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THE NORMAL REPAIR OF EXPERIMENTAL FRACTURES

A Histo-quantitative Study of Rats

By

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The repair of a fracture of a long bone is brought about by the osteogenic tissue present in the periosteum and the endosteum, the osteogenic cells of the Haversian system and presumably also by undifferentiated surrounding mesenchymal cells (*Urist & McLean* 1941, *Pritchard & Ruzicka* 1950, *Ham & Harris* 1956). The initial proliferative response in the periosteum is seen 16 hours after the trauma (*Tonna & Cronkite* 1961) and calcified bone is histologically recognizable two or three days after the fracture, being laid down under the periosteal collar at a distance from the fracture site (*Urist & McLean* 1941). In experimental animals, the callus gains considerable tensile strength within 20–30 days, although the remodelling of the new bone takes place at a much slower rate (*Falkenberg* 1961). Both in experimental animals (*Bohr* 1955), and in man (*Wendeberg* 1961) the remodelling of the callus, as judged from studies with bone-seeking isotopes, continues for several months.

Although the histological course of events in the repair of fractures is well documented, few reports are available regarding the quantitative relations of the various tissue components in the callus during the repair process. *Koskinen* (1959) determined the amounts of the different tissue components in the callus under various experimental conditions, using the line-sampling method (*Uotila* 1940). The method was further developed by us in a previous paper and enables determination of the tissue components with a methodical error or less than 5 per cent

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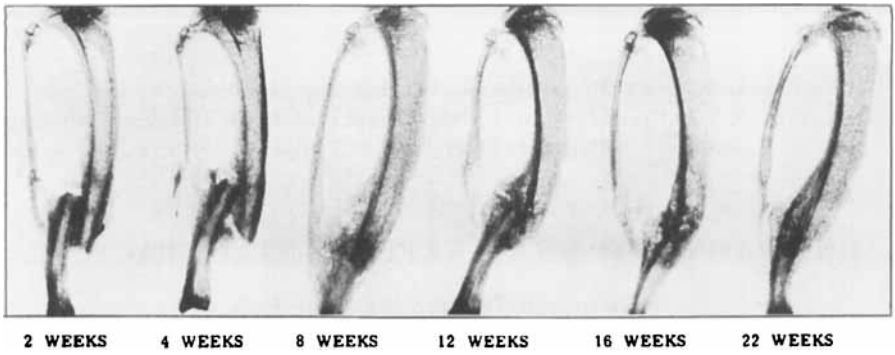

Fig. 1.
Radiographs of the fracture specimens at various times after the fracture. Mineralized tissue can be demonstrated mainly on the muscular side of the bone fragments. The density of the callus increases rapidly, whereas the fracture line between the fragments is still visible at 12 weeks. Note the re-modelling of the fracture area during 8–22 weeks with rearrangements of the trabecular network in accordance with the static load on the bone.

units (*Rokkanen & Slätis* 1964). In the present paper a histo-quantitative analysis of the normal repair of fractured long bones is presented.

MATERIAL AND METHODS

Sixty white adult female rats were used. The right lower leg was fractured subcutaneously according to the technique of *Urist & McLean* (1941). The leg was not splinted. The animals were sacrificed 1 to 22 weeks later, the fracture specimen was cleaned of soft tissues, radiographed and examined histologically. Prints were made of the radiographs at × 5 magnification, the boundaries of the visible callus outlined and the area thus obtained measured by planimetry. The callus was histologically examined and quantitative determination of the callus components made according to the line-sampling method previously described (*Rokkanen & Slätis* 1964).

RESULTS

Radiographic Changes

Plain radiographs of the fracture revealed visible callus 2 weeks after the trauma. The bulk of this callus was found on the posterior facies of the tibia. At 4 weeks the density of the callus had increased, although the fracture line was still clearly visible. At 8 weeks the fracture line was less marked and the bone ends had become rounded and partly resorbed. At 16 weeks the fracture was roentgenologically united and at 22 weeks the remodelling of the bone at the fracture site was well advanced (Fig. 1).

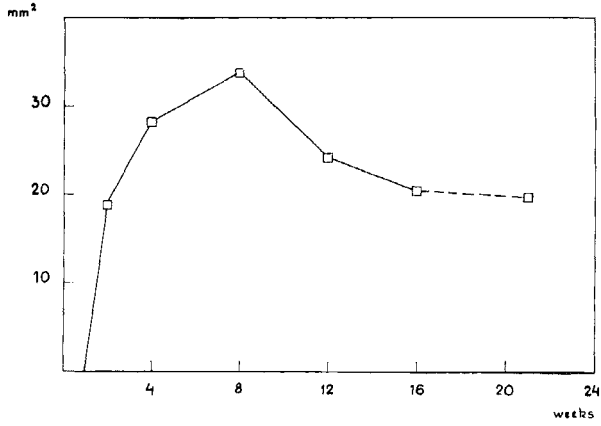

Fig. 2.
Planimetry of the radiographs at various times after the fracture.

Planimetry of the Radiographs

The size of the roentgenologically demonstrable callus at various times after the fracture is graphically depicted in Fig. 2. There was a steep increase in the callus area during the first weeks after the trauma, the peak value being recorded at 8 weeks. After this point, the callus area gradually diminished, although it was still of considerable magnitude at the end of the experiment.

Histological Examination

The histological changes observed were essentially the same as those reported in a previous paper (*Rokkanen & Slätis* 1964), to which the reader is referred for details. The main changes observed may be summarized as follows:

During the first 4–8 weeks the most distinctive feature of the repair process was the intense proliferation of the osteogenic layer of the periosteum, giving rise to the main part of the initial callus around the fractured bone ends. This callus was predominantly composed of cartilaginous and fibrous tissue and to a lesser degree of new bone, first demonstrable under the periosteal collar. The process of mineralization then proceeded gradually towards the central part of the callus, the cartilaginous and fibrous tissues being successively replaced by spongious bone.

From 8 to 22 weeks after the fracture there was little change in the histological picture of the callus. The mineralization of the central parts

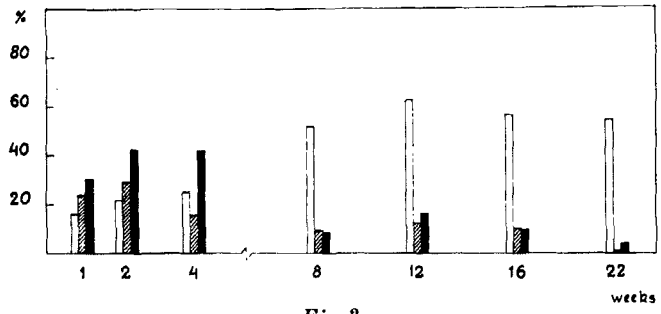

Fig. 3.

Histo-quantitative analysis of the callus tissue at various times after the fracture of a long bone, expressed as percentages of the total area.
White column: new bone. Hatched column: cartilaginous tissue.
Black column: Fibrous tissue.

continued, although cartilaginous tissue was still clearly demonstrable between the broken bone ends 16 weeks after the fracture. At this time, however, bony union between the fragments was regularly observed in the periphery of the callus. The spaces between the trabeculae became narrower as the new bone tightened up around the fractured bone ends. This remodelling of the callus was the most noticeable histological feature during the late stage of fracture repair.

TABLE 1

Relative Amounts of Main Tissue Components in the Callus, Expressed as Percentage at Various Times after the Fracture of a Long Bone.

Time after fracture	New bone (*per cent*)	Cartilaginous tissue (*per cent*)	Fibrous tissue (*per cent*)
1 week	16	24	30
2 weeks	22	29	42
4 weeks	25	16	42
8 weeks	52	10	9
12 weeks	62	12	16
16 weeks	56	10	10
22 weeks	55	0.3	4

Histo-quantitative Analysis of the Callus

The relative amounts of the main tissue components present in the callus at different times after the fracture are graphically depicted in Fig. 3. During the first four weeks the proportion of new bone was low

but increased continuously and reached a peak value at 12 weeks. At this point the new bone was the major constituent (62 per cent) of the total callus and this dominance persisted during the subsequent course of the experiment.

During the first four weeks the cartilaginous and fibrous tissue made up more than half the callus. The proportion of cartilaginous and fibrous tissue diminished rapidly, however, from the fourth to the eighth week and gradually fell to a value of less than 5 per cent of the total amount of callus tissue by the end of the experiment.

DISCUSSION

The advantage of histo-quantitative studies in connection with investigations on fracture repair is two fold. First, it provides information on the composition of the callus at various times after the fracture. Secondly, it permits comparison of callus tissue formed under different experimental conditions (*Koskinen* 1959, *Rokkanen & Slätis* 1964). In the present investigation, a quantitative examination of the main callus components was performed without any attempt at an analysis of the specific cellular reactions during fracture repair. Special problems regarding the mineralization of bone and the ultrastructure of bone formation and resorption are obviously better studied with the aid of bone-seeking isotopes and a microradiographic technique.

The histo-quantitative analysis of the callus at various times following a fracture showed that the proportion of new bone increases steadily during the first 12 weeks. This corresponds well with earlier investigations (*Nilsonne* 1959, *Falkenberg* 1961). During the first month, however, the part played by newly formed bone is small and during this stage the callus is predominantly composed of cartilaginous and fibrous tissue. By contrast, after the first month the new bone becomes dominant, whilst the proportion of cartilaginous and fibrous tissue rapidly decreases.

Thus, from a histo-quantitative point of view, fracture repair seems to be biphasic in character. During the first phase of fracture repair the bone ends are held together by a mass of soft tissue. During the second phase mineralization becomes the dominant feature. The spongious bone laid down is gradually tightening up around the fragments, eventually forming a structure closely resembling the surrounding cortical bone.

Falkenberg (1961), investigating the tensile strength of the callus in

rabbits, demonstrated that fracture healing is achieved in the first place by a relatively rapid increase in the absolute tensile strength of the callus, while the specific tensile strength of the callus increases at a slower rate. Correspondingly, *Nilsonne* (1959) showed that the final bony union between the fractured bone ends occurs at a late stage, and this was also confirmed histologically in the present investigation.

The biphasic nature of fracture repair obviously reflects the functional properties of two callus tissues of different composition. During the first phase, as judged from the histo-quantitative analysis, a provisional callus is rapidly formed between the fractured bone ends, allowing early weight-bearing at a time when the bone bridges between the bone ends are still incomplete. The second phase of fracture repair, including the remodelling of the spongious callous bone, is a slow process lasting several months and occurs at a time when, functionally, the fracture has already healed.

SUMMARY

The repair of experimental fractures of the long bones has been investigated in 60 female white rats. The fracture area was investigated roentgenologically, histologically and histo-quantitatively from 1 to 22 weeks following the trauma. The results may be summarized as follows:

1. Radiographically, new bone can be detected in the callus areas 2 weeks after the trauma. Planimetry of the radiographs reveals a continuous increase of the visible callus area for 8 weeks, after which the callus area gradually decreases.

2. Histologically, the essential feature in fracture repair is the intense proliferation of the osteogenic tissue covering the bone, especially of the periosteum. This gives rise to a mass of tissue around the fractured bone ends. This callus is mainly composed of cartilaginous and fibrous tissue although new bone is laid down during the first week under the periosteal collar. Subsequently, mineralization proceeds towards the central part of the callus area, bringing about a trabecular network of spongious bone around the fracture.

3. Histo-quantitative analysis of the main tissue components in the callus makes possible a division of the repair process into two phases.

During the first phase the immature callus is composed mainly of cartilaginous and fibrous tissue, whereas the proportion of new bone, although steadily increasing, remains small.

During the second phase new bone constitutes the prevalent tissue component, while the proportion of cartilaginous and fibrous tissue is minute and diminishes still further during the late stages of repair.

RESUME

La restauration de fractures expérimentales des os longs a été étudiée chez 60 cobayes femelles. La surface de la fracture a été examinée radiologiquement, histologiquement et histo-quantitativement entre 1 et 22 semaines après le trauma. Les résultats obtenus peuvent être résumés comme suit:

1. Au point de vue radiologique, une nouvelle formation osseuse peut être décelée dans la surface du cal 2 semaines après le trauma. La planimétrie des radiographies révèle une augmentation continue de la surface visible du cal pendant 8 semaines, après quoi la surface du cal diminue graduellement.

2. Au point de vue histologique, le fait essentiel de la restauration de la fracture est l'intense prolifération du tissu ostéogénique qui couvre l'os, spécialement dans le périoste. Cela donne lieu à une masse de tissu autour des extrémités osseuses fracturées. Le cal se compose principalement de tissu cartilagineux et fibreux, bien que la nouvelle formation osseuse se fasse pendant la première semaine sous le collier périosteal. Une minéralization subséquente s'opére vers la partie centrale de la surface du cal, formant un réseau trabéculaire d'os spongieux autour de la fracture.

3. Une analyse histo-quantitative des principaux composants du tissu du cal permet de diviser le processus de restauration en deux phases. Durant la première phase, le cal précoce se compose essentiellement de tissu cartilagineux et fibreux, alors que la proportion d'os nouveau reste faible bien qu'augmentant constamment.

Durant la seconde phase, le tissu osseux constitue le composant dominant, alors que la proportion de tissu cartilagineux et fibreux est faible et continue à diminuer pendant la dernière phase de la restauration.

ZUSAMMENFASSUNG

Die Heilung von experimentellen Brüchen der langen Knochen von 60 weissen weiblichen Ratten wurde untersucht. Das Bruchgebiet wurde röntgenologisch, histologisch und histo-quantitativ von der 1. bis 22.

Woche nach dem Trauma untersucht. Die Ergebnisse können in folgender Weise zusammengefasst werden.

1. Röntgenologisch kann neuer Knochen im Kallusgebiete 2 Wochen nach dem Trauma entdeckt werden. Planimetrie zeigt eine fortlaufende Zunahme des sichtbaren Kallusgebietes während 8 Wochen. Hernach nimmt die Kallusfläche gradweise ab.

2. Histologisch ist der wesentliche Zug der Bruchheilung die intensive Proliferation des den Knochen deckenden osteogenetischen Gewebes, besonders des Periostes. Dies führt zu einer übermässigen Gewebsbildung um die gebrochenen Knochenenden. Dieser Kallus besteht hauptsächlich aus kartilaginösem und fibrösem Gewebe obwohl auch neuer Knochen während der ersten Woche unter dem Periostkragen angelegt wird. Nachfolgend schreitet die Mineralisierung gegen die zentralen Teile des Kallusgebietes fort und lässt ein trabekuläres Netzwerk von spongiösen Knochen um den Bruch entstehen.

3. Gewebs-quantitative Analyse der hauptsächlichen Gewebskomponenten im Kallus ermöglichen eine Aufteilung des Heilungsprozesses in zwei Phasen. Während der ersten Phase besteht der unreife Kallus hauptsächlich aus kartilaginösem und fibrösem Gewebe, während der Anteil von neuem Knochen, obwohl ständig zunehmend, gering bleibt.

Während der zweiten Phase stellt neuer Knochen die vorwiegende Gewebskomponente dar. Der Anteil von kartilaginösem und fibrösem Gewebe ist dagegen sehr klein und veringert sich noch mehr während der späten Heilungsstadien.

REFERENCES

Bohr, H. H.: Studies in fracture healing. J. Bone Jt Surg. *37-A*: 327–337, 1955.

Falkenberg, J.: An experimental study of the rate of fracture healing. Acta orthop. scand. Suppl. 50, 1961.

Ham, A. W. & Harris, W. R.: Repair and transplantation of bone. In: The Biochemistry and Physiology of Bone. Bourne, G. H. Editor, New York, Academic press Inc. Publishers, 1956.

Koskinen, E. V. S.: The repair of experimental fractures under the action of growth hormone, thyrotropin and cortisone. Ann. Chir. Gynaec. Fenn. Suppl. 90, 1959.

Nilsonne, U.: Biophysical investigation of the mineral phase in healing fractures. Acta orthop. scand. Suppl. 37, 1959.

Pritchard, J. J. & Ruzicka, A. J.: Comparison of fracture repair in the frog, lizard and rat. J. Anat. *84*: 236–261, 1950.

Rokkanen, P. & Slätis, P.: The repair of experimental fractures during long-term anticoagulant treatment. Acta orthop. scand. 35: 21–38, 1964.

Tonna, E. A. & Cronkite, E. P.: Cellular response to fracture studied with tritiated thymidine. J. Bone Jt Surg. *43-A*: 352–362, 1961.

Uotila, U.: Einfaches Verfahren zur Bestimmung des Volumens und Gewichts des Schilddüsenkolloids. Acta Soc. Med. "Duodecim" Ser. A 23: 77–83, 1940.

Urist, M. R. & McLean, F. C.: Calcification in the callus in healing fractures in normal rats. J. Bone Jt Surg. 23: 1–15, 1941.

Wendeberg, B.: Mineral metabolism of fractures of the tibia in man studied with external counting of Sr^{85}. Acta orthop. scand. Suppl. 52, 1961.