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Fracture healing in paraplegic rats

In paraplegic rats, histomorphometric and chemical methods were used to evaluate callus production in tibial fractures stabilized by intramedullary nails. There were no differences in the mean sizes of fracture callus between paraplegic rats and their non-weight-bearing and weight-bearing controls. However, the variance of callus size was large in paraplegic rats. The concentration of nitrogen was high in calluses of paraplegic rats during the cartilaginous stage of healing. The hydroxyproline concentration did not differ between the groups. The rate of callus ossification was more rapid in paraplegic rats than in controls, but fracture calluses of paraplegic rats showed delayed accumulation of calcium and incomplete maturation of woven new bone. The results suggest that there are both mechanical and non-mechanical factors affecting callus formation in fractures below a spinal lesion.

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Long bone fractures of paralyzed limbs in spinal-cord-injured patients may be accompanied by a rapid formation of excessive callus (Comarr et al. 1962, Eichenholtz 1963, Freehafer & Mast 1965). However, McMaster & Stauffer (1975) and Nottage (1981) found an unexpectedly high rate of delayed unions and non-unions. Consequently, it is still doubtful whether spinal denervation *per se* accelerates the healing of fractures.

Mindell et al. (1971) observed delayed and depressed osteogenic callus response in non-immobilized femoral fractures of paraplegic rats. Our previous study (Aro et al. 1981) showed that tensile strengths of immobilized tibial fractures increased more rapidly in paraplegic rats than in non-weight-bearing controls in the early phase of callus formation. In addition, radiographic union by bridging callus occurred in paralyzed legs earlier than or at the same time as in control limbs. In the present study, we have further evaluated the effect of acute spinal paralysis on the formation of fracture callus by means of histomorphometric and chemical methods.

Material and methods

Experiments were performed on 66 female Wistar rats, aged 3–4 months. The surgical procedures were identical to those in our previous study (Aro et al. 1981). Standardized bilateral tibial fractures stabilized by intramedullary nailing were produced in both hind legs of the rats. The thickness of the intramedullary nail was selected with a view to good longitudinal alignment without preventing external callus formation (Aro et al. 1982). The type of the fracture and the position of the intramedullary nail were determined radiographically at the end of the experiments.

Twenty-four rats were subjected to spinal cord transsection *(paraplegic rats)*. The spinal cord was sectioned at the level of the upper lumbar region immediately before production of bilateral fractures. Persistent flaccid paralysis of both hind legs regularly occurred after anesthesia. The paraplegic rats were housed in plastic cages with wood chips on the floors to minimize the development of pressure sores. Twenty-one rats were subjected to bilateral surgical hip dislocations *(non-weight-bearing controls)*. The rats with hip dislocations were incapable of weight-bearing up to 2 weeks after operation. The functions of the legs were recovered gradually. No attempt was made to affect the function of the fractured legs in 21 rats *(weight-bearing controls)*. These controls walked almost normally within a few days after fracture.

The animals were killed 7, 9, 15 and 28 days after operation. The external calluses formed in the fractures were analyzed by histomorphometry or chemical analysis. Only undisplaced fractures with a proper position of the intramedullary nail were accepted for analysis of callus formation. Two paraplegic rats and two non-weight-bearing control rats died postoperatively. The total number of acceptable fractures was 44 in paraplegic rats, 36 in non-weight-bearing controls and 32 in weight-bearing controls.

Histomorphometry was carried out as previously described (Aro et al. 1985). A portion of the tibio-fibular bone including the fracture area with intact external callus was sectioned longitudinally, and the slice cut through the center of the medullary cavity was selected for histomorphometry. The areas of fibrous tissue, cartilage and new bone were measured with a computerized planimeter (Hewlett-Packard, Model 9864 A). The area occupied by fracture hematoma above the cortex was measured, if present. The measurement of the callus component was carried out on both sides of the bone and the mean value of these areas was regarded as the true area of the component.

External calluses were removed from the bones and cut into small pieces, stored at −20°C and analyzed for nitrogen, hydroxyproline and calcium contents by standard methods (Penttinen 1972). The results of the assays were expressed as concentrations per dry weight. Chemical analyses were carried out on fractures at 7, 9 and 15 days.

The results were assessed by two-way analysis of covariance, with the weight of the animal as the covariate and by one-way analysis of variance and Student's *t*-test. The computations were made by a DEC-20 computer using BMDP statistical software (1981).

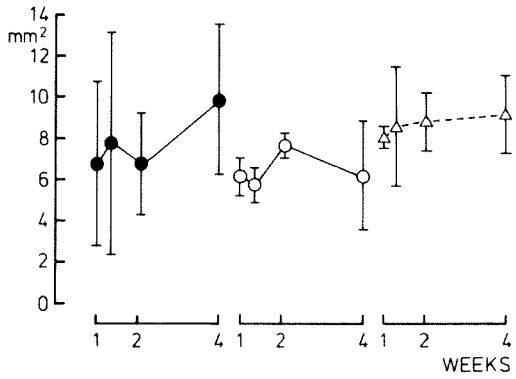


Figure 1. Callus size measured with histomorphometry. Values are mean ± SD, n = 4–8. ●—● paraplegic rats, ○—○ non-weight-bearing controls, △---△ weight-bearing controls.

A correlation was found between the initial weight of the animals and the histomorphometric size of their calluses (linear regression, $r = 0.66$, $n = 32$, $p < 0.001$). Therefore, the significance of differences between denervated and control calluses was assessed by the two-way analysis of covariance using the weight of the animals as the covariate.

Results

Body weight. Paraplegic rats continuously lost (18–32 per cent) weight over 28 days, whereas the controls showed only a transient decrease in weight at 7 and 9 days.

Callus size. There were no significant differences in the mean size of external calluses between the groups (Figure 1). The variance of callus size was, however, greater in paraplegic rats than in controls. No correlation was found between callus size and the ultimate weight of paraplegic rats.

Table 1. The total amounts of callus tissue components (mm^2) per fracture (mean ± SD) n = 4–8

	Day	Paraplegic rats	Non-weight-bearing controls	Weight-bearing controls
Fibrous tissue[a]	7	2.94±1.71	2.50±0.54	4.52±0.95
	9	1.50±1.47	0.80±0.84	3.46±1.70
	15	0.36±0.04	2.32±0.40	2.62±1.27
	28	0.21±0.10	0.48±0.20	0.76±0.46
Cartilage	7	1.74±1.37	1.48±1.06	0.46±1.04
	9	2.98±2.71	1.02±0.19	1.72±1.22
	15	0.51±0.39	1.04±0.06	1.05±0.04
	28	0.18±0.12	0.44±0.01	0.55±0.86
New bone	7	1.94±0.72	2.07±0.52	2.44±0.56
	9	3.24±1.40	3.50±1.91	2.52±0.24
	15	5.82±2.30	4.36±1.39	4.93±0.03
	28	9.38±3.62	5.43±2.79	7.58±1.56

[a] $p < 0.02$, paraplegic rats compared with weight-bearing controls, two-way analysis of covariance.

Ossification. The fracture hematoma appeared similar in size in paralyzed and control legs and was replaced by callus tissue by the 15th day. Fractures of paralyzed legs contained less fibrous tissue than those of weight-bearing controls ($p < 0.02$ for absolute amounts and $p < 0.01$ for relative amounts). The differences in the amounts of cartilage were not significant (Table 1), but the relative amount of cartilage was largest in paraplegic rats before endochondral ossification. There were no differences in the absolute amounts of new bone per fracture between the groups (Table 1). The relative amount of new bone increased more rapidly in paraplegic rats than in controls ($p < 0.05$). At 15 days, the relative amount of new bone was 91 ± 5 per cent (mean ± SD) in the calluses of paraplegic rats, 62 ± 23 per cent in those of non-weight-bearing controls ($p < 0.01$), and 59 ± 13 per cent in those of weight-bearing controls ($p < 0.01$).

Matrix production and mineralization. The concentration of hydroxyproline was similar in fracture calluses of paraplegic and control legs

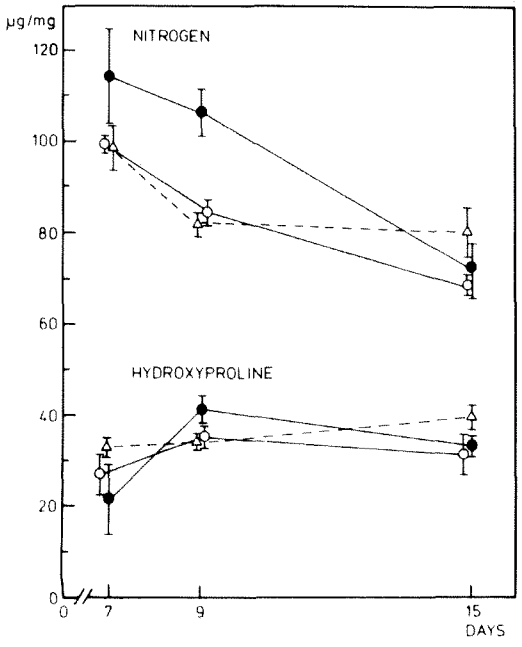
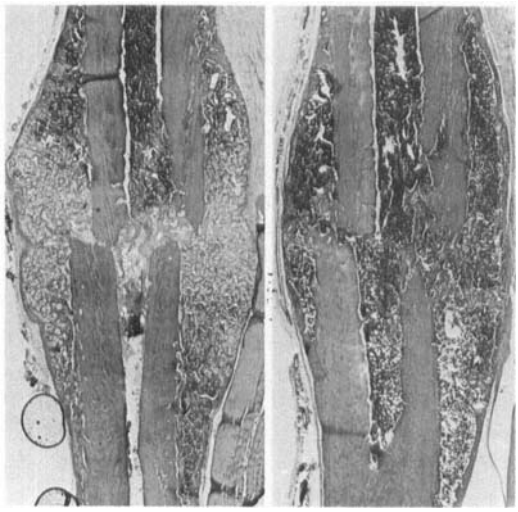


Figure 2. Concentrations per callus dry weight of nitrogen and hydroxyproline in fracture calluses. Values are mean ± SD, n= 4–8. The same symbols as in Figure 1.

Table 2. Concentrations (µg/ callus dry weight) of calcium (mean ± SD) n = 4–8

Day	Paraplegic rats[a]	Non-weight-bearing controls	Weight-bearing controls
7	8.2± 5.6	27.7±21.4	35.2± 3.4
9	41.9±13.1	66.3±12.8	55.0± 6.0
15	70.0± 7.7	82.2± 3.4	51.4±16.7

[a] $p < 0.01$, compared with non-weight-bearing controls, one-way analysis of variance.



A B

Figure 3. Healing of tibial fractures in paraplegic rats.
A Callus consisting of woven new bone at 2 weeks.
B Rapid replacement of new bone by red marrow at 4 weeks. (van Gieson, 6×).

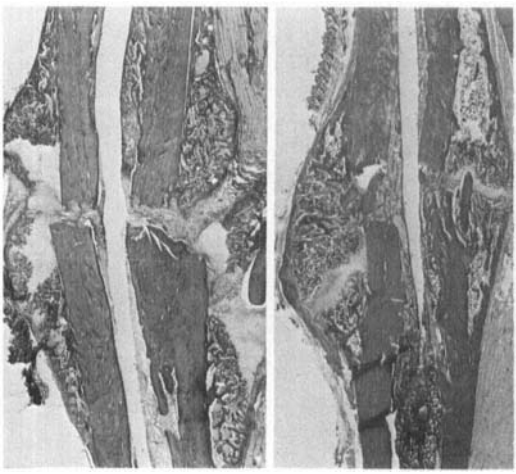


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Figure 4. Healing of tibial fractures in non-weight-bearing control rats.
A Cartilaginous union at 2 weeks.
B Advanced ossification at 4 weeks. (van Gieson, 6×).

(Figure 2). By contrast, the concentration of nitrogen was higher in calluses of paraplegic rats than in those of controls at 7 and 9 days ($p < 0.01$ and $p < 0.001$, Figure 2). The concentration of calcium (Table 2) and the ratio of calcium to hydroxyproline increased more rapidly in calluses of non-weight-bearing controls than in those of paraplegic rats ($p < 0.01$).

Remodeling. The bone trabeculae of woven new bone were thin and irregular in calluses of paraplegic rats (Figure 3 and 4), and large red marrow spaces tended to form between the thin trabeculae. Densitometry of bone structure was not performed.

Discussion

The present study showed that a spinal lesion increases the rate of callus ossification and the variance of callus size in tibial fractures of rats, while it does not produce an increase in the mean size of fracture calluses and does not increase hematoma formation at the fracture site.

Intramedullary fixation maintained longitudinal alignment of the fracture. It is natural that the mechanical environment in the fractures of paralyzed and control legs was not identical. The calluses of paraplegic rats showed a rapid replacement of fibrous tissue. It is possible that the enhanced ossification of calluses in paraplegic rats was only a result of delayed replacement of fibrous tissue in the calluses of control legs with greater fracture motion.

The degree of fracture stability generally determines the size of fracture callus. If the fractures of paralyzed legs were more stable than those of control legs, as suggested above, it is difficult to explain why the calluses of the paralyzed legs were, on average, as large as the control calluses. There may be a non-mechanical factor/factors increasing the mean size of fracture calluses of paralyzed legs. The great variance of callus size in paralyzed legs shows that such an effect was not present in all paraplegic rats. On the other hand, the relatively stable conditions in fractures of paralyzed legs could explain why the calluses of paralyzed legs, whether the callus was large or small, ossified rapidly.

Spinal transsection produced a qualitative change in the chemical composition of early calluses. It is unlikely that such a change resulted from mechanical factors, because the two control groups did not show differences in nitrogen and hydroxyproline concentrations. The studies of Lane et al. (1982) also clearly demonstrated that mechanical factors do not produce qualitative differences in the chemical composition of fracture calluses in the rat. Bone blood flow is increased in unfractured bones of paralyzed legs of paraplegic rats (Verhas et al. 1980). Hyperbaric oxygen treatment increases the content of nitrogen more than that of collagen in early calluses of rat tibial fractures (Penttinen 1972). Spinal paralysis did not increase the concentration of callus collagen, although there was a significant increase in the nitrogen concentration. The cause of the chemical change in the calluses of paraplegic rats and the significance of this change in the ossification processes of calluses remain to be investigated.

The recovery of mechanical strength is the main objective of the healing process of fractures. Our previous study (Aro et al. 1981) showed an increased tensile strength of fractures in paraplegic rats compared to those of non-weight-bearing controls in the early phase of healing. The tensile strength of experimental fractures is influenced by numerous structural and material properties of the uniting callus, which are dependent on one another. We have not tried to correlate our biomechanical, biochemical and histomorphometric measurements on fracture calluses.

Mindell et al. (1981) suggested that poor physical condition affects callus production in paraplegic rats. Our studies, however, agree with those of Urist & McLean (1950) in showing that callus formation in experimental fractures is independent of weight loss after multiple trauma and that the fracture site gives the highest priority to tissue-building materials.

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