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Effect of nerve injury on fracture healing

Callus formation studied in the rat

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In an attempt to determine the effects of peripheral nerve lesions on fracture healing, radiographic, histomorphometric and chemical methods were used to evaluate callus formation in tibial fractures of rats with sciatic denervation. Fracture union by bridging external callus was more rapid in denervated limbs than in controls. By contrast, external calluses of denervated fractures were smaller and less dense and contained less collagenous matrix (hydroxyproline) and minerals (calcium, phosphorus) than controls. The RNA/DNA ratio decreased more rapidly in denervated calluses than in controls. Mineralization of collagenous matrix (estimated from the calcium/hydroxyproline ratio) was not affected by denervation.

Periosteal nerves (Ralston et al. 1960, Miller & Kasahara 1963) probably have nociceptive, mechanoreceptive and vasomotor functions (Miller & Kasahara 1963, Aro et al. 1981, Thurston 1982). The effects of peripheral nerve lesions on callus formation at the fracture site are incompletely known. Peripheral denervation is claimed to delay healing of experimental fractures (Smith & Dunsford 1955, Reyes-Cunningham et al. 1971), but opposing opinions have also been presented (Frymoyer & Pope 1977, Aro et al. 1981). The effect of denervation may be dependent on the timing of bone damage (Becker 1974, Quilis & González 1974). The purpose of the present study was to perform quantitative analyses on callus formation in the tibial fractures of rats with peripheral nerve lesions.

Material and methods

Experiments were performed on 98 female Wistar rats, aged 3–4 months. Standardized tibial fractures were produced in both hind legs of the rats. Thirty-four rats were subjected to a unilateral sciatic paralysis. Fracture calluses formed in their paralyzed and control legs were used for determination of callus mass and nucleic acid contents (Experiment 1). Thirty-two rats were subjected to bilateral sciatic paralysis, and 32 rats without any paralysis served as their controls. The fracture calluses of these rats were either used for determination of callus mass and organic and inorganic matrix components (Experiment 2) or prepared for histomorphometric analysis of callus quantity and composition (Experiment 3).

The method of producing fractures has been described in detail elsewhere (Aro et al. 1985a). Fractures were stabilized with a 0.5-mm stainless steel nail, and a short wire was used to lock the nail (Figure 1). The short, locking wire was not used in Experiment 3. The sciatic nerve was cut at the level of the hip joint, as previously described (Aro et al. 1981). The surgical procedures in the control legs were identical, but the sciatic nerve was left intact.

Two radiographic projections were taken of each fracture and evaluated. The total number of acceptable fractures was 161 out of 196 (84 fractures of denervated legs and 77 fractures of control legs). A complete bridging callus was regarded as the radiographic criterion of union. External calluses formed in the fractures were isolated and prepared for chemical analysis (Aro et al. 1985a). Nitrogen, hydroxyproline, hexosamines, calcium and phosphorus, and

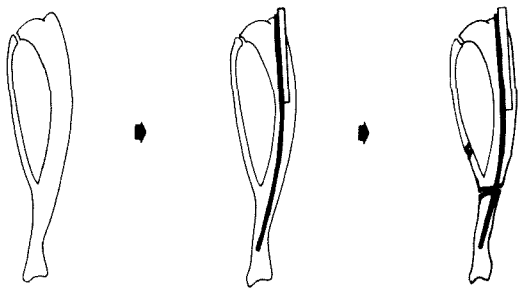


Figure 1. The fracture of the rat tibio-fibular bone was stabilized by intramedullary fixation. The tibia was prenailed and then fractured.

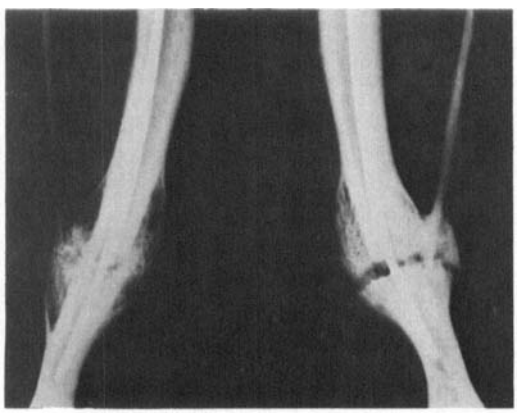
DNA and RNA contents of the calluses were determined by standard methods (Penttinen 1972). Chemical analyses were carried out on calluses at 7, 14 and 28 days. Histomorphometry was carried out on fractures at 7, 9, 15, 28, and 56 days, as previously described (Aro et al. 1985a). The results were assessed by two-way analysis of covariance, using the weight of the animals as the covariate, and partly by Student's *t*-test and the chi-square test.

Results

Radiographic union by bridging callus was more rapid in denervated legs than in controls (at 28 days, $p < 0.01$). However, the radiodensity of denervated calluses was lower than that of controls (Figure 2).

The size, height and width of the fracture callus were similar in denervated and control legs at 7 days. Later on, denervated fractures had smaller calluses than controls (−35 per cent at 56 days, $p < 0.05$). The dry weights of denervated calluses were already lower than those of controls at 28 days ($p < 0.05$). The differences in the wet weights of denervated and control calluses and, on the other hand, the differences in the effects of unilateral and bilateral paralysis on the callus weights were not significant.

Fracture hematoma was usually replaced by callus tissue by the 9th day in denervated legs and by the 15th day in controls. Denervated fractures also showed early formation of cartilage (Figure 3). The differences between denervated and control fractures in the rate of callus ossification, estimated from the ratio of new bone to callus size, were not significant (Figure 3).



A B

Figure 2. Rat tibial fractures at 4 weeks.
A Osteoporotic bony callus in a denervated fracture.
B Dense callus of a control fracture with cloudy areas of mineralization between advancing frontiers of the bridging callus.

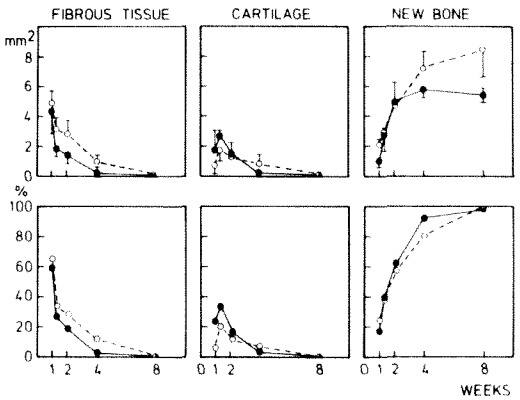


Figure 3. Absolute and relative amounts of the main callus tissue components in fracture calluses measured from longitudinal histological sections. Absolute amount expressed as mean ± SEM (n = 4–6). Relative amounts expressed as mean percentages of callus size. ●—● denervated fractures, ○--○ control fractures.

The chemical composition of denervated and control calluses did not differ at 7 and 14 days (Figure 4 and Table 1). However, the RNA contents of denervated calluses were lower than those of controls during ossification ($p < 0.01$ for the amount of RNA per callus and $p < 0.001$ for the RNA/DNA ratio, Figure 5). In addition, denervated fractures contained less hydroxyproline than controls at 28 days, expressed as the total amount per callus (−46 per cent, $p < 0.02$) or as the concentration per callus dry weight (−18 per cent, $p < 0.01$). The total amounts of calcium and phosphorus per callus were also lower in denervated fractures than in controls at 28 days ($p < 0.05$ for both, Figure 4). The concentrations of these minerals (Figure 4) and the calcium/hydroxyproline ratio (Table 2) were not affected by denervation.

Woven new bone of fracture calluses in denervated legs was not as dense as in controls. Densitometry of bone structure was, however, not carried out. The maturation of new bone to lamellar bone was also incomplete in the denervated legs, and bone marrow partially replaced bone trabeculae inside the external calluses (Figure 6 and 7).

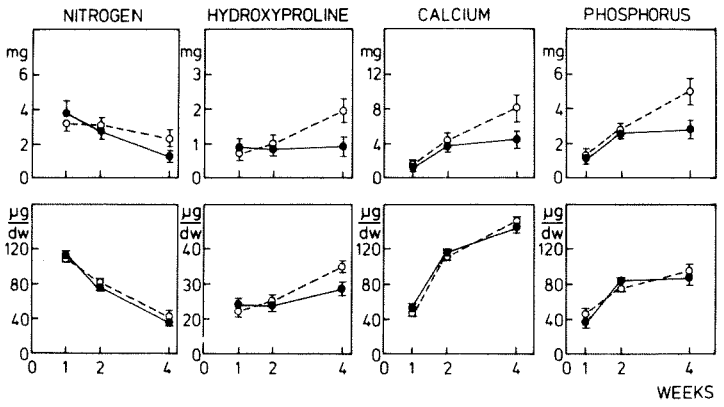


Figure 4. Nitrogen, hydroxyproline, calcium and phosphorus contents of fracture calluses, expressed as milligrams per callus or as concentrations per callus dry weight. Values are mean ± SEM, n = 7–8. The same symbols as in Figure 3.

Table 1. Hexosamine contents of fracture calluses. Means ± SEM are indicated. Numbers of fractures are shown in brackets. The differences between denervated and control calluses were not significant (Student's t-test)

	Weeks after fracture[a]			
	1		2	
	µg/callus	µg/mg dry weight	µg/callus	µg/mg dry weight
Controls	310±27 (8)	12.0±1.2 (8)	531±74 (7)	14.0±1.2 (7)
Denervated	412±49 (8)	11.4±0.9 (8)	388±80 (8)	10.9±1.3 (8)

[a] Four-week-old fractures were not analyzed.

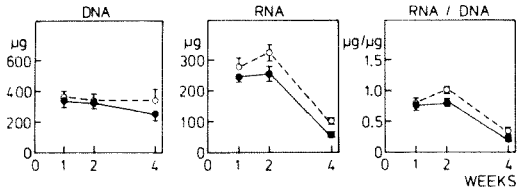
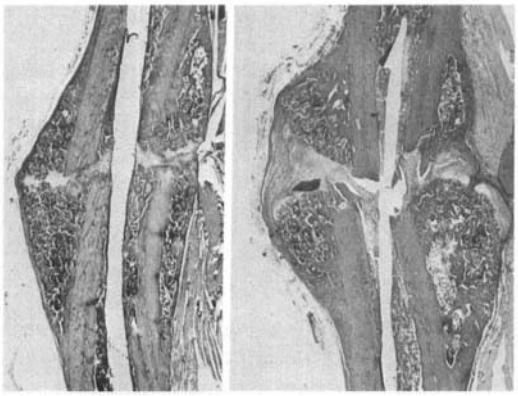


Figure 5. The DNA and RNA contents of fracture calluses. Values are mean ± SEM, n = 10–12.. The same symbols as in Figure 3.

Table 2. Ratio of calcium to hydroxyproline in fracture calluses. Means ± SEM are indicated. Numbers of fractures are shown in brackets. The differences between denervated and control calluses were not significant (two-way analysis of covariance)

	Weeks after fracture		
	1	2	4
Controls	2.46±0.62 (8)	4.54±0.26 (7)	4.42±0.13 (8)
Denervated	1.55±0.29 (8)	5.28±0.68 (8)	4.95±0.26 (8)



A B

Figure 6. Longitudinal sections through the fracture region of rat tibia at 4 weeks. (×5.5, van Gieson).
A Denervated fracture with cartilaginous union.
B Control fracture with fibrous-cartilaginous union.

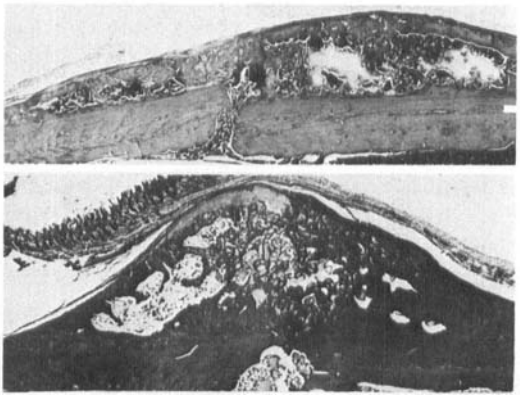


Figure 7. Longitudinal section through the external callus of rat tibial fracture at 8 weeks. (×5.5, van Gieson).
Upper. Denervated fracture with a flat bony callus with wide red bone marrow areas.
Lower. Control fracture with a large, dense bony callus.

Discussion

Our previous experiments (Aro et al. 1981) showed that sciatic paralysis increases the tensile strength of rat tibial fractures in the early phases of callus formation. The present analysis confirmed a significant difference in the radiographic healing of denervated and control fractures. Histomorphometry showed no significant change in the rate of callus ossification. This finding was not surprising since radiographic ossification (i.e. the disappearance of the radiolucent zone) produces only minor changes in the histomorphometric composition of the callus (Aro et al. 1985a). The size of the fracture callus in paralyzed legs decreased during conversion of fibrous-cartilaginous callus to bony callus. This indicates that the size of the bony callus does not necessarily indicate the extent of primary callus growth.

New matrix production by osteoblasts and mineralization are normally coupled (Frost 1966). The coupling of matrix production and mineralization was not disturbed after denervation, since there were no significant differences in the ratio of calcium to hydroxyproline between denervated and control calluses.

Klein et al. (1977) found that the turnover of bone collagen is increased in the sciatically denervated legs of rats. The formation of wide red marrow spaces between bone trabeculae during the remodeling processes of denervated fractures shows a high rate of bone turnover, since trabeculous bone is remodeled at red marrow sites at a much higher rate than the bone of fatty marrow sites (Wronski et al. 1980). Denervated calluses contained constant amounts of hydroxyproline, whereas in control calluses the amounts continuously increased. This suggests a difference in the ratio of collagen production to breakdown between denervated and control calluses. On the other hand, the decreased RNA content of denervated calluses during endochondral ossification suggests that denervation may primarily affect the capacity of callus cells for matrix production.

Sciatic paralysis, as already demonstrated in previous studies (Smith & Dunsford 1955, Hulth & Olerud 1965, Reyes-Cunningham et al. 1971), prevents formation of dense bone structure. Hulth & Olerud (1965) emphasized that these very thin-walled calluses did not show refractures. Bony calluses formed in paraplegic rats were also osteoporotic (Aro et al. 1981, 1985b). These findings show that the bony calluses of paralyzed legs rapidly adapt their structure to functional requirements.

Our studies showed that the effects of spinal cord and peripheral nerve lesions on fracture healing are parallel but not completely identical. Spinal and sciatic denervation improved the strength of calluses in the early phase of healing, promoted union by bridging callus, and induced osteoporotic transformation of external calluses. Spinal denervation increased the variance of callus size, which was not evident after sciatic denervation. Spinal denervation increased the concentration of non-collagen nitrogen in fracture callus before endochondral ossification. Such a response was not seen after sciatic denervation.

Acknowledgements

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References

Aro, H., Eerola, E., Aho, A. J. & Penttinen, R. (1981) Healing of experimental fractures in the denervated limbs of the rat. *Clin. Orthop.* **155**, 211–217.

Aro, H., Eerola, E. & Aho, A. J. (1985a) Determination of callus quantity in 4-week-old fractures of the rat tibia. *J. Orthop. Res.* **3**, 101–108.

Aro, H., Eerola, E. & Aho, A. J. (1985b) Fracture healing in paraplegic rats. *Acta Orthop. Scand.* **56**, 228–232.

Becker, R. O. (1974) The significance of bioelectric potentials. *Bioelectrochem. Bioenerg.* **1**, 187–199.

Frost, H. M. (1966) Bone dynamics in metabolic bone disease. *J. Bone Joint Surg.* **48**–A, 1192–1203.

Frymoyer, J. W. & Pope, M. H. (1977) Fracture healing in the sciatically denervated rat. *J. Trauma* **17**, 355–361.

Hulth, A. & Olerud, S. (1965) The healing of fractures in denervated limbs. An experimental study using sensory and motor rhizotomy and peripheral denervation. *J. Trauma* **5**, 571–579.

Klein, L., Dawson, M. H. & Heiple, K. G. (1977) Turnover of collagen in the adult rat after denervation. *J. Bone Joint Surg.* **59**–A, 1065–1067.

Miller, M. R. & Kasahara, M. (1963) Observations on the innervation of human long bones. *Anat. Rec.* **145**, 13–23.

Penttinen, R. (1972) Biochemical studies on fracture healing in the rat. *Acta Chir. Scand.* Suppl. 432.

Quilis, A. N. & González, A. P. (1974) Healing in denervated bones. *Acta Orthop. Scand.* **45**, 820–835.

Ralston, H. J. III, Miller, M. R. & Kasahara, M. (1960) Nerve endings in human fasciae, tendons, ligaments, periosteum and joint synovial membrane. *Anat. Rec.* **136**, 137–148.

Reyes-Cunningham, A., Marquez-Monter, H., Guerrero, L. M. B. D., Flores-Martinez, A., Lopez-Nosthe, F. & Mejia, S. (1971) Estudio del callo oseo en extremidades denervadas. Trabajo experimental en ratas. *Arch. Invest. Med.* **2**, 15–24.

Smith, W. S. & Dunsford, E. R. Jr. (1955) Healing of fractures in denervated limbs in rats. *Surg. Forum* **6**, 559–562.

Thurston, T. J. (1982) Distribution of nerves in long bones as shown by silver impregnation. *J. Anat.* **134**, 719–728.

Wronski, T. J., Smith, J. M. & Jee, W. S. S. (1980) The microdistribution and retention of injected ^{239}Pu on trabecular bone surfaces of the beagle: implications for the induction of osteosarcoma. *Radiat. Res.* **83**, 74–89.