0	0	0	0	0	1	0
	84	4	0	1	0	0	0	1	0

for the parabola $y = A + B \cdot (t - 2.8) + C \cdot (t - 2.8)^2$. In the regression curve for AD the time point for the parabolic maximum for the ventral sector was 2.6, for the dorsal sector 2.7, for the tibial sector 2.6 and for the fibular sector 3.3. The difference between the time points for the parabolic maximum within the different sectors was not significant. No evident difference was found in the form of the regression curve for AD between the sum of the reamed sectors (ventral + dorsal) and the sum of the non-reamed sectors (tibial + fibular).

Since no marked difference was found between the different sectors or pairs of sectors, it seemed suitable to study all sectors combined. The regression curve for MAD (Table 6, Fig. 31) was an ascendingly convex, almost significant parabola with its maximum at time point 2.75. At time

Table 6. *Results of regression analysis for growing animals, with activity difference (AD, MAD) as dependent variable and length of observation time as independent variable*

The regression equation is written: (AD, MAD) = A + B $(t - 2.8)$ + C$(t - 2.8)^2$. A, coefficient of intercept at $t = 2.8$; B, regression coefficient for the linear term $(t - 2.8)$; C, regression coefficient for the term $(t - 2.8)^2$; The results are given for the difference in each pair of sectors and for the sum of the differences within all 4 pairs of sectors divided by 4 (Mean, MAD)
* denotes the degree of significance.

	Sectors									
	Tibial		Dorsal		Fibular		Ventral		Mean	
	Coeff.	±S.E.	Coeff.	±S.E.	Coeff.	±S.E.	Coeff.	±S.E.	Coeff.	±S.E.
A	0.31*	0.10	0.19*	0.08	0.29*	0.10	0.34*	0.11	0.29**	0.06
B	−0.06	0.06	−0.01	0.05	0.08	0.06	−0.05	0.06	0.01	0.03
C	−0.14*	0.05	−0.06	0.04	−0.07	0.05	−0.13*	0.05	0.10*	0.03

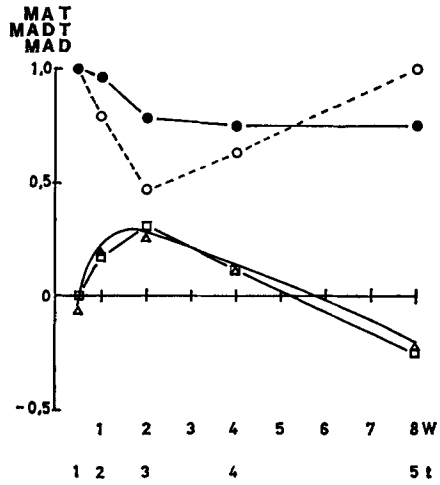


Fig. 31. Growing animals. The mean activity value (MAT) and activity difference value (MADT) in each experimental group at observation times of 3 days to 8 weeks, and the regression curve for the mean activity difference (MAD) at time points 1 to 5. ●—●, MAT for treated tibia; ○–○, MAT for control tibia; □—□, MADT; △—△, regression curve for MAD.

point 2.8, MAD was 0.29, which is a significantly increased difference value.

A study of the relations of the individual experimental groups shows that at an observation time of 3 days, MAT = 1 both on the treated and on the control side (Fig. 31). On the control side, MAT = 0.47 at an observation time of 2 weeks. The reduction from 1 to 0.47 was highly significant. MAT increased to 0.63 at an observation time of 4 weeks and to 1.0 at 8 weeks. This increase is not significant.

On the treated side, MAT = 0.78 at an observation time of 2 weeks. The decrease from 1.0 at an observation time of 3 days to 0.78 at 2 weeks is almost significant. At observation times of 4 and 8 weeks, MAT = 0.75. The decrease in MAT was so much greater on the control side than on the treated side that MADT was significant at an observation time of 2 weeks.

Adult animals

For the control side the regression curve for the activity value (A) of the individual sectors lacked a parabolic shape throughout. At time point 2.8 the curve showed a significant downward slope for the dorsal and fibular sectors and a tendency to a downward slope for the ventral sector. At this time point none of the sectors on the control side showed a significant A value.

For the treated side the regression curve for A lacked a significant parabolic shape throughout. At time point 2.8, the curve showed no significant

Table 7. *Results of regression analysis for adult animals*

For notation see Table 6.

	Sectors									
	Tibial		Dorsal		Fibular		Ventral		Mean	
	Coeff.	±S.E.	Coeff.	±S.E.	Coeff.	±S.E.	Coeff.	±S.E.	Coeff.	±S.E.
A	0.66*	0.15	0.61*	0.17	0.50*	0.13	0.63*	0.16	0.60*	0.11
B	0.09	0.07	0.06	0.08	0.06	0.06	0.14	0.08	0.09	0.05
C	−0.15*	0.06	−0.10	0.07	−0.14*	0.05	−0.06	0.07	−0.11*	0.04

slope throughout, but in all sectors significant activity values were found at time point 2.8.

In the regression curves for AD (Table 7) the activity values (A) on the treated side at time point 2.8 and around this point predominate so that an almost significant AD value was then found in all sectors. For the tibial and fibular sectors the curve had an almost significant parabolic contour, but not for the ventral and dorsal sectors. For the tibial sector the parabola had its maximum at time point 3.1 and for the fibular sector at time point 3.0. No evident difference was found in the shape of the regression curve for AD between the sum of the reamed sectors (ventral + dorsal) and the sum of the non-reamed sectors (tibial + fibular).

The regression curve for MAD (Table 7, Fig. 32) was an upwardly convex almost significant parabola with a maximum at time point 3.2. Already

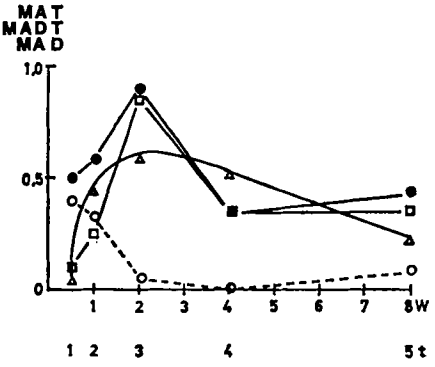


Fig. 32. Adult animals. The mean activity value (MAT) and activity difference value (MADT) in each experimental group at observation times of 3 days to 8 weeks, and the regression curve for the activity difference (AD) at time points 1 to 5. ●—●, MAT for treated tibia; ○--○, MAT for control tibia; □—□, MADT; △—△, regression curve for MAD.

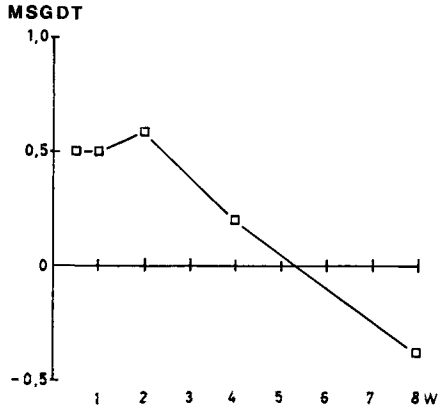


Fig. 33. Growing animals. Mean standardized growth difference value (MSGDT) in each experimental group at observation times of 3 days to 8 weeks.

at time point 2.8, MAD was 0.6, which is an almost significant difference value.

A study of the relations of the individual experimental groups showed that on the control side MAT was 0.4 at an observation time of 3 days, 0.05 at 2 weeks, 0 at 4 weeks and 0.08 at 8 weeks (Fig. 32). The decrease between the observation times of 3 days and 2 weeks was not significant. On the treated side MAT was 0.5 at an observation time of 3 days, 0.9 at 2 weeks, 0.35 at 4 weeks and 0.43 at 8 weeks. The increase from 0.5 to 0.9 is not significant. The decrease from 0.9 to 0.35 is almost significant. There was a highly significant positive activity difference at an observation time of 2 weeks.

Studies of the Standardized Growth Difference

Growing animals (Fig. 33)

At observation times of 3 days and 1 week, almost significant positive MSGDT values were found, and at 2 weeks, highly significant positive MSGDT values. An almost significant positive difference in MSGDT was found between the combined animal groups observed at 3 days, 1 week and 2 weeks compared with the groups observed at 4 and 8 weeks.

Adult animals (Fig. 34)

Almost significant positive MSGDT values were found in the animal group observed at 1 week and highly significant at 2 weeks. The group observed at 2 weeks showed a significantly higher MSGDT than the group observed at 3 days and an almost significantly higher value than the group observed at 4 weeks.

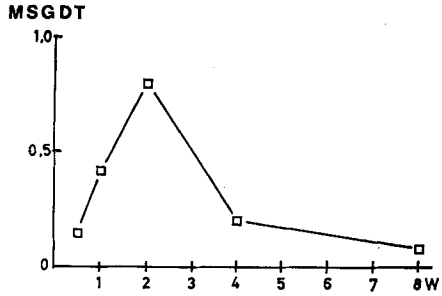


Fig. 34. Adult animals. Mean standardized growth difference value (MSGDT) in each experimental group at observation times of 3 days to 8 weeks.

Study of the Absolute Growth Values

Growing animals (Fig. 35)

On the control side MGT was 3.8 U at an observation time of 3 days, 0.9 at 2 weeks, 2.0 at 4 weeks and 3.5 at 8 weeks. On the treated side MGT was 13.1 U at 3 days, 4.8 at 2 weeks, 5.0 at 4 weeks and 2.8 at 8 weeks. On the control side a significantly lower MGT value was found in the animals observed at 2 weeks than in those observed at 3 days, and an almost significantly higher MGT value in the animals from the combined groups observed at 4 and 8 weeks compared with those observed at 2 weeks.

On the treated side no significant difference in MGT was seen between the different experimental groups, but the values showed a distinct tendency

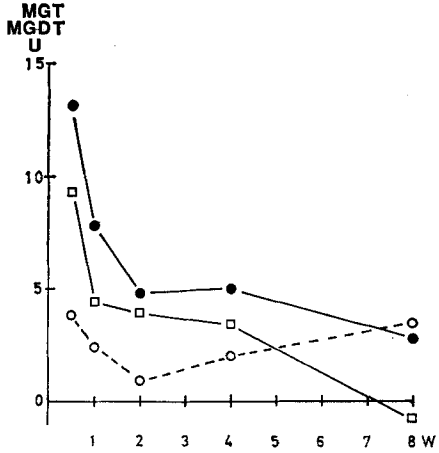


Fig. 35. Growing animals. Mean growth value (MGT) and growth difference value (MGDT) in each experimental group at observation times of 3 days to 8 weeks. ●—●, MGT for treated tibia; ○—○, MGT for control tibia; □—□, MGDT.

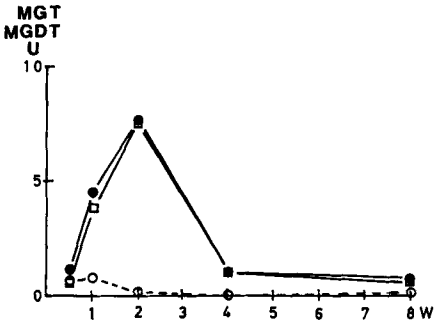


Fig. 36. Adult animals. Mean growth value (MGT) and growth difference value (MGDT) in each experimental group at observation times of 3 days to 8 weeks. ●—●, MGT for treated tibia; ○—○, MGT for control tibia; □—□, MGDT.

towards lower values at longer observation times. There was an almost significant MGDT between the treated and control side at observation times of 1 and 2 weeks. The highest MGDT was found at 3 days, but this value was not significantly increased. The value is largely accounted for by a high GD in 8 pairs of sectors in the three animals 125, 3 and 81.

Adult animals (Fig. 36)

On the control side MGT was 0.6 U at an observation time of 3 days, 0.1 at 2 weeks, 0 at 4 weeks and 0.1 at 8 weeks. On the treated side this value was 1.1 U at 3 days, 7.6 at 2 weeks, 1.0 at 4 weeks and 0.7 at 8 weeks. The maximal MGT on the treated side and the maximal MGDT were found at an observation time of 2 weeks. At this time MGDT was almost significant.

Study of the Resorption Values (Fig. 37)

A significantly positive MRT was found both on the treated side and on the control side at an observation time of 2 weeks. MRT was so much higher on the control side than on the treated side that at this observation time MRDT was almost significantly negative. At observation times of 3 days and 1, 4 and 8 weeks MRDT was not significant.

Discussion

In previous studies on the reaction of the periosteum after disturbances of the endosteal circulation (Röhlich, 1941; Küntscher 1957; Flatmark, 1967; Trueta & Cavadias, 1955; Richany *et al.,* 1965; Mital & Cohen, 1966; Zucman *et al.,* 1968), the total amount of bone which has newly formed

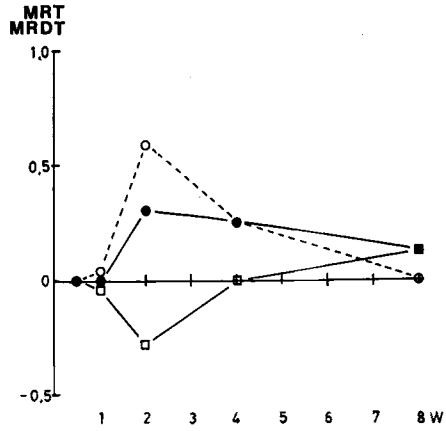


Fig. 37. Growing animals. Mean resorption value (MRT) and resorption difference value (MRDT) in each experimental group at observation times of 3 days to 8 weeks ●—●, MRT for treated tibia; ○—○, MRT for control tibia; □—□, MRDT.

during different periods of observation has been assessed. Their observations have been combined with studies of the morphology of the bone at the end of each observation period and from these findings conclusions have been drawn on the course of the periosteal bone growth.

In the present investigation this course has been studied by other methods, as follows.

(1) Qualitative recording of the bone formation capacity of the periosteum some days before the angiographic examination was performed by determining the activity values. The development of the activity values during the whole period of observation, eight weeks, was studied by means of regression analysis.

(2) Quantitative recording of the bone formation was performed by measuring the thickness of the bone and osteoid tissue which had formed during the last days of each observation period. The large variation in the bone formation rate between different animals and different sectors was evened out by calculating the standardized growth difference. For this purpose the quantitative measurement value was converted to a qualitative unit.

(3) A recording was made of whether the sectors showed superficial resorption or not.

By studying these variables for the treated side, for the control side and for the difference between the treated and control sides the development of the subperiosteal bone formation activity could be assessed both qualitatively and quantitatively.

In the growing animals all sectors were active both on the treated and the control side (MAT = 1) at an observation time of 3 days. Since all animals in this group were given tetracycline 1 day after the operation, these activity values actually reflect the situation about 1 day postoperatively. The high activity which was observed at that time on the control side may either reflect the normal bone formation activity of the animal or be a result of stimulation from the operation on the contralateral bone. The results obtained by Tonna & Cronkite (1961) appear to contradict the second possibility. On study of the cell proliferation in the periosteum after experimental fracture on the rat tibia, they found no stimulation of the cell division on the contralateral bone.

The high activity value on the control side meant that no activity difference occurred at an observation time of 3 days despite the fact that the activity value on the treated side was then maximal. At 3 days the absolute growth on the treated side (MGT) and the absolute growth difference (MGDT) showed their highest values. But at that time point the increase in MGDT was not significant since it referred to a relatively small number of sectors which showed a very high degree of growth. The standardized growth difference (MSGDT) was almost significantly increased, however, at an observation time of 3 days. This indicates that a difference existed between the growth on the treated side and on the control side at this time point even though this was not expressed in an activity difference or an absolute growth difference.

Compared with the animals observed at 3 days those observed at 2 weeks showed a lower activity value (MAT) and a lower absolute growth value (MGT) on the treated side. The absolute growth difference (MGDT) was also smaller at 2 weeks, while the standardized growth difference (MSGDT) was not significantly changed. The activity difference (MADT), the standardized growth difference (MSGDT) and the absolute growth difference (MGDT) showed almost significantly positive values, however, at an observation time of 2 weeks. The high activity difference (MADT) which was found at that time point was due to a large reduction of the activity value (MAT) on the control side, and the significant activity difference occurred despite the fact that the activity value on the treated side was lower then than that at 3 days. Also for the occurrence of the significant positive absolute growth difference (MGDT) at an observation time of 2 weeks a significant reduction of the absolute growth (MGT) on the control side played a decisive role. Even though the absolute growth on the treated side at an observation time of 2 weeks was thus only slightly greater (4.8 U)

than the absolute growth on the control side at 3 days (3.8 U), a significant absolute growth difference was found at 2 weeks.

The study of sectors undergoing resorption (MRT) showed that a significantly increased number of sectors were undergoing resorption at an observation time of 2 weeks, both on the treated and control side. The resorption was so much greater on the control side that an almost significant negative resorption difference (MRDT) was found at 2 weeks. The analysis thus showed that the bone formation activity both on the treated and on the control side at an observation time of 2 weeks was distinctly lower than at 3 days. In growing animals the periosteum on the treated side thus seems to be activated to bone formation most strongly on the days immediately following reaming of the medullary cavity — no distinct maximum was recorded. The difference between the treated and the control sides was greatest at an observation time of 2 weeks. This rapid increase in the periosteal bone formation following medullary trauma corresponds well with the results of Tonna & Cronkite (1961). They studied the DNA synthesis in the osteogenic layer of the tubular bone after fracture and found that away from the fracture this began to increase 16 hours after the fracture and reached a maximum at 32 hours. In the present material a later phase in the bone formation was studied than in the material reported by Tonna & Cronkite.

At an observation time of 4 weeks a partial elimination of the activity difference, the standardized growth difference, the absolute growth difference and the resorption difference took place in the growing animals. At an observation time of 8 weeks a tendency was observed to more active bone formation on the control side than on the treated side.

Adult Animals

As in the growing animals, the control side in the adult rabbits showed its maximal activity value (MAT) at an observation time of 3 days. In spite of this relatively high activity value, only a very small absolute growth value (MGT) was found. The activity value on the control side then decreased and reached a minimum at 2–4 weeks. The activity value (MAT) and absolute growth value (MGT) on the treated side, the activity difference (MADT), the standardized growth difference (MSGDT) and the absolute growth difference (MGDT) all increased to a maximum at an observation time of about 2 weeks. As in the growing animals, there seemed to be a partial elimination of the differences between the treated and control sides at 4 weeks. No compensatory phase with greater bone formation activity on the control side than on the treated side was observed in the adult animals.

Conclusion

In growing animals the bone formation activity on the treated side seemed to be greatest on the days immediately after reaming of the medullary cavity. This activity then decreased on the treated side so that at 2 weeks it was on the same level as that on the control side at 3 days. Since, however, the reduction of the bone formation activity was at the same time relatively greater on the control side, there was still a significant difference between the treated and control sides at 2 weeks. At an observation time of 4 weeks the bone formation difference was almost eliminated and at 8 weeks there was a tendency to a negative difference.

In adult animals there was somewhat greater bone formation activity on the treated side than on the control side at an observation time of 3 days. This difference was maximal at about 2 weeks and was partly eliminated at 4 weeks.

STUDY OF THE HISTOLOGICAL SECTIONS

The histological picture in periosteal bone formation, the reconstruction of the cortex and the reformation of the structure of the medullary cavity after disturbances of the endosteal circulation have been described in detail previously in histological studies by Axhausen & Bergmann (1937), Ham & Harris (1956) and Brånemark (1964), among others. In the present study the interest was therefore focused on the extent of the cortical necrosis. The appearance and stainability of the osteocytic nuclei were thus correlated to the Indian ink filling of the intracortical vessels in the area.

Method

The histological sections were stained with haematoxylin and eosin. The Indian ink filling of the intracortical vessels was assessed both on the histological and the angiographic sections prepared from the same paraffin-embedded material.

Results

The osteocytic nuclei could best be studied on the histological cross-sections. From each main experimental group at observation times of 3 days up to and including 4 weeks, sections were obtained from at least one animal in which the nuclear staining was satisfactory. The examination was therefore concentrated on these sections. In sections from several other animals, however, there was no nuclear staining at all, either in the cortex or in the surrounding soft tissues.

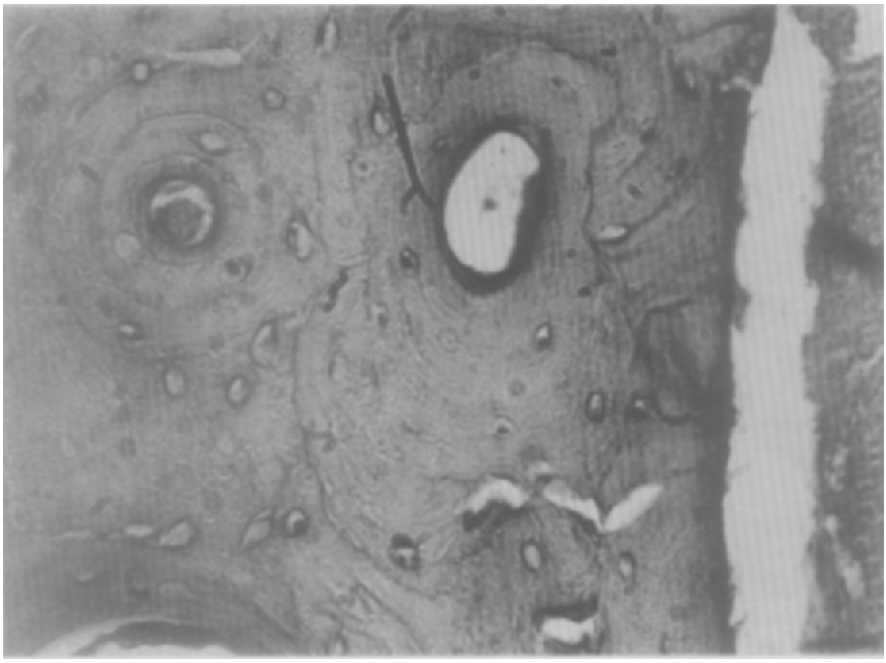


Fig. 38. Histological cross section of dorsal part of cortex, observation time 2 weeks. Growing animal 45. Stained osteocytic nuclei are present in the inner avascular part of the cortex.

Shrinkage of the osteocytic nucleus was seen in bone tissue also on the control side and could not therefore be used as a sign of bone necrosis.

In sections from animals observed at 3 days, small scattered areas were observed in the inner and middle part of the cortex where the osteocytic lacunae lacked stainable material, while in other parts of the cortex and in the surrounding soft tissues the nuclear staining was good. No difference between the treated and the control side was observed, however.

Sections from animals observed at 1 week showed similar pictures to those from animals observed at 3 days.

In sections from animals observed at 2 weeks, scattered areas, larger than on the control side, were seen in the middle and inner parts of the cortex with practically no stainable osteocytic nuclei, on the treated side. These areas sometimes comprised more than one-quarter of the surface area of the section. In sections from one animal (45), the vessels in the middle and inner parts of the cortex showed no filling with Indian ink. In these areas, however, several stained osteocytic nuclei were seen (Fig. 38). In a few bone fragments, which lay freely in the medullary cavity, which completely lacked Indian ink-filled vessels, stained nuclei were observed in several osteocytic

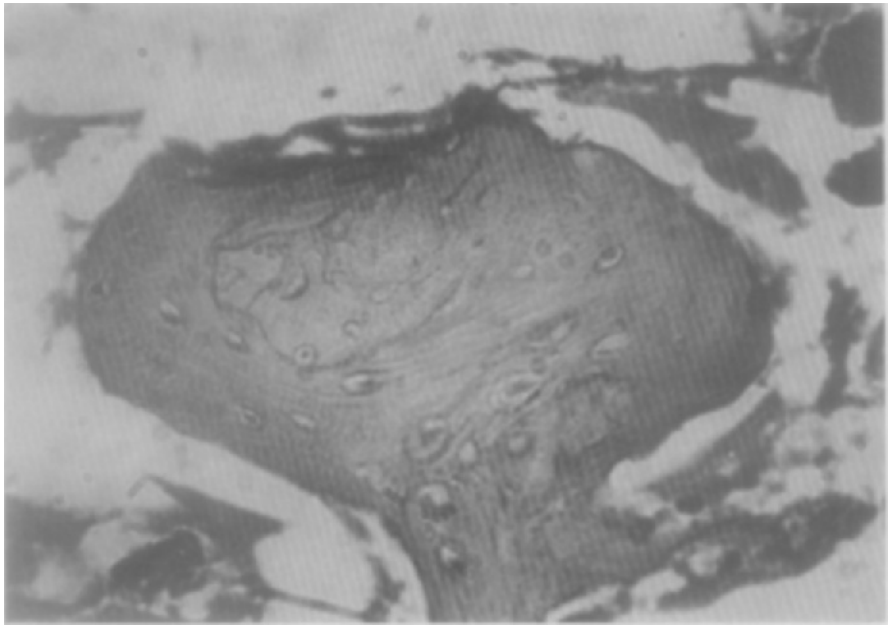


Fig. 39. Hematoxylin-eosin stained histological section, observation time 2 weeks. Growing animal 45. A fragment of cortical bone is lying freely in the medullary cavity. In the surrounding area there are no newly formed blood vessels. Stained nuclear material is seen in several of the osteocytic lacunae.

lacunae (Fig. 39). On fluorescence microscopy of the adjacent sections it was found that the bone fragments in the medullary cavity did not fluoresce.

In sections from the treated side of animals observed at 4 weeks, scattered areas larger than those observed at 2 weeks were sometimes found in the middle and inner parts of the cortex, where most of the osteocytic lacunae lacked stainable nuclei. Corresponding angiography showed that some of the vessels here were filled with Indian ink. In the cortex no sharp boundary was seen between the areas without and those with nuclear staining. Nearest to the medullary cavity, nucleus-bearing osteocytic lacunae were observed, where no Indian ink-filled vessels were seen.

Discussion

Necrosis occurs in cortical bone a short time after the circulation to the cortex has been arrested (see literature reference, p. 21). The first histological sign of bone necrosis is shrinking of the osteocytic nucleus. The necrotic nucleus becomes fragmented and nuclear material is carried away when the

fluid circulation to the area has been restored (Axhausen & Bergmann, 1937). In certain cases this can take place only a few days after the occurrence of the necrosis, but in other cases it can take a very long time. The shrinking of the osteocytic nucleus in necrosis has been utilized by many investigators in the diagnosis of bone necrosis. In the present material, however, the preparatory procedure gave rise, as a rule, to shrinkage of the osteocytic nucleus even in bone tissue which was not necrotic. It was therefore not possible to determine the extent of the bone necrosis from the shrinkage of the osteocytic nuclei. Instead, an attempt at such determination was made by observing whether or not the osteocytic lacuna contained stainable nuclear material. The absence of such material is usually a definite sign that the bone is necrotic (Axhausen & Bergmann, 1937), but falsely empty osteocytic lacunae can occur in that the nucleus does not occupy the whole lacuna and that the section cuts through the lacuna without encountering the nucleus.

In the specimens from this series of experiments there was no sharp borderline between nucleus-bearing and non-nucleus-bearing regions of the cortex. The latter regions visible at 2 weeks and increasing with observation time were observed especially on the treated side, and there within the inner and middle part of the cortex. Often, however, stainable nuclear material remained in the cortex even just adjacent to the medullary cavity at least up to 4 weeks after the reaming, in the area where angiography showed that no vessels were filled with Indian ink.

In loose bone fragments in the medullary cavity, stainable nuclear material was observed 2 weeks after the reaming of the medullary cavity. Corresponding fluorescence specimens showed that the bone fragments in the medullary cavity were not fluorescent, which, provided that no vessels were seen in the medullary cavity, can be regarded as a sign that no fluid diffusion to the area had taken place two days before angiography.

Thus, stainability of osteocytic nuclei by no means constitutes a guarantee of the absence of necrosis even 4 weeks after operation.

In the present material the extent of the bone necrosis could thus not be determined by the histological method used within 4 weeks after the operation. This seems to be due to the fact that all necrotic nuclear material has not then been transported away from the osteocytic lacunae.

It would seem, therefore, that the extent of the cortical damage after reaming of the medullary cavity can better be evaluated from the angiographic findings in specimens with a short observation time than from a study of the histological picture.

CHAPTER 4

Marrow Embolism as a Cause of Intracortical Circulation Block after Surgery of the Medullary Cavity

On reaming of the medullary cavity the intracortical circulation in the diaphysis is disturbed. This has been demonstrated by Indian ink angiography and is discussed on page 74. The blood vessels do not fill with Indian ink within large areas, the extent of which varies greatly between different animals and between different sectors in the same animal. The reason for the deficient filling with Indian ink would therefore seem to be not only the elimination of the medullary circulation, but also a simultaneous direct effect on the intracortical blood vessels. One possible cause of the intracortical circulatory disturbance is that the Haversian canals are occluded by medullary cavity contents which are squeezed into the canals when a surgical operation is performed in the medullary cavity.

In man, fat emboli in the lungs often occur after fracture of the long tubular bones (for literature, see Bergentz, 1961). Kjerstell (1969) has shown that pulmonary fat emboli occurring in experimental femoral fractures in the dog originate in the traumatized fat tissue, especially in the medullary cavity. Stained fat which is injected into the medullary cavity of a tubular bone gives rise to multiple emboli in the lungs when the bone is fractured (Glas *et al.,* 1956), but also without fracture if the contents of the medullary cavity are mobilized by the introduction into it of a steel wire (Busch, 1866). Bisgard & Baker (1940) claim that medullary fat enters the circulatory system for the reason that on such trauma the fat is released from the fat cell and becomes fluid, that it accumulates under pressure on bleeding in the medullary cavity and that the open veins through the cortex do not collapse.

Bone marrow can thus leave the medullary cavity on intramedullary trauma, but no investigations on the pathway of the marrow from the medullary cavity through the cortex, and its possible influence on the intracortical circulation appear to have been reported previously. A series of experiments was thus carried out with the aim of answering the following questions:

1. Do different degrees of medullary trauma give rise to different degrees of influence on the intracortical circulation?

Table 8. *Observation time, number of animals and kind of treatment in the animal group used for study of fat emboli in the intracortical canals*

Observation time	No. of animals	Treated tibias		
		Suction	Reaming	Brushing
1 day	13	5	5	5
1 week	1		1	1
2 weeks	3		2	3
4 weeks	3		2	3

2. Are medullary emboli the cause of the disturbance of the intracortical circulation?

Material

The material comprised 27 growing and adult non-pregnant rabbits of both sexes. Five of these animals (Table 9), which also served as controls, were taken from the material presented in Table 1, namely 109, 114, 123, 124 and 125, all of which belonged to the group studied at an observation time of 3 days. The sizes of the other animal groups, the lengths of the observation times and the kind of treatment are given in Table 8. Two animals died during the operation.

Methods

The anaesthesia and operation were performed as described in chapter 2.

The medullary cavity of the tibia was evacuated by one of the following three methods, which gave rise to different pressure increases in the medullary cavity and, partly as a result of these pressure increases, was considered to be exposed to different degrees of medullary trauma (see p. 40): (1) suction, (2) reaming, (3) brushing.

Groups of animals were studied after observation times of 1 day and 1, 2 and 4 weeks (Table 8). The circulatory system was filled with Indian ink, as described in chapter 2. In a deep-frozen state the tibia was divided 2.5 and 7.5 cm from the tibiotalar joint. From each end of the middle part, which was decalcified, $^1/_2$ cm was sawn off and used for Spalteholz preparations from which a drawing was made with the vascular front outlined, according the method described previously (see p. 29). On these drawings the percentage proportion of the cortical cross-section with no Indian ink filling of the vascular system was calculated and named avascular cortex. From the intermediate part of the diaphysis, 10–15 μ thick frozen sections were prepared. The sections were stained with Sudan 3 and haematoxylin and then mounted under a cover glass with Permount. Since the contents of the medullary cavity in the tibial diaphysis in the rabbit consist to a large part of fat, fat staining with Sudan 3 was used to indicate the presence of bone marrow.

From the five animals from the material presented in Table 1, bone material for frozen-sectioning was taken from the distal part of the diaphysis.

Table 9. *Mean number of marrow emboli per section from control and reamed tibias and the percentage of avascular cortex in the 5 animals from Table 1*

Animal no.	Fat emboli per section		Percentage avascular cortex
	Control	Reamed	
109	0.3	2.0	19
114	0.3	3.7	26
123	0.7	20.0	61
124	0.0	2.3	9
125	0.7	8.0	31
Mean	0.5	7.2	

Results

Both tibiae from the five animals from Table 1 were studied, three Sudan-stained histological sections being studied from each tibia. In each section from the untreated control tibia an average of 0.5 fat emboli were found in intracortical vessels. The range of variation was 0–1 fat droplet per section. In sections from the reamed tibia an average of 7.2 fat droplets per section were found. The results are presented in Table 9. The table shows that the larger the amount of avascular cortex on the treated side, the greater the number of fat droplets in the intracortical canals on that side.

Result of Differentiated Medullary Trauma

The percentage of avascular cortex 1 day after operation can be seen in Table 10. The table shows that reaming of the medullary cavity gave somewhat greater intracortical vascular damage than suction of the contents of the medullary cavity, both in the distal and the proximal part of the diaphysis. The difference was not significant. Brushing out of the contents of the medullary cavity gave somewhat greater vascular damage than reaming or suction of the contents. The difference in this respect in the distal sections was significant at the 10 per cent level. A study of the Sudan-stained sections showed that Sudan-stained material was present in many Haversian canals in the areas of the cortex that lacked Indian ink-filled vessels. (Color pl. 3 *b, c*). As a rule, the Sudan-stained material obliterated the entire Haversian canal, which then did not fill with Indian ink. In some cases only small fat droplets were found in the canals, and Indian ink-filled blood vessels passed these droplets. This was rather unusual, however, and seemed to occur only in the outer part of the cortex. In these cases there were possibly two blood vessels in the same Haversian canal, of which one was obliterated by fat. Haversian canals which were obliterated by fat were observed in

Table 10. *Mean weights, mean values and ranges of variation of avascular cortex for the tibias of each group of 5 rabbits, 1 day after suction, reaming or brushing of the medullary cavity*

P indicates the proximal section taken about 5.7 cm above the tibiotalar joint, and *D* the distal section from about 2.7 cm above the same joint.

		Suction	Reaming	Brushing
Percentage avascular cortex	P	44 (8–61)	56 (23–77)	64 (46–85)
	D	23 (6–34)	37 (14–54)	81 (64–93)
Mean weight kg.		3.5 (3.3–3.9)	3.4 (3.1–3.9)	3.3 (3.1–3.7)

greatest abundance close to the medullary cavity. In animals in which angiography preparations showed avascularity of the outer parts of the cortex, Sudan-stained material in Haversian canals was also observed, however, in the outer part of the cortex. In some preparations, especially from the fibular part of the proximal area of the diaphysis, a layer of marrow up to some mm thick was seen under the periosteum. (Color pl. 3 *d*.)

In preparations from animals studied at observation times of 1 and 2 weeks, the same pattern of fat in the Haversian canals was found as at an observation time of 1 day. At an observation time of 4 weeks some of the canals which contained Indian ink-filled vessels were wider than those observed at 2 weeks. In some cases (Color pl. 3 *f*) it was found that a Haversian canal had increased in calibre but that fat was still present in the canal at the side of an Indian ink-filled blood vessel. Color pl. 3 *e* shows how a cutter head is revascularizing an osteone whose canal in front of the cutter head is obliterated by fat.

Two of the animals died during brushing out of the medullary contents. They showed cyanosis, tachypnoea and tachycardia followed by bradycardia and died despite artifical respiration. At autopsy fat emboli were observed in Haversian canals of the treated tibia, in many pulmonary vessels and in one renal vessel.

Discussion

The normal occurrence of fat in cortical bone was studied by Conklin *et al.* (1956). They found only small quantities of lipids in osteocytes, osteocyte lacunae, canaliculi and in organic matrix, and no fat droplets in the Haversian canals. Jones *et al.* (1965) found in two patients with idiopathic avascular necrosis of the femoral head multiple fat droplets in the subchondral arterioles and in the capillaries in the necrotic bone. Karlström *et al.* (1939) considered that one cause of avascular bone necrosis might be fat emboli in the intracortical blood vessels.

This series of experiments showed that when the contents of the medullary cavity were removed by different methods, which gave rise to different degrees of medullary trauma, varying degrees of vascular damage were obtained in the diaphyseal cortex lying outside. Suction of the contents of the medullary cavity gave the least vascular damage, while brushing out of the contents, which caused the largest increase in the intramedullary pressure, gave the greatest vascular damage. In those areas of the cortex in which the blood vessels did not fill with Indian ink at angiography, an abundance of Sudan-stained droplets was often found in the Haversian canals. Sudan staining demonstrates the presence of fat and was used to indicate medullary tissue. In the areas in which almost every blood vessel filled with Indian ink, no or only a very small number of fat droplets were observed in the Haversian canals. In no section from control tibias, which were not operated on, was more than one Haversian canal obliterated by fat. The fat in the control tibias may have constituted emboli from the other, treated tibia. On the histological sections it could not be determined whether the fat lay inside or outside the blood vessels. In preparations studied at an observation time of 4 weeks it was found that fat was still present in the Haversian canals and that it offered resistance to revascularization of the bone. Jones & Sakovich (1966) injected Lipiodol intravenously in the rabbit and observed Lipiodol in intracortical vessels during an observation time of 5 weeks. Cohen & Harris (1958) reported that the canals in cortical bone were wider nearer to the medullary cavity than peripherally under the periosteum. This could contribute to the fact that fat droplets on their way from the medullary cavity were stopped in the Haversian canals.

The rather high mean percentage of avascular cortex in the suctioned group may depend on the fact that high pressure was produced in the medullary cavity in some animals by the awl.

Conclusion

Intracortical emboli of the bone marrow are thus an important cause of intracortical vascular damage after medullary trauma. The contents of the medullary cavity can be squeezed out subperiosteally where they can be observed macroscopically as a haemorrhage.

CHAPTER 5

Periosteal New Bone Formation Correlated to Endosteal Bone Removed, Cortical Vascular Damage and Subperiosteally Squeezed out Bone Marrow

There would seem to be several causative factors in periosteal new bone formation. Phemister (1930), Axhausen & Bergmann (1937) and Trueta (1963) consider that vascularly disturbed cortical bone causes local stimulation of periosteal osteogenic cells to new bone formation. Frost (1963) and Schenk (1967) claim that mechanical factors contribute to stimulation of the periosteum. Zucman *et al.* (1968) have shown that bone marrow which after an operation in the medullary cavity has become localized subperiosteally has a strong stimulatory effect on bone formation. The high osteogenetic potency of bone marrow has been demonstrated previously by several investigators, including Urist & McLean (1952) and Burwell (1964), especially in connection with transplantation experiments.

The experimental methods used in the present study give prerequisites for new bone formation according to all of the above possibilities. In this chapter studies are described on the relationship between avascular bone or bone removed on reaming, and the amount of new bone formed subperiosteally. The effect of subperiosteally localized bone marrow must, however, be evaluated indirectly.

For the studies of this relationship statistical methods of analysis were used.

Material

Twenty-four albino male and non-pregnant female growing rabbits were used. The ages of the animals were stated by the breeder to be between 14 and 18 weeks. They were divided into two groups, 1 and 2. Three animals were excluded from group 1 owing to fracture of the treated tibia, and 4 animals were excluded from group 2 because of death in connection with the operation. After these exclusions group 1 comprised 7 animals and group 2, 10 animals. The mean weight of group 1 was 2340 g with a range of variation of 1600–2850 g, and of group 2, 2470 g, range 1700–2650 g.

Methods

The anaesthesia and operation were performed as described in chapter 2. In group 1 the medullary cavity of the left tibia was evacuated by reaming and that of the right tibia by suction. In group 2 the medullary cavity of the left tibia was evacuated by brushing and that of the right tibia by suction. For both groups of animals the left tibia was called the treated tibia and the right the control tibia.

Bone Labelling

The animals were given approximately 50 mg tetracycline/kg body weight intraperitoneally on the day of operation and 5 days postoperatively.

Angiography

Angiography was performed as described in chapter 2, 7 days after the operation.

Preparation of the Sections for Analysis

After fixation in formalin the tibia was divided in the deep-frozen state 2.5 cm above the tibiotalar joint. From the distal part of the proximal fragment, three consecutive 1.2 cm long preparations were sawn. These were named, from the distal end, preparations *a*, *b* and *c*. Preparations *a* and *c* were embedded in methyl metacrylate and preparation *b* was decalcified and embedded in paraffin. From the distal and proximal parts of preparation *a* and from the distal part of preparation *c*, $^1/_2$ mm thick slices were sawn and used for the preparation of ground sections for fluorescence microscopy. Further $^1/_2$ mm thick slices were then sawn from the same areas and used for Spalteholz preparations after removal of the methyl metacrylate with chloroform and decalcification of the specimen. (See description of methods on p. 29). In this way three pairs of preparations were obtained. Each pair comprised one fluorescence preparation and one Spalteholz preparation, taken from immediately adjacent bone. The pairs of preparations were named, from the distal end, section 1 (SN1), section 2 (SN2) and section 3 (SN3). All three sections together were called SNT. SN1 was thus obtained from the distal part of preparation *a*, SN2 from the proximal part of preparation *a* and SN3 from the distal part of preparation *c*. The distance between SN1 and SN2 was about 11 mm, and between SN2 and SN3 about 14 mm. From each section an image drawing was made in the way described on p. 28. Each image drawing was divided into four sectors, and each sector into the areas AB, CD, CE and DE, after which the sizes of these areas were calculated. Area AB in sector 1 was called AB1, in sector 2 AB 2, and so on.

Corresponding designations were used to denote the localization of the areas CD, CE and DE.

Analytical Methods

Between the values obtained for corresponding AB, CD, CE and DE areas on the treated and control sides, the difference, the quotient and the difference between the logarithms were calculated. Analysis showed that difference calculation gave better correlation between the different sections than calculation of the quotient or of the difference between the logarithms. Difference calculation was therefore used throughout. The differences between the areas were denoted ABd, CDd, CEd and DEd. The values obtained were expressed in mm^2.

By, for example, the abbreviation CDd2SN3 is thus meant the difference between the sizes of areas CD on the treated and control sides in sector 2 in section 3.

Calculations of the mean values of ABd, CDd, CEd and DEd were performed separately for the groups of animals treated by reaming (group 1) and by brushing (group 2) (Table 11). For groups 1 and 2 together, each ABd value was correlated with the ABd values of all other sectors and certain formed sums of sectors (Table 12). By simple correlation analysis (Table 13) and by multiple regression analysis (Table 14) the ABd values were correlated with the corresponding values for CDd and DEd.

Results

General Reaction of the Animals

In no case did wound infection or wound rupture occur. The mean change in weight during the experimental period of 1 week was −52 g in group 1, with a range of variation of +8 to −197 g, and −34 g in group 2,with a range of variation of +310 to −300 g.

The animals bore weight normally on both legs a few days after the operation.

Mean Value Calculation of ABd, CDd, DEd and DEd (Table 11)

Calculation of the mean value of each sector in the three sections was performed separately for the "reamed" and "brushed" groups of animals. The mean ABd value was positive in all sectors in both the reamed and brushed groups and reached the almost significant level in sectors 1 and 2 in the reamed and in sector 2 in the brushed animals. The mean ABd value was somewhat higher throughout in the brushed animals than in the reamed, but this difference did not reach the level of significance.

The mean CDd value was highly significantly raised in all sectors in the brushed animals. In the reamed animals the mean CDd value was highly significantly raised in sectors 1 and 3 and almost significantly raised in sectors 2 and 4.

On comparing the mean CDd values for the reamed and brushed animals it was found that these were higher throughout in the latter. The difference was almost significant in sectors 2 and 4 but not significant in the other sectors.

The mean CEd value was highly significantly increased in all sectors in both the reamed and brushed animals. In sectors 2 and 4 in which most bone had been removed on reaming the mean CEd value was somewhat higher in the reamed than in the brushed animals, but in the other sectors somewhat lower. The difference between the reamed and brushed groups did not reach the level of significance.

Table 11. *Mean values ($\bar{X}$ in square mm) and standard errors* (S.E.) *for ABd, CDd, CEd and DEd in sectors 1 to 4 for all sections in the reamed and brushed groups and the difference values between the groups*

The degree of significance is denoted by asterisks.

Sector	Reamed		Brushed		
	$\bar{X}_R$	±S.E.	$\bar{X}_B$	±S.E.	$\bar{X}_R - \bar{X}_B$
ABd1 SNT	0.12*	0.03	0.39	0.28	−0.17
ABd2 SNT	0.26*	0.09	0.27*	0.11	−0.01
ABd3 SNT	0.24	0.28	0.30	0.18	−0.06
ABd4 SNT	0.19	0.20	0.20	0.11	−0.01
CDd1 SNT	1.53***	0.26	2.16***	0.36	−0.63
CDd2 SNT	0.61*	0.20	1.51***	0.23	−0.90*
CDd3 SNT	1.10***	0.13	1.65***	0.35	−0.55
CDd4 SNT	0.62*	0.21	1.39***	0.20	−0.76*
CEd1 SNT	1.88***	0.21	2.16***	0.36	−0.29
CEd2 SNT	1.63***	0.20	1.51***	0.23	+0.12
CEd3 SNT	1.26***	0.19	1.65***	0.35	−0.39
CEd4 SNT	1.55***	0.21	1.40***	0.20	+0.16
DEd1 SNT	0.35**	0.06			
DEd2 SNT	1.02***	0.10			
DEd3 SNT	0.16	0.07			
DEd4 SNT	0.93***	0.06			

The mean DEd value calculated in the reamed group of animals was highly significantly increased in sectors 2 and 4 and significantly increased in sector 1.

Calculation of Correlation between ABd Values in Individual Sectors and Certain Sums of Sectors (Table 12)

The three sections in sector 1 were all highly significantly correlated to each other. In sector 2, section 3 differed greatly from the other two sections. In sector 3, section 2 differed greatly from section 3.

Sector 1 was the most homogeneous sector. The three sections in this sector were therefore suitable for use as reference sections. All three sections in sector 1 showed a weak correlation not only with ABd2SN3, which was very weakly correlated to all other sectors and sums of sectors, but also with ABd3SN2 and ABd4SN2. ABd3SN2 and ABd4SN2 were highly significantly correlated with each other.

Almost all other sectors and sums of sectors were highly significantly or almost significantly correlated with each other.

Table 12. *The correlation coefficient (r) between the ABd values in individual sectors and certain sums of sectors*

The following significance limits, taken from *Documenta Geigy*, 6th edn, p. 61, were used with 15 degrees of freedom: $|r| \leqq 0.48$, not significant; $0.48 < |r| < 0.61$, almost significant; $0.61 < |r| < 0.73$, significant; $|r| \geqq 0.73$, highly significant. These limits held stringently under certain idealized conditions.

Sectors	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ABd1 SN1	1																		
ABd1 SN2	2	0.95																	
ABd1 SN3	3	0.85	0.94																
ABd1 SNT	4	0.94	0.99	0.98															
ABd2 SN1	5	0.85	0.82	0.72	0.80														
ABd2 SN2	6	0.68	0.63	0.53	0.61	0.92													
ABd2 SN3	7	−0.22	−0.07	0.12	−0.01	−0.19	−0.16												
ABd2 SNT	8	0.50	0.56	0.61	0.59	0.68	0.72	0.56											
ABd3 SN1	9	0.80	0.75	0.61	0.72	0.93	0.86	−0.13	0.67										
ABd3 SN2	10	0.38	0.27	0.11	0.22	0.65	0.74	−0.07	0.55	0.80									
ABd3 SN3	11	0.91	0.92	0.84	0.90	0.72	0.54	0.02	0.54	0.72	0.38								
ABd3 SNT	12	0.82	0.74	0.60	0.71	0.83	0.75	−0.02	0.65	0.92	0.79	0.85							
ABd4 SN1	13	0.74	0.67	0.53	0.64	0.89	0.85	−0.13	0.65	0.99	0.86	0.67	0.91						
ABd4 SN2	14	0.49	0.36	0.29	0.37	0.57	0.68	0.00	0.54	0.68	0.79	0.52	0.76	0.67					
ABd4 SN3	15	0.83	0.80	0.74	0.80	0.60	0.42	0.18	0.56	0.63	0.37	0.96	0.84	0.59	0.51				
ABd4 SNT	16	0.72	0.64	0.49	0.61	0.79	0.75	0.03	0.67	0.91	0.87	0.77	0.99	0.92	0.79	0.77			
ΣABdSn1 + 2 + 3	17	0.89	0.87	0.79	0.86	0.89	0.79	0.09	0.77	0.91	0.66	0.91	0.95	0.88	0.68	0.87	0.92		
ΣABdSN3	18	0.81	0.88	0.90	0.90	0.65	0.47	0.34	0.71	0.62	0.25	0.93	0.74	0.56	0.43	0.93	0.67	0.87	
ΣABdSN1 + 2	19	0.80	0.72	0.57	0.68	0.92	0.90	−0.12	0.69	0.98	0.85	0.74	0.96	0.98	0.76	0.67	0.95	0.93	0.62

Table 13. *The correlation coefficient (r) between the dependent variable ABd and the corresponding independent variables CDd and DEd for all individual sections and sectors in the 17 animals*

For the evaluation of significance, see Table 12.

	Correlation	
	ABd/CDd	ABd/DEd
SN1	0.09	0.08
SN2	0.16	0.29
SN3	0.63*	0.45
Sector 1	0.42	−0.16
2	0.39	−0.07
3	0.27	−0.24
4	0.05	−0.15

Correlation between Dependent Variable ABd and Corresponding Independent Variables CDd and DEd (Table 13)

A correlation analysis was performed both for the sum of all sectors in each individual section and for the sum of each individual sector with the same localization in all three sections.

The correlation between the ABd values and the CDd values were positive throughout. An almost significant value was attained in section 3. This could be explained by the fact that one animal differed greatly from the rest, and the significance was therefore of no explanatory value.

The correlations between the ABd and DEd values were positive throughout for individual sections, but negative throughout for individual sectors. The values did not reach the level of significance.

Regression Analysis with the Size of Differently Localized ABd Areas as Dependent Variable and the Size of Similarly Localized CDd and DEd Areas and also the Type of Operation as Independent Variables (Table 14)

The total explanatory value expressed as R^2 was low throughout for the variables studied, the maximal value being 0.47.

On regression analysis an almost significant negative relationship was found between ABd and DEd in section 1 and in sector 4. On closer analysis of these relationships it was found, however, that they could be explained by the fact that the results from one animal differed greatly from the others. The almost significant correlation was therefore of no explanatory value.

Table 14. *Regressors with coefficients significantly different from 0 and coefficients of determination (R^2) in an analysis with the sizes of ABd areas of different localizations as dependent variable and similarly localized CDd and DEd areas and also the type of operation as independent variables*

Reamed = 1; Brushed = 0; $n = 17$.

Dependent variable	Regressors with significance according to multiple regression analysis	R^2
ΣABd *in:*		
SN1	DEd (*neg)	0.43
SN2	None	0.30
SN3	None	0.45
SN1 + 2	None	0.23
SN1 + 2 + 3	None	0.19
Sector 1	None	0.20
2	None	0.23
3	None	0.18
4	DEd (*neg) Reamed (*pos)	0.47

An almost significant positive correlation was found between ABd and the type of operation in sector 4. This correlation was found at DEd = 0, but not at those values for DEd which were obtained for the reamed animals. This almost significant correlation therefore lacked conclusive value.

Discussion

When the bone marrow in the rabbit tibia was removed by reaming or by brushing, greater intracortical vascular damage and somewhat greater periosteal new bone formation occurred than when the contents were removed by suction. With all three types of operation the medullary circulation was completely destroyed in the diaphysis of the bone. It would seem, therefore, that factors other than the loss of the medullary circulation should constitute the reason for the fact that somewhat more bone was formed subperiosteally on reaming or brushing than on suction.

In the studies described in chapter 4 it was shown that the intracortical vascular damage was due essentially to the fact that the intracortical blood vessels were obliterated by bone marrow which had been forced in as a result of the increase in pressure in the medullary cavity during the operation.

Correlation analysis and multiple regression analysis of the relationships between the ABd values and the corresponding CDd and DEd values showed that the amount of avascular bone in, and the amount of bone removed on reaming from the underlying cortex had no apparent explana-

tory value for the increase in the ABd area. This could be due either to the fact that this increase was not influenced by changes in the size of the CDd and DEd areas, or to the fact that an existing relationship was obscured by other, more dominating factors. It should also be emphasized that a true relationship might be difficult to demonstrate in view of the relatively small size of the material.

On analysis of the correlation between the ABd values in individual sectors, it was found that the increase in the ABd values in certain sectors differed considerably from that in the other sectors. This was true in particular for sector 2 (dorsal) in section 3, and to a smaller degree for sector 3 (fibular) and sector 4 (ventral) in section 2. It seems probable that the increase in the ABd values in these three sectors was highly influenced by other factors than those which produced an effect in the other sectors. The difference was most clearly evident when the three sectors in question were compared with the sections in sector 1, which were highly correlated with one another. The deviating reactionary pattern in these sections was found to be due to anatomical conditions. On dissection of this area it was found that the dorsal sector of section 3 was taken from the area immediately distal and dorsal to the outer opening of the primary nutrient foramen in the bone, above the fibulo–tibial synostosis. A fascia is attached ventral to the foramen and this should mean that marrow which was forced out through the foramen became localized dorsally on the tibia in the area from which the dorsal sector of section 3 was taken. The fibular and ventral sectors of section 2 were taken from the area immediately distal and ventro-distal to the outer openings of the secondary nutrient foramen and emissary foramen. A fascia shields these openings here from the dorsal sector and this should mean that marrow forced out through these canals would have become localized fibularly and ventrally and may possibly have influenced the increase in the ABd values in these two sectors. The high correlation between these two latter sectors supports the assumption that they were influenced by the same dominating factors. The weak correlation between the dorsal sector in section 3, on the one hand, and the fibular and ventral sectors in section 2, on the other, supports the view that the ABd increase in these two groups of sectors was influenced by different dominating factors, probably by bone marrow which was forced out through *different* canals in the bone.

The analysis of the correlation between the three ABd values discussed above and the remaining ABd values thus supports the assumption that the increase in these three ABd values was influenced by forced-out bone marrow. The results of the morphological studies reported in chapter 3 also support this view.

In this material it could neither be demonstrated nor precluded that forced-out bone marrow was of importance for the increase in the ABd area in the other sectors.

On comparison between animals treated by reaming and by brushing, it was found that in the most reamed sectors 2 and 4 the "reamed" animals showed an almost significantly lower difference between the size of the CD areas on the treated and control sides than the "brushed" animals. In the other sectors no such difference was found.

This indicates that the reaming in itself gives no essential increase of the intracortical avascularity compared with operations which give the same degree of trauma in the medullary cavity.

CHAPTER 6

The Effect of Reaming and Brushing of the Medullary Cavity on the Diaphysis of the Dog Femur

In chapter 3 a study was described of the effect of reaming of the medullary cavity and of brushing out of the bone marrow in the tibial diaphysis in the rabbit. In order to determine whether there were any differences in this respect between the rabbit and the dog, corresponding investigations were also performed on the dog. The femur was used for these experiments.

Material

For the experiments 11 adult mongrel dogs were used (Table 15). The femur was chosen as the experimental bone since the tibia was used in these animals for studies of the healing pattern in compression osteosynthesis.

Methods

The dogs were anaesthetized with Nembutal® (Abbott, for veterinary use), given intravenously until the cough reflex was eliminated. They were then intubated and ventilated with a mixture of oxygen and nitrous oxide.

After shaving the skin and washing with spirit, the trochanter major was exposed through a lateral incision. The medullary cavity was opened with an awl medially to the trochanter major and was then widened 3–5 mm with hand-driven reamers of the Küntscher model (see Küntscher, 1962). The reaming was performed down to the distal metaphysis of the femur.

In two dogs the contents of the medullary cavity were removed with a bottle-brush about 2 cm thick, which was moved up and down in the cavity three times.

In order to label the newly formed bone Terramycin® (Pfizer) was injected intraperitoneally, in a dose of 50 mg/kg body weight. The time points of the injections in relation to the operation can be seen in Table 15.

At the end of the experiment the animal was again anaesthetized and was given an intravenous injection of 500 U Heparin (Vitrum) per kg body weight. A catheter with an inner diameter of 1.5 mm was inserted in the external carotid artery in the direction towards the heart. Through this catheter a suspension consisting of 200 ml Pelikan Indian ink in 800 ml isotonic NaCl was infused under pressure with an ordinary Record syringe. After infusion of 100–200 ml the animal usually died. The infusion was then continued for a further few hours at a slow rate until 5 litres of the Indian ink mixture had been given. During the infusion the dog was kept in an

Table 15. *Dogs used for morphological studies. The observation times and the times of labelling after the operation are presented*

L indicates the left femur; *R*, the right femur.

Animal no.	Observ. time	Terramycin labelling on days after the operation		
		label. 1	label. 2	label. 3
40	1 day			
44	2 days			
7*L*	7 days	5		
19	13 days	7	13	
14	14 days (2 weeks)	6	12	
7*R*	21 days (3 weeks)	7	19	
8	28 days (4 weeks)	7	26	
9	35 days (5 weeks)	7	33	
13	42 days (6 weeks)	9	39	
12	49 days (7 weeks)	10	47	
10	56 days (8 weeks)	7	21	40
11	70 days (10 weeks)	10	56	

upright position so that the grains of Indian ink would be deposited in as many blood vessel branches as possible in the freely dependent legs.

After the Indian ink infusion the femur was exarticulated at the hip joint and freed from soft tissues. After fixation for a few days in neutral 10% formalin the bone was frozen and in this state was divided, the middle 5 cm being taken for examination. From each end of this 5 cm, a 1 cm thick slice was sawn off and used for the preparation of histological sections and angiographic sections according to the Spalteholz method. A further 1 cm slice was then sawn off from each end of the specimen, and these parts were embedded in methyl metacrylate and used for the preparation of fluorescence sections. The remaining part of the middle specimen was used in some cases for the preparation of Sudan-stained frozen sections.

The Spalteholz angiography sections were studied in a stereomicroscope and the fluorescence sections in an ordinary microscope under ultraviolet illumination as described in chapter 2.

Results after Reaming of the Medullary Cavity

General Reaction

As a rule the animals began to stand carefully on the treated leg a few days after the operation and were able to bear full weight on the leg about 1 week postoperatively. In no case was there any clinically manifest infection.

Fluorescence Microscopic Studies

Observation time: 7 days

Labelling on day 5 after the operation.

Periosteum. Around the diaphysis of the femur, there was a narrow, flu-

orescent band of newly formed bone, of almost even thickness. Outside this band, non-fluorescent bone and osteoid was seen. The largest amount of unstained bone was found in the tibial part, where trabecular bone up to 250 μ high had been formed.

Cortex. In the middle part of the cortex numerous small resorption cavities which contained blood clots were seen. In some of these cavities, there were narrow, Indian ink-filled blood vessels. All resorption cavities lacked fluorescence labelling of the walls.

Medullary cavity. Newly formed bone trabeculae, the central parts of which were fluorescent, were found in the dorsal part of the medullary cavity adjacent to the endosteum. In other parts of the medullary cavity, where newly formed blood vessels filled with Indian ink had invaded the medullary cavity from the cortex, bone fragments were found which had been released from the cortex by the reaming. The surfaces of the bone fragments were rough and many showed a thin fluorescent layer on the surface.

Observation time: 13 days

Labelling on days 7 and 13.

This dog died during the intravenous infusion of alizarin, which in this experiment was used for labelling 2. Angiography was therefore not performed.

Periosteum. Active bone formation was observed around the entire diaphysis. The new bone consisted mainly of trabecular bone, but primary osteones were also found in some areas. From labelling 1, trabecular bone was labelled around a large part of the diaphysis, but in some areas primary osteones or circumferential lamellae were labelled. The peripheral parts of the trabecular bone showed diffuse alizarin labelling but in the inner parts there were ring-shaped labelled areas around the blood vessels. Adjacent to the surface of the original cortex several large cavities were found, some of which continued into the original cortex.

Cortex. In several areas adjacent to the medullary cavity resorption cavities were found, the rough surfaces of which showed a thin zone of alizarin labelling, which was considered to be a sign of resorption. In other parts of the cortex osteones were found with an outer even fluorescent yellow Terramycin ring and an inner, relatively thick, even red ring of alizarin. These formations were osteones which were becoming closed. Some cavities showed yellow fluorescent bone deposits in one part of the wall and red resorption fluorescent layers in other parts.

Medullary cavity. In the dorsal area broad bone trabeculae were found, communicating with the original cortex, and between these there were some

blood vessels with thick walls. The bone trabeculae, as well as the endosteal surface of the cortex in the same area, were doubly labelled, with an inner yellow fluorescent and an outer red fluorescent band. In this area the blood vessels and endosteal bone trabeculae had thus not been destroyed completely by the reaming. In the medullary cavity central to this region, newly formed bone trabeculae were seen. Of these, the trabeculae located nearest to the endosteum showed massive yellow fluorescence in the central regions and were covered with a red fluorescent band. Further inside the medullary cavity were other bone trabeculae, the central parts of which showed massive red fluorescence.

Observation time: 2 weeks

Labelling on days 6 and 12.

Periosteum. The periosteum was active and formed trabecular bone around almost the entire diaphysis. The newly formed bone was up to 2.2 mm thick. From labelling 1 a layer of newly formed bone with a maximal thickness of 400 μ was labelled. In the peripheral parts of the trabecular bone the trabeculae were narrow and surrounded by an abundance of perivascular soft tissue. In this area the trabeculae were homogeneously fluorescent from labelling 2. In the middle layer of the newly formed bone the trabeculae were wide, the perivascular soft tissue sparse and the blood vessels narrow. The central parts of the trabeculae were unlabelled, while ring-shaped labelling was seen around the vessels. In the inner layer of the trabecular bone the trabeculae were homogeneously fluorescent from labelling 1. In this part there were numerous narrow, long cavities, the walls of which were partly unlabelled by labelling 2 and in the unlabelled parts the walls were often rough—a sign of bone resorption.

Çortex. In the cortex, especially in its inner areas, resorption cavities were observed, the walls of which were partly covered by newly formed fluorescent bone and partly by narrow labelled areas indicating resorption. The newly formed bone was often seen on the peripheral wall.

Medullary cavity. There was an abundance of newly formed trabecular bone in the medullary cavity. Many of the bone trabeculae adjacent to the endosteum showed superficial resorption.

Observation time: 3 weeks

Labelling on days 7 and 19.

Periosteum. Superficial resorption was in progress around the entire diaphysis. From labelling 1 a band of even thickness around more than half of the diaphysis, as well as occasional beams between the blood vessels,

had become labelled. From labelling 2, ring-shaped osteones which were becoming closed, were labelled. The periosteal reaction appeared to have been strongest over the lateral part of the bone corresponding to the area where the bone had been affected most by the reamer from the inside.

Cortex. In the cortex there were rather few small resorption cavities, some of which showed active bone formation on the wall. Other cavities were unlabelled or exhibited a thin area of labelling indicating resorption. Three fairly large pear-shaped resorption cavities were observed, which from the periosteal surface were eroded into the original cortex under the area where considerable periosteal bone formation had taken place previously. No doubly labelled osteones were seen in the cortex.

Medullary cavity. In the dorsal part, doubly labelled endosteal bone and some doubly labelled newly formed bone trabeculae were seen. There was active bone formation on the bone trabeculae in towards the centre of the medullary cavity in several areas. Close to the endosteum, many parts of the bone trabeculae were undergoing resorption, at the same time as other parts of the trabeculae showed active bone formation.

Observation time: 4 weeks

Labelling on days 7 and 26.

Periosteum. Superficial resorption was taking place around the entire diaphysis. The periosteal reaction appeared to be strongest fibularly and tibioventrally. From labelling 1 the cross section showed fluorescent spicules between intermediate vessels and in some cases bridges over the vessels. On labelling 2 most of the primary osteones which had formed after the operation had become closed, but in some of them a small fluorescent ring had formed close to the blood vessels. The bone which was fluorescent from labelling 1 also showed extensive superficial resorption. In those areas where the callus was thickest, long narrow resorption cavities were found adjacent and parallel to the original cortex.

Cortex. Pronounced reconstruction was taking place in the entire cortex. In longitudinal sections several "cutter heads" were observed. A small number of doubly labelled osteones were seen in the outer and middle parts of the cortex. In the inner part of the cortex mainly single-labelled osteones were found.

Medullary cavity. In the central parts of the medullary cavity active trabecular bone formation was seen in towards a homogeneous, almost structureless area which was invaded by narrow vessels. In the peripheral parts of the medullary cavity there was extensive resorption of trabecular bone, but bone formation on the trabeculae was also seen.

Observation time: 5 weeks

Labelling on days 7 and 33.

Periosteum. In the fibular part there was a prominence of newly formed bone. The periosteum over its peak was inactive, but slight periosteal new lamellar bone formation was seen on the one side of the prominence. The prominence consisted of primary osteones, in which some of the narrow blood vessel canals were surrounded by a narrow, ring-shaped fluorescent area, while other osteones were completely closed at labelling 2.

Cortex. In the cortex numerous small cavities were seen, which were completely or partly lined with newly formed fluorescent bone.

Medullary cavity. In the central part of the medullary cavity there was a structureless, sparsely vascularized area. This was partly surrounded by a thin border of trabecular bone, the bone trabeculae of which were directed inwards towards the structureless area. On the tips of the trabeculae, which were directed in towards the centre, an active deposition of bone was taking place, while the peripheries of the trabeculae were undergoing resorption. In the remaining parts of the medullary cavity there were only widespread fragments of bone trabeculae undergoing resorption, on which sporadic bone formation was also observed.

Observation time: 6 weeks

Labelling on days 9 and 39.

Periosteum. Superficial resorption was taking place around the entire diaphysis. The fluorescence labelling showed that after the reaming primary osteones had formed, but these had been largely reabsorbed.

Cortex. Very active reconstruction was taking place in the cortex.

Medullary cavity. The picture was similar to that from the animal observed at 5 weeks. The resorption of the bone trabeculae in the centre of the medullary cavity was so active that even the bone which was labelled by labelling 2, given 3 days before the angiography, was undergoing extensive resorption.

Observation time: 7 weeks

Labelling on days 10 and 47.

Periosteum. Superficial resorption was taking place around the whole diaphysis. Fluorescent, ring-shaped structures around the blood vessels were seen from labelling 1. At the time of labelling 2 the first-formed primary osteones were completely closed.

Cortex. Very active reconstruction of the entire cortex was taking place, but no doubly labelled osteones were observed in the inner part of the cortex.

Medullary cavity. The preparation had about the same appearance as that observed at 6 weeks.

Observation time: 10 weeks

Labelling on days 10 and 56.

Periosteum. Periosteal bone formation with the development of lamellar bone was seen in a few areas, but otherwise extensive superficial resorption was taking place. From labelling 1 there was fluorescence of the centres of the bone trabeculae which were formed after the reaming. At labelling 2 the newly formed osteones were closed.

Cortex. Pronounced reconstruction of the entire cortex was taking place.

Medullary cavity. As in the preparations observed at 4, 5, 6 and 7 weeks there was a structureless area in the medullary cavity, penetrated by occasional blood vessels. The structureless area bordered onto normal fatty bone marrow or the endosteal surface of the original cortex.

Microangiographic Studies

Observation time: 1 day

Periosteum. The blood vessels in the periosteum were well filled with Indian ink, rather tortuous and appeared wider than normally, especially in the fibular part of the preparation.

Cortex. Approximately one-third of the inner part of the cortex was avascular. The vascular damage was greatest in the fibular part where most bone had been removed from the endosteal surface on reaming. The blood vessels in the outer part of the cortex were usually well filled. The same Haversian canal often contained two blood vessels, of which one, which was narrower and massively filled with Indian ink, was considered to be an arteriole, while the other, which was wider and often only lined with Indian ink, was considered to be a venule.

Medullary cavity. The medullary cavity was filled with a blood clot. Bone appeared to have been removed from the entire endosteal surface by the reaming. In the ventral and dorsal parts there were blood vessels which penetrated the whole cortex up to but not into the medullary cavity. In front of the mouth of the blood vessel there was a minor extravasation of Indian ink into the medullary cavity.

Observation time: 7 days

Periosteum. In the tibial sector of the preparation an intensive periosteal vascular reaction was observed. The blood vessels were mutually parallel and ran at right angles to the surface of the cortex. They were convoluted

and showed calibre variations. On several levels there were anastomoses between adjacent vessels. In the remaining parts of the periosteum there were tortuous, well filled blood vessels which ran mainly parallel with the bone surface.

Cortex. In the tibial sector of the bone about $^1/_{10}$ of the cortex was avascular. In the remaining parts the cortex was vascularized and the intracortical blood vessels were usually well filled with Indian ink.

Medullary cavity. About one-quarter of the medullary cavity contained blood vessels. Blood vessels grew from the cortex within the ventral and dorsal sectors in the areas where the endosteum did not appear to have been damaged by the reamer. Several types of blood vessels were found in the medullary cavity. Nearest to the endosteum a small number of anastomosing vessels of even calibre and up to 200 μ wide, which were lined with grains of Indian ink, were seen. Central to these were blood vessels about 40 μ wide, of even calibre and lined with Indian ink, which anastomosed abundantly. In the area bordering on the non-revascularized part of the medullary cavity there were convoluted vessels about 5–40 μ wide and massively filled with Indian ink, of similar appearance to those vessels observed in the rabbit at an early phase of revascularizaton of the medullary cavity and subperiosteal haematomas (see p. 69).

Observation time: 2 weeks

Periosteum. There was a very strong vascular reaction around almost the entire diaphysis. The blood vessels in the up to 2 mm thick vascular layer ran mainly perpendicular to the surface of the cortex and showed numerous mutual anastomoses. In the outermost part of the vascular layer the blood vessels were convoluted and varied in calibre. In the middle part of the layer the vessels were usually of equal calibre, about 20 μ wide and straight. Nearest to the original cortex, in the areas where the thickest periosteal vascular reaction occurred, there were blood vessels of equal calibre, about 100 μ wide, lined with Indian ink. These vessels ran mainly parallel with the surface of the original cortex. They were of the same type as were observed during resorption in periosteal and endosteal callus in the rabbit.

Cortex. About half of the cortical cross section area was avascular. In some areas the entire cross section was avascular, but in the dorsal sector the cortex was completely vascularized. Some of the blood vessel canals in the vascularized part of the cortex were considerably wider than in the non-vascularized part. The canals often contained two blood vessels. In longitudinal sections loops of blood vessels leading to "cutter heads" and to broom-shaped vascular proliferations were observed.

Medullary cavity. Approximately half of the medullary cavity was revas-

cularized. The same types of blood vessels were seen as in preparations observed at 1 week.

Observation time: 3 weeks

Periosteum. The blood vessels in the periosteum were tortuous and dilated.

Cortex. About half of the fibular sector of the cortex was avascular. In some preparations blood vessels had grown through the cortex into the medullary cavity, even in areas where the reamer had removed bone from the endosteal surface.

Medullary cavity. The medullary cavity was almost completely revascularized. Nearest to the endosteum there was a sparse number of the approximately 150 μ wide blood vessels which were described in preparations observed at 1 week. In front of these, vessels of similar appearance but about 40μ wide were seen. Central to these vessels, on the border with non-revascularized parts of the medullary cavity, anastomosing vessels were seen which were massively filled with Indian ink and which gave an impression of greater maturity than the blood vessels seen in corresponding areas in the animal observed at 1 week, in that they were of more equal calibre and less tortuous.

Observation time: 4 weeks

Periosteum. Dilated and tortuous periosteal vessels were observed in the fibular sector, which was most affected by the reamer. In other parts there was no definite, increased vascular reaction.

Cortex. The cortex was almost completely vascularized. In longitudinal sections vascular components of many "cutter heads" were observed.

Medullary cavity. The medullary cavity was completely revascularized. In the peripheral parts there were large blood vessels up to 200 μ wide. In the middle layer blood vessels about 40 μ wide were seen. In the centre of the medullary cavity there was an almost structureless area, which was sparsely invaded by fairly straight blood vessels about 20 μ wide and massively filled with Indian ink, as well as sparsely anastomosing vessels about 30 μ wide and lined with Indian ink.

Observation time: 5 weeks

Periosteum. In the lateral sector there was a moderate number of dilated periosteal vessels. Otherwise no vascular reaction was observed.

Cortex. The cortex was almost completely vascularized. The intracortical blood vessels appeared narrower and of more equal calibre than the vessels in preparations from animals at shorter observation times.

Medullary cavity. The medullary cavity was completely revascularized. Adjacent to the endosteum the vascular structure was as in normal bone marrow, and inside this the same types of vessels as described at an observation time of 4 weeks were seen.

Observation time: 6 weeks

Periosteum. No periosteal vascular reaction was observed.

Cortex. Active reconstruction was taking place in the cortex, which was almost completely vascularized.

Medullary cavity. The medullary cavity was revascularized. The angiographic picture was similar to that at an observation time of 5 weeks.

Observation time: 10 weeks

Periosteum. No definite periosteal vascular reaction was observed.

Cortex. The entire cortex was vascularized. Pronounced reconstruction of the whole cortex was taking place and several vessel loops in "cutter heads" were seen.

Medullary cavity. The medullary cavity was revascularized. In the peripheral parts of the medullary cavity the vascular structure was normal. More centrally there were fragments of bone trabeculae, which lay in close contact with blood vessels up to 200 μ wide and lined with grains of Indian ink. In the central part of the medullary cavity there was a structureless area criss-crossed with vessels of the previously described type. A normal vascular structure was seen in some areas in direct contact with the homogeneous, central region.

Studies of Histological Azan-stained Sections

The histological studies were concentrated to the central area of the medullary cavity in animals with an observation time of more than 3 weeks. On the fluorescence-microscopic and angiographic preparations an almost homogeneous area containing a sparse number of relatively narrow blood vessels was seen at this location.

Azan-stained histological sections showed that the structureless area consisted of densely situated, essentially parallel, collagen fibres in which occasional cells, cell aggregations and blood vessels were interspersed. Between this connective tissue and the surrounding bone trabeculae there was a layer of cells with large nuclei. Collagen fibres ran from the bone trabeculae into the fibrous tissue. In other areas at an observation time of more than 5 weeks practically normal bone marrow or endosteal surface of the cortex bordered directly on to the fibrous tissue without any intervening layer of cells. (Color pl. 2 *b*.) At an observation time of 10 weeks the active bone formation at the border to the fibrous scar tissue had stopped.

Results after Brushing-out of the Medullary Content

Microangiographic Studies

Observation time: 1 day

Periosteum. The periosteal blood vessels were somewhat narrower and less tortuous than the vessels in preparations from the left femur, which had been reamed.

Cortex. About $^1/_{10}$ of the inner part of the cortex was avascular. Many Indian ink-filled vessels, especially in the dorsal and ventral parts, penetrated the entire cortex to the medullary cavity. A little extravasation of Indian ink was seen in the medullary cavity just in front of their opening.

Medullary cavity. The medullary cavity was filled with a blood clot and no Indian ink-filled vessels were observed.

Observation time: 2 days

Periosteum. The periosteal vascular reaction was stronger than in preparations observed at 1 day. The blood vessels were tortuous and massively filled with Indian ink.

Cortex. About two-thirds of the cortex was avascular. In the dorsal sector blood vessels were seen to penetrate the entire cortex as far as the medullary cavity.

Medullary cavity. The medullary cavity was filled with a blood clot, and no Indian ink-filled vessels were observed.

Histological Results after Reaming and Brushing

Studies of Fat-stained Histological Frozen Sections

Observation time: 1 day

In every cross-sectional preparation from the right femur, where the content of the medullary cavity was removed with a bottle-brush, there were about 10 fat droplets, which obliterated Haversian canals in the inner part of the cortex. In preparations from the left femur, where the contents of the medullary cavity were removed by reaming, approximately the same number of fat droplets were seen, localized in the same way.

Observation time: 2 days

In preparations from the control femur, which was not operated on, occasional fat droplets were observed, obliterating Haversian canals in the inner part of the cortex. In five cross-sectional preparations a total of only three fat droplets was seen. In preparations from the other femur, from which

the contents of the medullary cavity were removed with a brush, about 20 fat droplets were observed in every section. Many of the canals in the cortex in this femur were completely obliterated by fat.

Summary and Discussion

Periosteum. After reaming of the medullary cavity the periosteal structures reacted in all animals. The blood vessels increased in calibre, became more tortuous and were filled massively with Indian ink on angiography. Vessels which ran perpendicular to the surface of the cortex were seen at observation times of 1 and 2 weeks. The vascular reaction began to decline at 4 weeks and appeared to be greatest and to persist longest in the area where the cortex had been most affected by the reaming from the inside.

The periosteal bone formation was very active throughout. In all animals primary osteones or trabecular bone were newly formed during the first few weeks postoperatively. The most abundant new bone formation appeared to take place in those parts of the cortex where most bone had been removed from the endosteal surface by the reaming. In these areas the vascular damage in the underlying cortex seemed to be greatest. Subsequently there was very rapid resorption of most of the newly formed bone. The bone resorption was most pronounced between 2 and 8 weeks postoperatively. At an observation time of 10 weeks some tendency to formation of lamellar bone was noted. The Terramycin labelling showed that about 1 week after the reaming, mineralized bone beams were formed between the blood vessels and in some cases bridges over the vessels. The so-formed primary osteones closed successively during the following 2–3 weeks.

Cortex. The extent of the intracortical vascular damage varied. The greatest damage to the vascular system was observed after brushing out of the bone marrow, when about two-thirds of the cortex was seen to lack Indian ink filled vessels at subsequent angiography. In one animal about one-third of the cortex became avascular after the reaming. The reason for the avascularity is, at least in part, that the contents of the medullary cavity are squeezed or embolized into the Haversian canals and occlude them. The cortex appeared to become revascularized fairly quickly, however. On prepations from animals studied 4 weeks or longer postoperatively, the cortex was almost completely vascularized. The revascularization took place with the aid of "cutter heads", but a broom-like vascular reaction which gave larger resorption cavities in the cortex was also observed. In some cases resorption cavities had formed also in the subperiosteal part of the original cortex. This occurred where there was a very strong periosteal reaction. It was probably due to the fact that the blood vessels in the outer part of the cortex had also been damaged. The rebuilding of the cortex, as reflected in the

number of secondary osteones, increased continuously throughout the experimental period up to 10 weeks after the operation. Practically the entire cortex, including the subperiosteal part, was reconstructed. In primarily avascular areas of the cortex the new bone formation in resorption cavities appeared to commence about 10 days after the operation.

Medullary cavity. In one case undamaged vessels were seen in the dorsal part of the medullary cavity after the reaming. This seems to be due to the fact that during the reaming the vessels were protected by endosteal bone trabeculae which were not removed by the reamer. Revascularization of the medullary cavity began quickly, usually first from blood vessels in the dorsal part of the cavity. The first vessels to invade the medullary cavity were abundantly anastomosing vessels of varying calibre and massively filled with Indian ink, of the type observed in the rabbit medullary cavity in the early revascularization phase. Koekenberg (1963) classified this type of blood vessel as type 3 (see p. 76). These vessels later underwent a change to relatively narrow, abundantly anastomosing vessels of more equal calibre, around which trabecular bone was formed. Newly formed bone in the medullary cavity became fluorescent from Terramycin which was given 5 days after the operation. Resorption of the newly formed bone trabeculae adjacent to the endosteum was observed 1 week postoperatively. These trabeculae were then surrounded by vessels of large calibre, which appeared to become transformed to normal sinusoids when the bone trabeculae had become resorbed. After an observation time of 2 weeks the type of immature vessel which was first seen in the medullary cavity was no longer present there, and instead more mature vessels were seen close to the central, as yet not revascularized part of the medullary cavity. After an observation time of 4 weeks an area of connective tissue interspersed by sparsely anastomosing, narrow vessels of which many were massively filled with Indian ink, was observed mainly localized to the central part of the medullary cavity. On preparations studied after a long observation time this fibrotic area seemed to be inactive. It bordered directly on the normal medullary tissue or to the endosteal surface of the original cortex. Whether the fibrous area would remain as a scar or whether it would be rebuilt later cannot be decided. The absence of cells between the fibrous area and normal marrow tissue or cortex supports the former alternative, however.

Comparison between the Reaction in the Rabbit and Dog on Reaming of the Medullary Cavity

In the rabbit the investigation was performed on the tibia, and in the dog on the femur. The musculature around the femur is considerably more

massive than around the tibia, but this should not have influenced the results essentially.

The periosteal reaction appeared to be stronger in adult dogs than in adult rabbits. In all dogs woven bone in primary osteones or trabecular bone formed subperiosteally, which is rather uncommon in adult rabbits. The resorption of the newly formed bone also appeared to take place considerably more intensively in the dog than in the rabbit.

The intracortical vascular damage in the dog, as also in the rabbit, varied greatly. It is reasonable to assume that the degree of intracortical vascular damage in the dog was dependent to a large extent on the pressure increase which occurred in the medullary cavity during the operation, which was found to be the case in the rabbit (see chapter 2). Thus an experiment with brushing out of the medullary cavity in the dog, when a thick bottle-brush was used, gave rise to considerable vascular damage in the cortex. The reamers used in dogs, and which were hand-driven, were so formed that they probably gave only a relatively small pressure increase in the medullary cavity. The reamers used in the rabbit experiments, on the other hand, resembled more the mechanically-driven reamers of the Küntscher or AO type, which are used in the clinic (See Müller *et al.* 1965). Revascularization of the cortex appeared to take place more rapidly in the dog than in the rabbit, but this opinion is uncertain, since the primary vascular damage varied greatly. The rebuilding of the cortex, with the formation of secondary osteones, appeared to be more intensive in the dog than in the rabbit.

Reaming of the medullary cavity did not always result in complete destruction of the blood vessels in the diaphyseal medullary cavity in the dog, which always seemed to occur in the rabbit. This is probably due to the fact that the dog has endosteal bone trabeculae which protect the vessels nearest to the endosteum, especially in the dorsal part of the medullary cavity, while in the rabbit the endosteal surface in the diaphysis is quite smooth. The medullary cavity becomes revascularized very rapidly in the adult dog and bone formation starts earlier than in the adult rabbit. The same types of blood vessel were observed in the medullary cavity in both animals during revascularization. In the central parts of the medullary cavity in the dog a fibrous, cicatricial tissue, sparsely interspersed by blood vessels, was often observed. This tissue was also seen in the rabbit but was less prominent there.

CHAPTER 7

General Discussion and Summary

Danis (1947) stated that in his experience diaphyseal fractures stably fixed with compression plate osteosynthesis heal without roentgenologically visible periosteal callus. Several authors have since found that the amount of periosteal callus is related to the degree of stability. The AO group aim also with their stable osteosyntheses at obtaining "callus-free" bone healing.

On healing of femoral fractures stably fixed by intramedullary nailing after reaming of the medullary cavity, a large amount of periosteal callus is often formed, however, even if, judging by clinical and roentgenological signs, the osteosynthesis remains stable. On intramedullary nailing there is therefore some additional factor which stimulates the periosteal new bone formation.

In order to study this problem under reliably stable conditions, different surgical procedures (brushing, suction, reaming) were carried out in the medullary cavity of the tibia in growing and adult rabbits and of the femur in adult dogs without the leg being fractured.

The traumatization of the bone and its circulation which was produced by the operation in the medullary cavity induced characteristic reactions in the periosteum and cortex and in the medullary cavity itself. Bone was formed subperiosteally, among other places, and the bone which was newly formed after the operation was labelled with tetracyclin, the ultraviolet fluorescence of which was studied microscopically in plastic-embedded ground sections. The microcirculation in the cortex was studied by angiography at the end of the experimental period and preparations treated according to the Spalteholz technique were examined by stereomicroscopy. The extent of the intracortical vascular damage and the amount of periosteal newly formed bone were quantitated in cross-sections. The development of the periosteal bone formation activity was evaluated by recording whether the surfaces of the bone sections were fluorescent or not, and by measuring the thickness of the bone and osteoid which were formed on the days preceding the angiographic examination. The pressure conditions in the medullary cavity were recorded on reaming and brushing, and the temperature increase on reaming. Conventionally stained histological preparations and Sudan-stained frozen sections of bone were studied.

The methods of recording and measurement were found on testing to have good reliability.

On suction, reaming or brushing of the medullary cavity, the medullary blood vessels in the diaphysis of the bone were destroyed. This was especially effective in the rabbit. Furthermore, on reaming, bone was removed from the endosteal surface of the diaphysis. On destruction of the medullary vessels the cortex was deprived of that part of its circulation which came from the medullary cavity. When this destruction resulted from reaming, between 10 and 60% of the cortical cross-sectional area became avascular. An approximately equal degree of avascularity occurred in the distal and proximal parts of the diaphysis. In a few isolated sectors in some animals, however, practically all intracortical vessels were filled with Indian ink. Suction, as a rule, resulted in considerably less cortical avascularity than reaming or brushing. The loss of the medullary circulation is thus not an essential cause of the cortical avascularity after operations in the medullary cavity, and there are other, more important factors, which are discussed below.

Friction heat produced by the reaming is of no or very little importance as a cortex-damaging factor under the given experimental conditions. On the other hand, the increase in pressure which can occur during operations in the medullary cavity can be of great importance. Pressure increases far exceeding the systolic blood pressure are produced very easily and one result is that bone marrow is forced into the intracortical canals and obstructs the blood circulation there. It is also conceivable that pressure increases may result in tearing of the intracortical vessels so that the circulation is directly occluded, or, with less damage, in the occurrence of secondary thrombosis.

A good correlation was found between extensive cortical avascularity, a large number of intracortical marrow emboli and a high intramedullary pressure produced by incautious reaming or brushing in the medullary cavity. On the other hand, only very slight cortical avascularity and few intracortical emboli were found, for example, on careful reaming or suction in the medullary cavity.

These results thus indicate that if the medullary circulation is occluded without the intracortical vessels being obstructed by marrow or being directly damaged, the periosteal vessels can maintain the circulation in almost the whole of the intracortical vascular bed. It is improbable, however, that at an early stage after the operation the intracortical circulation is sufficient to nourish all bone tissue, especially not in the cortex nearest to the medullary cavity. The incomplete filling of the functioning intracortical blood vessels at observation times of less than 24 hours, support this assumption.

My conclusions on the ability of the periosteal vessels to take over the intracortical nutrition when the medullary vessels have been destroyed thus

differ somewhat from those of many previous investigators. Macnab (1958), McAuley (1958) and Gustilo *et al.* (1964) thus considered that the periosteal vessels were unable to supply any part of the cortex, and Trueta & Caladias (1964) claimed that only the outer third of the cortex could be supplied by periosteal vessels. de Marneffe (1951, 1953) considered that in the proximal part of the tibial diaphysis in the rabbit the periosteal vessels could only supply the outer part of the cortex, but in the distal part of the diaphysis the whole cortex. Göthman (1961) and Rhinelander & Baragry (1962) found that during the first days up to one week after destruction of the medullary vessels the intracortical vessels were filled very incompletely from periosteal vessels (according to the latter authors due to the fact that normally many intracortical vessels are resting), but that the periosteal vessels were then able to take over the circulation in the cortex.

The differences in the conclusions may be due to the fact that these investigators used Micropack as perfusion medium, which seems to have difficulty in passing through the vessels in the subperiosteal bone before they have increased in calibre about one week after the operation. They may also be due to the fact that a pressure increase was produced in the medullary cavity in their experiments, resulting in marrow emboli in the intracortical vessels, but that sufficient consideration was not taken of these factors.

On increase in pressure in the medullary cavity, bone marrow can be forced through canals in the cortex and deposited subperiosteally, or transported further into the venous system to be filtered mainly in the lungs. The transport of bone marrow through the cortex takes place most easily through the canals for the large vessels (canals for the primary and secondary nutrient arteries and the emissary vein). The marrow can often be observed macroscopically as a subperiosteal haematoma around the outer openings of these canals. Embolism in the lungs can, especially on brushing of the medullary cavity, be so extensive that the animal dies during the operation.

Operations in the medullary cavity induce characteristic reactions in the periosteum, cortex and medullary cavity, and these will be discussed separately.

Periosteal Reaction

In the periosteum the blood vessels react earlier than the osteogenic cells (Wray, 1963; Trueta, 1963). The morphology of the newly formed bone in the present study varied with the vascular reaction. Convoluted vessels in one or a few layers, parallel with the cortex and massively filled with Indian

ink were found during the formation of circumferential lamellae. Several layers of similar vessels developed during the formation of primary osteones. Relatively straight, frequently anastomosing vessels at right angles to the cortex and often of considerable length, preceded the formation of trabecular bone. The vascular reaction was observed in the periosteum as long as the new bone formation or bone destruction there was increased, but during the process of maturation of the bone the vessels became narrower and straighter.

The periosteal newly formed bone could be either lamellar or woven bone. Lamellar bone which was present in circumferential lamellae and in some primary osteones was not reconstructed during the course of maturation. In woven bone, which was found in certain primary osteones and especially abundantly in trabecular bone, resorption cavities were sometimes observed. These cavities gave the impression of migrating in a central direction towards the medullary cavity. Subperiosteally formed woven bone was not observed during the normal periosteal growth in rabbits of the ages studied. This could therefore be regarded as pathological bone formation which only occurred during the first 2–4 weeks after the operation. In some cases after partial superficial resorption it was later covered by deposition of lamellar bone. The perivascular spaces between the primary trabeculae of woven bone decreased by the formation of lamellar bone on the trabeculae. In this way the primary osteones closed during the course of about 4 weeks.

The periosteal bone formation activity was of varying intensity at different times after reaming of the medullary cavity. In the rabbit the activity in the treated tibia, which in growing animals was maximal a few days after the operation, had decreased at an observation time of 2 weeks to almost the same level as was noted at 4 and 8 weeks. Also on the control side the periosteal bone formation activity varied. A few days postoperatively it was high, it decreased to a minimum at 2 weeks and then again increased. The reduction of the activity in the control tibia meant that the difference in the bone formation activity between the treated and control tibias was greatest at an observation time of 2 weeks, despite the relatively low activity observed at that time point in the treated tibias. Owing to a subsequent increase in the activity in the control tibias, there was a partial equilibration with the treated tibia at an observation time of 4 weeks. Extensive superficial resorption at 2 weeks, most pronounced in the control tibia but also considerable on the treated side, also indicated that the activation to periosteal bone formation which was induced by the operation in the medullary cavity had almost ceased 2 weeks postoperatively. In adult rabbits, in which there was no appreciable spontaneous periosteal bone formation, the formation of bone on the treated side was activated more slowly than in the

growing animals and reached its maximum about 2 weeks after the operation.

All rabbits, both growing and adult, formed more bone subperiosteally in the treated tibia than on the control side. The amount of newly formed bone varied greatly between different animals. In the growing animals bone was formed on the treated tibia mainly during the 2nd week postoperatively, and on the control tibia during the 3rd and 4th weeks. In the adult animals a considerably smaller amount of bone was formed than in the growing animals, and an almost significant increase in bone formation on both tibias was only obtained when animals observed at 1–4 weeks were compared with those observed at 8 weeks.

The cause of the increased periosteal bone formation could only be partially elucidated in this investigation, and appeared to some extent to differ for the formation of lamellar bone and the formation of woven bone.

The formation of lamellar bone, which is the most usual type of periosteal bone formation in the rabbit after operations in the medullary cavity, appears to be an acceleration or revival of normal bone formation, where the age of the animal and the degree of stimulation determine whether circumferential lamellae or primary osteones are formed. Mature lamellar bone is homogeneous, is not rebuilt and appears also in other respects to be primarily adapted to mechanical demands on the skeleton. It is not clear in what way this bone formation is stimulated, but no findings have contradicted the view that it takes place by the mediation of growth and thyroid hormones (Frost, 1963) or space polarizing factors (Frost, 1963; Schenk, 1965). Local stimulating factors cannot be excluded, however.

Woven bone, especially when it was formed in a large quantity, which occurred, for example, in trabecular bone, was found most commonly in those areas where, as a result of the increased intramedullary pressure during the operation, considerable amounts of bone marrow were sometimes forced out subperiosteally. The formation of woven bone, especially when it occurs in trabecular bone, thus seems to be promoted by this subperiosteally forced out bone marrow. Urist & McLean (1952) and Burwell (1967) showed, in transplantation experiments, that cells in the bone marrow, stimulated by necrosing cortical bone, could be induced to form ossicles. These ossicles have a great resemblance to the cavities in trabecular callus tissue which were observed in the present material. Zucman *et al.* (1968) found that bone marrow which had become localized subperiosteally was accompanied by considerable local bone formation. Frost claimed (1963) that the formation of woven bone is governed by local factors and not by growth and thyroid hormones, and that it has to be reconstructed in order to attain full resistance to mechanical stress.

It has been considered that stimulating substances or the loss of inhibitory substances from the underlying cortex, whose circulation has deteriorated, may initiate periosteal bone formation. Such a stimulating substance, named by Lacroix (1951) osteogenin, has been considered by several investigators to be capable of stimulating osteogenesis even from immature mesenchymal cells (Levander, 1938; Annersten, 1940; Goldhaber, 1961; Urist, 1965; Trueta, 1963; Burwell, 1964; Puranen, 1966).

On statistical analysis of a material studied at an observation time of 1 week, in which the medullary cavity of the right tibia was evacuated by suction and of the left tibia by brushing or reaming, it was not found, however, that the extent of the intracortical vascular damage or the amount of bone removed on reaming had any explanatory value for the amount of new bone formed by the overlying periosteum. This was in spite of the fact that the amount of subperiosteally formed bone was, on the average, greatest on the left tibia where the cortical vascular damage was also the most extensive. Since the medullary cavity was evacuated from both the left and right tibia, however, the results indicate that other factors than loss of the medullary circulation—which was postulated by Richany *et al.* in 1965—were of importance for the periosteal bone formation. One significant factor was probably the bone marrow which was squeezed out subperiosteally.

Cortical Reaction

The cortical necrosis could not be delimited by histological methods. Nuclear pyknosis occurred even in viable bone, and even when the number of empty osteocytic lacunae was found to have increased in the central and inner parts of the cortex with increasing observating times, nuclear material was still observed in the inner avascular part of the cortex during an observation period of at least 4 weeks. Neither could any well-defined borderline be drawn between nucleated and non-nucleated areas in the cortex. The cortical damage caused by the reaming and other operations in the medullary cavity was therefore evaluated on the basis of the Indian ink filling of the intracortical vessels in preparations studied at short observation times.

In that part of the cortex which had retained its circulation after the operation, many of the blood vessels increased in calibre during the first week postoperatively, and during the second week the bone canals around these vessels widened. Many of the bone canals began to close again, however, after an observation time of about 4 weeks, and in this way the circulation from the periosteum through the outer part of the cortex to its inner part and to the medullary cavity became concentrated to a number

of larger vessels, which thus took over the function of the destroyed nutrient artery. Those parts of the cortex which had become avascular at the operation were revascularized by "cutter heads" and broom-shaped vascular proliferations. The main revascularization did not appear to take place until after 2 weeks postoperatively. Shortly afterwards bone formation began in resorption cavities often located in parts of the cortex rendered avascular by the operation. After 8 weeks the cortex in growing animals was essentially revascularized, while non-revascularized areas were still found in the inner part of the cortex in adult animals at the longest observation time of 12 weeks. In cases with very extensive intracortical vascular damage there was a tendency to sequestration of the innermost part of the cortex.

Medullary Cavity Reaction

After brushing or suction, the medullary cavity was filled with a clot which contained fragments of disintegrated bone marrow and, after reaming, also bone fragments. Occasional blood vessels were sometimes seen to penetrate the cortex from the periosteum, and appeared to be responsible for some local fluid circulation in the medullary cavity. On revascularization of the medullary cavity the blood vessels usually grew out first from those areas of the cortex which had not been directly damaged by the reamer. The first newly formed, immature vessels were observed in the medullary cavity about 1 week postoperatively. The formation of bone trabeculae of woven bone began to take place close to more mature vessels about 30–40 μ in width, which could be seen after an observation time of 3 weeks. Resorption of the newly formed bone trabeculae, which began in the peripheral areas of the medullary cavity, took place in the close vicinity of blood vessels about 100 μ in calibre, which after the resorption of bone trabeculae appeared to become transformed to normal sinusoids. The same type of blood vessel was observed close to trabecular, subperiosteally located bone, when callus cavities developed there. A fibrous tissue, which was localized as a rule within the central areas of the medullary cavity, and penetrated by narrow blood vessels, often developed in adult animals. The cicatricial tissue did not appear to become completely rebuilt. Foster (1951) observed such medullary fibrosis after a severe circulatory disturbance of the cortex and medullary cavity in adult rabbits, while Brånemark (1954) found that the bone marrow was completely restored in adult rabbits, after scraping out of the contents of the medullary cavity.

Comparision between Reaction in Rabbits and Dogs

A comparative investigation between the reaction on evacuation of the medullary cavity of the femur in adult dogs and the corresponding reaction

in the tibia of adult rabbits showed that the periosteum in the dogs reacted more intensively and that it always formed woven bone, which was less common in adult rabbits. After an observation time of 2 weeks there was very active resorption of the newly formed periosteal bone in the dog, so that often only small fragments remained at an observation time of 4 weeks. The intracortical vascular damage seemed to have the same origin in the dog as in the rabbit. The reamers which were used in the main experiments, viz. hand-driven reamers of the Küntscher model (see Küntscher, 1962) probably gave rise to considerably smaller pressure variations in the medullary cavity, however, than those used in the rabbit experiments. It is probably for this reason that the intracortical vascular damage seemed to be considerably less extensive in the dogs than in the rabbits after reaming of the medullary cavity. Brushing in the medullary cavity could result in avascularity of two-thirds of the cortex, however. The reconstruction in the cortex was more active in the dog than in the rabbit. The bone trabeculae in the medullary cavity in the dog appeared to be able to protect some of the endosteal blood vessels during the reaming. Revascularization of the medullary cavity seemed to be initiated more quickly in the dog than in the rabbit, but otherwise the courses were similar. Fibrotic tissue, which seemed to be at least partly permanent, was found in the medullary cavity in all dogs at an observation time of more than 3 weeks.

Complementary Investigations

A logical continuation of this work seemed to be to study how a cortical defect healed with intact and reamed medullary cavities. I made such a study on the rabbit tibia. It was found that when the medullary cavity was intact the defect was bridged over and filled in rapidly by woven endosteal bone. Periosteal callus, which also contained cartilage, also quickly bridged over the defect, and was concentrated to the area outside it. When the medullary cavity had been destroyed, the defect was first bridged over by periosteal callus, which took place considerably later than when the medullary cavity was intact. Callus was then often found around almost the entire bone, but cartilage only outside the defect. During the first 2 weeks of observation the amount of periosteal callus outside the defect was usually greatest when the medullary cavity was intact, but at longer observation times it was greatest when the medullary cavity had been destroyed. After destruction of the medullary cavity the defect was revascularized by blood vessels from the periosteum and outer soft tissues, but was filled with callus considerably more slowly than when the medullary cavity was intact. A detailed report of the results will be published in a separate paper.

The increase in pressure in the medullary cavity and the subsequent intracortical and subperiosteal marrow emboli was found to be a very important factor for the occurrence of cortical vascular damage and periosteal new bone formation on operations in the medullary cavity. A reduction of the pressure in the medullary cavity during the operation should reduce the marrow emboli and thereby decrease the intracortical vascular damage and the periosteal new bone formation. Such an investigation, in which healing of an osteotomy in the rabbit tibia, fixed stably by compression nailing, was studied, has been carried out (Olerud, Danckwardt–Lilliestrom, Lorenzi 1969). The pressure in the medullary cavity was reduced during the operation by suction through a drill-hole in the distal tibial metaphysis and the results were compared with a series without such a distal hole. In the series with a peripheral drill-hole to reduce the pressure, there was less cortical vascular damage and less periosteal new bone formation than in the series in which the pressure was not reduced. Primary bone healing over the fracture gap also occurred in the former cases.

CHAPTER 8

Conclusions

From the series of experiments described, in which the medullary cavity of the tibia in growing and adult rabbits and of the femur in adult dogs was destroyed by reaming, brushing or suction, the following conclusions could be drawn on the reaction of the diaphysis to these operations.

1. The periosteal blood vessels were able to maintain circulation in practically the whole cortical vascular bed when the medullary vessels were destroyed.

2. Pressure increase in the medullary cavity, which was very easily produced at surgery to this cavity, forced bone marrow into the intracortical canals and obliterated these to varying extents. When large pressure increases occurred in the medullary cavity, this took place within considerable areas of the cortex.

3. The periosteal vessels reacted with increased filling with contrast medium and became tortuous. Blood vessels which were mutually parallel and perpendicular to the surface of the cortex were present when trabecular bone was being formed.

4. The periosteum reacted with increased bone formation. In growing rabbits the periosteal bone formation activity increased to a maximum within a few days after the operation and the largest amount of new bone was formed subperiosteally during the second week postoperatively. Adult animals reacted more slowly and showed maximal bone formation activity 2 weeks after the operation. At an observation time of 2 weeks there was pronounced superficial resorption of the newly formed bone in growing rabbits.

5. The periosteum formed either mature lamellar bone which was considered to be the result of an acceleration or revival of normal bone formation, or woven bone, which was believed to result from callus formation promoted at least partly by subperiosteally forced-out bone marrow. The woven bone appeared to be a provisional callus, in which reconstruction often began quickly, and in which cavities with angiographically normal medullary sinusoids were formed. These cavities seemed to migrate in a central direction with increasing observation times.

6. The cortical necrosis could not be outlined by histological methods owing to the fact that nuclear material could remain in necrotic bone during an observation period of at least 4 weeks.

7. The extent of the intracortical vascular damage and the amount of bone removed on reaming were not found to have any explanatory value for the amount of newly formed bone in the overlying subperiosteal are.

8. Those parts of the cortex which had been rendered avascular by the operation were revascularized from the outer vessel-containing parts of the cortex by means of "cutter heads" and broom-shaped vascular proliferations, and thereby underwent extensive reconstruction. The areas of the cortex with residual circulation were partly rebuilt, the bone canals around many of the blood vessels first becoming wider and then again decreasing in width when the circulation to the inner part of the cortex and to the medullary cavity became concentrated to a smaller number of vessels. The cortex adjacent to the medullary cavity was revascularized also from vessels which had newly formed in the medullary cavity.

9. The medullary cavity was revascularized mainly from vessels which, in the diaphysis, penetrated the cortex from the periosteum. In the medullary cavity trabecular bone was formed close to 30–40 μ wide thin-walled vessels, and was subsequently quickly resorbed in the close vicinity of vessels approximately 100 μ wide which were later transformed into angiographically normal sinusoids. Small areas of dense fibrous tissue appeared to remain permanently in the medullary cavity.

10. On comparison between the reaction of the diaphysis to destruction of the medullary cavity in adult rabbits and adult dogs, it was found that the periosteum reacted more intensively in the dog, and always formed woven bone within some area, which was relatively unusual in the adult rabbit. The newly formed subperiosteal bone was resorbed very rapidly in the dog. The cortical vascular damage appeared to have the same origin as in the rabbit, but the cortical reconstruction was more extensive. In the medullary cavity the same rebuilding process was seen as in the rabbit.

CHAPTER 9

Statistical Methods

Notation

Number of cases: $N=n$

$$\text{Mean: } M=\bar{X}=\frac{\sum X_i}{n}$$

where X_i denotes the value for the ith case.

Standard deviation:

$$\text{S.D.}=\sqrt{\frac{\sum(X_i-\bar{X})^2}{n-1}}$$

Standard error of the mean:

$$\text{S.E.}=\frac{\text{S.D.}}{\sqrt{n}}$$

Standard error of the difference between two means $\bar{X}$ and $\bar{Y}$:

$$\text{S.E.}_{\bar{X}-\bar{Y}}=\sqrt{\text{S.E.}_{\bar{X}}^2+\text{S.E.}_{\bar{Y}}^2}$$

Correlation analysis. The correlation coefficient, r of x and y is defined by the expression:

$$r=\frac{\sum(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2\sum(y_i-\bar{y})^2}}$$

where $\bar{x}$ and $\bar{y}$ denote the means for the x and y series, respectively.

Significance tests

1. In testing whether a mean value differs from 0 the following approximately t-distributed ratio has been formed:

$$t=\frac{\bar{x}}{\text{S.E.}_x}$$

2. In testing whether the regression coefficients differed from 0, the method described by Cramér (1945) and Hald (1948) was used:

A t- value was produced from the expression: $\frac{b_j}{\text{S.E.}\,b_j}$

3. In testing whether a correlation coefficient differed from 0, the following ratio was produced:

$$\frac{r}{\sqrt{1-r^2}}\sqrt{n-2}$$

which— if the true correlation was zero—was considered to be distributed like Student's t, with n-2 degrees of freedom.

4. In testing differences between means, the following approximately t-distributed ratio was formed:

$$t=\frac{\bar{x}-\bar{y}}{\text{S.E.}_{\bar{x}-\bar{y}}}$$

Significance levels

The term "significant" is used in accordance with the following convention. If an observed difference between two means is of such magnitude that the probability, P, of obtaining a difference at least as great as the observed value is greater than 0.05 (where the null-hypothesis is assumed to hold), then that observed difference is said to be non-significant.

If $0.01<P<0.05$, the difference is said to be almost significant and is marked*.

If $0.001<P<0.01$, the difference is said to be significant and is marked **.

If $P<0.001$, the difference is said to be highly significant and is marked***.

Multiple regression analysis used in the present study

For a series of n experimental animals we wish to study the relation between a dependent variable, y, called the regressand and constituting different *ABd* areas, and a set of explanatory variables, $x^{(1)}$, $x^{(2)}$ and $x^{(3)}$. The explanatory variables were similary localized *CDd* and *DEd* areas and also the type of operation.

In order to study the relation between the y and x variables, a type of equation must first be laid down. Individual numerical values must also be available for each variable. In the present work equations of the following form have been studied:

$$y=b_0+b_1x^{(1)}+b_2x^{(2)}+b_3x^{(3)}$$

where b_0, b_1, b_2, b_3 are the coefficients which are to estimated. In estimating these coefficients the principle of least squares has been applied.

The principle of least squares has been described in detail by Cramér (1945) and Hald (1948), who also explain the method of calculating S.E. for the coefficients.

Predicted value

The y value for an individual (i), predicted with the aid of the regression equation, is defined as:

$$b_0 + b_1 x_i^{(1)} + b_2 x_i^{(2)} + b_3 x_i^{(3)}$$

R = Correlation between observed y-value and predicted y-value.

The regression analysis program

In treating the data, as stated above, the following regression analysis program was used: Hodson Thornber. Manual for (B34T, 8Mar 66) a stepwise regression program. Report 6603. Univ. of Chicago, March 1966 (stencil).

All statistical analyses were performed in collaboration with Associate Professor G. Eklund, Ph. D. at the Department of Statistics, University of Stockholm.

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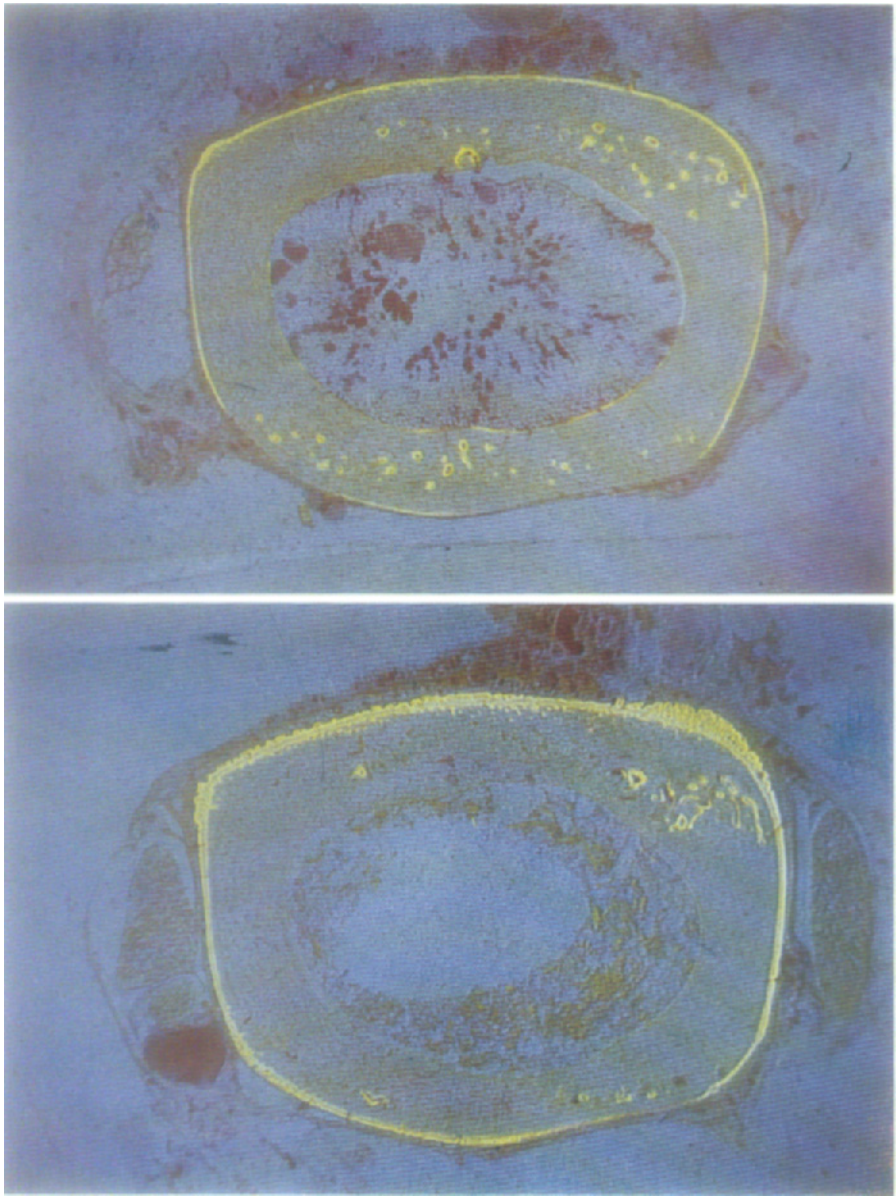

Plate 1. Distal fluorescence cross section, observation time 2 weeks. Growing animal 211, labelled on days 0 and 12 postoperatively.

(*a*) Control side. Subperiosteally, circumferential lamellae are formed around two-thirds of the section. Reconstruction is taking place in the cortex, mainly new formation of bone.

(*b*) Reamed side. Subperiosteally, circumferential lamellae, primary osteones or trabecular bone are formed around almost the entire section. More bone has been formed subperiosteally than on the control side. Several areas in the central and middle parts of the cortex lack signs of new bone formation, where such signs are present on the control side. This is a sign of avascularity there. In the ventro-tibial corner of the section (above right) there are larger resorption cavities than on the control side, partly with bone formation taking place on the walls.

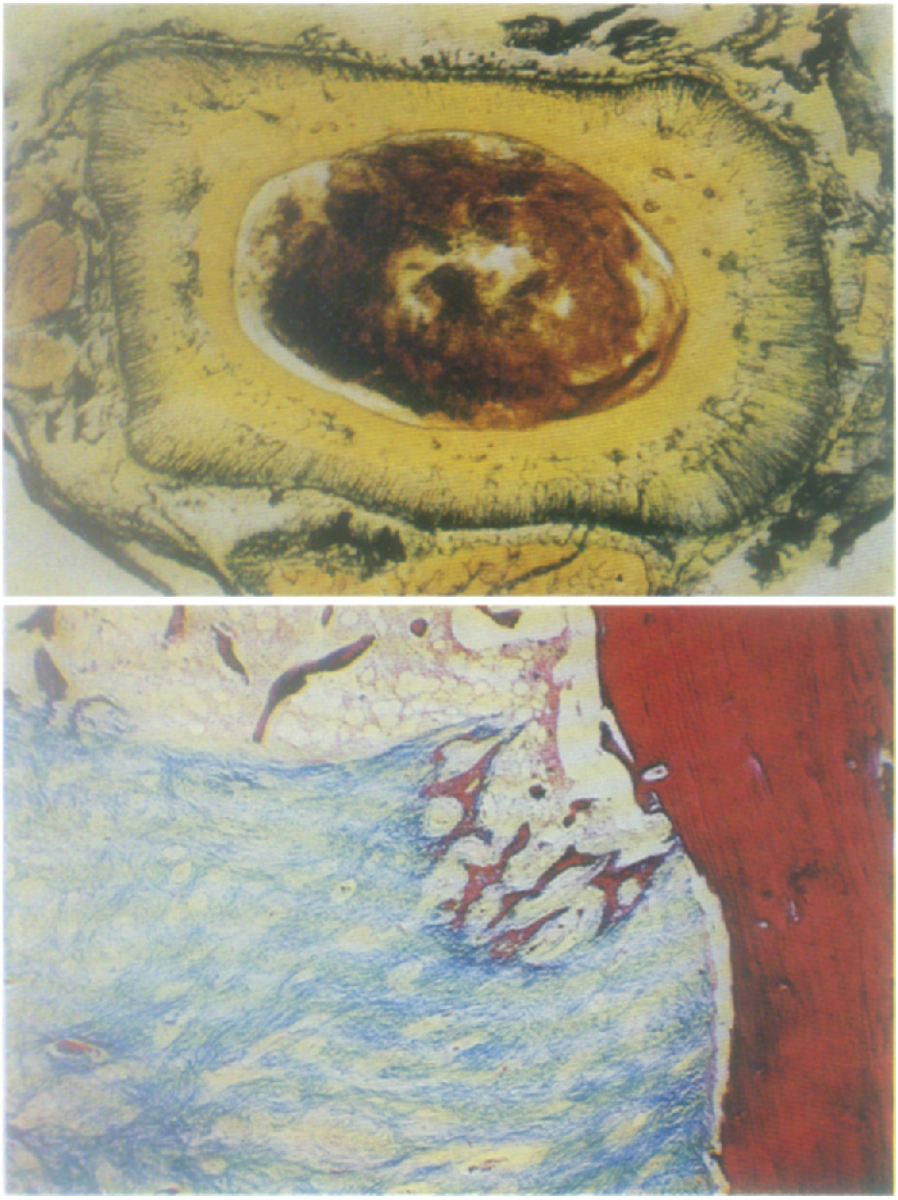

Plate 2. (*a*) Distal Spalteholz cross section, observation time 2 weeks. Growing animal 45. There is a strong periosteal vascular reaction with the formation of vessels perpendicular to the original cortex (formation of trabecular bone) except in the central part of the ventral sector (above), where several layers of convoluted vessels have formed (formation of primary osteones). Under the palisade-formed blood vessels a large part of the original cortex is avascular and in several areas is undergoing resorption from the surface of broom-shaped vascular proliferations. Under the convoluted vessels ventrally, only the central part of the cortex is avascular. Revascularization is taking place here, largely by "cutter heads".

(*b*) Azan-stained histological section from a dog, 5 weeks after reaming of the medullary cavity. In the middle of the section red-stained trabecular bone is formed close to the blue-stained fibrous area. In other parts of the section the fibrous area borders on old cortical bone or newly formed almost normal marrow. This may indicate that the fibrous tissue will remain in its present state.

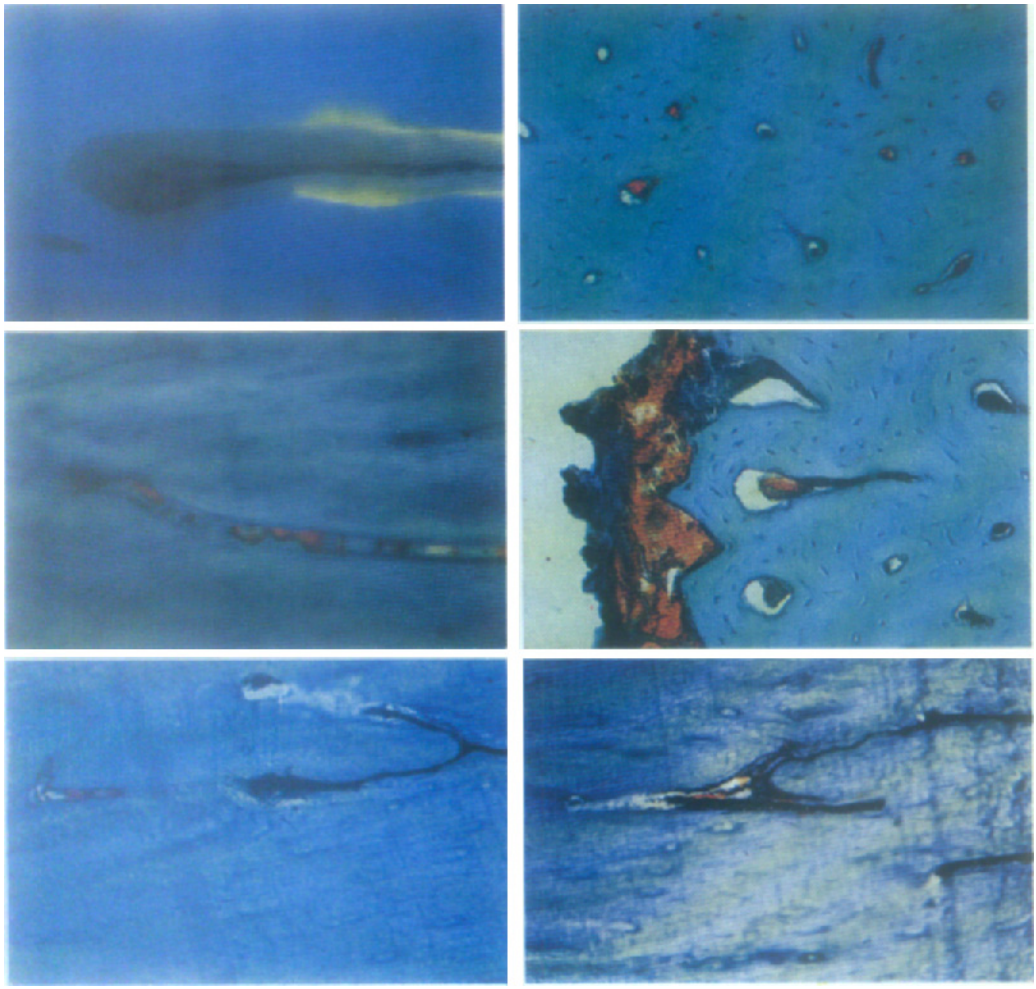

Plate 3. *(a)* Longitudinal fluorescence section of the diaphysis from an untreated tibia. Animal 124. Labelled 2 days before angiography. A "cutter head" with a vessel loop forming a secondary osteone.

(b) Sudan-stained cross section of rabbit tibia 1 day after brushing of the medullary cavity. Several of the intracortical canals are filled with red fat stained material. Other canals are empty or contain Indian ink-filled vessels.

(c) Sudan-stained longitudinal section of rabbit tibia 1 week after reaming of the medullary cavity. An intracortical canal is partly filled with Sudan-stained material.

(d) Sudan-stained cross section of rabbit tibia 1 day after brushing of the medullary cavity. Much Sudan-stained material is located below the periosteum and in some intracortical canals.

(e) Sudan-stained longitudinal section of rabbit tibia 4 weeks after reaming of the medullary cavity. A "cutter head" is revascularizing an osteone whose canal in front of the cutter head is obliterated by fat.

(f) Sudan-stained longitudinal section of rabbit tibia 4 weeks after reaming of the medullary cavity. The intracortical canals have become widened. Indian ink-filled vessels have passed by the fat droplets which have not yet been resorbed.