

The role of periosteal tension in the growth of long bones

E. WARRELL AND J. F. TAYLOR

Department of Orthopaedics, University of Liverpool

(Accepted 2 February 1978)

INTRODUCTION

The activity of growth plate chondroblasts is thought to be genetically pre-determined (Owen, 1970), though they are responsive to changes in the micro-environment. This activity is primarily dependent on the endocrine system (Siffert, 1966), but local factors play an important part in determining the bone growth rates. Thus limb length discrepancy is known to follow fractures and Ollier (1867), Truesdell (1921), Levander (1929) and Hedstrom (1969) have suggested that the growth stimulus on the fractured side results from an increase in blood flow to the growth plate.

Growth plate activity is inhibited by applied mechanical compression (Hert, 1969), which may be dynamic due to body weight and muscular activity, or static such as the restraint by the periosteum which forms a fibro-elastic tube attached on the epiphysial side of the growth plate. Crilly (1972) made circumferential and vertical incisions in the periosteum of chicken radii and found greater relative stimulation of bone growth after the former procedure. He concluded that anisomelia after fracture results from a release of periosteal tension and 'decompression' of the growth plate. However, the periosteum is firmly bound to the bone by cellular adhesions and blood vessels, which may restrict its movement over the diaphysis of the bone. In this work we have sought to investigate the concept of growth plate decompression by examining the effect of periosteal division on the length of the rat tibia.

MATERIALS AND METHODS

The experiments were carried out on the right hind leg of outbred Wistar male rats. Operation was undertaken at 28 days and the animals killed 34 days later.

Before operation the animals were weighed and identified with an ear mark. All were anaesthetized with intraperitoneal Nembutal, up to 60 mg/kg body weight, and were then allocated to one of five experimental groups, using a table of random numbers, as follows:

- (A) Circular periosteal division.
- (B) Circular periosteal division plus periosteal stripping.
- (C) Vertical periosteal division.
- (D) Vertical periosteal division plus periosteal stripping.
- (E) Control intact rats.

There were ten rats in each group, and for those in A, B, C and D, the rat's right tibia was exposed by a vertical 8 mm anterior incision extending proximally from the ankle centred about 6 mm above the malleoli. The nutrient artery was exposed on the posteromedial aspect 12 mm above the ankle, but was not damaged. Then the tendons and muscle distal to the nutrient artery were separated from the periosteum

using the cutting edge at the point of a hypodermic needle (16×0.5 mm diameter). Following a similar procedure in group B the periosteum was elevated for 2 mm on each side of the incision. In group C two parallel vertical 4 mm periosteal incisions were made, centred about 6 mm above the lower growth plate. Similar incisions were made in group D, but with circumferential elevation of the periosteum over a 4 mm length of diaphysis. This was done using a 25 gauge needle with a bent end. Three cutaneous silk sutures were inserted following the operation, and the animals allowed to recover. Group E consisted of control rats which were allowed to recover from the anaesthetic without operation. All animals were subsequently maintained on a standard diet.

Thirty four days after operation (when aged 62 days) the rats were killed with chloroform. The legs were excised, excess muscle removed, and the bones fixed in 10% formal saline. After fixation the length of the bones was measured from the articular cartilage of the medial condyle to the most distal point of the medial malleoli, using Vernier callipers. Radiographs were taken of the legs with a tube-film distance of 61 cm and the measurements repeated. In our hands there was less variation between repeated gross measurements of the tibiae than there was in repeated measurements of radiographs of the same bones and so the gross measurements have been used for the purpose of this paper. Measurements were made without prior knowledge of the experimental group to which the animal had been allocated.

Similar operations were undertaken on a sub-group of eight animals. However, at 60 days of age each received an intraperitoneal injection of 2% alizarin red (S) (sodium sulphalizarate, 100 mg/kg; Hoyte, 1957), and oxytetracycline, 10 mg/kg body weight. After death these bones were embedded first in celloidin and then in wax and sectioned at $60 \mu\text{m}$ without decalcification.

RESULTS

The right tibial lengths differed significantly from the left in both groups which had had circumferential periosteal division and also in group D in which vertical division was combined with periosteal elevation. In all three groups the side subjected to periosteal division was longer (Table 1). The significance of the difference was estimated using the paired readings from right and left legs, and a dependent t test. The greatest difference (0.69 mm) was seen in group B – in which circumferential division had been combined with stripping of the periosteum. As a percentage of leg length this represents a 1.9% discrepancy between right and left legs. In group E, having anaesthesia alone, and in group C, having vertical periosteal division, no significant difference was found between right and left tibial lengths.

The tibial lengths of the rats in each group were compared to those of rats in the control group E by means of the independent t test.

When compared to rats subjected to anaesthesia only (group E), the right and left mean leg lengths were found to be increased in the groups having had operations, with the exception of the control (left) leg in those animals having circular periosteal division alone (group A). However the only significant increase was observed in the right legs of group B (circumferential division plus stripping).

There was no significant difference between the weights of the rats in the experimental groups and those of the control animals (Student's independent t test). However, the weights of group A did differ significantly from the weights of animals

Table 1. *Showing the difference between mean right and left leg lengths in each experimental group*

Experimental group ... (10 rats per group)	A	B	C	D	E
	Circular division	Circular division plus periosteal stripping	Vertical division	Vertical division plus periosteal stripping	Control. Anaesthetic but no operation
Initial mean weight (g)	53.1	66.4	63.3	67.8	60.7
Mean leg lengths (mm \pm S.D.)					
Right	35.62 \pm 1.5	36.69 \pm 1.2	36.13 \pm 1.1	36.07 \pm 0.79	35.23 \pm 1.3
Left	35.21 \pm 1.4	36.00 \pm 1.1	36.10 \pm 1.1	35.72 \pm 0.81	35.22 \pm 1.1
Mean difference (right minus left)	+0.41	+0.69	+0.03	+0.45	+0.01
Significance*	$P < 0.02$	$P < 0.001$	$P > 0.05$	$P < 0.001$	$P > 0.05$

* In this table the significance of the differences between the right and left legs of the animals in each group has been evaluated using the paired Student's *t* test.

Table 2. *Alignment of blood vessels in the cortex of the proximal tibiae of rats having had circumferential periosteal release on the right leg (Group A)*

Distance from the proximal growth plate (mm)	Percentage of vessels making exit towards the proximal growth plate	
	L leg (control)	R leg
5	30	25
10	39	61
15	100	100
20	89	94

in all the other groups. The relatively short tibiae in this group may not be related to their weight, however, as Schemmel, Mickelsen & Mostosky (1969) have shown that age and genetic potential are the primary determinants of rat skeletal size, the rate of weight increase having little influence. In the present study the percentage increase in weight was similar in all groups, being over 400%.

Histological examination confirmed previous observations on the normal micro-anatomy (Theunisson, 1973). The tibiae from control animals showed regular osteonal development. Cortical blood vessels ran outwards in a neutral area between the growth plates, but in the metaphyses ran out towards the nearby growth plate (Table 2).

Following circular division of the periosteum the alignment of cortical blood vessels in the proximity of the operation site was found to be irregular. Examination of undecalcified sections of tibiae taken from animals injected with alizarin showed that circular periosteal release had been followed by a change in vascular orientation, diaphysial vessels that had been running outwards then bending towards the proximal growth plate (Fig. 1). In order to quantify the difference in alignment we estimated the direction of vessels in tibiae from ten rats which had had circumferential periosteal division. At a site 10 mm from the proximal growth plate 61% of vessels on the right, and 39% of vessels on the left pointed proximally ($P < 0.05$; Fisher's exact probability test). At other sites the difference was insignificant (Table 2).

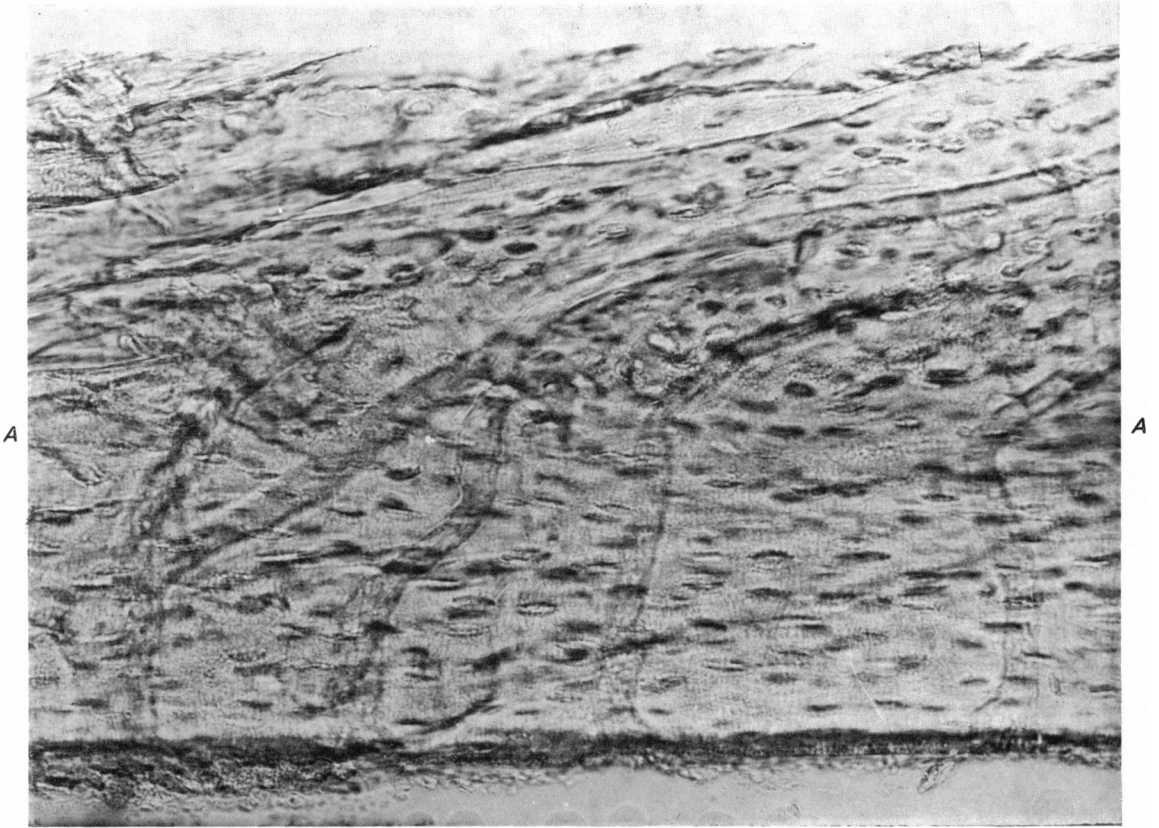


Fig. 1. This shows the endosteal surface of the tibial cortex below, the periosteal surface being at the upper edge. *A-A* marks an alizarin stain which was injected on the day of operation. The proximal growth plate was to the right at a distance of 20 mm and the periosteum was incised circumferentially 7 mm to the left. The cortical blood vessels were seen to run directly outwards until they reach the alizarin line. They then streamed towards the proximal growth plate. It is postulated that the periosteum pulled them to the right as it retracted.

DISCUSSION

Experimental fracture of the leg in young animals is followed by regional and systemic effects. Wray & Goodman (1961) found that immediately after fracture of one tibia in young rats, femoral growth was retarded on both sides. This was followed by overgrowth on both sides which was significantly higher on the operated side. There is a generalized increase in bone formation throughout the body (Wray & Schneider, 1969). The systemic effects probably represent a rise in the output of somatomedin during this period, and a corresponding increase in weight has been noted in the endocrine glands (Chatterjee, Prasad & Udupa, 1970).

However, the anisomelia which follows either fracture or periosteal division must result from a disturbance of local growth controlling mechanisms. This study of periosteal division in the rat hind leg has confirmed that circumferential release alone, or release accompanied by localized stripping, produces a tibial length discrepancy. Vertical division alone produced no such effect. These findings support the concept that release of the static load on the growth plate accelerates new bone formation.

The histological study of vessel alignment under the periosteum was partly confounded by the orientation in diaphysial vessels brought about by normal development (Theunissen, 1973). The periosteum normally behaves as an elastic membrane stretched between two epiphyses. In the tibia there is a neutral zone in the distal diaphysis where movement of the periosteum in relation to the diaphysis is negligible. Proximally the activity of the growth plate stretches the periosteum between the neutral zone and the proximal epiphysis (Brookes, 1971). This leads the cortical vessels to make exit towards the proximal growth plate in the normal animal, and periosteal release produces little change in their orientation. Subjacent to the growth plate the bone is of recent origin and the vessels disorientated. However, at the junction of metaphysis and diaphysis in control animals the vessels show no particular orientation, and it is in this region that we found significant change after periosteal release, demonstrating that the periosteal tube does move proximally after distal release. It appears that the fibres anchoring the periosteum to the bone remain intact and movement is brought about by change in orientation of new subperiosteal bone trabeculae as they are deposited.

These findings lead us to favour growth plate decompression as a local mechanism responsible for growth plate stimulation, in agreement with the finding of Crilly (1972). Added support for this hypothesis is found in the work of Hert (1969) who was able to accelerate the rate of growth in the rabbit tibia by graduated distraction of the proximal epiphysis and diaphysis. Moreover, Noel & Wright (1972) noted an increase in the growth rate of rat vertebrae which were transplanted to a non-load-bearing site.

The addition of periosteal stripping appeared to augment anisomelia after circumferential release, and whereas vertical incision produced no discrepancy, vertical incision and stripping caused significant tibial inequality. We conclude that this must result from the influence of another control mechanism. Though direct damage to the nutrient artery was avoided, movement of the periosteum may have produced vasoconstriction or obliteration of this vessel. This is known to produce hyperaemia of both epiphysis and growth plate (Yabsley & Harris, 1965).

Alternatively, the disturbance of the skeletal electric field (Bassett & Becker, 1962) may have resulted in growth plate stimulation. Lokietek, Pawluk & Bassett (1974) have shown that both muscle damage and periosteal stripping produce electric potentials in the underlying bone.

Regardless of the mode of action, circumferential periosteal division is seen as a potentially useful means of correcting clinical leg length discrepancy. In comparing it with more major procedures used for children with anisomelia, it must be remembered that the quadruped rat is little affected by trauma to one limb, while tibial osteotomy confines a child to bed and leads to a period of negative calcium balance. Though Jenkins, Cheng & Hodgson (1975) have successfully produced relative elongation with total stripping of the diaphysial periosteum, circumferential division should be considered as a simpler procedure.

SUMMARY

Vertical or circumferential periosteal incisions were made over one tibia of anaesthetized rats, supplemented by localized periosteal stripping in two of the experimental groups. The rats were killed 5 weeks later and tibial lengths measured.

Circumferential periosteal division plus periosteal elevation produced the greatest

ipsilateral increase in tibial length. In this group of rats the alignment of cortical blood vessels provided evidence that sliding of the periosteum and a release of periosteal tension are associated with the gain in length. However, anisomelia, though not seen following vertical periosteal division alone, did occur after vertical division and periosteal stripping. This supports suggestions that both growth plate decompression and vascular phenomena influence skeletal growth rates after trauma.

This work was supported by grant no. 352 from Merseyside A.H.A. (T.) Research Committee. The authors are grateful to Mrs C. Oliver for the histological preparations, Mr A. Taunton for the microphotograph and Miss L. Marshall for the typescript. They are also indebted to Professors R. Roaf and G. Burwell and to Dr R. Crilly for help with the study. They wish to thank Professor G. Bentley for advice during the preparation of the paper.

REFERENCES

- BASSETT, C. A. & BECKER, R. O. (1962). Generation of electric potentials by bone in response to mechanical stress. *Science* **137**, 1063–1064.
- BROOKES, M. (1971). *The Blood Supply of Bone*. London: Butterworths.
- CHATTERJEE, S., PRASAD, G. C. & UDUPA, K. N. (1970). Changes in endocrine gland during fracture repair and effect of their ablation. *Journal of Trauma* **10**, 890–899.
- CRILLY, R. G. (1972). Longitudinal overgrowth of the chicken radius. *Journal of Anatomy* **112**, 11–18.
- HEDSTROM, O. (1969). Growth stimulation of long bones after fracture or similar trauma. A clinical and experimental study. *Acta orthopaedica scandinavica*, Suppl. **122**, 7–134.
- HERT, J. (1969). Acceleration of the growth after decrease of load on epiphyseal plates by means of spring distractors. *Folia morphologica* **17**, 194–203.
- HOYTE, D. A. N. (1957). The interpretation of the results of alizarin staining of bone in growing animals. *Journal of Anatomy* **91**, 591.
- JENKINS, D. H. R., CHENG, D. H. F. & HODGSON, A. R. (1975). Stimulation of bone growth by periosteal stripping. *Journal of Bone and Joint Surgery* **57B**, 482–484.
- LEVANDER, G. (1929). Treatment of fractures of the shaft of the femur. *Acta chirurgica scandinavica* **65**, Suppl. 12, 1–237.
- LOKIETEK, W., PAWLUK, R. J. & BASSETT, C. A. L. (1974). Muscle injury potentials: a source of voltage in undeformed rat tibia. *Journal of Bone and Joint Surgery* **56B**, 361–369.
- NOEL, J. F. & WRIGHT, E. A. (1972). The growth of transplanted mouse vertebrae. *Journal of Embryology and Experimental Morphology* **28**, 633–645.
- OLLIER, L. (1867). *Traité Experimental et Clinique de la Regeneration des Os et de la Production Artificielle du Tissue Osseux*, vol. I. Paris: Victor Masson et Fils.
- OWEN, M. (1970). The origin of bone cells. *International Review of Cytology* **28**, 213–238.
- SCHEMMELE, R., MICKELSEN, O. & MOSTOSKY, U. (1969). Skeletal size in obese and normal weight littermate rats. *Clinical Orthopaedics and Related Research* **65**, 89–96.
- SIFFERT, R. (1966). The growth plate and its affections. *Journal of Bone and Joint Surgery* **48A**, 546–563.
- THEUNISSON, J. J. W. (1973). The fibrous periosteum. Doctoral thesis, Catholic University, Nijmegen.
- TRUESDELL, E. D. (1921). The inequality of the lower extremities following fracture of the shaft of the femur in children. *Annals of Surgery* **74**, 498–500.
- WRAY, J. B. & GOODMAN, H. O. (1961). Post fracture vascular phenomena and long bone overgrowth in the immature skeleton of the rat. *Journal of Bone and Joint Surgery* **43A**, 1047–1055.
- WRAY, J. B. & SCHNEIDER, A. J. (1969). Skeletal changes after tibial fracture in the rat. *Journal of Surgical Research* **9**, 433–439.
- YABSLEY, R. H. & HARRIS, W. R. (1965). The effect of shaft fractures and periosteal stripping on the vascular supply to the epiphyseal plates. *Journal of Bone and Joint Surgery* **47A**, 551–566.