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BIOCHEMICAL CHANGES IN BONE GRAFTS STABILIZED WITH RIGID PLATES

I. Cancellous Grafts

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The effect of rigid plate fixation on the chemical composition of cancellous interposition grafts was studied in rabbit tibio-fibular bones.

The concentrations of hexosamines and, to a lesser degree, of hydroxyproline and nitrogen, were high in the graft for the first 6 weeks, decreased from weeks 6 to 12, but remained higher than the corresponding values for the controls throughout the experiment (52 weeks). The ratio of hexosamines to hydroxyproline was highest for the graft at 3 weeks, indicating formation of cartilage and osteoid.

The initially low calcium concentration of the graft increased by 35 per cent from weeks 1 to 6, decreased from weeks 6 to 12, and remained below normal thereafter in comparison with corresponding values for the cortical host bone. The ratio of calcium to hydroxyproline increased throughout the experiment, reflecting maturation of the graft to lamellar bone.

Thus, biochemically the early incorporation of rigidly fixed cancellous interposition grafts resembles the healing of unimmobilized fractures by callus formation.

Key words: bone grafts; bone plates; calcium; fracture fixation; hexosamines; hydroxyproline; nitrogen; phosphorus

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The connective tissue of cartilage and bone consists mainly of organic fibres (collagen), cement substance (glycosaminoglycans) and water. In bone, inorganic apatite crystals displace the water during mineralization (Robinson 1975).

Water accounts for about 20–30 per cent of the wet weight of cortical bone and about 75–88 per cent of the wet weight of cartilage (Herring 1968, Wuthier 1969). In bone, minerals make up approximately 70–77 per cent of the tissue dry weight, and organic substances account for the rest (Herring 1968, Carter & Spengler 1978).

Collagen forms about 90–95 per cent of the organic matrix of bone and can be measured by determination of hydroxyproline. The hexosamines are the main components of glycosaminoglycans, i.e. polusaccharide chains linked covalently to protein as proteoglycans, which make up about 1–2 per cent of the organic dry weight of bone (Robinson 1975, Carter & Spengler 1978).

In cartilage, the collagen content of the dry organic matrix is lower (only 55–64 per cent) and the content of glycosaminoglycans higher (24–40 per cent) than in bone (Campo & Tourtellotte 1967, Herring 1968).

During the endochondral ossification of epiphyseal cartilage, the collagen content increases and the proteoglycan content progressively decreases (Wuthier 1969). The same sequence of events has also been noted for callus tissue during normal fracture repair (Solheim 1965, Penttinen 1972, Ketenjian et al. 1978).

During fracture repair without concomitant internal fixation the periosteal callus stabilizes

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the bone. Morphologically and chemically, the maturation of the callus can be differentiated into fibrous, cartilaginous and ossification phases (Slätis & Rokkanen 1965 and 1967, Penttinen 1972).

After rigid plate fixation, simple osteotomies (Olerud & Danckwardt-Lilliestrom 1968), and cortical (Olerud & Danckwardt-Lilliestrom 1971, Hutzschenreuter 1972) and cancellous (Düker et al. 1975) interposition grafts heal by direct intraosseal bridging of the fracture gaps without external cartilaginous callus. Chemical changes in the bone are minimal during the primary healing of plated osteotomies (Paavolainen et al. 1979b).

The scaffolding texture of cancellous grafts facilitates their early incorporation and remodelling (Siffert 1955, Boyne 1970). The chemical changes occurring during this process have not yet been assessed, and the present study was undertaken to fill this gap. For this purpose the chemical composition of cancellous interposition grafts fixed by rigid plates was determined at specified intervals between 1 and 52 weeks after the operation.

MATERIAL AND METHODS

Operative technique

A 6-mm long tubular segment of the midshaft of rabbit tibio-fibular bone was removed with a double-bladed circular saw. The defect was filled with a 6-mm long cortico-cancellous graft from the iliac crest. Osteosynthesis was achieved with a 6-hole DCP/ASIF stainless steel plate (for details, see Waris 1981).

Sampling technique

Animals were killed 1, 3, 6, 12, 24, 36 and 52 weeks after the operation. Both tibio-fibular bones were removed, the periosteum and any callus tissue being left *in situ*.

A total of 55 successful operations were performed on adult rabbits. Samples from 31 grafted tibio-fibular bones were taken for chemical analysis. From each bone, a disc-shaped specimen was removed from both the proximal and the distal host bone, and a third from the distal area of the graft (Figure 1). One disc taken from the opposite unoperated tibia served as a control specimen. Reference values for the biochemical composition of the iliac cancellous bone were determined from six samples.

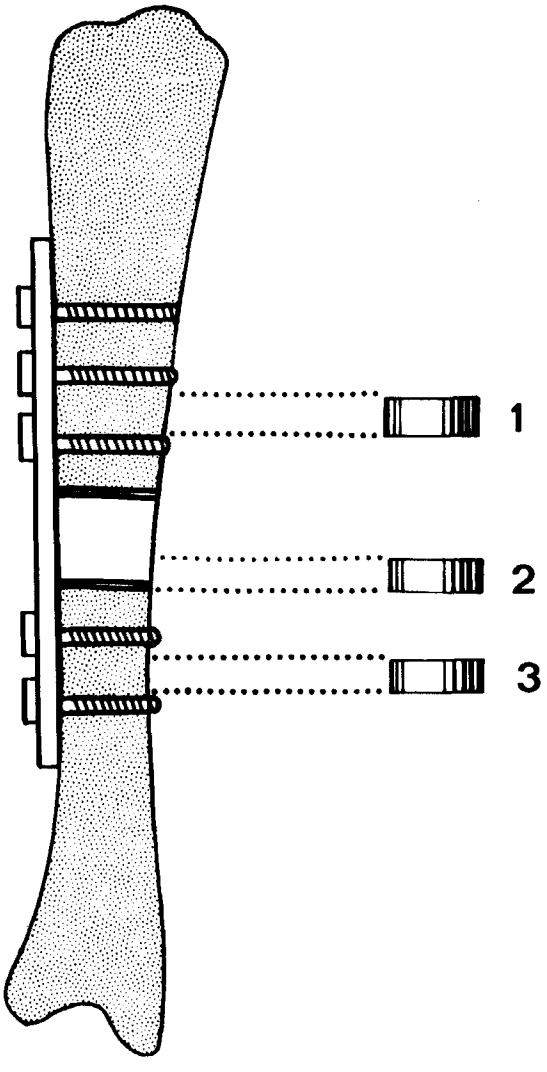

Figure 1. Sites of specimens taken from the host bone (1 and 3) and graft (2).

Chemical analysis

The samples were lyophilized, weighed and ground to powder in a mortar. The chemical analyses were carried out according to the methods of Penttinen (1972). For determination of hexosamines the samples were hydrolysed in 2 N HCl for 16 hours at 105°C. A portion from each hydrolysate was taken for the hexosamine assay, and the residue was further hydrolysed in 6 N HCl for 3 hours at 130°C. Nitrogen and hydroxyproline were assayed as described by Pikkarainen (1968). The remainder of the hydrolysate was used for calcium determination in 1 per cent $LaCl_3$ with an atomic absorp-

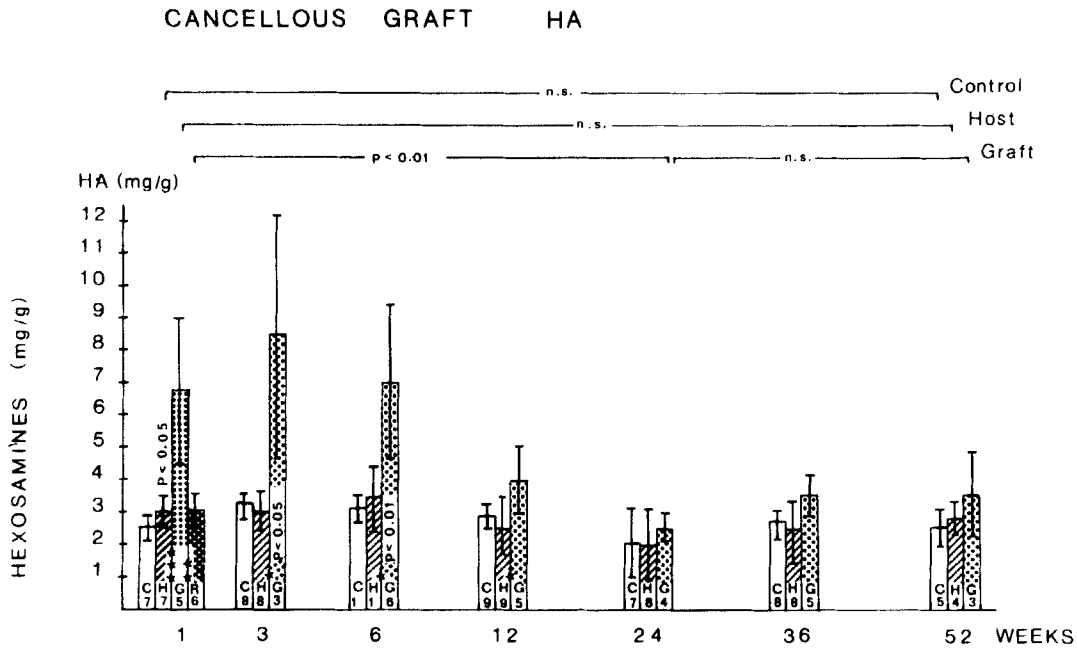

Fig. 2a

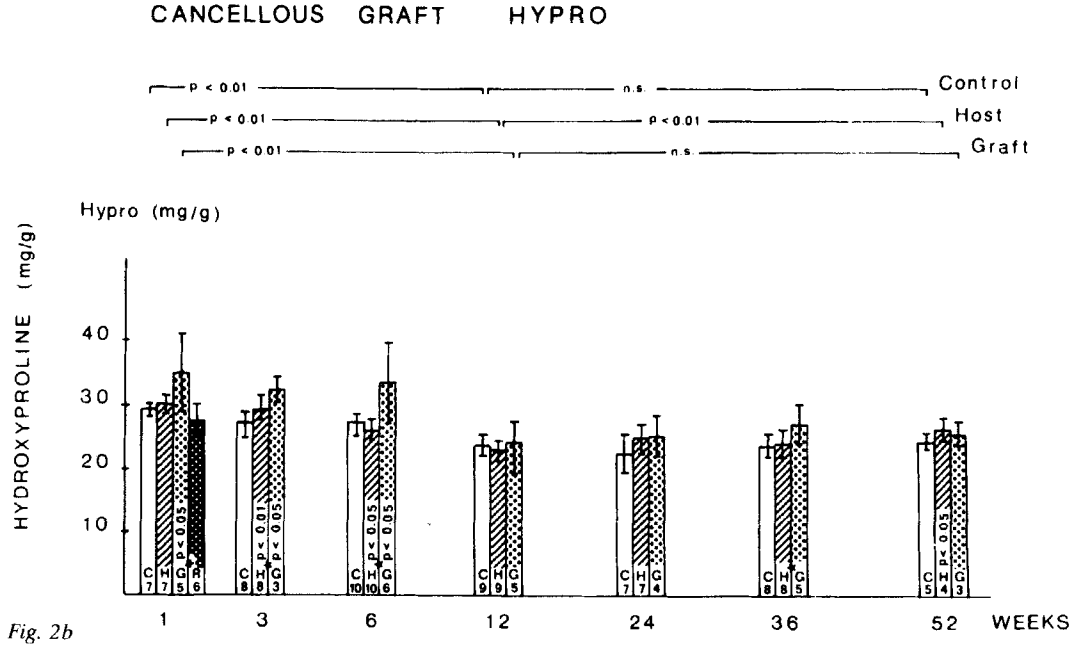

Fig. 2b

Figure 2a–d. Concentration of hexosamines (HA), hydroxyproline (Hypro) and calcium (Ca) and the ratio of calcium to hydroxyproline (Ca/Hypro) in the contralateral control bone (column C), proximal host bone (column H), distal graft specimens (column G) and reference cancellous bone from the iliac crest (column R). (Vertical bar = standard deviation; number at base of column = number of specimens; P value (x= < 0.05, xx = < 0.01, xxx = < 0.001): within column = comparison with control value (t test); between columns = comparison between values of respective columns; top of figure = comparison between values for time intervals indicated).

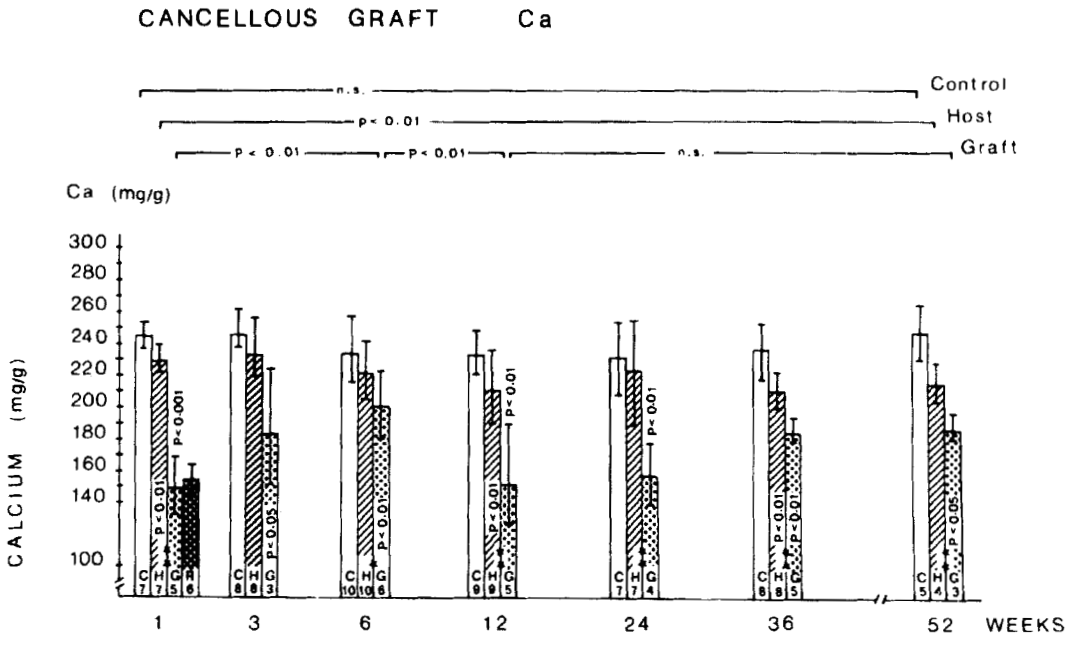
CANCELLOUS GRAFT Ca
n.s.
Control
P < 0.01
Host
P < 0.01
P < 0.01
n.s.
Graft
Ca (mg/g)
CALCIUM (mg/g)
300
280
260
240
220
200
180
160
140
100
P < 0.001
P < 0.01
P < 0.05
P < 0.01
P < 0.01
P < 0.01
P < 0.01
P < 0.05
C 7 H 7 G 5 R 6
C 8 H 8 G 3
C 10 H 10 G 6
C 9 H 9 G 5
C 7 H 7 G 4
C 8 H 8 G 5
C 5 H 4 G 3
1
3
6
12
24
36
52
WEEKS

Fig. 2c

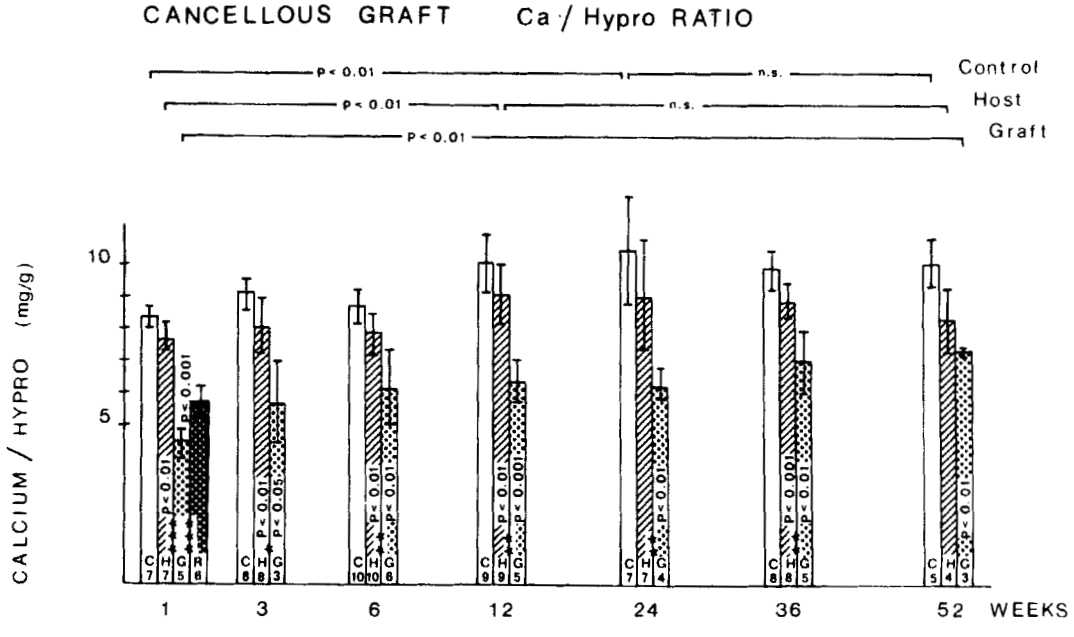
CANCELLOUS GRAFT Ca / Hypro RATIO
P < 0.01
n.s.
Control
P < 0.01
n.s.
Host
P < 0.01
Graft
CALCIUM / HYPRO (mg/g)
10
5
P < 0.001
P < 0.01
P < 0.01
P < 0.05
P < 0.01
P < 0.01
P < 0.001
P < 0.001
P < 0.001
P < 0.01
P < 0.01
P < 0.01
C 7 H 7 G 5 R 6
C 8 H 8 G 3
C 10 H 10 G 6
C 9 H 9 G 5
C 7 H 7 G 4
C 8 H 8 G 5
C 5 H 4 G 3
1
3
6
12
24
36
52
WEEKS

Fig. 2d

tion spectrophotometer (Varian Techtronit Pty. Ltd., Model 1100, Melbourne, Australia).

Statistical analysis was performed on the 5 per cent level with Student's *t* test for comparisons between two means and with a paired *t* test for comparisons of paired-bone values.

RESULTS

A macroscopic and radiographic examination showed that all the grafts were solidly united to the host bone by primary bone healing without any noteworthy formation of stabilizing callus (Waris et al. 1980).

The results of the chemical analysis of the specimens were correlated with the dry weight of the material. The concentrations of hexosamines, hydroxyproline and calcium, and the ratio of calcium to hydroxyproline are given in Figure 2a–d, and the concentrations of nitrogen and phosphorus, and the ratio of hexosamines to hydroxyproline are given in Table 1.

At week 1 the concentrations of the organic elements (nitrogen, hydroxyproline and hexosamines) were always higher in the proximal than in the distal host bone (sections 1 and 3 in Figure 1). Likewise, the depletion of minerals in the host bone, first detectable at 12 weeks, always started proximally. At other observation times the differences between the chemical content of the proximal and distal host bone were insignificant. Hence, only data for the proximal host bone are presented in detail.

Table 1. Concentration of nitrogen and phosphorus and the ratio of hexosamines to hydroxyproline (mean and SD) in the cancellous interposition grafts and host bone (proximal specimens) after rigid plating, and of contralateral intact bone. (Reference values (mean ± SD) for fresh iliac cancellous bone: nitrogen: 41.86 ± 3.14; phosphorus: 83.73 ± 7.22; ratio of hexosamines to hydroxyproline: 0.11 ± 0.1). For number of specimens see Figure 2

Type of bone	Graft Mean	SD	Host Mean	SD	Control Mean	SD
Nitrogen (mg/g)						
Week 1	57.88	20.31	41.29	11.23	35.21	8.11
Week 3	48.47	7.79	38.94	2.83	35.06	3.45
Week 6	50.45	10.58	36.77	6.18	31.29	4.48
Week 12	38.60	5.51	37.02	4.39	34.92	2.23
Week 24	34.55	3.07	34.91	4.75	34.56	5.06
Week 36	38.56	4.05	35.59	6.10	33.35	4.94
Week 52	40.30	3.27	38.90	3.10	36.16	2.54
Hexosamines/hydroxyproline (mg/g)						
Week 1	0.19	0.05	0.10	0.02	0.09	0.01
Week 3	0.26	0.13	0.10	0.02	0.12	0.01
Week 6	0.22	0.11	0.12	0.04	0.12	0.02
Week 12	0.16	0.03	0.11	0.04	0.12	0.02
Week 24	0.10	0.01	0.08	0.05	0.10	0.06
Week 36	0.13	0.02	0.11	0.04	0.12	0.02
Week 52	0.14	0.05	0.11	0.01	0.10	0.02
Phosphorus (mg/g)						
Week 1	83.54	10.15	125.26	4.28	129.01	6.19
Week 3	92.63	20.96	118.75	5.46	126.28	12.58
Week 6	108.83	15.23	115.89	7.44	127.27	7.45
Week 12	80.66	12.41	107.79	11.58	123.14	3.97
Week 24	83.15	9.18	116.69	29.81	121.16	11.35
Week 36	100.83	8.47	109.44	4.39	127.10	7.84
Week 52	97.53	4.58	116.00	2.10	127.30	3.94

Organic components

The concentrations of *hexosamines* (Figure 2a) in the graft were higher than in the host or control bone after 1 week, and by the third week had increased by an additional 25 per cent. Compared with these values, the hexosamine concentration in the graft decreased significantly ($P < 0.01$) thereafter, but still remained higher than in the host or control bone up to the end of the observation period. The differences in hexosamine concentration for the graft as compared with the host and the control bones were significant at weeks 1, 3 and 6, as was the difference between the corresponding values for the host and control bones at week 1.

The changes in the *hydroxyproline* (Figure 2b) and *nitrogen* concentrations (Table 1) ran closely parallel. The concentrations of both chemicals in the graft were highest from weeks 1 to 6, when they differed significantly from those of the host and control specimens. From weeks 1 to 12 the hydroxyproline concentration diminished in both the graft and host samples as well as in the control bones. After week 12 the graft and host bones again showed a gradual increase in their hydroxyproline content.

The *ratio of hexosamines to hydroxyproline* (Table 1) reflected the changes in hexosamine concentration; the values for the graft were high from weeks 1 to 6 and thereafter declined.

Minerals

The initial concentrations of *calcium* (Figure 2c) and *phosphorus* (Table 1) in the cancellous graft were about 65 per cent of those in the cortical host and control bones. These concentrations increased in the graft by 35 and 30 per cent, respectively ($P < 0.01$) from weeks 1 to 6. Between weeks 6 and 12 the mineral content of the graft declined sharply ($P < 0.01$), but thereafter slowly increased again.

The *ratio of calcium to hydroxyproline* (Figure 2d) increased continually in the graft specimens from weeks 1 to 52 and in the host and control bones from weeks 1 to 24. The values for the graft and host samples were always lower than those for the controls.

DISCUSSION

In the present study the incorporation of the cancellous graft showed a biphasic pattern. During the first 6 weeks new bone was laid down on the internal cancellous trabeculae and subperiosteally around the graft. From 12 weeks onwards a new medullary canal opened within the graft, which was remodelled into thin-walled lamellar bone (Waris et al. 1980, Waris et al., to be published). These morphological changes were accompanied for the first 12 weeks by an increase, and thereafter by a decrease, in the strength of the host-graft bond (Waris 1981).

The chemical changes found in the graft in the present study showed a similar sequence of events. The osteoproliferative phase of graft incorporation at 1 to 6 weeks was characterized by an increase in organic components. The early increases of hexosamines, hydroxyproline and nitrogen resembled the changes noted in callus tissue surrounding unimmobilized fractures in other studies (Solheim 1965, Penttinen 1972, Ketenjian et al. 1978). In callus the concentration of hexosamines increases during the fibrous and cartilaginous phases and decreases during the ossification phase. The increases in collagen (hydroxyproline), calcium and phosphorus concentrations continue throughout the cartilaginous and ossification phases.

In this study unstable fracture specimens with external cartilaginous callus were excluded, but histological analyses showed cartilaginous areas in the middle of the grafts at 1 to 6 weeks (Waris et al., to be published). Some of the hexosamine may have originated from blood and mesenchymal tissue between the graft trabeculae, but most of it originates from new cartilage and osteoid (Wuthier 1969).

In fracture callus, mineralization of the osteoid parallels the increase in collagen content. Similarly, the collagen content of the epiphyseal plate increases rapidly and hexosamines progressively decrease as the epiphyseal cartilage is transformed into calcified cartilage and bone (Wuthier 1969). The same sequence of changes was noted in this study. The mineral contents of the graft increased during the first 6 weeks, while the concentration of hexosamines decreased. The ratio

of calcium to hydroxyproline rose throughout the experiment and reflected the transformation of the graft to lamellar bone.

During the remodelling phase, from 6 weeks on, the concentrations of organic components were slightly higher in the graft than in the contralateral bone. Simultaneously, the mineral content of the graft declined significantly and thereafter remained below the control level.

In the plated host bone the hydroxyproline and hexosamine concentrations were slightly elevated for the first 3 weeks, a sign of new bone formation in the area under the plate. The concentrations of calcium and phosphorus in the host bone gradually decreased throughout the observation period. This decline can be interpreted as reflecting the reaction of the plated bone to the loss of mechanical load, the plate having shielded the bone from stress.

This finding agrees with the results of Paavolainen et al. (1978, 1979b), who studied intact and osteotomized rabbit tibio-fibulae. Their studies revealed only a slight increase in hydroxyproline concentration and an initial loss of calcium of about 15 per cent during the first 3 weeks after plating.

Rigid plate fixation of tubular bone causes porotic transformation of cortical bone (e.g. Slätis et al. 1978, Strömberg & Dalen 1978). The morphological changes are associated with loss of bone minerals (Tonino et al. 1976, Strömberg & Dalen 1978) and a decrease in bone strength (Strömberg & Dalen 1978, Paavolainen et al. 1979a).

The present results indicate that rigid plate fixation provides conditions conducive to early incorporation of cancellous interposition grafts. The biochemical changes in the graft resemble the changes in normal fracture callus. However, the rigid plate protects the graft from normal mechanical stress and consequently the functional remodelling of the graft, as measured by its mineral concentration, remains incomplete.

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