Acta orthop. scand. *50*, 375–383, 1979

THE HEALING OF EXPERIMENTAL FRACTURES BY COMPRESSION OSTEOSYNTHESIS

II. *Morphometric and Chemical Analysis*

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The effects of rigid plate fixation on the structure and chemical composition of bones during healing of experimental fractures were studied by morphometric and chemical analysis at intervals of 3 to 24 weeks after attachment of six-hole AO plates to osteotomized rabbit tibiae.

After fracture union gradual porotic transformation could be observed from 9 weeks onwards, with rapid excavation and breakdown of the cortical wall. During the study over 24 weeks the degree of porosity increased from 9.0 ± 4.8 per cent to 37.5 ± 10.2 per cent ($P < 0.001$). This osteoporosis was accompanied by formation of new subperiosteal bone. The changes in the tubular bone led to a progressive increase in overall diameter and in the area occupied by the medullary cavity throughout the experiment.

In the osteotomy area increased values were found for the content of hexosamines and the ratio of hexosamines to hydroxyproline at 3 weeks, indicating formation of connective tissue in the fracture area. Later on, no chemical signs of callus formation could be detected. In spite of the slight increase in the content of hydroxyproline, reflecting the formation of new bone subperiosteally, the chemical composition of the unresorbed cortical bone remained unchanged.

Key words: bone; bone plates; fracture fixation; calcium; hydroxyproline

Accepted 20.xi.78

The pattern of fracture healing varies within wide limits. Usually, the more stable the fixation, the less the periosteal reaction. Danis (1947) pointed out that rigid plate fixation secured union of fracture ends without formation of subperiosteal callus, calling the process "*soundure autogene*" (primary healing). Morphologically this primary bone healing is accompanied by widening of the Haversian canals, formation of resorption cavities and subsequent formation of intraosseal new bone across the fracture gap (Andersson 1965, Müller et al. 1963, Olerud & Danckwardt-Lilliestrom 1968, Schenk & Willenegger 1964). Additional axial compression applied to the implant and conveyed to the underlying bone does not alter the mode of repair (Perren et al. 1969).

In secondary healing the stabilizing factor is callus. The cartilaginous and ossification phases can be distinguished, both morphologically and chemically, by the degree of maturation of the callus (Penttinen 1972, Slätis & Rokkanen 1967).

0001–6470/79/040375–09$02.50/0

Rigid metallic implants on cortical bone act as stress protectors in the bone (Lanyon et al. 1976). This leads to increased porotic transformation, with widening of the Haversian canals and formation of resorption cavities in dense lamellar bone (Gördes et al. 1975a, Slätis et al. 1978, Uhthoff & Dubuc 1971).

The attenuation technique has been used to show reduction of the mineral mass of bone after compression plating (Gördes et al. 1975b, Strömberg & Dalen 1976, Tonino 1974). On the other hand, as shown in our earlier reports concerning the chemical content of calcium and hydroxyproline, there is only a slight decrease in the mineral content of cortical bone (Paavolainen et al. 1978b,c).

The chemical composition of the callus formed in fractures treated without any internal fixation has been thoroughly analysed (Penttinen 1972), but little is known about the chemical changes during fracture repair after rigid plate fixation.

The aim of the present study was to analyse the morphometric and chemical changes after rigid plate fixation of experimental osteotomies and to compare these alterations with those earlier reported for fractures treated without any internal fixation (Penttinen 1972). The effect of these alterations on the reduced mechanical strength of bone after rigid plate fixation of fractures (Braden et al. 1973, Paavolainen et al. 1978a) will be discussed.

MATERIAL AND METHODS

Operative techniques

Forty-one rabbits weighing from 2800 to 4400 g were used. On the right tibia a midshaft osteotomy was made near the tibio-fibular junction with a circular saw cooled with saline. A stainless steel (AISI 316 L) six-hole dynamic compression plate (AO/DCP) was attached to the anterolateral face of the bone. The length of the plate was 53 mm, width 7 mm and thickness 1.5 mm. Compression was applied between the most proximal and the most distal screw and the other two pairs of screws were tightened in a neutral fashion (Figure 1).

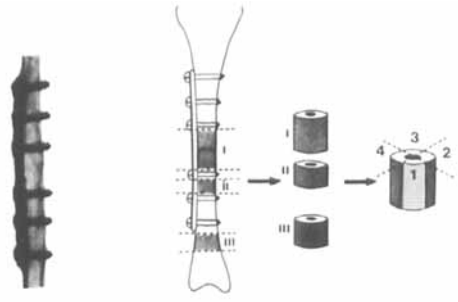

Figure 1. A diagram showing the discs of plated osteotomized rabbit tibio-fibular bone selected for chemical analysis. Two of the discs were taken from the bone beneath the plate; one of these at the level of osteotomy (I), the second distal to the osteotomy but beneath the plate (II) and the third beyond the plate (III). Each of these discs was further divided radially into four sectors, one of which contained the cortical area beneath the plate.

On the left tibia a sham operation was made, exposing the periosteum but without osteotomy or plate fixation.

Postoperatively, the animals were housed in separate cages where they were allowed to move freely, with water *ad libitum* and a standard laboratory diet. No external splints or bandages were applied after the operation.

Fifteen animals had to be excluded from the analysis because they sustained re-fractures or other postoperative complications.

Sampling techniques

The animals were divided into five subgroups killed 3, 6, 9, 12 and 24 weeks postoperatively. The tibio-fibular bones were exarticulated and freed from any soft tissue, but the periosteum and any callus tissue were preserved intact. Bones were radiographed before and after removal of the plates.

One animal from each subgroup (five animals) was randomly selected for chemical analysis. From each tibia three discs containing the entire cross section of the tubular bone were removed at predetermined levels.

From the plated tibia, two of the discs were taken from the bone beneath the plate, one of these at the level of osteotomy (I), the second distal to the osteotomy but beneath the plate (II)

and the third beyond the plate (III). Comparable discs were sawn from the control bones. Each of these discs was further divided radially into four sectors, one of which contained the cortical area beneath the plate (Figure 1).

Twenty-one animals were left for morphometric analysis of bone remodelling. A section through the tubular bone was removed at level II, under the plate but distal to the osteotomy. These samples were embedded in methylmethacrylate and ground to 80 µm with carborundum paper. Contact microradiographs of these undecalcified specimens were taken on films exposed with a Machlett AEG-50 tube at 10 kV, 10 mA, for 1 s, FFD 20 cm.

Morphometric measurements and estimation of resorption on cross sections of bone

The entire cross-sectional area and the area occupied by the medullary cavity were determined from ten-fold enlargements of contact microradiographs with planimetry (HAFF 317 planimeter). The values obtained were reduced mathematically to the actual size of the bone (Slätis et al. 1978). The percentage increase in the outer circumference as compared with the paired control bones was calculated for each pair of bones and indicated the degree of subperiosteal new bone formation. The area of the medullary cavity as a percentage of the entire cross-sectional area was calculated for the pairs of bones.

Photographs of the detailed structure of the cortical bone were made from the microradiographs at a magnification of × 100, with the resorption area in black and the cortical bone in white. The microradiographs were analysed by random point-sampling of each enlargement using a transparent grid on which there were lines and crossbars. The number of intersections over both black and white areas in the whole cortex were counted three times, and the number of so-called hits on the black areas was expressed as a percentage of all hits scored on cortical bone (Slätis et al. 1978). This technique permitted measurement of the proportion of cortical bone resorbed, and the results were reproducible with an error of less than 1 per cent.

A paired t test was performed to test the significance of the differences between the paired bones. $P > 0.05$ was taken to be non-significant.

Chemical analysis

The samples were lyophilized, weighed and ground to a fine powder in a mortar. The results were correlated with the dry weight of the material. The chemical analyses were carried out according to the methods used by Penttinen (1972). For determination of hexosamines the samples were hydrolysed in 4.0 ml of 2 N HCl for 16 hours at 105°C in sealed test tubes. A 1.0 ml portion was taken for the hexosamine assay and 2.0 ml of concentrated hydrochloric acid was added to make the solution 6 N. Hydrolysis was continued for 3 hours at 130°C in an N_2 atmosphere and the hydrochloric acid was evaporated in a boiling water bath. The specimens were dissolved in 8.0 ml of distilled water and small portions were used for determination of total nitrogen, hydroxyproline, calcium and phosphorus.

Calcium was determined in 1 per cent $LaCl_3$ with an atomic absorption spectrophotometer (Varian Techtronic Pty. Ltd., Model 1100, Melbourne, Australia). The t test was used for establishing the significance of differences between the values for the paired bones and between the means for every possible pair of subgroups. $P > 0.05$ was taken to be non-significant.

RESULTS

Technically successful preparations for morphometric analysis were obtained from 19 animals: at 3 weeks postoperatively (four animals), 6 weeks (five animals), 9 weeks (four animals), 12 weeks (four animals) and 24 weeks (two animals).

Planimetry of transverse sections of diaphyseal bone

Planimetry of transversal sections of the plated diaphyseal bone demonstrated subperiosteal new bone formation with increase in the overall cross-sectional area of the tubular bone. This was accompanied by resorption of subendosteal cortical bone leading to an increase in the area occupied by the medullary canal (Figure 2A–C).

Table 1 shows the percentage increase of the entire cross-sectional area in the plated bone when compared to the intact controls, and the size of the medullary canal in plated and control bones expressed as a percentage of the entire cross-sectional area of the tubular bone.

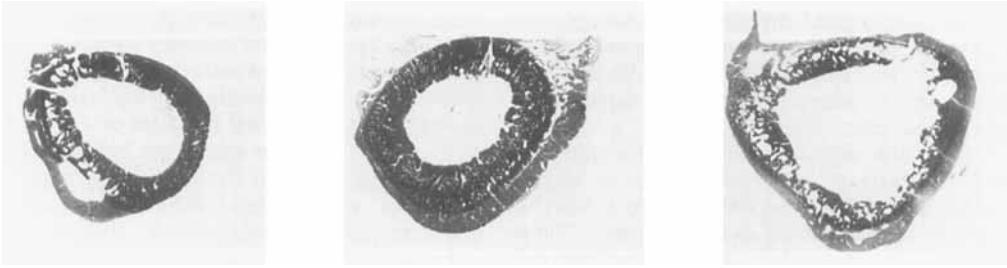

Figure 2A–C. Microradiographs of transverse sections from plated and osteotomized rabbit tibiofibular bone at 3 (A), 12 (B) and 24 (C) weeks after the operation. Note the subendosteal resorption, porotic transformation and the increase in the overall cross-sectional area of the tubular bone.

At 3 weeks postoperatively, the percentage increase of the entire cross section in plated bones was 16.4 ± 6.8 per cent. The difference between the size of the medullary canal expressed as a percentage of the entire cross-sectional area was not yet significant. At 6 and 9 weeks, the increase of the entire cross-sectional area was 24.4 ± 2.5 and 18.7 ± 7.9 per cent, respectively. At this stage the medullary cavity in the plated bones occupied a smaller percentage of the entire cross-sectional area than in the control bones ($P < 0.02$). Later on, at 12 and 24 weeks, the increase of the entire cross-sectional area in the plated bones was 16.4 ± 5.4 and 49.7 ± 11.5 per cent, respectively. At the same time, however, the area occupied by the medullary cavity had increased so much that the difference between the paired bones was no longer statistically significant. The increase in the total area of the transverse sections and the increase in the area of the medullary canal both reached a significant level ($P < 0.01$) at 24 weeks when compared with the values at 3 weeks postoperatively. A tubular bone had by then been achieved, with increased circumference, but with almost the initial ratio between the entire cross section and medullary cavity.

Morphometric measurement of the porotic changes in the cortical bone

Table 2 shows the results of the random point analysis made from microradiographs of transverse sections of the bones.

Table 1. Planimetry of transverse sections cut from the mid-diaphysis of the plated and control bones. The percentage increase of the overall cross-sectional area in the plated bones when compared with the control bones is expressed. The size of the medullary cavity is expressed as a percentage of the entire cross-sectional area in plated and control bones

Time after operation	Number of animals	Percentage increase of the cross-sectional area in plated bones (per cent) (mean ± s.d.)	Intramedullary area in relation to entire cross section (per cent) (mean ± s.d.)		*t*-test*
			Plated bone	*Control bone*	
3 weeks	4	16.4± 6.8	31.9±10.5	39.0±5.6	N.S.
6 weeks	5	24.4± 2.5	29.6± 4.6	37.4±8.1	$P < 0.02$
9 weeks	4	18.7± 7.9	29.3± 5.6	35.9±7.5	$P < 0.02$
12 weeks	4	16.4± 5.4	47.7± 5.8	48.4±3.3	N.S.
24 weeks	2	49.7±11.5	39.0± 0.1	40.8±3.0	N.S.

* Paired *t*-test of the differences in intramedullary area in relation to entire cross section between plated and control bones (N.S. means not significant).

Table 2. Porotic transformation of diaphyseal bone during fracture repair with rigid plate fixation

Time after operation	Porosity (per cent, mean ± s.d.)			
	Plated bone			*Control bone*
Weeks	Beneath the plate	Opposite cortex	(average)	(average)
3	5.8 ± 1.1	11.4 ± 5.2	(9.0 ± 4.8)	6.3 ± 1.5
6	12.5 ± 4.0	18.6 ± 4.5	(15.6 ± 5.1)	8.0 ± 1.9
9	12.2 ± 5.9	11.8 ± 7.8	(12.2 ± 5.9)	5.5 ± 2.5
12	31.0 ± 15.8	20.0 ± 14.5	(25.5 ± 15.2)	9.6 ± 2.9
24	39.3 ± 10.2	33.8 ± 11.2	(37.5 ± 10.2)	7.5 ± 2.0

During the first 3 weeks porosity in the cortex adjacent to the plate remained normal, but the cortex opposite the plate already showed signs of incipient porotic transformation (porosity 5.8 ± 1.1 and 11.4 ± 5.2 per cent, respectively). After this the proportion of resorbed bone increased rapidly. At 9 weeks postoperatively resorption of the cortical bone beneath the plate was equivalent to the degree of resorption at the opposite cortex (12.2 ± 5.9 and 11.8 ± 7.8 per cent, respectively). Later on, the porosity under the plate seemed to exceed the values for the opposite cortex. By 24 weeks after the operation the porosity under the plate had increased to 39.3 ± 10.2 per cent and in the opposite cortex to 33.8 ± 11.2 per cent. This increase in mean porosity in the plated bone during follow-up was statistically highly significant ($P < 0.001$).

Chemical analysis

The data obtained from chemical analyses were combined in subgroups according to the time of observation after the operation.

No significant differences could be observed between the plated bones and their controls at any of the three levels sectioned along the tubular bone (Figure 1) in any subgroup.

The values for section I, containing the line of osteotomy in plated bones and the comparable level from control bones, were used for comparisons between the various subgroups (Figure 3). In the plated bones only two of the parameters analysed showed statistically significant changes during follow-up: hexosamines (increase, $P < 0.01$) and the content of hydroxyproline (increase, $P < 0.05$). When these values were compared with the corresponding control values in the various subgroups, the differences between the paired bones were only significant for the content of hexosamines and the ratio of hexosamines to hydroxyproline at 3 weeks ($P < 0.01$ and $P < 0.05$, respectively). This may reflect formation of connective tissue in the fracture area at an early stage of fracture union. Otherwise the changes seemed to be negligible.

Whether chemical differences existed between the cortical bone adjacent to the plate and the bone in the opposite cortex was ascertained by comparing the isolated sectors I and III of the plated bones (Figure 1) in the various subgroups. These differently treated sectors showed no statistically significant differences in any of the parameters analysed.

DISCUSSION

The present study indicates that rigid plate fixation of experimental osteotomies in tibiofibular bone of rabbits induced profound structural changes in the cortical bone. The main alterations were formation of resorption cavities in the cortical wall and changes in

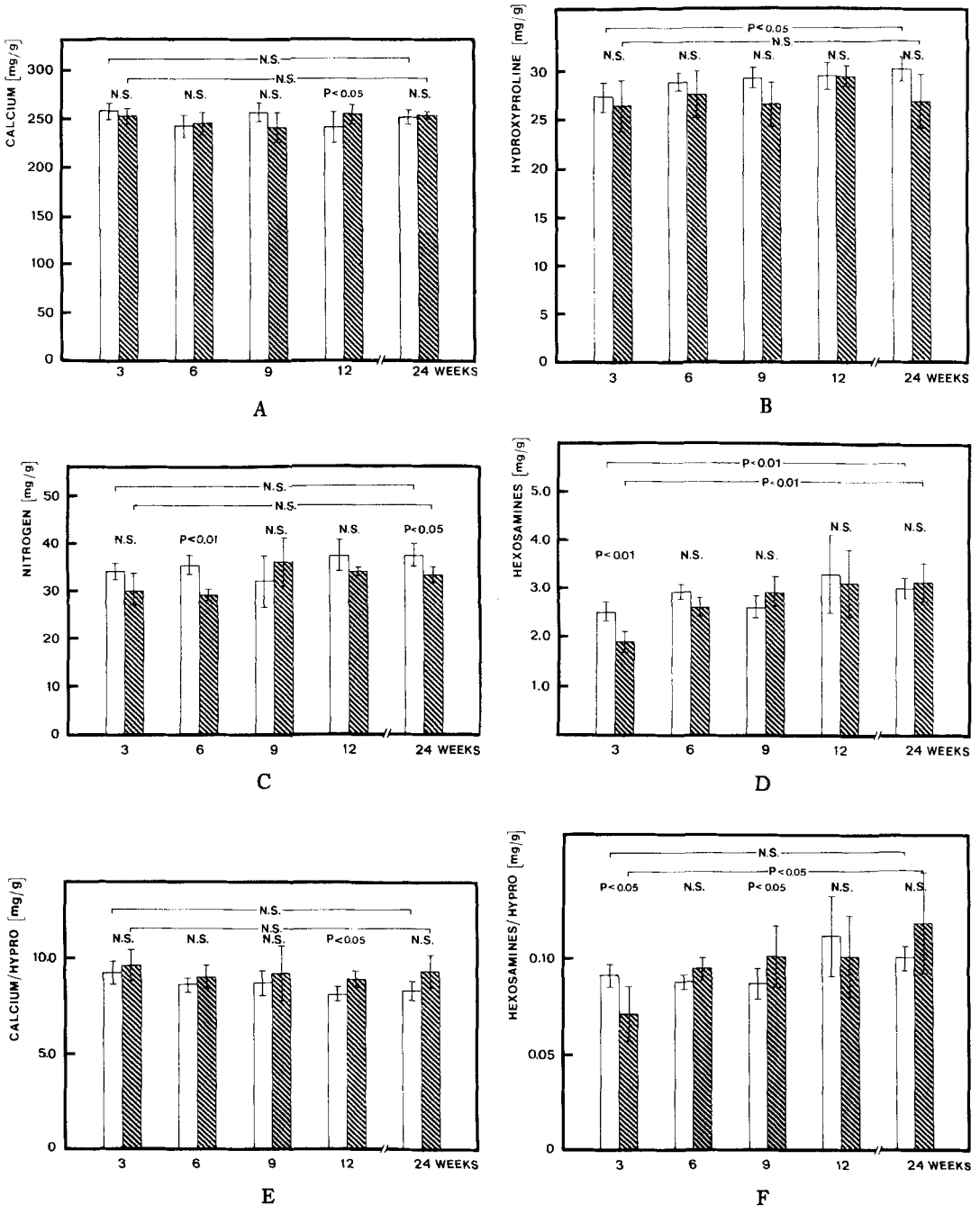

Figure 3A–F. The content of calcium (A), hydroxyproline (B), nitrogen (C), hexosamines (D) and the ratio of calcium to hydroxyproline (E) and hexosamines to hydroxyproline (F) in osteotomized rabbit tibio-fibular bones 3 to 24 weeks after the attachment of rigid plates. White columns indicate the plated bones and shaded columns the sham-operated contralateral bones. The vertical bars represent one standard deviation in each direction (± one s.d.). Statistical significances between the paired bones and bones at 3 to 24 weeks are presented.

the structure of the tubular bone. This may be partly due to the fact that the material applied was of excessive dimensions when compared with the underlying bone. Yet, despite these marked structural alterations, there were only slight changes in chemical composition in the area of fracture healing or in other parts of the cortical bone.

A uniform biphasic series of events could be observed in the structure of the specimens. At the first stage the outer circumference of the bone increased with subperiosteal formation of new bone, and this increase was followed by enlargement of the medullary cavity due to endosteal resorption of the cortical wall. At the end of the experiment, a tubular bone was achieved with increased circumference but with almost the initial ratio between the entire cross section and the medullary cavity.

Transformation of the bone begins with resorption of osteons in the cortical bone opposite the rigid plate. Later on, alterations beneath the plate exceeded those in the opposite cortex, producing a porotic bone with resorption of osteons in the cortical bone shapes separating the remaining bridges of cortical bone. The porotic transformation observed in the present study agrees with the changes previously described during fracture repair after compression osteosynthesis in the dog (Andersson 1965, Schenk & Willenegger 1967, Uhthoff & Dubuc 1971), sheep (Perren et al. 1969) and rabbit (Gördes et al. 1975a, Rahn & Perren 1971), and in man (Refior et al. 1975). On the other hand, similar observations have been reported even in intact bone after rigid plate fixation (Slätis et al. 1978). In intact bone the resorptive phase started slowly 3 weeks after plating, and rapid excavation and breakdown of the cortical wall occurred from 12 weeks onwards. In 36 weeks the degree of porosity increased from 7.3 to 40.9 per cent, which is equivalent to the present series with an observation period of 24 weeks.

The torsional properties of the healing fracture after rigid plate fixation (Paavolainen et al. 1979) indicated that the torque capacity and energy absorption increased linearly up to 9 weeks, reflecting fracture repair. At 24 weeks postoperatively, however, these parameters were again significantly reduced as compared with those of the control bones, reflecting the stress-protective effect of the rigid metallic implant. The effect of porotic transformation, with increased sites for crack initiation, seemed to exceed the mechanical effect of the increased cross section, with reduced shearing stress on the outer surface of the tubular bone (cf. Carter & Hayes 1977, Wright & Hayes 1977).

Previous studies concerning the chemical changes after rigid plate fixation in intact bones (Paavolainen et al. 1978a, 1978b) revealed only a slight increase in the content of hydroxyproline, reflecting formation of new bone subperiosteally and endosteally. Loss of calcium content of about 15 per cent occurred during the first 2 weeks postoperatively, but there was no evidence of progressive loss of calcium, as might have been expected from the histological observations on progressive structural derangement of the bone towards the end of the experiment.

The results of the chemical analysis in the present study accord with the previous observations. On the other hand, the results also agree with the observations made by Mattsson (1972) and Sevastikoglou et al. (1976) of the chemical composition of bone after experimental osteoporosis due to disuse.

As the loss of calcium beneath the plate might have been counterbalanced by formation of appositional new bone (Yu et al. 1975) sectors of bone adjacent to the plate and from the opposite cortex were analysed separately. No differences in chemical composition were found between these two sectors. In the area of the osteotomy the content of hexosamines and the ratio of hexosamines to hydroxyproline were slightly increased at 3 weeks, reflecting formation of connective tissue between the fracture ends. Later on, there were no significant changes in the computed ratios of calcium to hydroxy-

proline, hexosamines to hydroxyproline or nitrogen to hydroxyproline, indicating no real signs of cartilaginous callus formation during the later stages of fracture repair (Penttinen 1972).

Bone mineral mass is reduced after rigid plate fixation, according to estimates made with attenuation techniques (Gördes et al. 1975b, Strömberg & Dalen 1976, Tonino 1974). However, the use of attenuation techniques may lead to overestimation of the loss of minerals from non-resorbed cortical bone, owing to the reduced density of the porotically transformed bone.

Our findings suggest that fixation with a rigid plate provides conditions for undisturbed union of the fracture ends without callus formation, but the rigid implant protects tubular bone from mechanical stresses and causes resorption in those parts of the diaphysis which have been deprived of their normal function (Lanyon et al. 1976). The reduction of mechanical strength seems not to be connected with mineral loss or other chemical changes in the bone tissue, but due to structural and porotic transformation of the bone.

REFERENCES

Andersson, L. D. (1965) Compression plate fixation and the effect of different types of internal fixation on fracture healing. *J. Bone Jt Surg.* **47**-A, 191–208.

Braden, T. D., Brinker, W. O., Little, R. W., Jenkis, R. B. & Butler, D. (1973) Comparative biomechanical evaluation of bone healing in the dog. *J. Amer. vet. med. Ass.* **163,** 65–69.

Carter, D. R. & Hayes, W. C. (1977) The compressive behaviour of bone as a two-phase porous structure. *J. Bone Jt Surg.* **59**-A, 954–962.

Danis, M. R. (1947) Le traitement operatoire des fractures. *J. int. Chir.* **7,** 311–314.

Gördes, W., Kossyk, W. & Holländer, H. (1975a) Histologische und histomorphometrische Veränderungen bei Plattenosteosynthesen nach Osteotomien an der Tibia des Kaninchens. *Arch. orthop. Unfall-Chir.* **82,** 123–133.

Gördes, W., Kossyk, W. & Bödefeld, P. (1975b) Versuche zur Kalksalzdichtenbestimmung an der osteotomierten und stabilisierten Tibia des Kaninchens. *Arch. orthop. Unfall-Chir.* **81,** 125–147.

Lanyon, L. E., Goodship, A. E. & Baggot, D. G. (1976) The significance of bone strain "in vivo". *Acta orthop. belg.* **42,** 109–122.

Mattsson, S. (1972) Reversibility of disuse osteoporosis. Experimental studies in the adult rat. *Acta orthop. scand.*, Suppl. 144.

Müller, M. E., Allgöwer, M. & Willenegger, H. (1963) *Operative Frakturbehandlung.* Springer, Berlin, Göttingen, Heidelberg.

Olerud, S. & Danckwardt-Lilliestrom, G. (1968) Fracture healing in compression osteosynthesis in the dog. *J. Bone Jt Surg.* **50**-B, 844–851.

Paavolainen, P., Slätis, P., Karaharju, E. & Holmström, T. (1979) The healing of experimental fractures by compression osteosynthesis. I. Torsional strength. *Acta orthop. scand.* **50,** 369–374.

Paavolainen, P., Slätis, P., Ahonen, J. & Karaharju, E. (1978a) Changes in calcium and hydroxyproline content of cortical bone after compression and neutral plate fixation. *Acta orthop. scand.* **49,** 506–511.

Paavolainen, P., Karaharju, E., Slätis, P., Ahonen, J. & Holmström, T. (1978b) Effect of rigid plate fixation on structure and mineral content of cortical bone. *Clin. Orthop.* **136,** 287–293.

Penttinen, R. (1972) Biomechanical studies on fracture healing in the rat. *Acta chir. scand.*, Suppl. 432.

Perren, S. M., Huggler, A., Russenberger, M., Allgöwer, M., Mathys, R., Schenk, R., Willenegger, H. & Müller, M. E. (1969) The reaction of cortical bone to compression. *Acta orthop. scand.*, Suppl. 125.

Rahn, B. & Perren, S. M. (1971) Primary bone healing. *J. Bone Jt Surg.* **53**-A, 783–786.

Refior, H. J., Meister, P. & Matzen, K. (1975) Untersuchungen zum Verhalten der Mikrostruktur der menschlichen Corticalis nach Druckplattenosteosynthese. *Arch. orthop. Unfall-Chir.* **81,** 45–56.

Schenk, R. & Willenegger, H. (1964) Zur Histologie der primären Knochenheilung. *Langenbecks Arch. klin. Chir.* **308,** 440–451.

Schenk, R. & Willenegger, H. (1967) Morphological findings in primary fracture healing. *Symp. Biol. Hung.* **7,** 75–86.

Sevastikoglou, J. A., Lemperg, R., Larsson, S.-E. & Mattsson, S. (1976) Chemical composition of the skeletal parts of the extremity of the adult rat following below-knee amputation and immobilisation in a plaster cast. *Acta chir. scand.*, Suppl. 467, 26–30.

Slätis, P., Karaharju, E., Holmström, T., Ahonen, J. & Paavolainen, P. (1978) Structural changes

in intact tubular bone after application of rigid plates with and without compression. *J. Bone Jt Surg.* **60-**A, 516–522.

Slätis, P. & Rokkanen, P. (1967) The mineral phase in the repair of experimental fractures. *Ann. Chir. Gynaec. Fenn.* **56,** 193–201.

Strömberg, L. & Dalen, N. (1976) Influence of a rigid plate for internal fixation on the maximum torque capacity of long bones. *Acta chir. scand.* **142,** 115–122.

Tonino, A. J. (1974) *Operatieve fractuurfixatie met plastic:* Academisch proefschrift. Amsterdam.

Uhthoff, H. K. & Dubuc, F. L. (1971) Bone structure changes in the dog under rigid internal fixation. *Clin. Orthop.* **81,** 165–170.

Wright, T. M. & Hayes, W. C. (1977) Fracture mechanics parameters for compact bone—effects of density and specimen thickness. *J. Biomechanics* **10,** 419–430.

Yu, W. Y., Siu, C. M., Shim, S. S., Hawthorne, H. M. & Dunbar, J. S. (1975) Mechanical properties and mineral content of avascular and revascularizing cortical bone. *J. Bone Jt Surg.* **57-**A, 692–695.

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