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CHANGES IN CALCIUM AND HYDROXYPROLINE CONTENT OF CORTICAL BONE AFTER COMPRESSION AND NEUTRAL PLATE FIXATION

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Calcium and hydroxyproline content of intact rabbit tibio-fibular bone was assayed chemically 1 day to 36 weeks after compression and neutral plate fixation with a 4-hole stainless steel ASIF/DCP plate.

During the first three postoperative weeks calcium content of the cortical bone decreased by an almost significant amount ($P < 0.05$); thereafter, calcium values remained subnormal but did not differ significantly from initial postoperative values. Hydroxyproline content increased steadily throughout the experiment ($P < 0.001$).

The changes in chemical composition of the bone were of the same magnitude after compression and neutral plate fixation. The results suggest that the cancellous transformation of cortical bone, known to take place under rigid plates, reflects an active remodelling process of the bone in which the calcium and hydroxyproline content changes only slightly.

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

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In the treatment of certain fractures of long bones, compression plate fixation provides considerable stability and enables subsequent closure of the fracture gap by primary fracture healing (Müller et al. 1963, Schenk & Willenegger 1964, Olerud & Danckwardt-Lilliestrom 1968). However, while providing conditions conducive to fracture repair, the attachment of rigid plates to cortical bone simultaneously calls forth a structural derangement of the underlying bone. Uhthoff & Dubuc (1971) drew attention to the profound cancellous transformation that occurred after plating of osteotomized canine femora, and Gördes et al. (1975b) reported similar changes after plate fixation of osteotomized rabbit tibio-fibular bones. Recent reports suggest that the cancellous transformation of plated cortical bone is accompanied by a decrease in the mineral content of the bone. Gördes et al. (1975a) reported that 24 weeks after rigid plate fixation of rabbit tibio-fibular bones the mineral content, as measured with a gamma-ray attenuation technique, had decreased 26 per cent. Using a similar technique, Strömberg & Dalén (1976) and Tonino et al. (1976) also found that the mineral content of intact canine femora had decreased after rigid plating.

Contrasting with these observations are those of Schenk & Willenegger (1964) who showed, with a fluorescence labelling technique, that the renewal rate in osteotomized cortical bone increases during primary bone healing. Coutts et al. (1973), by measuring

the rate of bone formation in canine dog tibia with tetracycline labelling, also observed a marked stimulus to new bone formation in cortical bone subjected to compression plating.

No figures are available for the chemically-assessed content of calcium and collagen in cortical bone subjected to rigid plate fixation. The aim of this study was to compare, in rabbit tibio-fibular bone, the effects of compression versus neutral plate fixation on the chemically determined content of calcium and hydroxyproline.

MATERIAL AND METHODS

Operative procedure

Forty adult rabbits weighing from 2,400 to 3,850 g were used. Both tibio-fibular bones were exposed through a straight incision and a stainless steel four-hole dynamic compression plate (ASIF/DCP) was firmly attached to the anterolateral face of the intact tibia with a specially devised plate holder. The plate, which was less than half as long as the bone, was positioned so that its midpoint lay directly over the tibio-fibular junction. On the right tibia compression was applied between the most proximal and the most distal screws, with the two middle screws being driven home in a neutral fashion. The entire tubular segment between the two outermost screws was thus subjected to compression. On the left tibia the four-hole plate was attached without compression. Postoperatively, the animals were housed in separate cages in which they were allowed to move about freely. Seven animals were excluded from the study because of infection (1) or a fracture under the plate (6). Fractures of the plated bones occurred bilaterally in four, in the right tibio-fibular bone in one and in the left in one.

A designated number of animals were killed 1 and 3 days, and 1, 3, 6, 12, 18, 24 and 36 weeks after the operation. Tubular sections from the midshaft of the tibio-fibular bone under the plate were taken for chemical analysis.

Chemical analysis

The samples were carefully freed of soft tissue with the periosteum being preserved intact. The bone samples were defatted in several changes of acetone and air-dried to a constant weight, and then hydrolysed in 6 N hydrochloride acid at 130°C for 3 hours. One portion of the hydrolysate was neutralized with NaOH and used to determine hydroxyproline content. The method described by Pikkarainen (1968) without benzene extraction was used. The coefficient of variation of the method was ± 2.7 per cent. The remainder of the hydrolysate, appropriately diluted, was used to determine calcium content with a Unicam SP 90 atomic absorption spectrophotometer (Unicam Instruments Ltd, Cambridge, England); calcium was measured in 1 per cent lanthanium chloride.

Sixty-two samples from 31 animals were analysed successfully; the samples from two animals were excluded because of technical failure. The 31 animals were divided into five groups based on when, postoperatively, they were killed: 1–3 days postoperatively (6 animals), 1–3 weeks (12 animals), 6–12 weeks (5 animals), 18–24 weeks (5 animals) and 36 weeks (3 animals).

Statistical methods

To test the statistical significance of the differences between the compression plated and neutrally plated bones the paired t-test was used. t statistics for two means were used to compare the significance of differences among several groups. $P > 0.05$ was taken to be not significant.

RESULTS

Table 1 shows the mean calcium and hydroxyproline content for the five groups of animals. Figure 1 adds to this the corresponding calcium/hydroxyproline ratios. During the first three postoperative weeks, the calcium content of both the compression and neutrally plated bones declined by an almost significant ($P < 0.05$) 10 per cent. Thereafter, calcium values remained subnormal in both groups of bones, but the initial postoperative value and that at 36 weeks did not differ significantly. In all bones hydroxyproline content increased steadily, the difference between the initial postoperative value and that at 36 weeks being highly significant ($P < 0.001$). Consequently, the ratio of calcium to hydroxyproline gradually declined, until at 36 weeks the decrease in this ratio for both groups of bones was almost significant ($P < 0.05$). At none of the five periods was there any significant difference either in calcium or hydroxy-

proline content or in the calcium/hydroxyproline ratios between the bones plated with compression and those plated with neutral fixation.

DISCUSSION

The attachment of a rigid metallic plate to the cortical bone can induce untoward changes in the underlying bone. Gördes et al. (1975b) observed that during the first 24 weeks after neutral rigid plate fixation of rabbit tibio-fibular bone cancellous transformation was progressive and profound. In our laboratory, the rigid plate fixation of intact rabbit tibio-fibular bone induced similar changes. Thirty-six weeks after compression plate fixation the frequency of cavities in cross sections of the tubular bone underneath the plate had increased sixfold: in neutrally plated bone cancellous transformation was of the same magnitude (Slätis et al. 1978).

Table 1. Concentration of calcium and hydroxyproline (mg/g dry weight of bone) in the tibio-fibular bones of 31 rabbits after compression and neutral plate fixation. Mean ± s.d.

Time after plate fixation	No. of animals	Calcium		Hydroxyproline	
		Compression	Neutral	Compression	Neutral
1–3 days	6	189.8 ± 20.6	193.5 ± 21.0	21.8 ± 1.1	22.1 ± 0.9
1–3 weeks	12	174.3 ± 12.0	171.6 ± 31.6	22.9 ± 0.7	23.0 ± 0.9
6–12 weeks	5	179.3 ± 19.0	183.1 ± 17.6	23.3 ± 0.8	23.4 ± 0.6
18–24 weeks	5	187.3 ± 16.5	176.2 ± 12.4	24.2 ± 1.6	24.3 ± 1.7
36 weeks	3	176.8 ± 26.4	182.2 ± 6.50	25.4 ± 2.5	24.7 ± 1.8

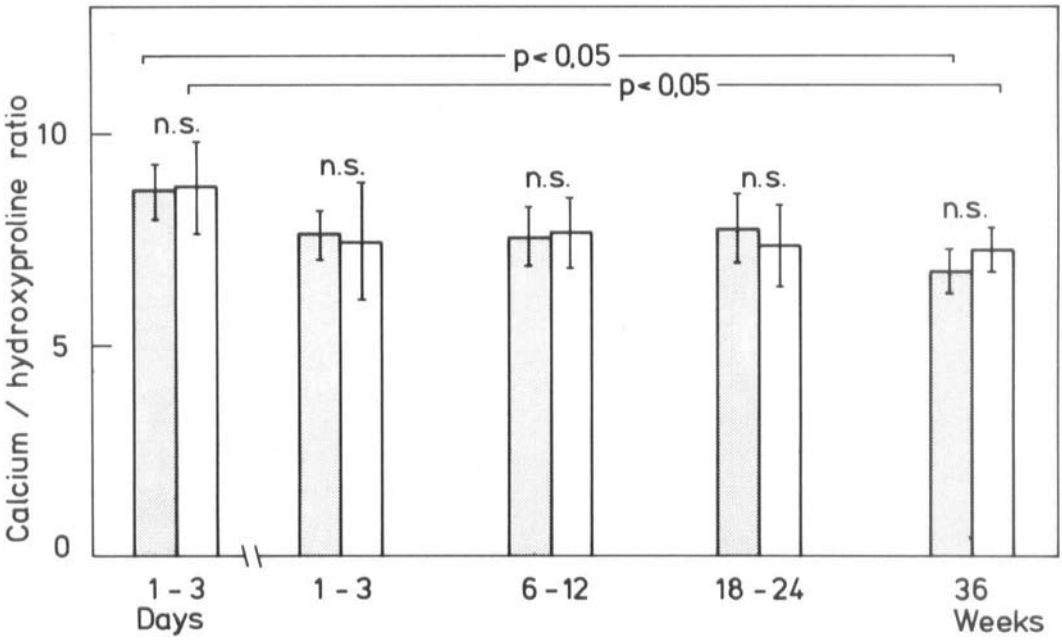

Figure 1. Calcium-hydroxyproline ratio in intact rabbit tibio-fibular bone after rigid plate fixation with compression plates (shaded columns) and with neutral plates (white columns). Closed bars represent one standard deviation.

Coutts et al. (1973) reported no differences either in porosity or in new-bone formation between bones plated with and without compression but otherwise plated identically. These authors attributed the observed changes not to compression but to the rigidity of the plate. Recently, the use of plates with various stiffness properties has shown that plates with bending properties closely resembling those of the underlying bone bring about less porotic changes in tubular bone than do rigid plates (Tonino et al. 1976).

During the first 3 weeks of this study the calcium content of all plated bone decreased and thereafter remained subnormal. The mineral loss was less profound than that reported by Gördes et al. (1975b). This may be because mineral loss is greater in the plated osteotomized bone these authors studied than in plated intact bone. Moreover, the method of assaying calcium content may also have a bearing on the estimate arrived at. Results achieved with the gamma-ray attenuation technique are apt to be influenced by changes in bone density. When cancellous transformation is accompanied by an increase in the amount of soft tissue elements in the porotic cavities, estimates of mineral content tend to be low (Meunier et al. 1970, Karjalainen 1973). Hence, the continuous decline in mineral content reported by Gördes et al. (1975a) may not only reflect a decrease in the amount of mineral in the plated bone, but also the cancellous transformation of the cortical wall adjacent to the implant. The steady increase we observed in the amount of hydroxyproline, together with the corresponding decrease in the calcium-hydroxyproline ratio, is evidence of an active turnover of collagen – the prerequisite for the remodelling of bone tissue and for the formation of immature new bone subperiosteally.

According to Perren et al. (1969), after plating, the compressive forces gradually diminish even in intact bone. This would mean that at the end of 36 weeks only slight, compressive forces were still acting on the cortical bone. Our observation that changes in the bones plated with and without compression were similar supports the view that the adverse changes in the plated bone are attributable not to the compression *per se*, but to the effect of the rigid plates.

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