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THE DISTRIBUTION OF MINERAL SALT IN NON-UNION OF FRACTURES

By

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The mineralisation of a callus area during the healing process of experimentally obtained diaphyseal fractures in rats and dogs has been studied previously by the author by means of microradiographic techniques (7). It was then shown that the distribution of mineral salt at the different stages of healing reflected and magnified the inhomogeneous distribution of bone salt which exists in normal bone tissue. Quantitative microradiography gave a determination of the average bone salt concentration in the various callus structures. The periosteal callus showed the most rapid mineralisation, whereas the intermediately situated callus was mineralised at a slower rate. The complete process from the fresh fracture to "full mineralisation" of the callus area took, however, a very long time. Thus in the rat fracture the difference in the average mineralisation of the callus and of the ends of the fractures were not equalised until 1½ years later. The corresponding period for dog fractures was more than two years.

The results obtained from these studies of mineralisation in the healing of experimental fractures are not immediately applicable to fractures in man; nevertheless, the skeleton of dogs, at least, closely corresponds with that of man in its microscopic structure and its reactions. It can therefore be expected that a microradiographic investigation of the conditions of mineralisation in clinical fractures will give results analogous to those in experimental fractures. On the other hand, for practical reasons it is difficult to carry out an investigation of this kind on clinical material. It would, for example, involve making consecutive biopsies on a callus area in order to obtain microradiographic specimens. This method would, however, mean a disruption of the biological process of healing, and consequently provide less significant information.

In contrast, by studying established pseudarthroses in clinical cases with microradiographical techniques, it is possible to observe the accumulation of bone salt in the callus. It is true that this method allows the investigation of only a stationary stage in the development of the callus, at a time when mineralised bone structures are no longer being formed across the fracture gap. It will be shown in the following, however, that a study of the distribution of mineral salt in the tissue of pseudarthroses permits certain indirect conclusions to be drawn concerning the dynamic processes involved in the calcification of a fracture callus.

The present investigation has been carried out on 10 clinical cases of non-union of diaphyseal fractures of the long-bones which have been admitted for orthopaedic treatment. From a strictly biological point of view this non-union should have manifested itself in the form of a real pseudarthrosis, which perhaps should be called ne-arthrosis. That is to say, the ends of the fracture are surrounded by some kind of synovial membrane, and the space between them is filled with synovial fluid. With modern methods of treating fractures, however, such a definite stage of non-union is rarely met; therapeutic action is now normally taken when it is apparent that delayed union is about to become non-union.

In the present investigation the term pseudarthrosis has therefore been given its clinical meaning. The material consists of fractures which, in spite of the usual methods of reduction and fixation, have not attained stability within a "normal" healing period, but have displayed mobility and given rise to pain in the callus area. Macroscopic X-ray pictures have shown typical changes including sclerosed, rounded fracture ends on either side of the pseudarthrosis gap. The pseudarthrosis areas have been resected in toto during reconstructive surgery, and a few centimetres of the fracture ends have been removed. This method has made it possible to study the microscopic distribution of mineral salt at varying distances from the topographical centre of the pseudarthrosis. The preparative and microradiographic methods used were identical with techniques described previously (7).

RESULTS

Fig. 1 shows the microradiographic cross section of an ulna some millimetres from the pseudarthrosis gap in an 8-months old fracture. The bone itself contains a comparatively large number of resorption

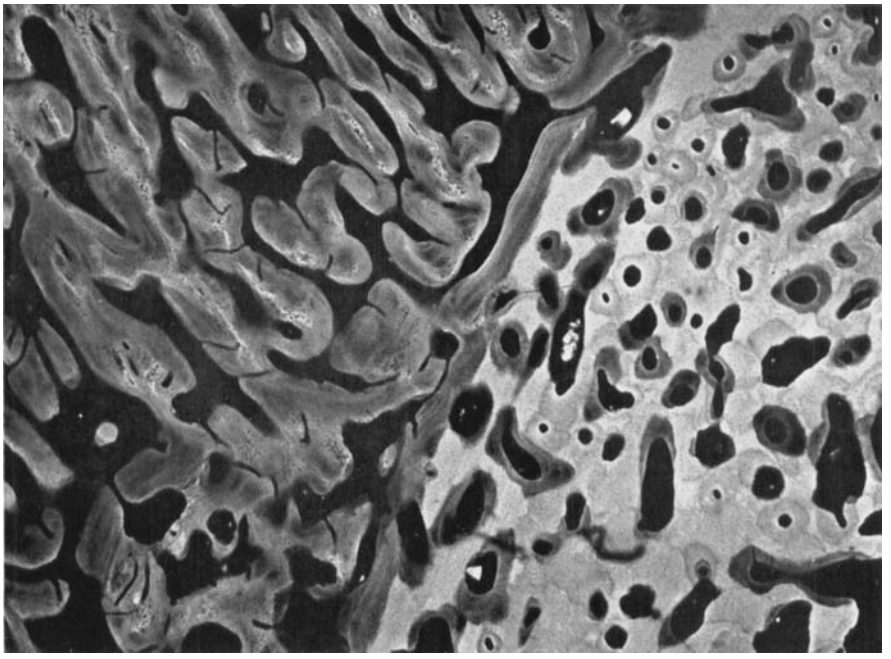

Fig. 1.
Microradiogram of a cross section some mm. from the fracture line in non-union of ulna 8 months after fracture. Ulna right, periosteal callus left. Magnification 23 ×.

zones but otherwise exhibits a normal structure and retained Haversian systems. The distribution of mineral salt in the bone does not depart from that found in undamaged bone. Thus it is possible to see typical Haversian systems showing varying degrees of mineralisation as well as a similar inhomogeneous distribution of bone salt in the intermediate zones. Judged microradiographically, the ends of the fractures do not, therefore, display any sign of homogeneiation either in the form of hyper- or hypo-mineralisation. The periosteal callus consists of coarse trabeculae closely packed together; they display varying degrees of mineralisation, usually with an increased accumulation of bone salt at the centre. The distribution of mineral salt is in fact identical with the distribution found in the periosteal callus during the later stages of healing of diaphyseal fractures in dogs (Fig. 2).

Fig. 3 is the microradiogram of a longitudinal section through the pseudarthrosis gap in the same case as in Fig. 1. The same mineralisation pattern can be observed in both specimens in the periosteal callus as well as in the outermost parts of the fracture

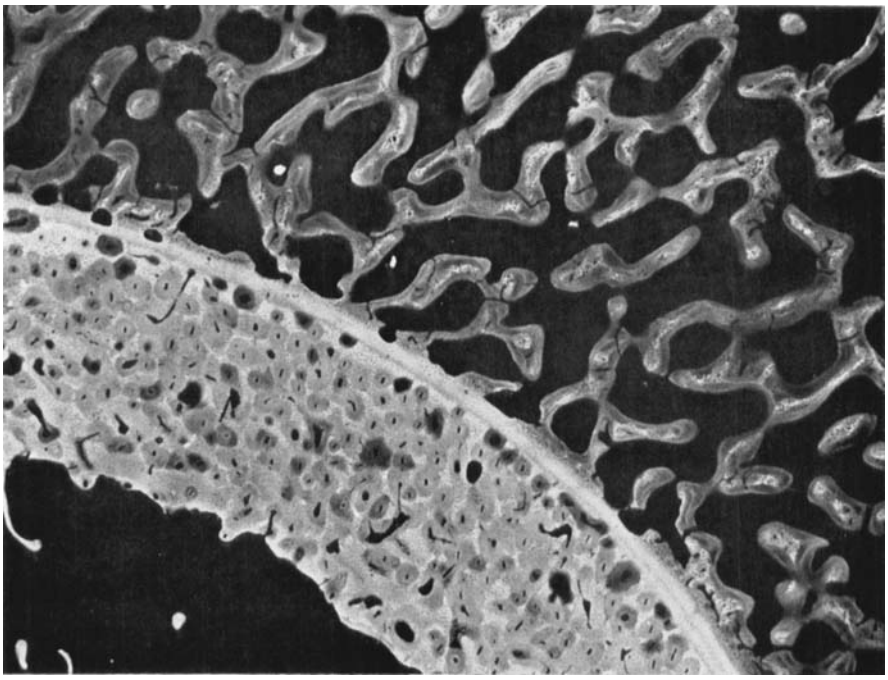

Fig. 2.
Microradiogram of a cross section of a 4 months old femur fracture in the dog. Bone left, periosteal callus right. Magnification 21 ×.

ends. In the intermediately situated callus a degree of mineralisation is displayed which is, on average, lower than in the periosteal callus. There are, however, wide local variations, so that even adjacent structures may reveal considerable differences in mineralisation. This type of mineral salt distribution in the intermediate callus can be observed as far out towards the pseudarthrosis gap as the mineralised structures are in fact visible. A comparison with the femur fracture during healing in a dog shows remarkable similarities in mineral salt distribution. (Fig. 4), notwithstanding the fact that this picture represents a progressive stage in healing, while Fig. 3 is of a stationary stage.

All the pseudarthroses investigated showed the same mineralisation pattern as the typical cases described above. From the results gained in connection with the reaction of the mineral phase in experimentally obtained callus tissue (7), it can thus be stated that analogous conditions exist between the distribution of mineral salt in normal

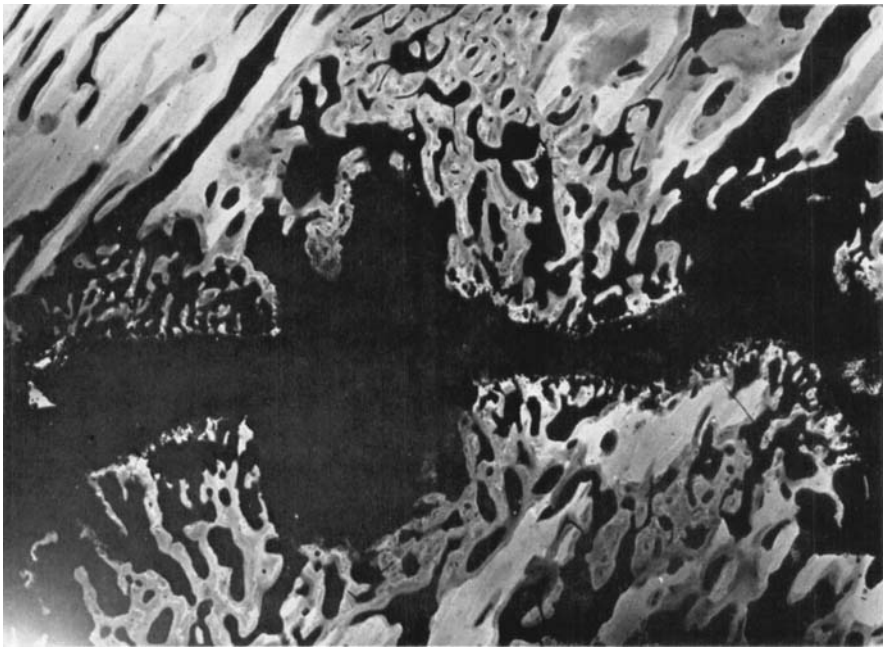

Fig. 3.
Microradiogram of a longitudinal section through the pseudarthrosis gap in the same case as in Fig. 1. The black area in the centre represents the unmineralised part of the pseudarthrosis. Magnification 10 ×.

callus tissue and its distribution in pseudarthrosis tissue. The mineralisation which was observed in the pseudarthroses corresponds in the experimental callus material to a comparatively late stage of healing. The quantitative microradiography which has been carried out also supports the observation that the average bone salt concentration in the mineralised structures of pseudarthrosis tissue corresponds to a stage which displays a relatively high degree of mineralisation.

DISCUSSION

In clinical studies of the causes of pseudarthrosis great interest has usually been paid to the primary methods of fracture treatment. This is natural enough, since, to mention one reason alone, the anatomical results of the method applied can be studied directly in the clinical X-ray picture. During the healing process it is also customary to let the macroscopic X-ray picture be decisive in the assessment of the callus development and its function. As previously shown (3, 7),

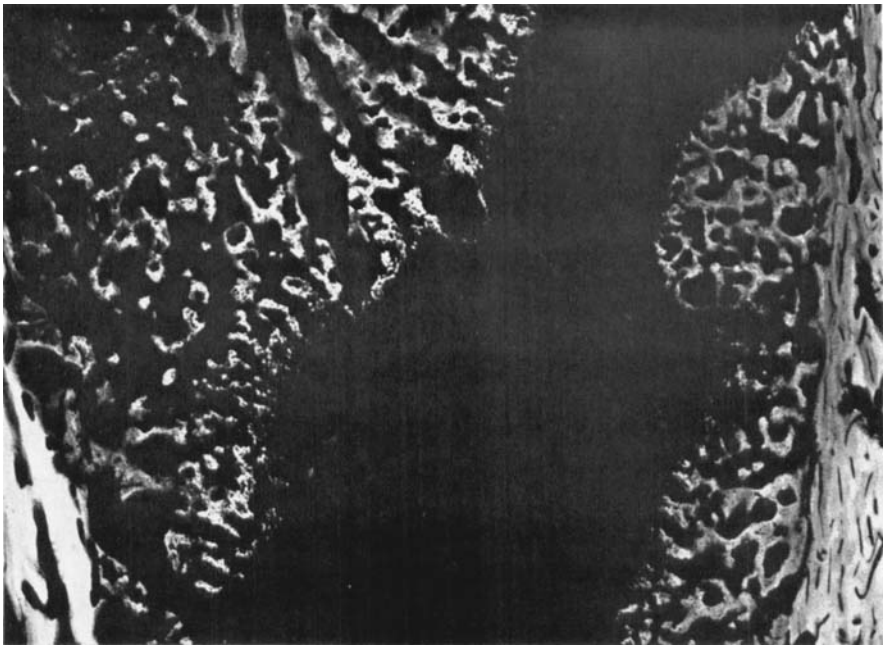

Fig. 4.
Microradiogram of a longitudinal section through the fracture region in a 4 months old femur fracture in the dog. The black area in the centre represents the not yet mineralised part of the callus. Magnification 17 ×.

however, macroscopic X-ray pictures give incomplete information about the mineral salt contents of callus tissue. The present microradiographic investigation, has on the other hand, made it possible to analyse in detail the distribution of mineral salt in pseudarthrosis tissue.

A macroscopic X-ray picture is also liable to lead to an assessment of fracture healing which is based solely on the degree of calcification; in this way non-union is interpreted as a defect in the mineralisation capacity as such. Consequently a vast number of experimental and clinical investigations have been carried out with the aim of discovering some abnormality in the calcium or phosphorus metabolism of healing fractures. No disturbance of these factors has, however, been revealed, either in normal experimental animals or in normal persons on normal diet (for a survey, see (2)). And *Urist* (8) states that "The humoral source of bone salt is essential for the normal progress of healing."

Thus, if the humoral factors can be assumed to be normal, it remains to see how they act in the callus tissue. In the building of this tissue

interaction takes place between the organic and the inorganic phases, which consist of organic matrix and the bone salt respectively. Ossification and calcification in normal embryonic and post-fetal bone formation have been shown (1, 5) to occur practically simultaneously. This means, therefore, that the organic matrix is ready to be mineralised at the same moment as it is formed; and the same condition would also seem to exist in the building of callus tissue. Thus *Urist & Mc Lean* (9) have found through histological investigations of tibia fractures in rats that the organic matrix in the periosteal callus is calcified as soon as it is formed. The same authors also write (6) that "The process of calcification of bone matrix in a healing fracture also closely resembles that of calcification of growing bone". Experimental data therefore indicates that osteogenesis and mineralisation appear simultaneously in normal bone growth and fracture healing.

Thus a necessary condition for the occurrence of pseudarthrosis is evidently a disturbance either of the organic or of the inorganic phase or of both simultaneously. In the present investigation it has been shown that the distribution of mineral salt in a pseudarthrosis area is analogous to the distribution found in healing stages of experimental fractures before mineralised structures have bridged the fracture gap. The mineralisation patterns have been found to be practically identical both in the fracture ends and in the periosteal and the intermediately situated callus. As far as the pseudarthrosis area is concerned this means that normal interaction has taken place between the organic matrix and the bone salt at those places where mineralised structures have been formed, that is, up to the pseudarthrosis gap. In the healing fractures which have been used here as comparative material (7), it must be assumed that the organic matrix is normally calcifiable, since a normal healing sequence has occurred. Thus, the non-mineralised area in the fracture gap, which can be seen in Fig. 4 contains normally calcifiable, organic matrix.

In microradiograms of cases of non-union it is possible also to see a central, non-mineralised area corresponding to the pseudarthrosis gap. (Fig. 3). The organic matrix in this area has evidently not been mineralised. Had osteogenesis taken place normally, as it should according to the preceding discussion, the process of calcification could have continued across this area as well. The analogous distribution of mineral salt in the callus in cases of non-union and of healing fractures indicates that the local mineralisation process has not been disturbed. This "normal" wave of mineralisation should, in other words, be capable

of spreading out between the fracture ends, provided that it meets an organic matrix which is normally calcifiable. It is evident that no such development takes place in cases of pseudarthrosis. The probable conclusion can therefore be drawn that non-union is not connected with a primary disturbance of the mineralisation process, but with a change in the organic matrix, which diminishes or prevents its calcifiability. Similar opinions as to the cause of pseudarthrosis have been expressed by *Karcher* (4), who found approximately the same uptake of isotopes in experimental fractures and pseudarthroses when using Ca^{45} and P^{32}. From these results he concluded that the local mineral salt contents was not affected in either case.

Further investigations, using biophysical methods, aim at studying the ultra-structure and change of mass of the organic phase during fracture healing, and its relation to the mineral phase.

SUMMARY

Microradiographic investigations have been carried out on clinical cases of non-union of fractures. The distribution of mineral salt in the pseudarthrosis area is described in detail. A comparison between experimental fractures and pseudarthroses reveals in both cases an analogous distribution of mineral salt in the callus areas. This fact makes it probable that non-union is not the result of a primary disturbance of the mineralisation process, but rather of a change in the organic matrix, which diminishes or prevents its calcifiability.

RESUME

Des investigations microradiographiques ont été pratiquées dans des cas cliniques de non-soudure de fractures. La distribution du sel minéral dans la pseudarthrose est décrité en détail. Une comparaison entre des fractures expérimentales et la pseudarthrose révèle dans les deux cas une distribution analogue du sel minéral dans le cal. Ce fait rend probable que la non-consolidation n'est pas le résultat d'un trouble primaire du processus de minéralisation, mais plutôt une altération da la matière organique qui diminue ou abolit son pouvoir de calcification.

ZUSAMMENFASSUNG

Mikroröntgenologische Untersuchungen wurden an klinischen Fällen von ungeheilten Brüchen ausgeführt. Die Verteilung von Mine-

ralsalzen im Pseudarthrosengebiete wird im Einzelnen beschrieben. Ein Vergleich zwischen experimentellen Brüchen und Pseudarthrosen zeigt in beiden Fällen eine gleichartige Verteilung von Mineralsalzen im Kallusgebiete. Diese Tatsache macht es wahrscheinlich, dass Nichtheilung nicht das Ergebnis einer Störung des primären Mineralisationprozesses ist, sondern eher einer Veränderung der organischen Grundsubstanz, welche deren Fähigkeit zur Kalkaufname herabsetzt oder verhindert.

REFERENCES

1. *Bloom, W.* & *Bloom, M.:* Calcification and ossification. Calcification of developing bones in embryonic and newborn rats. Anat. Record, *78*: 497, 1940.
2. *Ham, A. W.* & *Harris, W. R.:* Repair and transplantation of bone. In The biochemistry and physiology of bone. Ed. G. H. Bourne. Acad. Press Inc. Publ., New York, N. Y. 1956.
3. *Ham, A. W., Tisdall, F. F.* & *Brake, T. G. H.:* Experimental non-calcification of callus simulating non-union. J. Bone and Joint Surg., *20*: 345, 1938.
4. *Karcher, H.:* Der Calcium- und Phosphorstoffwechsel bei der normalen und gestörten Knochenbruchheilung sowie in frischen und konservierten Transplantaten. Langenbecks Arch. klin. Chir., *275*: 1, 1953.
5. *McLean, F. C.* & *Bloom, W.:* Calcification and ossification. Calcification in normal growing bone. Anat. Record, *78*: 333, 1940.
6. *McLean, F. C.* & *Urist, M. R.:* Newer knowledge of post-fetal osteogenesis. Bull. Joint Diseases, *12*: 431, 1951.
7. *Nilsonne, U.:* Biophysical investigations of the mineral phase in healing fractures. Acta Orthopaed. Scand. Suppl. 37, 1959.
8. *Urist, M. R.:* The rôle of local transfer of bone salt in the calcification of the fracture callus. J. Bone and Joint Surg., *24*: 47, 1942.
9. *Urist, M. R.* & *McLean, F. C.:* Calcification in the callus in healing fractures in normal rats. J. Bone and Joint Surg., 23: 1, 1941.