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BIOCHEMICAL CHANGES IN BONE GRAFTS STABILIZED WITH RIGID PLATES

II. Cortical Grafts

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

The concentration of hexosamines increased both in the graft and in the host bone during the first 6 weeks, but thereafter decreased. The concentrations of hydroxyproline and nitrogen increased throughout the experiment. The ratio of hexosamines to hydroxyproline increased in the graft for the first 6 weeks, indicating formation of immature osteoid and bone during this period, but thereafter decreased.

The calcium concentration of the graft decreased significantly during the first 12 weeks and remained below normal (–10 to –19 per cent). The mineralization of the graft, assessed by the ratio of calcium to hydroxyproline, decreased continually.

The results suggest that cortical bone grafts stabilized with rigid plates heal with only slight chemical signs of callus formation. The demineralization of the graft reflects the porotic changes that take place under the plate.

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

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In clinical pratice, both cortical and cancellous orthotopic bone grafts are used in connection with rigid osteosynthesis. After rigid plating both heal through intraosseal formation of new bone across fracture gaps without the appearance of external callus. Cancellous grafts are usually completely replaced in a short time (Siffert 1955, Boyne 1970), whereas remodelling of cortical grafts takes several months and remains incomplete (Albrektsson 1971, Enneking et al. 1975).

In a previous study (Waris et al. 1981) we reported the changes that occur, during healing, in the chemical composition of cancellous interposition grafts stabilized with rigid plates. The concentrations of the organic components of the graft were high from 1 to 6 weeks, but decreased significantly thereafter. The initially low mineral concentration of the cancellous graft increased for 6 weeks, but thereafter decreased again to a subnormal level, as compared with controls. The changes resembled those known to take place in callus tissue forming around non-stabilized fractures.

The purpose of the present study was to analyse the changes that occur in the chemical composition of cortical interposition grafts stabilized with rigid plates and to compare these changes with those occurring in cancellous grafts under similar experimental conditions.

MATERIAL AND METHODS

Operative and sampling techniques

The procedures were the same as in the preceding paper (Waris et al. 1981), except that the 6-mm long

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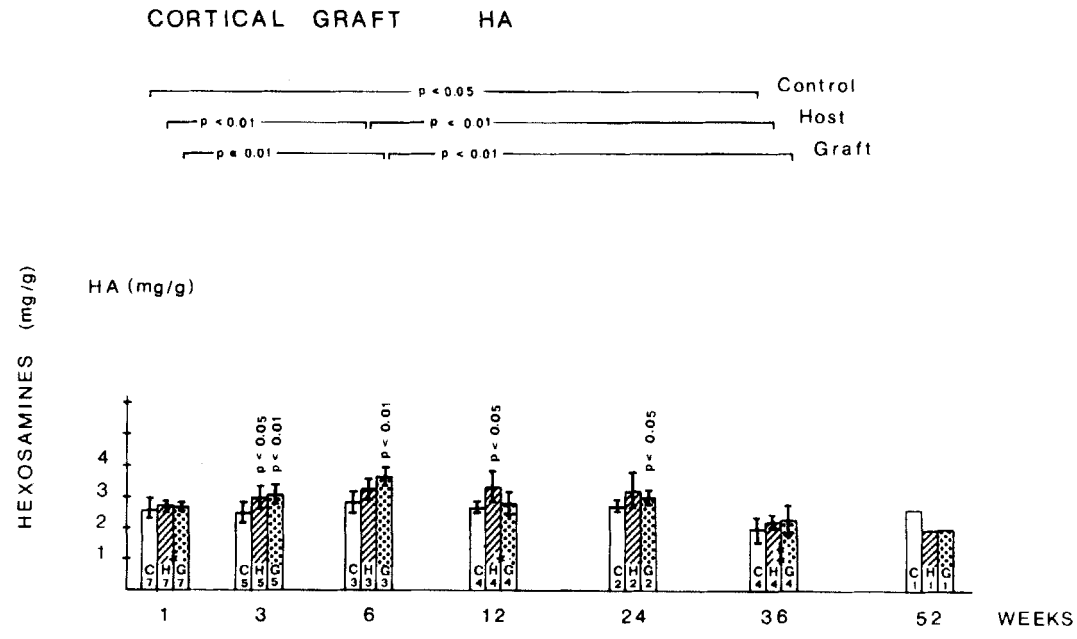

Fig. 1a

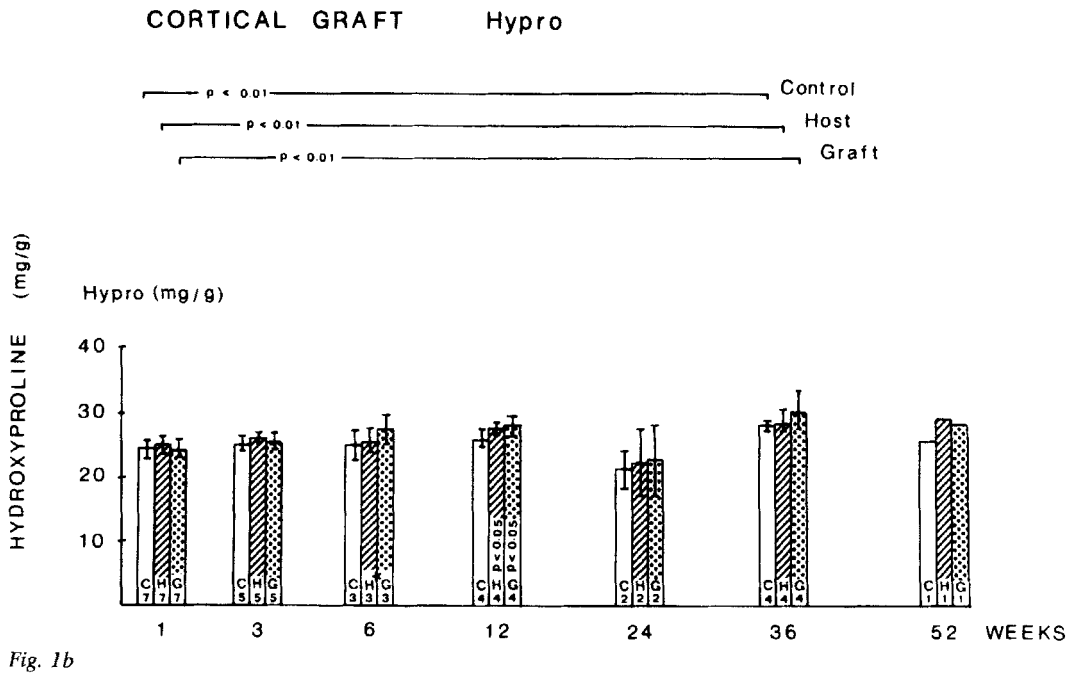

Fig. 1b

Figure 1a–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) and cortical graft specimens (column G). (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 the time intervals indicated).

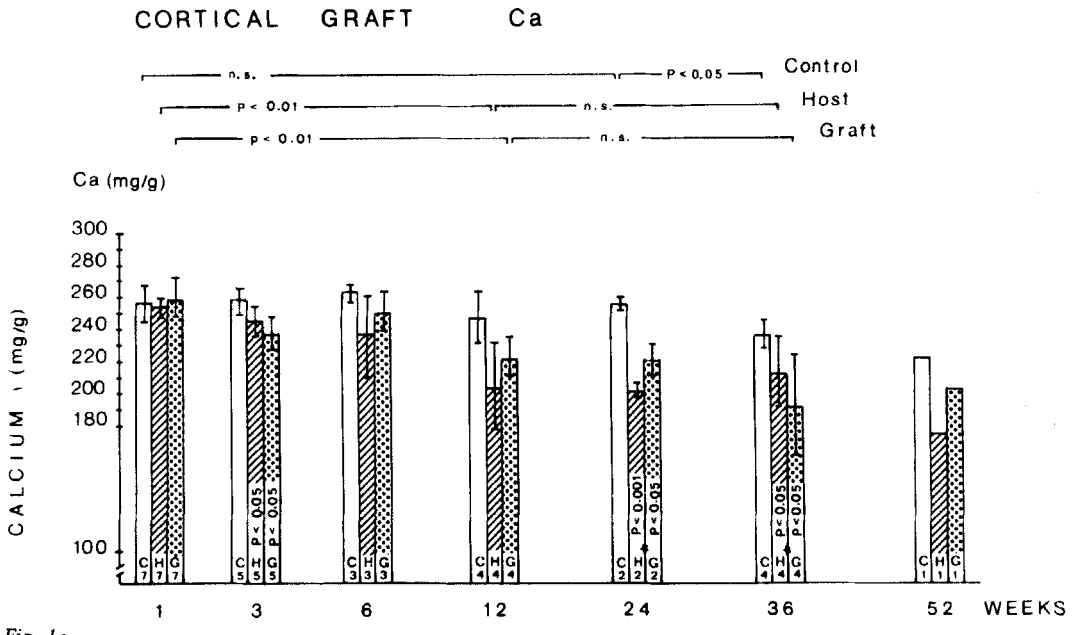

Fig. 1c

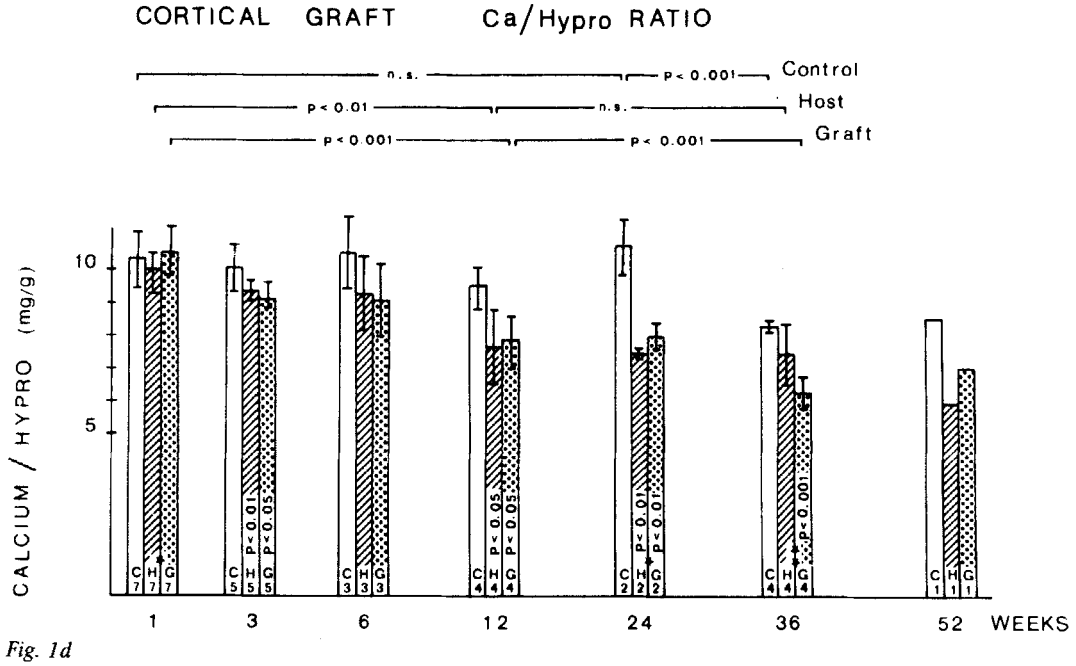

Fig. 1d

resected segment of rabbit tibio-fibular bone was turned through 180° and replaced in the defect instead of being replaced by a cancellous graft.

Of 70 operations on adult rabbits 50 succeeded (Waris 1981). Samples from 26 grafted tibio-fibular bones were used for the chemical analysis. Cross-sectional discs were taken from the proximal and distal host bone, from the distal graft area, and, for control, from the contralateral tibia (Waris et al. 1981). The contents of calcium, phosphorus, hydroxyproline, hexosamines and total nitrogen were determined using the techniques described by Penttinen (1972). The concentrations were expressed as milligrams per gram of dry weight of the material. In addition, ratios of calcium

to hydroxyproline and hexosamines to hydroxyproline were calculated.

Standard statistical methods were used, and the 5 per cent probability level was accepted as significant.

RESULTS

Macroscopic and radiographic examinations showed that the cortical graft mended by primary bone healing with only a slight periosteal callus reaction (Waris et al. 1980).

The results of the hexosamine, hydroxyproline and calcium analyses, and the ratio of calcium to hydroxyproline are given in Figure 1a–d, and Table 1 presents the results for nitrogen and phosphorus, and the ratio of hexosamines to hydroxyproline.

The increase in organic components at 1 week and the decrease in mineral concentration at 12 weeks were always greater in the proximal than in the distal fragment of the host bone. This delay in chemical changes in the distal fragment was similar to that observed in studies on cancellous interposition grafts (Waris et al. 1981). At later observation times the samples from the distal and proximal fragments did not differ significantly in chemical composition. Therefore, only the results for samples from the proximal fragment are reported here.

Organic components

The concentration of *hexosamines* (Figure 1a) increased in the first 6 weeks by 17 per cent in the host bone and by 37 per cent in the graft. The increase started earlier in the host bone; the difference between the paired samples at 1 week was significant ($P < 0.05$). After the 6 weeks the hexosamine level diminished gradually in both host and graft bones.

In the control specimens there was a gradual decrease in the hexosamine values between 1 and 36 weeks ($P < 0.05$). The differences between the host and graft bones and their paired controls were significant from weeks 3 to 24.

The concentrations of *nitrogen* (Table 1) and *hydroxyproline* (Figure 1b) in the graft, host and control specimens increased slowly from weeks 1 to 36. The increase was most marked in the graft.

The calculations of the ratio of *hexosamines to hydroxyproline* (Table 1) showed an increase of 18 per cent from weeks 1 to 6 for the graft and host specimens; thereafter the ratio gradually declined.

Minerals

The concentrations of *calcium* (Figure 1c) and *phosphorus* (Table 1) in the host and graft specimens declined significantly from weeks 1 to 12 and thereafter remained below normal ($P < 0.05$). The decline was slower for the graft samples. In the control bone the calcium concentration decreased after 24 weeks.

Consequently, for the graft and host bone, the *ratio of calcium to hydroxyproline* (Figure 1d) declined between weeks 1 and 12 ($P < 0.01$–0.001) and was below normal throughout the observation period.

The results of the analyses of cancellous (Waris et al. 1981) and cortical (present study) graft specimens are compared in Figure 2a–d. Accumulation of organic components, especially hexosamines, occurred earlier and was more marked in the cancellous grafts (Figure 2a–b). As regards mineral content (Figure 2c–d), that of the cancellous grafts increased, while that of the cortical grafts decreased. At 36 and 52 weeks, however, the two types of graft were almost identical in bone composition.

DISCUSSION

The cortical bone graft, stabilized by a plate, healed without noteworthy callus formation. The chemical changes in the graft were small, cortical grafts differing markedly in this respect from plated cancellous interposition grafts (Waris et al. 1981). The chemical changes found in the specimens of bone during the incorporation of the cortical bone graft resembled those observed during the repair of plated osteotomies of rabbit tibio-fibular bone (Paavolainen et al. 1979). Paavolainen et al. found an increase in hexosamine content of about 20 per cent in the osteotomy area at 3 weeks. In our graft the in-

Table 1. Concentration of nitrogen and phosphorus and the ratio of hexosamines to hydroxyproline (mean and SD) in the cortical interposition grafts and host bone (proximal specimens) after rigid plating, and of contralateral intact bone. For number of specimens see Figure 1

Type of bone	Graft Mean	SD	Host Mean	SD	Control Mean	SD
Nitrogen (mg/g)						
Week 1	32.09	2.63	33.67	4.79	31.71	3.52
Week 3	34.92	2.70	35.94	4.01	33.44	4.37
Week 6	36.63	3.46	34.33	4.54	30.00	3.26
Week 12	40.48	4.04	40.75	4.39	36.93	6.77
Week 24	42.05	4.17	38.55	10.64	33.40	5.52
Week 36	51.50	30.27	44.03	14.14	43.63	8.89
Week 52	36.50		39.90		32.90	
Hexosamines/hydroxyproline (mg/g)						
Week 1	0.11	0.01	0.11	0.01	0.11	0.01
Week 3	0.12	0.02	0.11	0.01	0.10	0.01
Week 6	0.13	0.01	0.13	0.02	0.12	0.03
Week 12	0.10	0.01	0.12	0.02	0.10	0.01
Week 24	0.11	0.01	0.12	0.02	0.11	0.01
Week 36	0.08	0.01	0.08	0.01	0.07	0.02
Week 52	0.09		0.07		0.01	
Phosphorus (mg/g)						
Week 1	123.81	2.22	122.47	2.80	123.43	3.91
Week 3	112.42	3.54	113.24	3.36	121.78	1.56
Week 6	117.07	3.75	107.80	7.97	117.93	2.63
Week 12	105.33	7.86	100.33	11.78	116.08	5.79
Week 24	102.80	7.64	95.95	0.21	116.45	7.43
Week 36	100.90	11.59	104.48	7.44	120.33	2.40
Week 52	119.20		107.90		115.70	

crease in hexosamine concentration was even more marked. This difference suggests that the early stage of incorporation of cortical grafts involves more abundant formation of callus tissue.

In the cortical as in the cancellous interposition grafts (Waris et al. 1981), organic components accumulated later in the graft and in the distal than in the proximal fragment of the host bone. This may reflect the less abundant blood supply to the distal area, a suggestion that is supported by a histological study of host/graft specimens (Waris et al., to be published).

The mineral concentration decreased in the host bone and in the graft; this process started earlier in the proximal fragment of the host bone, but the ultimate loss was greater in the graft. The result was a continual decline in the ratio of calcium to hydroxyproline in both fragments of host bone and in the graft.

The changes noted in the composition of the control bones (decrease in concentrations of hexosamines and minerals and increase in concentrations of hydroxyproline and nitrogen) may reflect the influence of age or immobilization on the composition of cortical bone. The hexosamine content of bone has been found to decrease with age (Sobel et al. 1954, Kazavina & Zenkevich 1961). Mattson (1972) noted an increase in the hydroxyproline and calcium content of rat cortical bone with advancing age, whereas others, e.g. Weidmann & Rogers (1958), Dickerson (1962) and Strandh & Norlén (1965), noted an increase in calcium and a decrease in nitrogen. On the other hand, in experimental osteoporosis, the composition of the remaining bone is generally considered to be unchanged (Larsson & Vejlens 1969, Sevastikoglou et al.

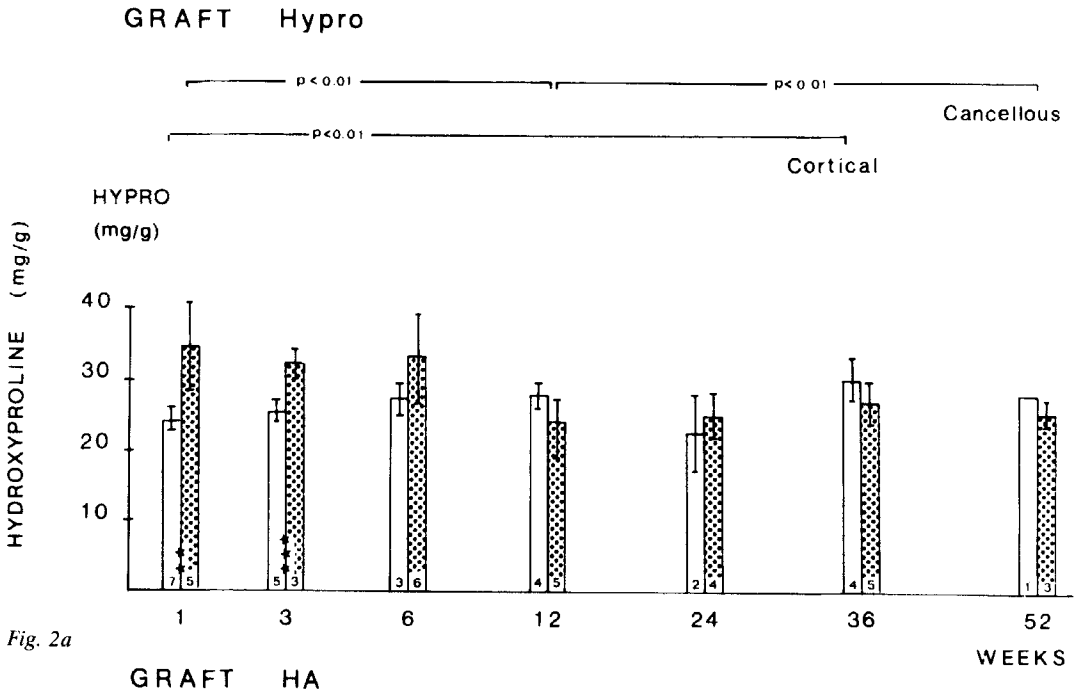

Fig. 2a

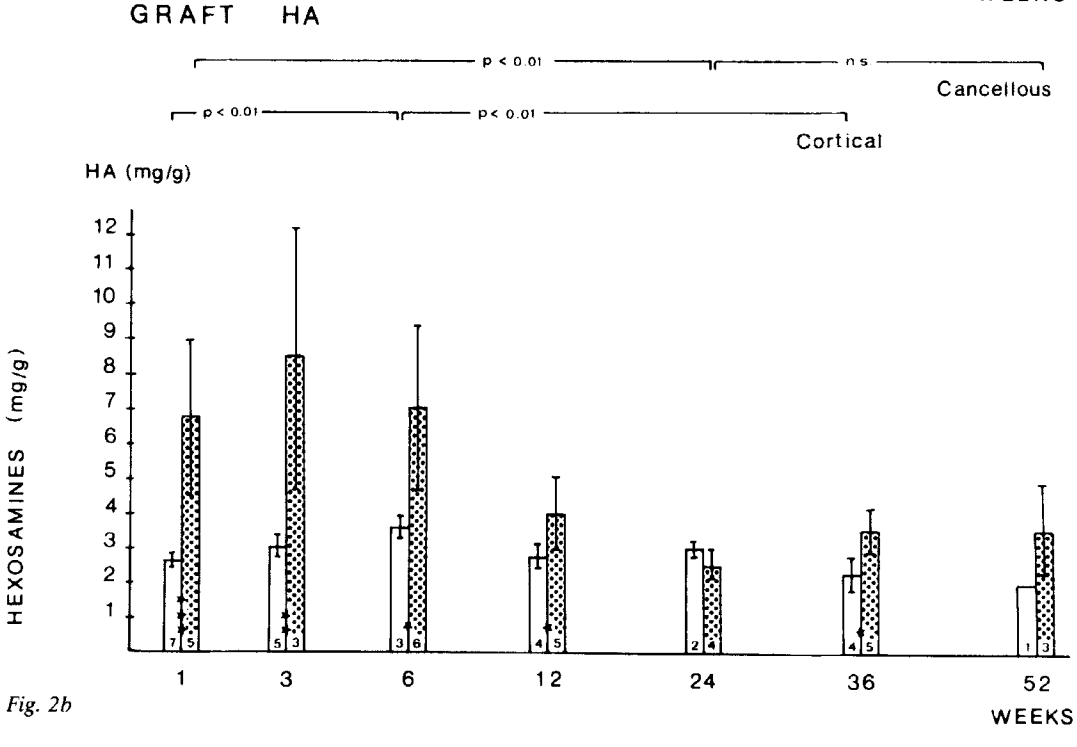

Fig. 2b

Figure 2a–d. Concentration of hexosamines (HA), hydroxyproline (Hypro) and calcium (Ca) and the ratio of calcium to hydroxyproline (Ca/Hypro) in cortical (white columns) and cancellous (stippled columns) interposition grafts after rigid plate fixation. (Vertical bar = standard deviation; number at base of column = number of specimens; P value (x = < 0.05, xx = < 0.01, xxx = < 0.001): between columns = comparison between column values; top of figure = comparison between values for the time intervals indicated).

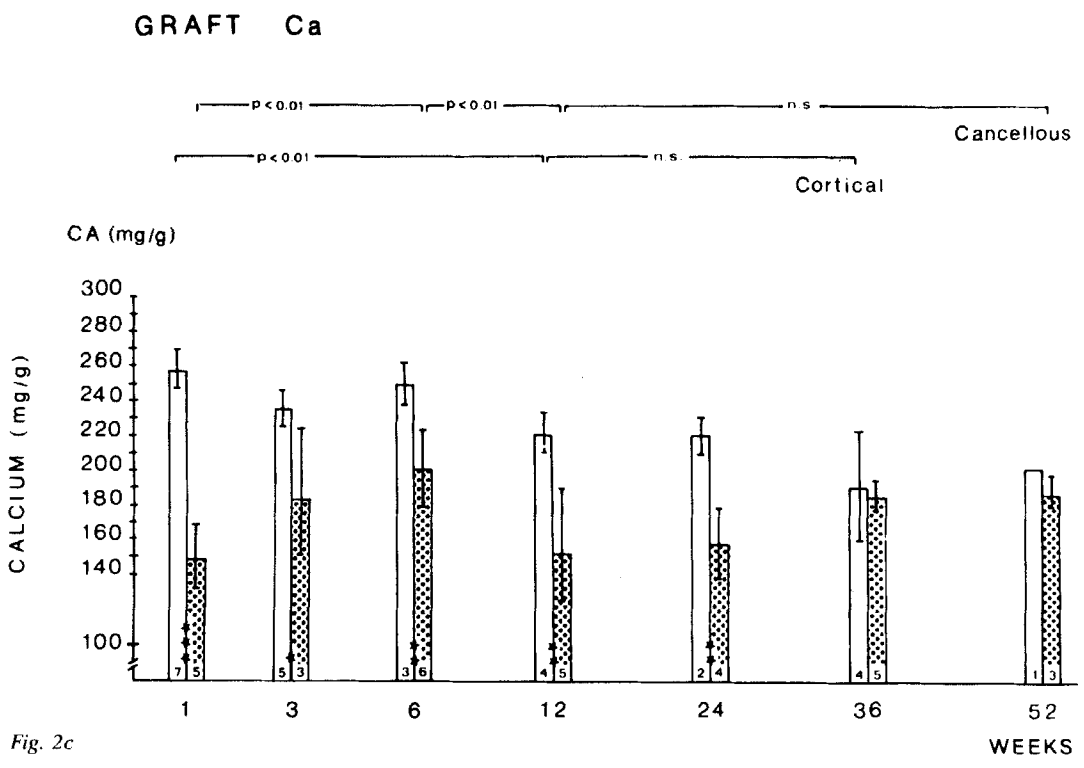
GRAFT Ca
p<0.01
p<0.01
n.s.
Cancellous
p<0.01
n.s.
Cortical
CA (mg/g)
CALCIUM (mg/g)
300
280
260
240
220
200
180
160
140
100
1
3
6
12
24
36
52
WEEKS

Fig. 2c

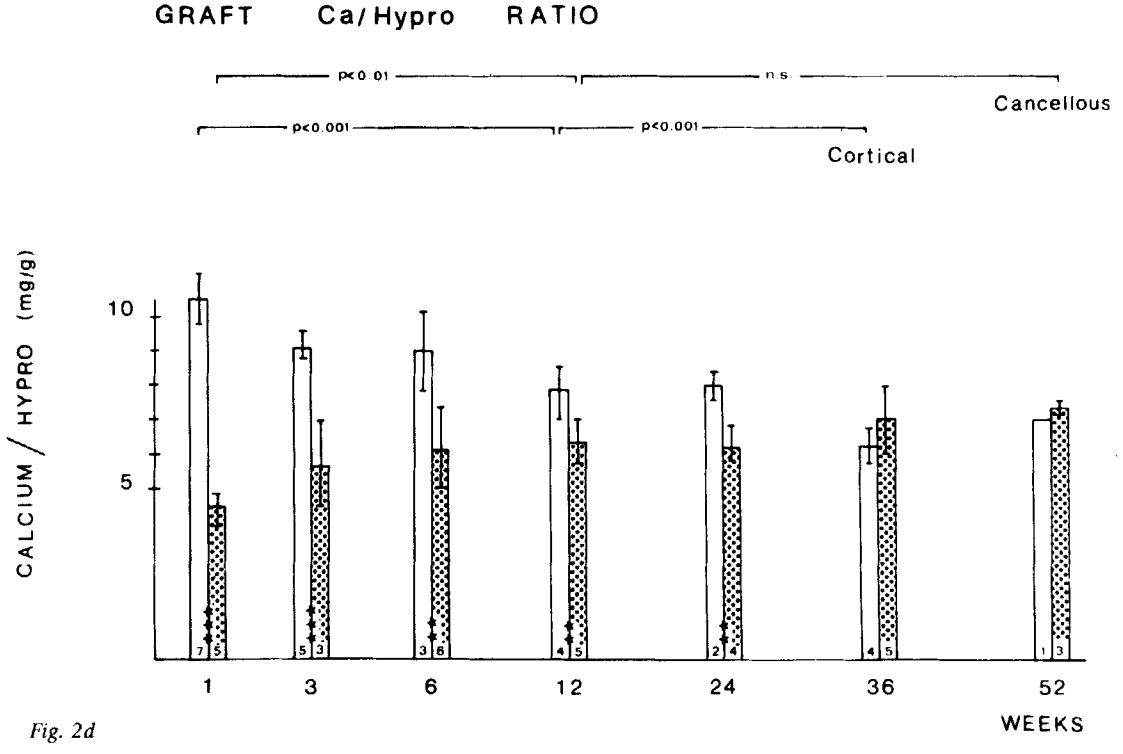
GRAFT Ca/Hypro RATIO
p<0.01
n.s.
Cancellous
p<0.001
p<0.001
Cortical
CALCIUM / HYPRO (mg/g)
10
5
1
3
6
12
24
36
52
WEEKS

Fig. 2d

1976), and the loss of minerals parallels that of bone matrix.

Comparison of the results for the cancellous and cortical grafts showed that the increase in organic components occurred earlier in the cancellous grafts and was more marked. This finding corresponds to those of radiographic (Waris et al. 1980) and histological (Waris et al., to be published) studies, which showed that formation of new woven bone occurred earlier and was more extensive in cancellous than in cortical grafts. The difference could be due to a greater osteogenic potential of the cancellous bone (Siffert 1955, Deleu & Trueta 1965).

The loss of minerals from the cortical grafts and their increase in the cancellous grafts resulted in a bone with almost identical chemical composition, which, however, differed markedly from that of normal bone. The plated graft was characterized by a slight increase in the amount of organic osteoid but a substantial loss of minerals. The results suggest that the induction of callus repair is restricted in the plated graft and that the protection from stress afforded by the rigid plate causes considerable loss of bone mass both in the graft and in the adjacent host bone.

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