Acta orthop. Scandinav. 42, 113–121, 1971

Orthopedic Hospital, Copenhagen, Denmark.

BONE FORMATION AND RESORPTION IN CASES OF DELAYED UNION AND PSEUDARTHROSIS

H. Bohr

Accepted 24. ii. 71

In failure of fracture healing one usually distinguishes between delayed union and pseudarthrosis. According to McLean & Urist (1955), spontaneous healing may occur until 18 months after the fracture; but following this time they use the term non-union, implying that the process of healing has stopped. On the other hand, it has been emphasized (Watson-Jones 1955) that almost any fracture will ultimately heal if adequate immobilization is accomplished.

Previous investigations have shown that the uptake of radioactive calcium, phosphate and strontium around a fracture is the same in normal and delayed union (Karsher 1953, Bohr 1955, Wendeberg 1961), indicating that the process of mineralization continues despite the failing consolidation. In order to illuminate this question further, the rate of bone formation and bone resorption has been studied in cases of delayed union and pseudarthrosis through microradiographic investigations and the uptake of Tetracyclines at the fracture site.

MATERIAL AND TECHNIQUE

During operations for delayed union or pseudarthrosis biopsies were made from the un-united bone fragments. In 31 cases the material obtained was sufficient for studying the healing process. These include 28 fractures and 3 osteotomies, as seen in Table 1; there were also 2 cases of congenital pseudarthrosis of the tibia in children, 4 and 5 years of age. All cases, except a fracture of the malleolus medialis, had previously been treated with osteosynthesis without success. Labelling with Tetracycline was performed before the operation in 14 cases using Ledermycin, (demethyl-chlor-tetracycline) Terramycin (Oxytetracycline) or Reverin (Pyrrolidino-methyl-Tetracycline). In 6 cases labelling was repeated with an interval of 2–4 weeks.

The bone samples were embedded in methyl-metacrylate and after cutting with

Table 1. 31 fractures and osteotomies in 29 cases of delayed union.

Fractures							Osteotomies
fem.	tib.	mall.	hum.	uln.	rad.	clav.	subthroch. fem.
4	8	2	2	5	5	2	3

a rotating saw and grinding under water, bone specimens with a thickness of about 50 microns were obtained. For microradiography a Machlett 50 A.E.G. X-ray tube with a wolfram anode, generated at 12 k.V. with 12 m.A., was used, exposures being made on Kodak spectroscopic plates 549–0 during 10 minutes at a focus-film distance of 15 cm. Examination of fluorescense in ultraviolet light was made with the use of Reichert filters G.G. 9/1 mm and O.G. 1/5 mm. In some cases specimens were prepared for ordinary histological investigation after further grinding to 20 micron thickness and staining with Haematoxylin and Eosin following pretreatment with Mollifex, and organic solvent from the British Drug House, Ltd.

Measurements of the surfaces of bone formation and bone resorption on microradiographs were performed with a map measurer on diagrams drawn during microscopic observations, according to Jowsey et al. (1965). The area of bone formed between double labelling was determined with a planimeter (OTT type 30011) on drawings during fluorescent microscopy.

RESULTS

Measurements of surfaces of bone formation and bone resorption on microradiographs are given in Figure 1 as per cent of the total bone surface, within an area of about 10 mm^2. It is seen that the extent of bone formation surfaces varied from 10 to 65 per cent, and that of the bone resorption surfaces from 5 to 53 per cent, with average values of 34 and 15 per cent respectively. These figures are increased in comparison with similar measurements on normal, adult, human bone, but in the two cases of congenital pseudarthrosis, measurements differed only little from normal values of that age (Jowsey et al. 1965). In Table 2 the average values of bone formation and bone resorption surfaces in the 13 cases, where union was delayed from 7 to 18 months, have been compared with the average values in the 16 cases, where union was delayed from 18 to 63 months, the mean age for the cases in the two groups being almost equal. It is seen that there was no significant difference as regards bone formation surface, whereas the bone resorption surface was somewhat greater in the last group. Figure 2 shows a microradiograph from a radial fracture with a 2-year healing delay. Bone formation and bone resorption surfaces

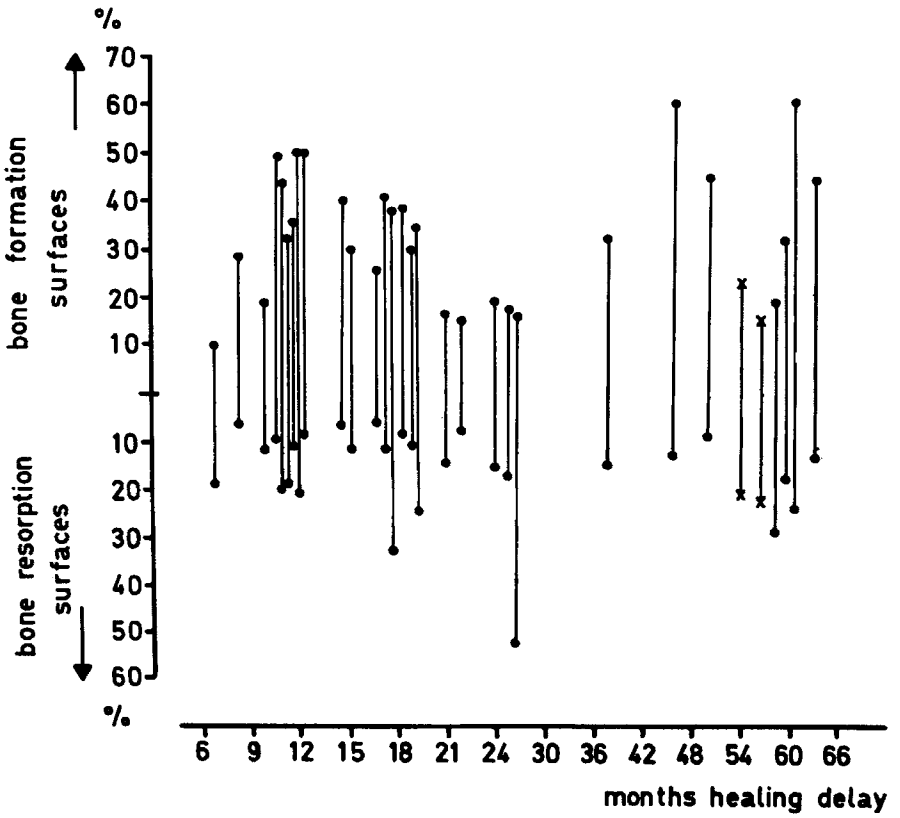

Figure 1. Measurements of surfaces of bone formation and bone resorption on microradiographs expressed as per cent of the total bone surface. Results in cases of congenital pseudarthrosis are indicated as crosses.

of secondary bone are increased, and callus formation as indicated by the presence of primary bone tissue, which is gradually transformed into secondary bone. The increased osteogenic activity is confirmed from the results of the uptake of Tetracycline, showing extensive labelling of bone surfaces at the bone ends. In Figure 3, which corresponds to Figure 2, it is seen that Ledermycin is deposited in sharp lines along the mineralizing surface of the secondary bone, while the primary bone tissue is diffusely labelled. By repeating the administration of Tetracycline the bone tissue deposited during the interval between labellings is demonstrated (Frost et al. 1960, Sissons & Lee 1964). This is shown in Figure 4 from a femoral osteotomy with a 19-month healing delay, where labelling with Ledermycin was per-

Table 2. Average values of bone formation surface and bone resorption surface, expressed in per cent of total bone surface, in 2 groups with delay of union from 7 to 18 months and 18 to 63 months, respectively.

Delay in union	Number of cases	Mean age	Bone form.	Bone resorp.
7–18 m.	15	40	37±4 %	11±2 %
18–63 m.	16	42	32±4 %	19±3 %

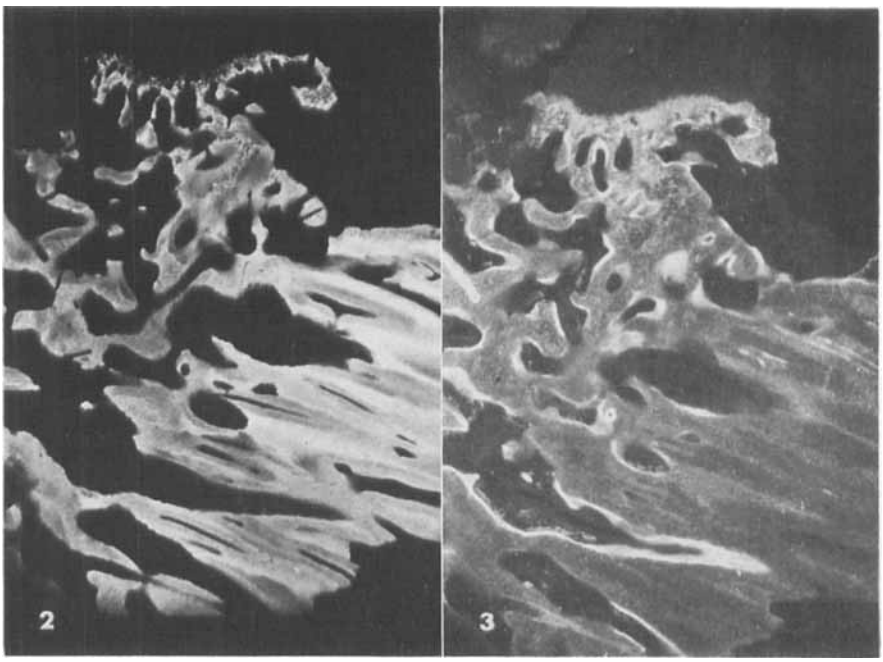

Figure 2. Microradiograph from an un-united radial fracture of 2 years' duration (case 253). Secondary bone with increased formation and resorption is seen below, and primary bone under gradual transformation into secondary bone above. Magnification × 30.

Figure 3. Fluorescense photo corresponding to Figure 1. Labelling with Ledermycin was performed one day before the biopsy. Sharp fluorescent lines are seen along the mineralizing surfaces of the secondary bone and diffuse labelling of the newly formed primary bone. Magnification × 30.

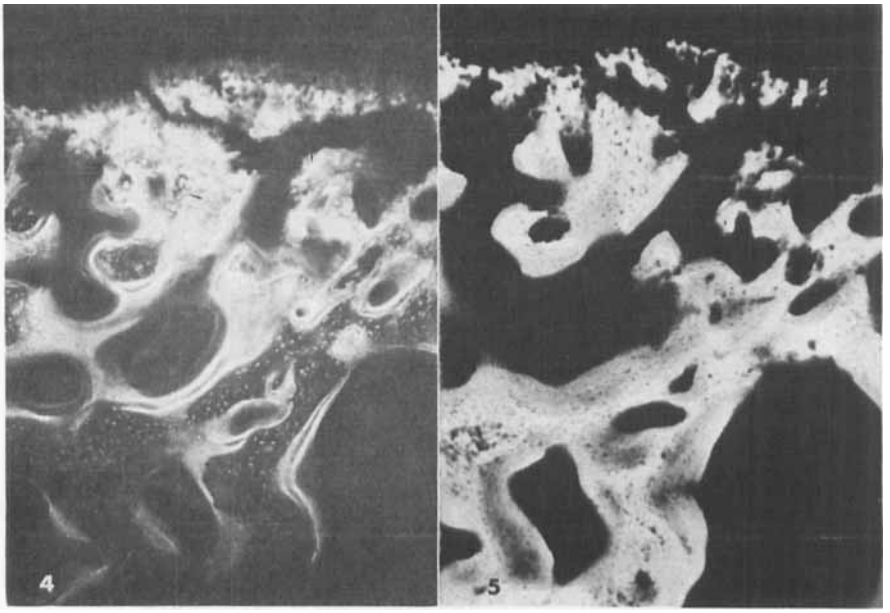

Figure 4. Fluorescense phote from an un-united femoral osteotomy of 19 months' duration (case 254) labelled with Ledermycin 30 days and 16 days before the biopsy. Double labelling of secondary bone is seen.
Magnification × 120.

Figure 5. Microradiograph corresponding to Figure 3, showing calcified cartilage above the primary bone tissue.
Magnification × 120.

formed 30 and 16 days respectively before the biopsy. Measurements of the area of bone between the corresponding fluorescent lines show the amount of bone deposited pr. day to be 0.7 per cent of the total bone area, and the appositional rate 1 pr. day. Measurements in a case of congenital pseudarthrosis showed the same bone formation rate, while the appositional rate was 3.3 pr. day.

From the microradiograph on Figure 5, which corresponds to Figure 4, it is seen that calcified cartilage is present above the primary bone, indicating that enchondral ossification takes place at the bone ends. This is further demonstrated in Figure 6 from an un-united femoral fracture of 5 years' duration, showing the typical arrangement of hypertrofic cartilage cells in columns. By employing polarization microscopy the fibrillar structure of the cartilage between the bone

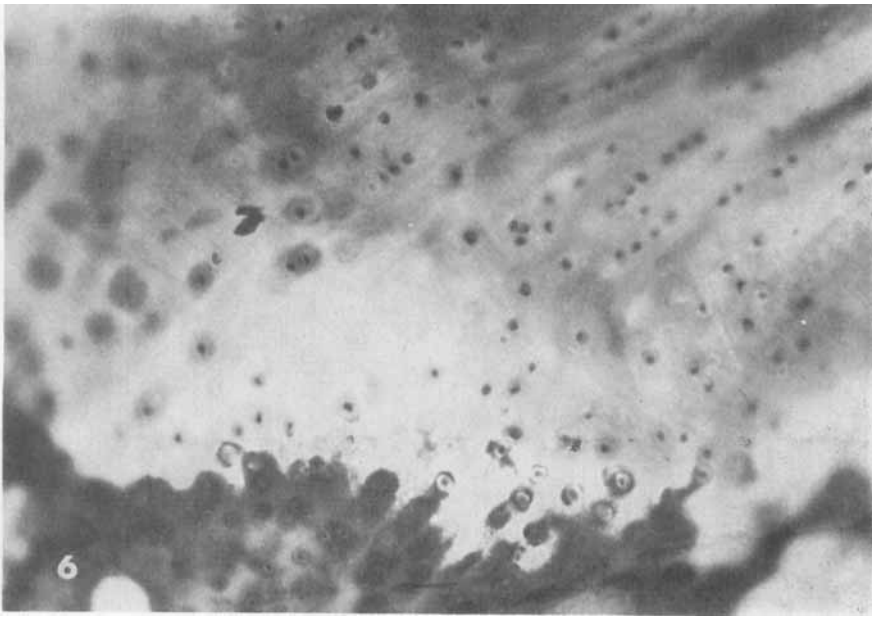

Figure 6. Undecalcified section from an un-united femoral fracture of 5 years' duration (case 251), stained with Haematoxylin and Eosin after pretreatment with Mollifex (B.D.H.) The arrangement of the hypertrofic cartilage cells in columns indicate enchondral ossification.
Magnification × 120.

fragments is demonstrated and seen radiating from the bone surface between the cartilage cells and deviating along the fracture gap, as demonstrated in Figure 7 from an un-united tibia fracture of 2 years' duration.

DISCUSSION AND CONCLUSIONS

Different histological methods have been used to determine bone formation surfaces. Measurements of the bone surfaces covered with osteoid tissue in normal adult human bone have shown values of about 5 per cent (Sissons et al. 1967), results which are consistent with the measurements of bone formation surfaces performed on microradiographs as well as with Tetracycline labelling (Jowsey et al. 1965). Through double Tetracycline labelling the appositional rate has been measured and from that also the rate of bone formation (Frost

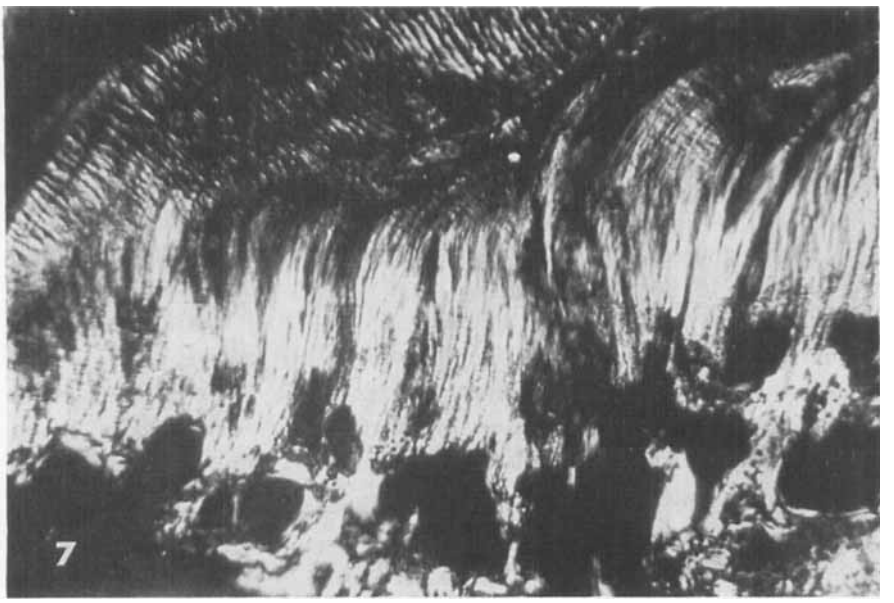

Figure 7. Polarization microscopy from an un-united tibial fracture of 2 years' duration (case 227) showing the fibrillar structure of the cartilage radiating from the bone ends and deviating along the fracture gap.
Magnification × 120.

et al. 1960, Amprino & Marotti 1964, Sissons & Lee 1964). For normal human ribs the amount of bone formed pr. day was determined to be about 0.05 per cent of the total bone mass in adults and 0.1 per cent in children (Frost 1969). The appositional rate showed values of about 1 pr. day in adults and 1.5 pr. day in children (Lee 1965, Frost 1969).

In the present investigation the results, although subject to some variation, seem sufficient to demonstrate that the rate of bone formation is increased in cases of delayed fracture healing and do not decrease with time, at least not within the first 5 years. In the case of congenital pseudarthrosis, bone formation rate was also increased, although less markedly compared with normal values of that age (Skou Andersen et al. 1968). The retained osteogenic activity is in accordance with the well-known effect of the Phemister operation in promoting healing of pseudarthrosis, when contact between the fragments is obtained through bone transplants (Phemister 1947). As spontaneous healing does not take place despite the increased bone

formation, it is probably not due to the simultaneous increase in bone resorption, since this was relatively small compared with the increase in bone formation. The presence of enchondral ossification demonstrated suggests that proliferation of cartilage cells takes place between the bone ends. The ossification of cartilage requires participation of blood vessels (Trueta 1963) and if penetration of capillaries is inhibited, as in the case of ineffective immobilization (Rhineländer et al. 1968), the cartilage will remain as a barrier, similar to the condition existing in the epiphyseal plate of the growing bone. While the proliferation of cartilage cells in the epiphyseal plate results in growth, the more limited phenomenon in pseudarthrosis causes sclerosis and broadening of the bone ends. Such a view seems to be supported by the favourable effect of compression forces on the healing of pseudarthrosis without interfering with the intervening tissue (Müller 1966, Witt & Jäger 1966, Mouritzen 1970).

SUMMARY

The rate of bone formation and bone resorption has been studied through microradiographic investigations and Tetracycline uptake in cases operated upon for delayed fracture healing and pseudarthrosis. It was increased compared with normal bone, and on the average the rate of bone formation was almost the same in the cases where union was delayed from 7 to 18 months as from 18 to 63 months, while bone resorption was somewhat more pronounced in the latter group. The presence of enchondral ossification suggests that the delay in healing may be due to a continued proliferation of cartilage cells between the bone ends.

ACKNOWLEDGEMENT

For valuable technical assistance I wish to thank Miss M. Bertran.

REFERENCES

Amprino, R. & Marotti, G. (1964) A topographic quantitative study of bone formation and reconstruction. *Bone and tooth*, ed. Blackwood, H.J.J., p. 21. (Proceedings of the First European Symposium on Calcified Tissues). Pergamon Press.

Bohr, H. (1955) Studies on fracture healing. *J. Bone Jt Surg.* **37-A**, 237–337.

Frost, H. M., Villenueva, A. R. & Roth, H. (1960) Measurements of bone formation in 57 year old man by means of tetracyclines. *Henry Ford Hosp. Bull.* **8**, 2, 239–254.

Frost, H. M. (1969) Tetracycline-based histological analysis of bone remodeling. *Calc. Tiss. Res.* **3**, 211–237.

Jowsey, J., Kelly, P. J., Riggs, L. B., Bianco, A. J., Scholtz, D. A. & Gershon-Cohen, J. (1965) Quantitative microradiographic studies of normal and osteoporotic bone. *J. Bone Jt Surg.* **47**-A, 785–806.

Karsher, H. (1953) Der Calcium und Phosphorstoffwechsel bei der normalen und geströrten Knochenbruchheilung. *Langenbecks Arch. klin. Chir.* **275**, 1–49.

Lee, W. R. (1965) A quantitative microscopic study of bone formation in a normal child and in two children suffering from osteogenesis imperfecta. *Calcified Tissues.* (Proceedings from the Second European Symposium on Calcified Tissues), ed. Richelle, L. J. & Dallemagne, M. J., p. 451. Université de Liège.

McLean, F. C. & Urist, M. R. (1955) *Bone*, p. 149. University of Chicago Press.

Mouritzen, V. (1970) Pseudarthrosis treated with compression-osteosynthesis. (Paper delivered at the meeting of the Danish Surgical Society, Oct. 1970). In press.

Müller, M. E. (1966) Die Behandlung der nicht infizierten Pseudarthrosen an der oberen Extremität mit Kompressionsplatte. *Akt. Chir.* **1**, 99.

Phemister, D. B. (1947) Treatment of ununited fractures by grafts without screws or fixation and without breaking down of the fibrous union. *J. Bone Jt Surg.* **29**, 946–960.

Rhineländer, F. W., Phillips, R. S., Steel, W. M. & Beer, J. C. (1968) Microangiography in bone healing II. *J. Bone Jt Surg.* **50**-A, 643–662.

Sissons, H. A. & Lee, W. R. (1964) Tetracycline studies in bone turnover. *Bone and tooth*, ed. Blackwood, H.J.J., p. 65. (Proceedings of the First European Symposium on Calcified Tissues). Pergamon Press.

Sissons, H. A., Holley, K. J. & Heighway, J. (1967) Normal bone structure in relation to asteomalacia. *L'osteomalacia*, ed. Hioco, D. J., p. 19. Masson & Co.

Skov Andersen, K., Bohr, H. & Sneppen, O. (1968) Congenital angulation of the lower leg. *Acta orthop. scand.* **39**, 387–397.

Trueta, J. (1963) The role of the vessels in osteogenesis. *J. Bone Jt Surg.* **45**-B, 402–418.

Watson-Jones, R. (1955) *Fractures and joint injuries*, 4th ed., p. 13. E. & S. Livingstone Ltd., Edinburgh.

Wendeberg, B. (1961) Mineral metabolism of fractures of the tibia in man, studied with external counting of Sr^{85}. *Acta orthop. scand.* Suppl. 52, p. 49.

Witt, A. N. & Jäger, M. (1966) Besondere Indicationen und Grenzen für die Verwendung des Instrumentariums der Arbeitgemeinschaft für Osteosynthese bei orthopädisch-chirurgischen Eingriffen. *Archiv. orthop. Unfall-Chir.* **60**, 49–70.