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EARLY FRACTURE CALLUS IN NORMAL AND CORTISONE TREATED RATS

A Study by a Combination of Tetracycline Labelling, Microangiography and Microradiography.

By

ANDERS HULTH and SVEN OLERUD

The healing process in experimental fractures has been the subject of comprehensive studies by various methods. The most important histological investigations in the last twenty years were those by *McLean & Urist* (13), *Pritchard & Ruzicka* (17) and *Ham & Harris* (7). These investigators showed primarily the dominant role of the periosteum in healing. Osteogenic cells in the deep layers of the periosteum proliferate and are differentiated into osteoblasts and bone salts are precipitated. The periosteal proliferation begins at some distance from the fracture line and then continues via the periosteum, which is raised from the corticalis, in towards the fracture line, forming a periosteal cuff. Callus is also formed from the endosteum. On a level with the fracture line, where the blood supply is poorer, cartilage is often formed instead of bone. The actual ends of the fracture are dead, the osteocytes there do not stain and even if they were alive, they could not take part in the osteogenesis, as they are enclosed in lacunae. It is an open question as to what extent undifferentiated cells from the bone marrow, the Haversian canals and the surrounding muscular tissue actively participate in osteogenesis. The precipitation of bone salts in the proliferation zone of the periosteum takes place at an early stage and can already be observed on a microradiogram on the fourth day (16). *Tonna & Cronkite* (21) investigated the cellular response with tritiated thymidine. They found that the cell proliferation took place mainly in the osteogenic layers of the periosteum but also in the mesenchymal

cells of the surrounding soft parts and that maximum proliferative response occurred 32 hours after the fracture.

Several authors have carried out microangiographic investigations on experimental fracture callus. *Lexer* and later *Koloday* found that local hyperaemia arises as the fracture heals. *Teneff* (20) found that the blood vessels derived mainly from the bone ends and from the pericallous soft tissue. *Ladányi & Hidvégi* (12) found that the most important vascularization came from the marrow cavity, whilst the periosteal vascularization was less pronounced. *Wray & Lynch* (25) made plastic casts of the blood vessels from the extremities of the rat and compared the number of vessels in the fractured and the uninjured legs after the soft parts had been removed with potassium hydroxide. There was a substantial increase in vascular response from the third day onwards, with the largest peak on the ninth day. *Göthman* (6) investigated by microangiography the blood vessels in operative fractures of the tibia in rabbits and monkeys and found, as a constant feature that it was primarily the vessels in the soft parts round the fracture that proliferated as bundles in towards the fracture haematoma. *Rhinelander et al.* (18) investigated by microangiography undisplayed closed fractures of the radius and ulna in adult mongrel dogs from one day to eight weeks after the fracture. They found that the medullary vessels were of greater importance than the periosteal in this type of fracture. The effect of cortisone on fracture healing was studied by *K. Kowalewski* (11), who found in humerus fractures in rats that the uptake of S^{35} was considerably less than in normal fractures. *Storey* (9), in a histological investigation of tibia fractures in rats, found that cortisone produced so-called dense callus. Cortisone does not prevent early callus but causes retarded resorption, leaving large amounts of cartilage callus. Bone of various degrees of maturity and non-union of the fracture were characteristic findings. *Duthie & Barker* (3) found that cartilage callus was rather calcified than ossified under the influence of cortisone.

The work reported here is an investigation using tetracycline-induced fluorescence of proliferating bone tissue during fracture healing, combined with indian-ink microangiography on the same preparations. These preparations were in addition examined microradiographically. *Milch* (14, 15) found that in man and in experimental animals tetracycline localized itself in the newly formed bone and could be traced there with the aid of ultraviolet light, emitting a golden-yellow fluorescence. Several investigations have since been made on tetracycline la-

belling, principally by *Frost* (4, 5) who has devoted a large number of papers especially to the conditions under which bone grows. *Harris et al.* (8) have recently published the results of a comparative investigation of labelling with different tetracyclines and autoradiography with Ca^{45}. They found that the tetracyclines marked all the points of active bone formation. The lacunae were also marked. *Vanderhoeft et al.* (24) carried out an investigation of tetracycline marking of the Haversian systems and studied their growth with repeated doses of tetracycline. They also made comparisons between tetracycline localization and the degree of mineralization using microradiography.

MATERIAL AND METHODS

Fractures of the tibia (left leg) were caused in 50 adult white weighing 200 g. by breaking by hand. The fracture was displaced *ad libitum;* this was considered more suitable than altering the local experimental conditions by an operative intervention in the fracture region. Cortisone was administered to half the rats; 5 mg. of Cortidrin® (Astra) per 100 g. body weight was given intramuscularly each day after the day of fracturing. After various periods (3, 7, 10, 14 and 17 days) five or six animals were taken from each group for examination. Two days previously the animals had received Terramycin® (Pfizer) in a dose of 50 mg. per kg. body weight. Under ether narcosis the abdominal aorta was exposed and a catheter inserted in it, through which Indian ink diluted with physiological saline (NaCl, 1: 3) was injected by hand with an ordinary syringe, after the animal had died. The injection was given instalments, with pauses after each 1–2 ml. in order to eliminate the risk of bursting blood vessels and capillaries. The animals were stored in the deep freeze until the whole experimental series was ready. The bodies were then thawed in 10 per cent formalin, the lower legs were detached and skinned and subjected to further fixation in formalin for another 24 hours. The remaining soft parts were left undisturbed. The specimens were then dehydrated in alcohol. Storage for 24 hours in unpolymerized methyl methacrylate preceded the final bedding in methyl methacrylate, to which 5 per cent of butyl phtalate had been added. After polymerization the block of plastic was sawn into slices 0.5 mm. thick in the longitudinal direction of the lower leg. The slices were photographed by ordinary X-ray and selected sections were ground by hand to a thickness of 100 μ. These sections were then examined by microradiography and afterwards mounted under coverslips in fluo-

rescence-free balsam (Permount). The mounted preparations were then studied in a fluorescence microscope fitted with an UV light source. One or two animals in each group were used for histological examination. Decalcification was carried out with formic acid, the specimens were bedded in paraffin and the sections, 10 μ thick, were stained with haematoxylin-eosin.

RESULTS

With one or two isolated exceptions, the fluorescence in our preparations was very good. There was, however, a certain reduction of intensity in the case of the animals treated with cortisone. The indian-ink filling of the preparations was likewise complete, with one or two isolated exceptions. In describing the healing of these dislocated fractures, it is possible and advantageous to distinguish three areas of regeneration, viz. the periosteal and the endosteal proliferation areas and the interfragmental area. In the first two areas callus is formed in connection with vascular hyperplasia and in the third there is an ingrowth of mesenchyme from the soft parts surrounding the fracture, bringing with it blood vessels for resorption of the fracture haematoma. In what follows we shall therefore deal with each osteogenic field separately and explain the differences we found between normal healing and healing under the influence of cortisone.

1. *The Periosteal Osteogenic Area.*

As early as the first few days, cell proliferation takes place in the deep layers of the periosteum (Fig. 1). The maximum proliferation takes place at a certain distance from the actual end of the fracture and then gradually decreases along the injured leg within an area of varying size. The normal vascular supply to periosteum and bone consists of longitudinal vessels outside the fibrous layer. From these vessels small branches pass in often perpendicularly through the periosteum and through the corticalis. The proliferation layer in the periosteum increases in width at a rapid pace, the distance of the longitudinal vessels from the corticalis increases and the perpendicular vessels in the proliferation layer of the periosteum increase especially in length but also in thickness. As early as the third day after the fracture, there appears on microradiograms from both normal and cortisone fractures a growth of poorly mineralized osseous spiculae in the basal part of the periosteal proliferation zone. These spiculae fluoresce very clearly

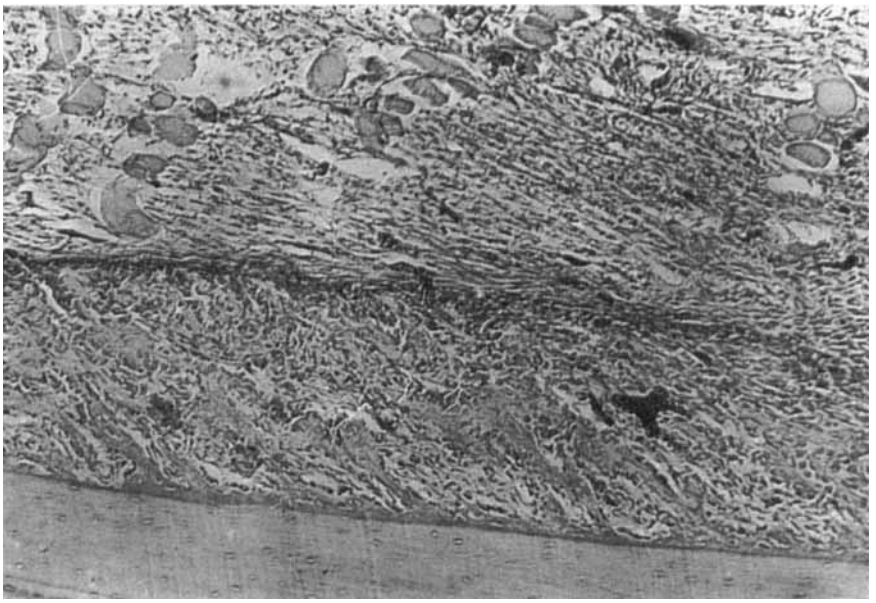

Fig. 1.
Microphotograph. Periosteal callus on the fourth day. At the bottom the corticalis, above it the periosteal proliferation with newly formed trabeculae. The periosteal zone is separated from the surrounding musculature by a fibrous layer. Haematoxylin-eosin. Magnification × 30.

with the Terramycin taken up (Figs. 2 a and b). After a week the periosteal callus has increased considerably as regards both mineralization and vascular hypertrophy, as is clear from the microradiograms and the fluorescence microscopy. In Figs. 3 a and b we see how the blood vessels penetrating the periosteum run in parallel through the callus and, after having changed direction somewhat, penetrate the corticalis, and then empty themselves in the veins of the marrow cavity (Fig. 3 b). We often see how the blood vessels penetrating the periosteum gather together as it were in the shape of a fan in the direction of the area of maximum proliferation (Fig. 3 b). The direction of these transverse periosteal vessels seems to be independent of the progress of the vessel through the corticalis.

In displaced fractures produced by the above method, the periosteal injury will as a rule be greater on the "convex" side of the displacement than on the "concave" side. On the former side the periosteum must necessarily be torn off, whilst on the latter side there is some possibility of its holding together like a bridge between the ends of the fracture.

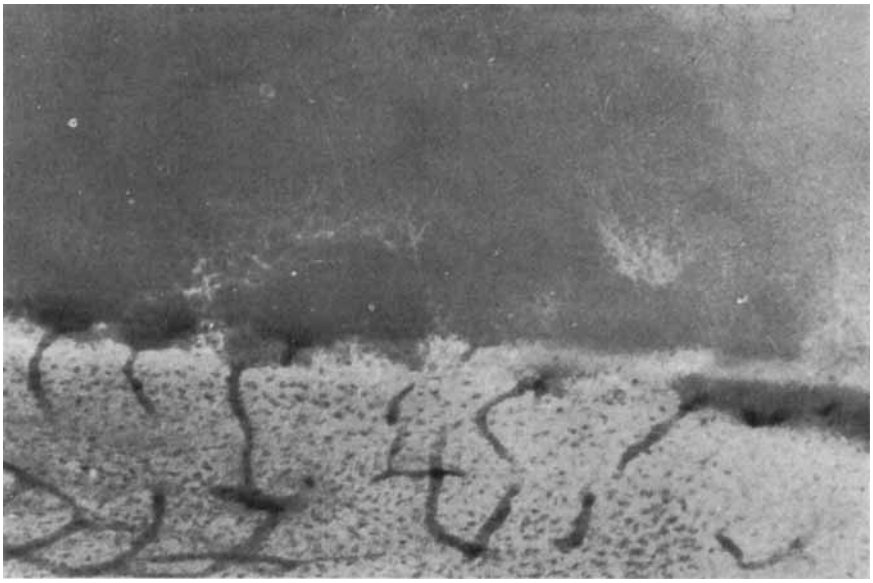

Fig. 2 a.
Microangiograph. Callus three days old in a cortisone-treated rat. Bone trabeculae with a low degree of mineralization are visible outside the corticalis. Thickness of section 100 μ. Magnification × 50.

On the more protected side the periosteum, in spite of the fact that it has been released from the corticalis, seems to form the regeneration centre for the callus, which often has the form of a bridge between the fragments. Several preparations show this state of affairs and there does not seem to be any difference in the principle of the bridge construction between normal rats and cortisone rats (Fig. 4). This bridge-building callus consists of trabeculated bone, is practically always developed without an intermediate cartilage stage and is therefore reminiscent of the purely periosteal, bone-callus formation. There are perhaps not sufficient periosteal vessels to supply this periosteal flap but it also seems to be possible to obtain a supply of blood through vessels newly formed from the environment and growing into the flap. This state of affairs seems also to be proved by Fig. 4. The superseded periosteum may therefore conceivably serve more or less as an autoplastic graft.

The rats under cortisone medication develop a periosteal callus which is in principle completely identical with the callus in the normal rats. However, in the cortisone rats there are remarkably often an irregular-

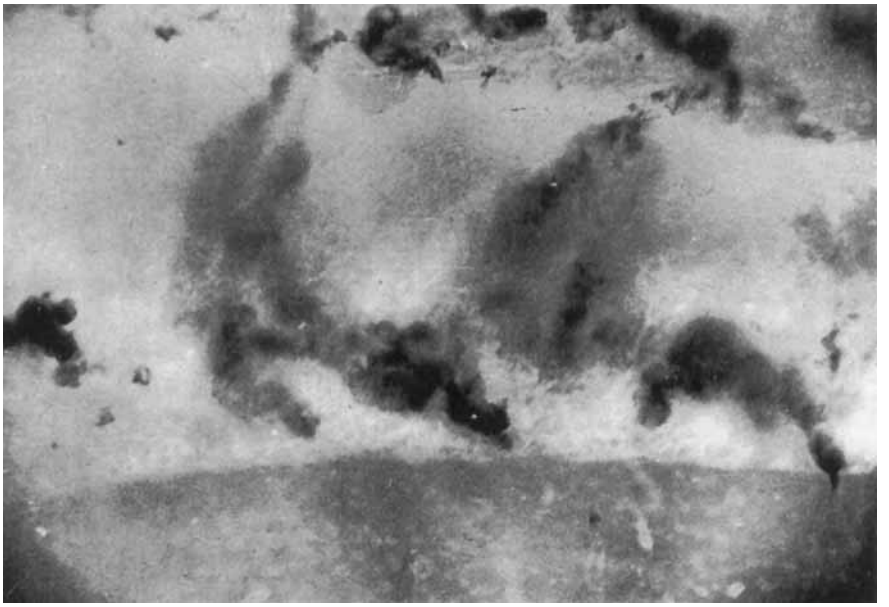

Fig. 2 b.

Microangiography + fluorescent microphotograph of the same spot as Fig. 2. a. The newly formed trabeculae fluoresce relatively distinctly. Vessels filled with Indian-ink pass perpendicularly through the callus. Thickness 100 μ. Magnification × 50.

ity and an absence of trabecular structure, together with avascular areas. In respect of time, there also seems to be a pronounced delay as regards callus development.

2. The Medullar Osteogenic Area.

Proliferation in the opening of one or both fragments is visible at the same early stage as proliferation in the periosteal zone. The micro-angiograms reveal vascular hyperplasia stretching out towards the opening of the fragment in the form of a bundle. Even on the third day a Terramycin fluorescence is observed round these vessels, indicating incipient mineralization. Callus is established here directly as trabecular bone without an intermediate cartilage stage. On the seventh day after the fracture there is already a large callus which begins a few millimetres inside the opening of the fragment and grows up to it (Fig. 5). The callus formation seems to be preceded by a vascular bundle pointing in the direction of the haematoma outside. The medullar callus formation may occur in both the proximal and the distal

Fig. 3 a.
Microangiography + fluorescent microphotograph of callus 7 days old in the immediate vicinity of the fracture site. The vessels run at right angles through the strongly fluorescing callus towards the corticalis. Section 100 μ. Magnification × 50.

fragment. It is sometimes absent in the distal fragment, probably owing to the fact that the vascularization there has been damaged. The marrow callus seems to be strictly confined to the actual marrow cavity. We never find this callus continuing outside the opening of the fragment into the interfragmental fracture zone. We have the impression that the callus proliferation does not take place from the endosteum to any particular extent. To judge from our figures, it seems more likely that bone formation develops in immediate connection with the growing blood vessels.

The entire marrow cavity in the upper fragment is always abundantly vascularized and seems to be so to an even greater extent than is the case in our formal preparations without fractures.

The medullar callus formation seems to develop in the same way in both the normal fractures and the cortisone-treated fractures. The formation of perivascular bone trabeculae takes place equally rapidly and on the same scale in both the series.

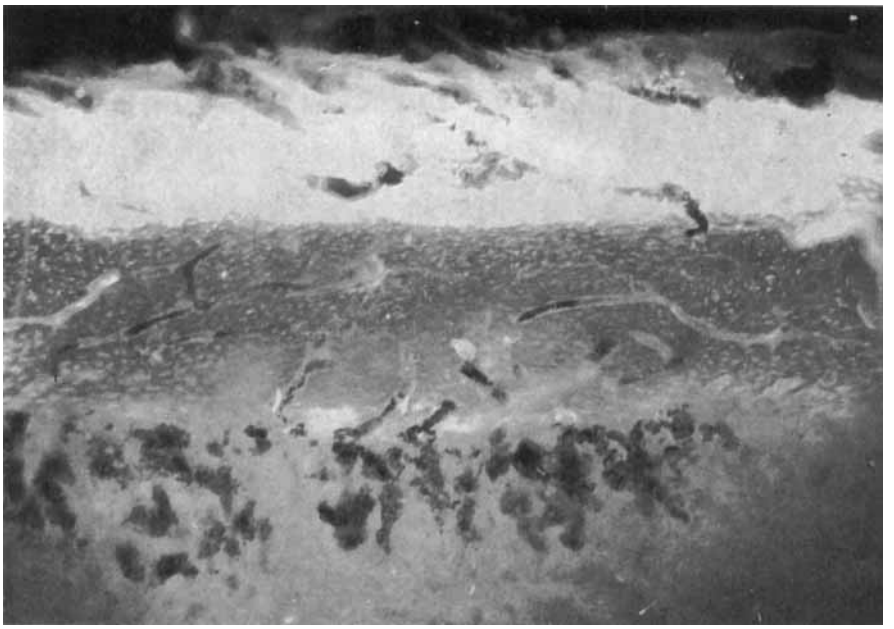

Fig. 3 b.
Microangiography + fluorescent microphotograph of callus 14 days old at a distance from the fracture site. Here the vessels run obliquely through the callus directed towards the fracture site (to the right in the picture). After passing the callus they change direction, penetrating the corticalis, into the veins of the marrow canal. 100 μ. Magnification × 50.

3. *The Interfragmental Area.*

In a displaced fracture there is formed around and between the two fragments a haematoma, in which small fragments of bone and also fragments of muscular and connective tissue are often to be found. In histological sections we find in this area, on the second and third days after the fracture, a cell proliferation which, after a few more days, has increased very rapidly in extent. In this mass of cells there arise in several places islands of cartilaginous tissue, probably on account of the fact that the vascularization does not proceed in step with the cell proliferation (Figs. 6 a and b).

Microangiograms from the third day after the fracture show a vascular proliferation from the soft parts of the environment, consisting mainly of musculature in towards the interfragmental fracture area. The blood vessels coming from the marrow cavity also turn in the direction of this interfragmental area. All these vessels are strictly di-

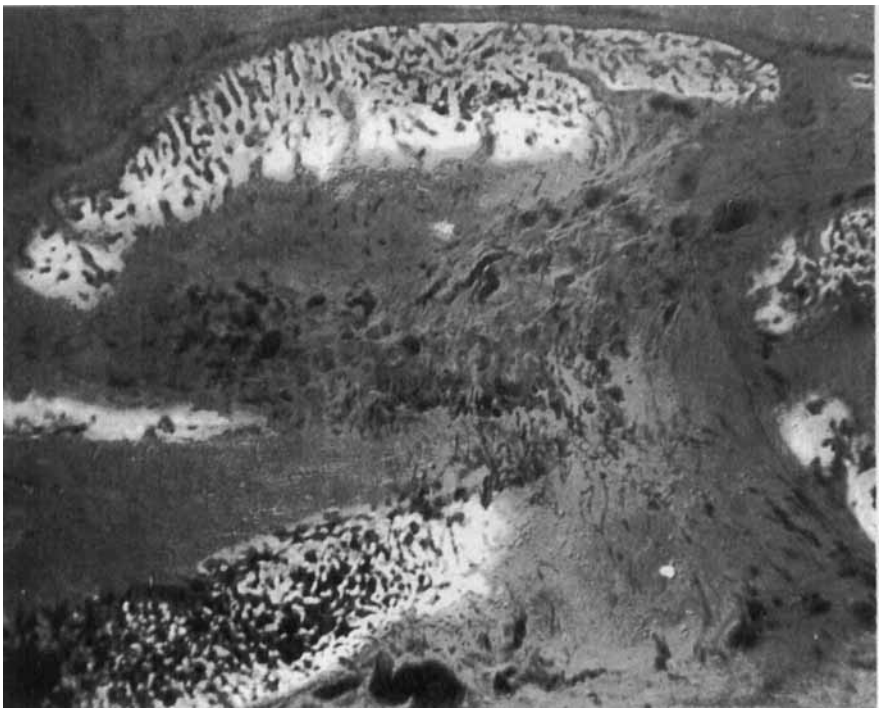

Fig. 4.
Microangiography + fluorescent microphotograph of periosteal bridging callus at the top. To the left periosteal callus is forming on the inferior side. The bridge is partly nourished by vessels coming from the interfragmental area. Normal fracture 18 days old. 100 μ. Magnification × 15.

rected to the apparently empty area, *i.e.* the haematoma, between the ends of the fracture. This phenomenon is particularly clear in cortisone preparations from the seventh day after the fracture (Fig. 7). The reason is probably that the haematoma is resorbed and transformed so much more slowly under cortisone medication.

A bone callus is developed in the cell proliferation which arises in the interfragmental area. In many places this transformation to bone takes place via cartilage tissue. In the mass of cells there arise islands of cartilage tissue which are calcified in their matrix. These islands of cartilage have no direct blood supply, but they are probably nourished by diffusion from surrounding vessels. The calcium deposited also probably obtains its tetracycline by a process of diffusion. The microangiograms demonstrate the existence of these mineralized islands of

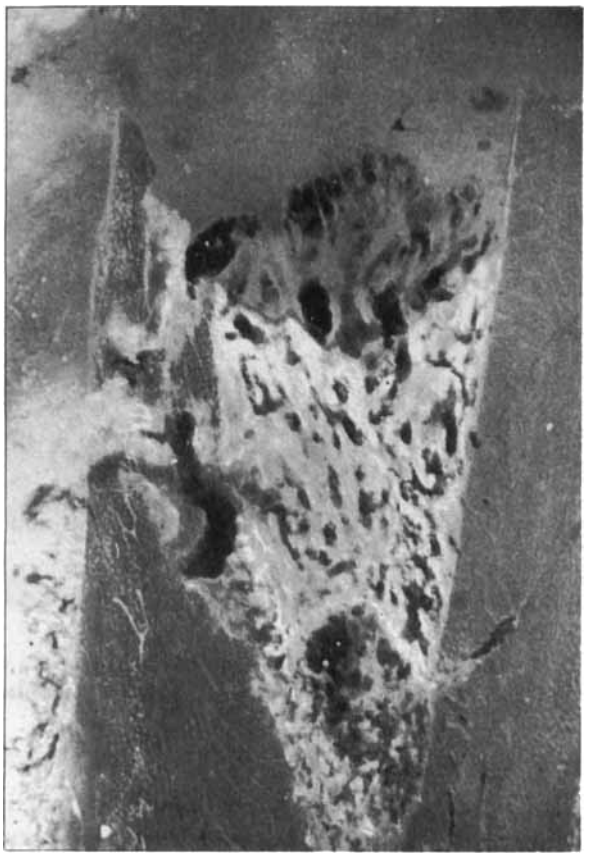

Fig. 5.

Microangiography + fluorescent microphotograph. Marrow callus 8 days old of cortisone-treated rat. From the opened marrow cavity outgrowth of osseous callus preceded by a vascular fan. To the left periosteal callus of poor trabecular structure. 100 μ. Magnification × 30.

cartilage by their very strong tetracycline fluorescence. The preparations likewise demonstrate the palisade-shaped pattern of blood vessels surrounding the cartilage, though not penetrating it. In a comparison of these microangiograms with the corresponding microradiograms we find that the mineralization seems to be of a remarkably low grade, in spite of the strong fluorescence in these areas (Fig. 9). Here and there, however, vessels seem to break through the islands of cartilage and at these points the mineralized cartilage is transformed into a trabecular bone tissue, though of irregular form (Fig. 8).

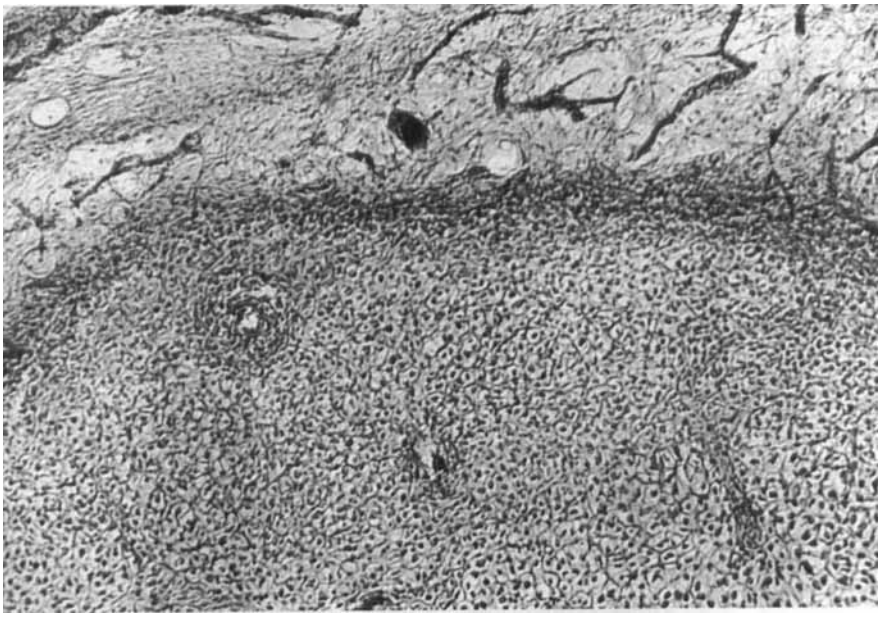

Fig. 6 a.
Microphotograph of interfragmental callus 8 days old. A large area of almost avascular cartilage is seen. Outside the cartilage an abundant vascular mesenchyme. Haematoxylin-eosin. Magnification × 50.

A comparison between normal fractures and the cortisone-affected fractures reveals a considerable difference in callus formation in the interfragmental area. The mineralized cartilage tissue is considerably more widespread and more irregularly shaped in the cortisone-affected fractures than in the normal fractures. The transformation of cartilage to bone takes place much more slowly and is more incomplete in the former. In the normal fractures, on the other hand, there is an often rapid development into trabecular bone. In many places this transformation seems to take place without an intermediate cartilage stage. In preparations from cortisone-treated rats there occur areas that are relatively large and apparently empty of blood vessels. Blood vessels are certainly directed towards these areas but they seem to have difficulty in growing further in towards the cartilage area. There seems to be far-reaching retardation of the whole healing process, particularly as regards the resorption of the haematoma.

Microradiographs of the healing of the fracture also show a great difference as regards the quantity of callus.

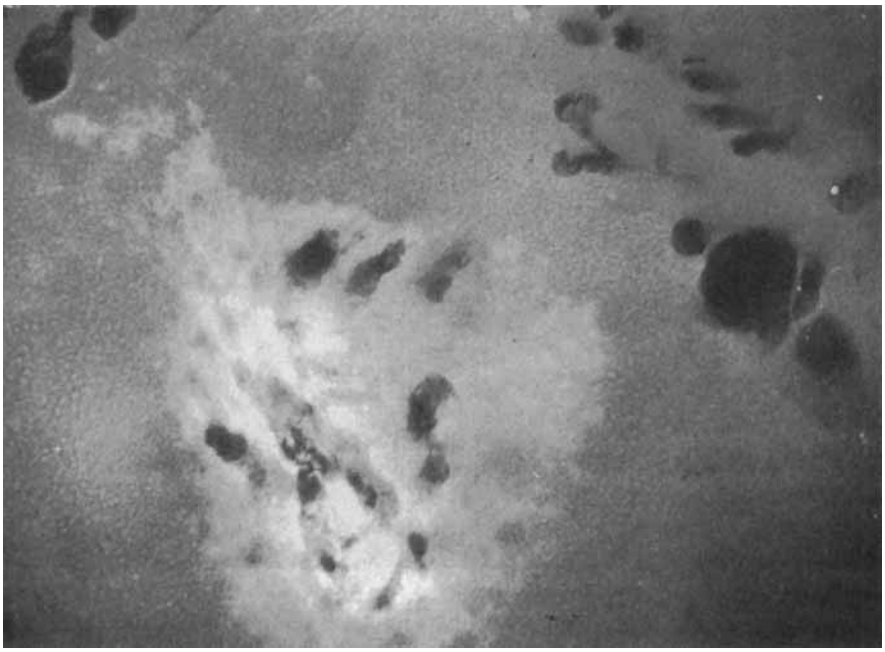

Fig. 6 b.
Microangiography + fluorescent microphotograph of an area similar to that in Fig. 6 a. Callus 7 days old of cortisone-treated rat. The cartilage is in the center perforated by vessels around which trabecular bone is being formed. At the top vessels forming a "palisade". No fluorescence in the cartilage but the newly formed bone is strongly fluorescing. 100 μ. Magnification $\times$ 50.

In several preparations non-vital osseous tissue which appeared to be undergoing resorption was found in the fracture area. In preparations from a week after the fractures there is found an abundance of parallel vessels directed towards apparently avascular bone fragments or parts of the corticalis (Fig. 10). This phenomenon occurs both in the normal and the cortisone-affected fractures. In preparations from our normal animals 14 days after the fracture, blood vessels reached the dead bone. These vessels or their terminal capillaries applied themselves to the bone surfaces in a way similar to amoebae. In the corresponding microradiograms there is revealed an incipient resorption of the bone corresponding to the site of the vascular phenomenon (Figs. 11 a and b). Other preparations also showed vascular perforations through the bone substance. Microradiograms with bone resorption were not found in the cortisone-treated fractures, nor was it possible

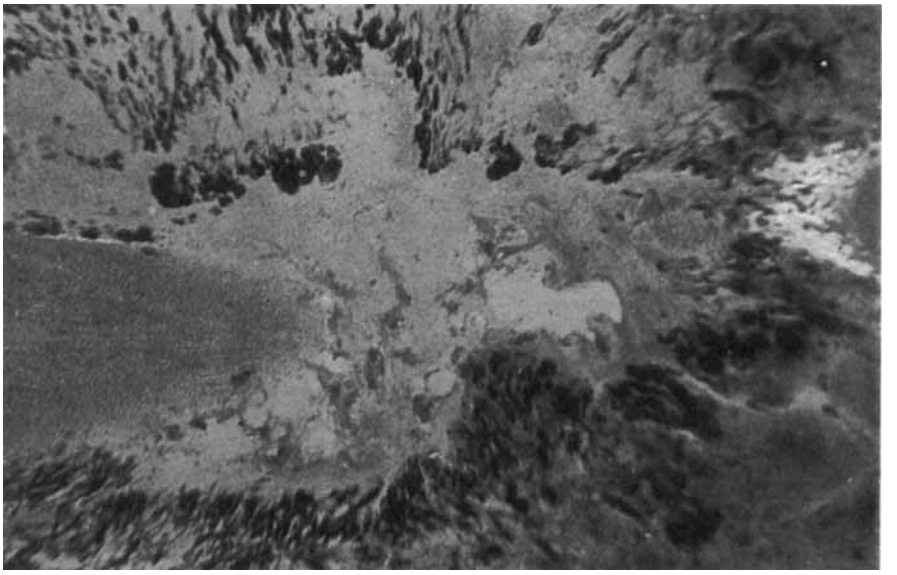

Fig. 7.
Microangiography + fluorescent microphotograph of the interfragmental area of a fracture 7 days old in a cortisone-treated rat. To the left and to the right the two fragments. Towards the haematoma vessels are growing in from the surrounding soft tissue. 100 μ. Magnification × 15.

to demonstrate in these portions of dead bone Terramycin fluorescence also indicating active bone deposition.

DISCUSSION

This investigation, using combined microradiography, microangiography and tetracycline-induced fluorescence, was carried out on tibia fractures in rats in order to study the relation between blood vessels and the reparative process in bone. We purposely chose fractures which could be freely displaced. All methods of standardizing a certain type of fracture involve further intervention besides the actual fracturing, which may disturb the mesenchyme's mode of reacting. Skin incisions and incisions through musculature for the purpose of producing an operative fracture may conceivably involve such disturbances; so too, and to a still greater extent, may application of osteosynthesis material of various kinds. The displacement is also necessary in order to obtain a sufficiently large fracture haematoma.

Three different osteogenic areas come into operation in a fracture of

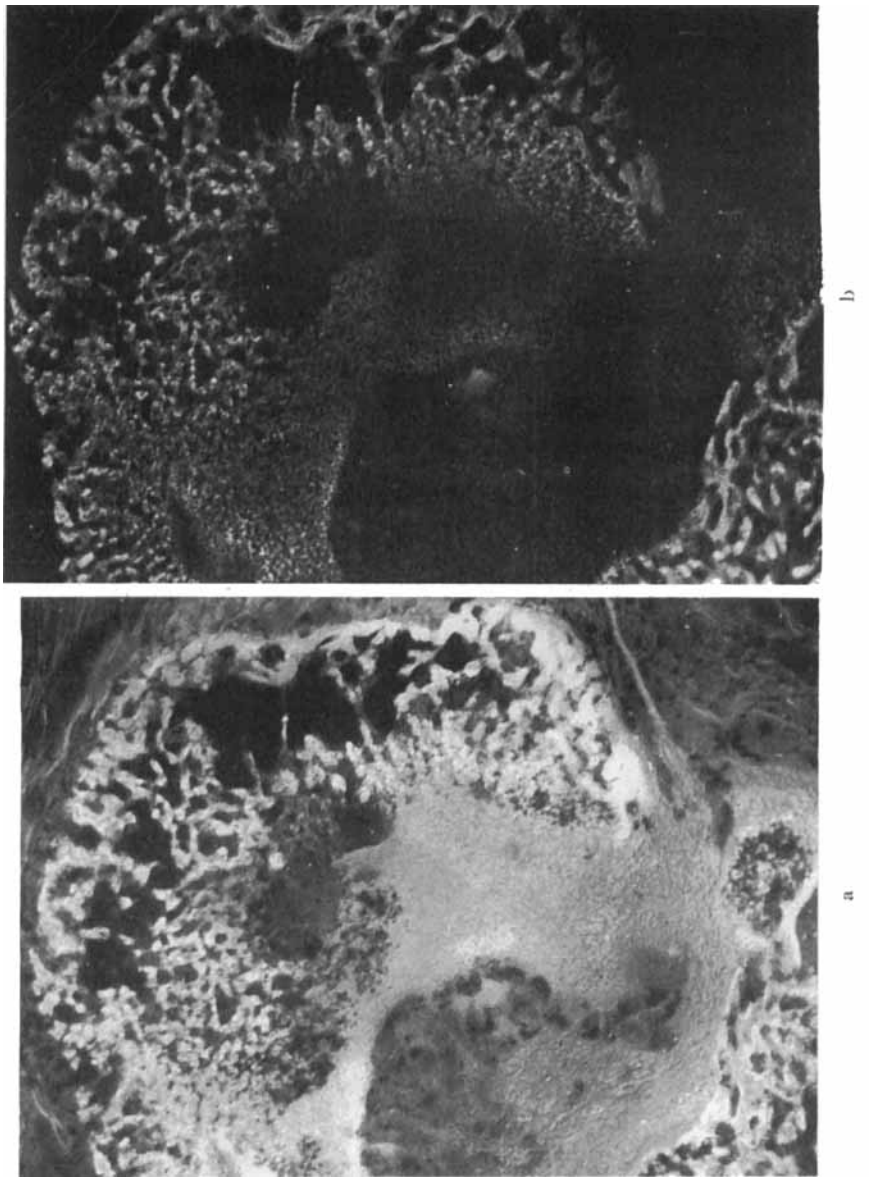

Fig. 8.

Microangiography + fluorescent microphotograph (8 a) compared with microradiography (8 b) of the same place in an interfragmental area. Normal fracture 17 days old. At the top trabecular bone with moderate fluorescence and mineralization. Centrally a cartilaginous area with vessels forming a "palisade" and relatively abundant fluorescence. This mineralization is calcified cartilage. 100 μ. Magnification $\times$ 30.

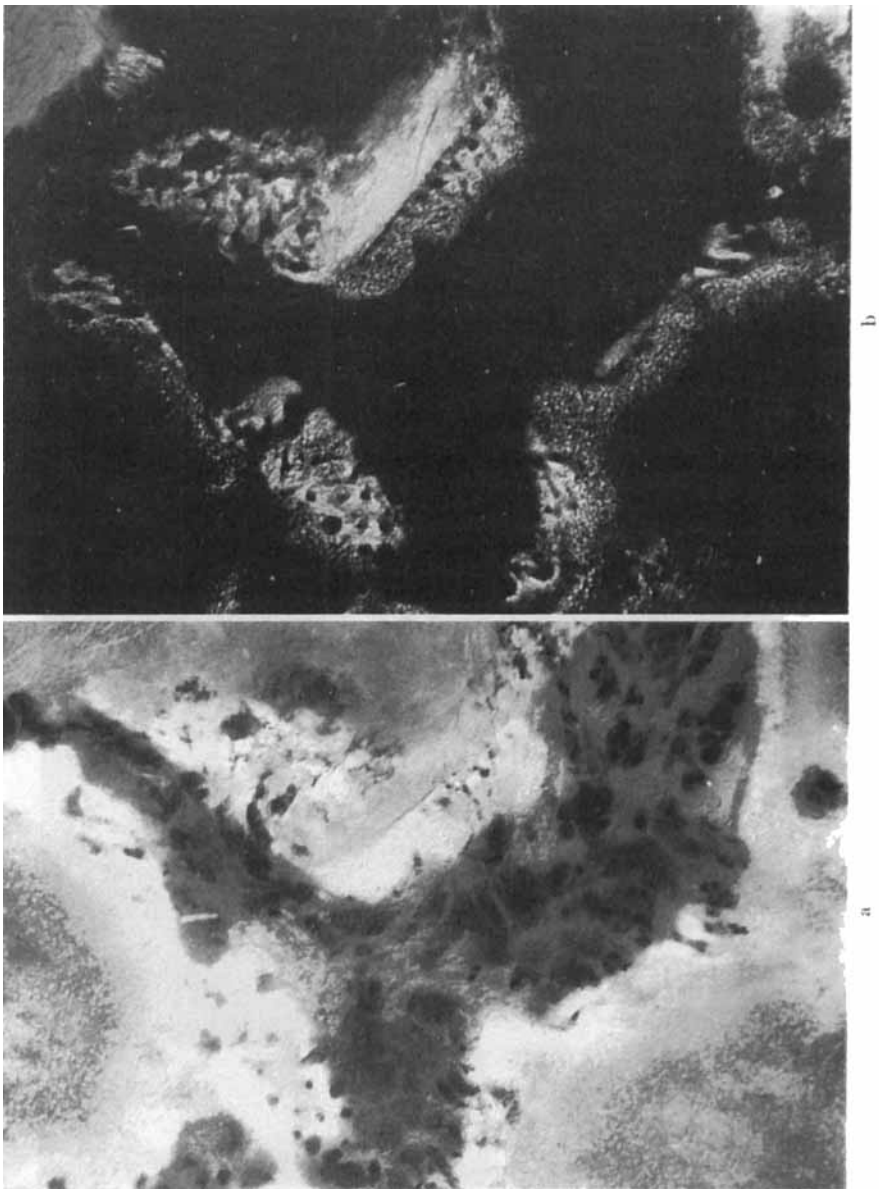

Fig. 9.

Microangiography + fluorescent microphotograph (9 a) compared with microradiography (9 b) of the same place in an interfragmental callus of a cortisone-treated rat. 17 days old. There is mainly cartilage with calcification of low degree taking up abundant fluorescence. Solely small areas of poorly trabecular bone. To the right old fragments highly mineralized but not fluorescent. 100 μ. Magnification × 30.

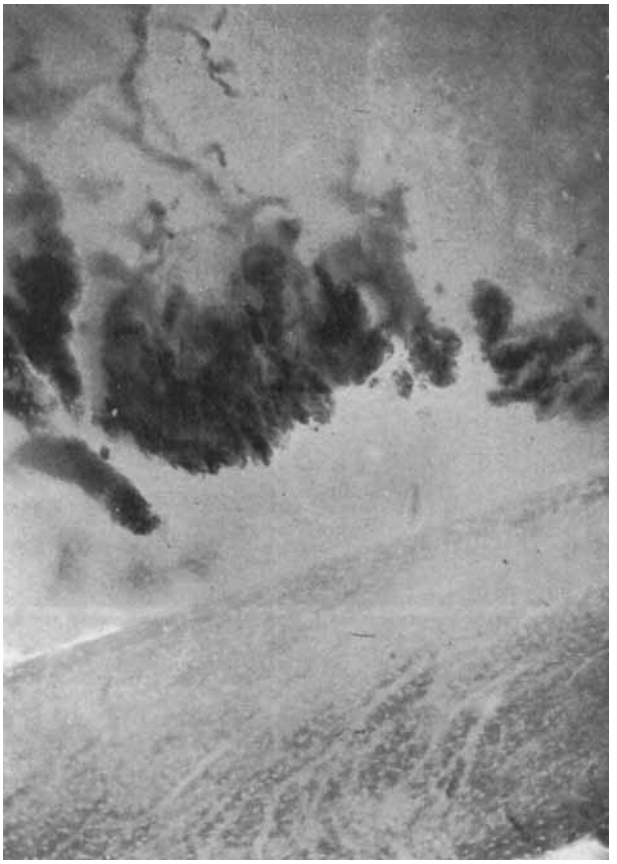

Fig. 10.
Microangiography + fluorescent microphotograph. Probably avascular part of corticalis, towards which newly formed vessels are directed. 100 μ. Magnification × 50.

this kind, two "natural", *i.e.* operative during the growth period (the periosteal and the marrow area), and a third which arises owing to ingrowth of blood vessels from the surrounding musculature. The marrow zone is not necessarily made up of proliferation from the endosteum but may instead be a perivascular osteogenesis originating from the mesenchyme of the bone marrow. This marrow osteogenesis forms the so-called sealing callus, which seals the open marrow cavity. In delayed healing of a fracture this sealing of the marrow cavity seems to be an evil, in that it prevents vascular contact between the marrow cavity and the interfragmental area and may thereby perhaps contri-

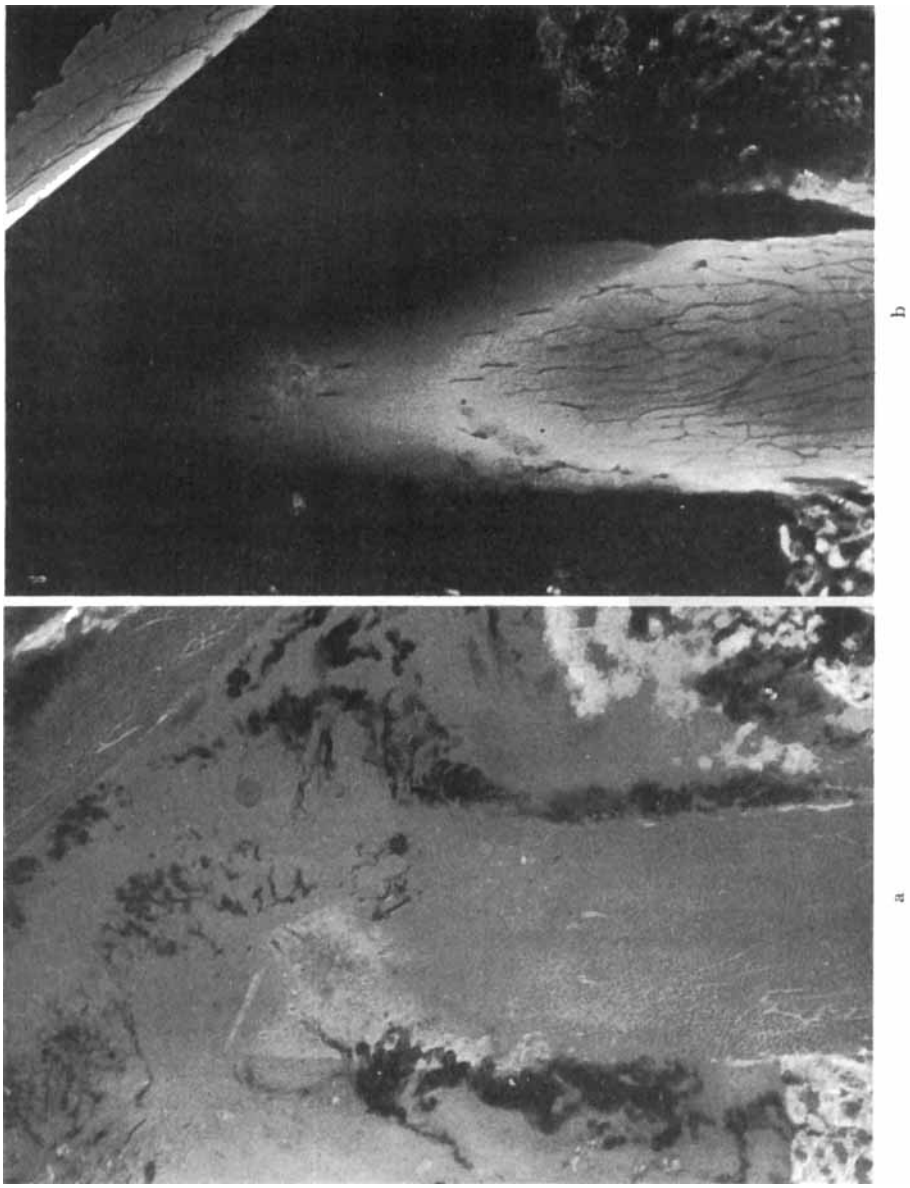

Fig. 11.

Microangiography + fluorescent microphotograph (11 a) compared with microradiography (11 b) of a necrotic bone fragment which is undergoing resorption by vessels applied to the bone surface. The microradiograph of the same spot shows incipient resorption. At the bottom fluorescent callus with a low degree of mineralization. 100 μ. Magnification × 30.

bute to the genesis of a pseudoarthrosis. We found a similar sealing of the marrow cavity with respect to the healing of amputation stumps in rabbits (9). That this callus can be formed directly from the blood vessels is to some extent confirmed by *Trueta*'s (22, 23) finding that osteogenesis takes place owing to the conversion of vascular endothelial cells to osteogenic cells.

The interfragmental osteogenic field arises through a substantial cell proliferation in the area of the fracture haematoma, which is thereby transformed. This transformation may take place owing to the ingrowth of blood vessels from the environment. The blood vessels contribute to the resorption of the fracture haematoma and nourish the developed cell proliferation. The vascular ingrowth directed towards the haematoma from the environment and from the marrow is probably due to some still unknown, vessel-stimulating factor (VSF) (*Trueta* (23)). This investigation cannot elucidate whether the actual proliferation derives from cells in the haematoma or from cells immigrating from the environment. *Tonna & Cronkite* (21) found, however, on labelling tritiated thymidine that cell division also took place in the surrounding mesenchyme, as well as in the periosteal field. These authors do not seem to have investigated in detail the interfragmental area.

The relatively substantial cortisone medication administered does not seem to have had any effect on callus formation within the marrow cavity. In the periosteal callus zone, on the other hand, the cortisone seems to have retarded the course of development. An irregularity and sometimes also avascular portions could be observed within the callus area. The interfragmental callus formation was affected by the cortisone to an even greater extent. The vascular proliferation in the direction of the haematoma seemed to be developed relatively quickly but, in spite of this, the haematoma did not seem to be resorbed in the normal way, *i.e.* largely disappearing in the course of a week. This was the case in all normal fractures. In the animals that received cortisone large areas were found consisting of poorly mineralized but strongly fluorescent cartilage tissue without any real tendency to conversion into trabecular bone callus. In the normal fractures osseous callus seemed to be largely developed directly from the osteogenic cells without a perceptible intermediate stage as cartilage. The fractures in the normal animals were healed in the course of 20 days from both the clinical and the radiographical point of view. This was not the case to any great extent with the cortisone-treated animals. Cortisone seems therefore to impede the specific osteogenic properties in the ingrowing

mesenchyme, possibly producing a defect in the collagen synthesis. The resorption of debris at the fracture site also seems to be reduced. Our findings respecting the noxious effects of cortisone on experimental callus are in good agreement with those of previous investigators (3, 19). By the method we used, the noxious effects of the cortisone could be localized to mainly two of the three proliferation areas, viz. primarily the intermediate and to a certain extent the periosteal. Probably this state of affairs is connected with the fact that the interfragmentary healing zone is that in which the callus is formed via a cartilage stage. It is this in the differentiation of the cartilage proliferation tissue to osseous callus that cortisone mainly introduces its noxious effects.

Interfragmentary callus formation is probably very important in fracture healing, especially in displaced fractures. Such fractures, as a rule, involve great injury to the periosteum and the soft parts. Even if the fracture is reset, healing is for this reason dependent to no small extent on the potency of the ingrowing mesenchyme. This may perhaps explain why disturbance of fracture healing in man is much more common in fractures that arise through high-energy direct trauma than in those that arise through indirect force (1, 10). The severe muscular and cutaneous injuries that then arise would seem to lead to the fracture environment losing its ability to mobilize potent mesenchyme for this intermediate callus formation. If, besides this, there is later a difficult, surgical resecting of such a fracture, perhaps with large osteosynthesis applications, further injury may conceivably arise in both the remaining periosteum and the soft parts. As regards the marrow callus, this does not seem to move outside the open orifice of the medullar cavity in the fracture site, but is probably of great importance in the healing of non-dislocated or slightly dislocated fractures (18). The task of the marrow callus seeems to be to seal the marrow cavity as quickly as possible rather than to take part in the re-uniting of the fracture ends, *i.e.* the healing of the fracture.

The above reasoning is based on an investigation of fractures in rats, but it seems improbable that the healing of fractures could take place in fundamentally different ways in animals and in man (except that the lower animal species, on account of their potent mesenchyme, heal their fractures so much more easily). An earlier author (6) found no difference in vascular proliferation in such different animal species as rabbit and monkey.

SUMMARY

Histologic, microangiographic, and combined tetracycline labelling and Indian-ink microangiographic techniques were used to study the healing of fractured tibia in rats. Half the animals received cortisone. In a displaced fracture there are three different sources of repair, viz. the periosteal, the marrow and the interfragmental areas. In the periosteal the marrow and the interfragmental areas. In the periosteal there occurs proliferation of the osteoblastic cell layer and hyperplasia of preexisting vessels. In the marrow area it is probably a matter of perivascular osteogenesis (not endosteal proliferation) and the callus does not grow past the opening of the fragment. The third field, viz. the interfragmental, is of great importance for healing. Numerous vessels grow from the surrounding soft tissues into the haematoma, where very rapid cell proliferation is brought about. Islands of cartilage arise, nourished by diffusion from end capillaries surrounding them. The cartilage is often poorly calcified but takes up plenty of fluorescing tetracycline, especially in the cortisone-treated animals. The cartilage is ossified through ingrowth of vessels. The most obvious difference between the normal and the cortisone-treated fractures is the delayed reconstruction of the fracture haematoma in the latter.

RESUME

Des moyens histologiques, microangiographiques et une combinaison d'étiquetage à la tétracycline et des techniques microangiographiques à l'encre de Chine ont été utilisés pour étudier la soudure des fractures du tibia chez les rats. De la cortisone a été administrée à la moitié des animaux. Dans une fracture déplacée, il existe trois sources de guérison, à savoir le périoste, la moelle et les surfaces interfragmentaires. Il se forme dans le périoste une prolifération de la couche des cellules ostéoblastes et une hyperplasie des vaisseaux préexistants. Dans la moelle, il est probablement question d'ostéogénèse périvasculaire (et non de prolifération endostéale) et le cal ne se développe pas par-dessus l'ouverture du fragment. Le troisième champ, c'est-à-dire la surface interfragmentaire est d'une grande importance pour la guérison. De nombreux vaisseaux surgissent à partir des tissus mous environnants dans l'hématome où des cellules de prolifération sont rapidement amenées. Des îlots cartilagineux apparaissent, nourris par diffusion des capillaires les entourant. Le cartilage est souvent pauvrement calcifié, mais absorbe bien la tétracycline fluorescente, spécialement

chez les animaux traités par la cortisone. Le cartilage est ossifié par la croissance des vaisseaux. La différence la plus apparente entre les fractures normales et celles traitées par la cortisone est le retard de reconstitution de l'hématome de la fracture dans le dernier cas.

ZUSAMMENFASSUNG

Histologische, mikroangiographische und kombinierte Tetracyclin-, Tuschemikroangiographische Verfahren wurden verwendet und die Heilung von Tibiabrüchen bei Ratten zu studieren. Die Hälfte der Tiere erhielt Cortison. Bei einem verschobenen Bruch findet man drei verschiedene Quellen der Wiederherstellung, nähmlich das periostale, das Mark- und das Gebiet zwischen den Bruchstücken. Im periostalen Bereich entsteht eine Proliferation der osteoblastischen Zellschichte und eine Hyperplasie vorhandener Gefässe. Im Markgebiete dreht es sich wahrscheinlich um perivaskuläre Osteogenese (nicht um endostale Proliferation) und der Kallus wächst nicht über die Bruchöffnung hinaus. Das dritte Gebiet, nähmlich das zwischen den Bruchenden ist von grosser Bedeutung für die Heilung. Zahlreiche Gefässe wachsen von den umgebenden Weichteilen in das Hämatom, wo eine rasche Zellvermehrung vor sich geht. Inseln von Knorpel, die mittels Diffusion von den sie umgebenden Endkappilaren ernährt werden, entstehen. Der Knorpel ist oft spärlich verkalkt, nimmt aber reichlich fluoreszierendes Tetracyklin, besonders bei den Cortison-behandelten Tieren auf. Der Knorpen wird durch das Hineinwachsen von Gefässen verknöchert. Der Ausfallendste Unterschied zwischen den normalen und den Cortison-behandelten Brüchen ist der verspätete Hämatomwiederaufbau bei den letzteren.

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