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Growth plate stimulation by diaphyseal fracture

Autoradiography of DNA synthesis in rats

Using autoradiography, I studied the effect of diaphyseal injuries on the DNA synthesis of growth plate cells in Swiss Albino rats and found that mitotic activity of germinal and proliferation zone cells of the growth plate and periosteal callus increased 24 hours after the fracture and peaked at 48 hours. The mitotic activity continued until the eighteenth day with gradual decline from the second day onwards. It appears as if the growth plate cells are stimulated by the same mitogenic activator as the periosteal cells. I therefore postulate that the overgrowth of long bones after fractures is due to local mitogenic stimulating factor(s), and is not due to an increased blood supply to the epiphyses, as is presently widely claimed.

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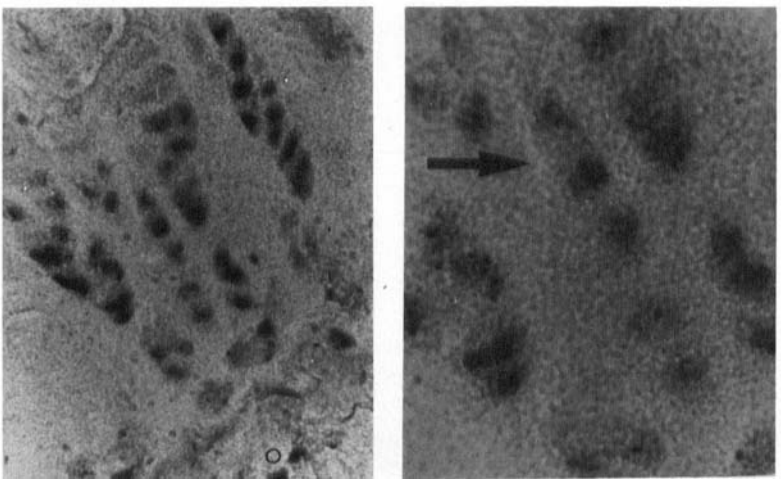
Increased length of a growing limb as a consequence of fracture was first reported by Truesdell (1921), and has generally been ascribed to metaphyseal hyperemia (Henry 1963, Trueta 1968, Kéry et al. 1980). However exact mechanism of this overgrowth is not known yet.

With an autoradiographic technique, I studied the DNA synthesis of the growth plate cells after diaphyseal injury.

Material and methods

Forty Swiss Albino rats (average weight 125 g) were used. Under ether anesthesia the right tibia of each animal was fractured manually. Tritiated thymidine was administered to the rat intraperitoneally in a dose of 0.5 mc per gram of body weight 2 hours before killing. Tissue samples were fixed in neutral buffered formaldehyde for 24 hours and then washed for an additional 24 hours in running tap water. Bones were decalcified in 10 per cent formic acid and embedded in paraffin. Sections 5 mc thick were wrapped with Kodak AR 10 stripping film, dried and exposed for 21 days in a refrigerator at 4°C. The autoradiographic preparations were stained with diluted hematoxylin and eosin and studied under the light microscope.

The tibia and femur of the uninjured limb were used as controls.



A B

Figure 1. A. Excessive labelling of growth plate cells 24 hours after diaphyseal fracture. "O" shows the osteoblasts in the metaphysis of the tibia (HE×480).
B. Increased mitosis on second day in proliferation zone. Arrow shows dividing cartilage cells (HE ×1200).

Results

In the growth plate of the fractured side, germinal and proliferation zone cells had excessive labelling from Day 1 onwards (Figure 1) and reached peak labelling by Day 2. The mitotic activity then gradually slowed down after 2 weeks and showed the same amount of labelling as the growth plate of the control tibia and femur by Day 18. The observations of the periosteal callus also confirmed high labelling of spindleshaped cells in the periosteum and disclosed a similar pattern of increase in mitotic activity. However, the labelling of cells with tritiated thymidine in the fracture callus was less than that of the growth plate. The decrease in labelling of callus cells was parallel to the decrease of labelling of the growth plate cells. The labelling index peaked simultanesusly in the growth plate and the callus cells (Figure 2).

The growth plate of the control tibia and femur showed no increased activity throughout the 20-day experiment.

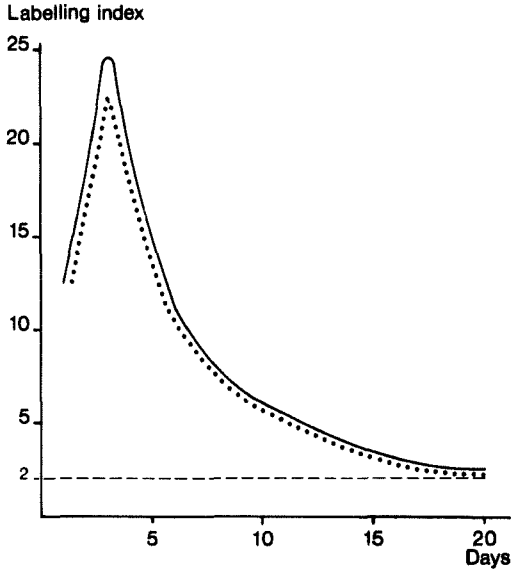


Figure 2. The labelling index of callus and growth plate cells. The index is the ratio of labelled cells to the total counted population of that cell expressed as percent.
= "... expressed as a percentage."
– – – control growth plate cells,
—— injured side growth plate cells,
· · · · · · callus cells.

Discussion

The mitotic activity after a tibial fracture was interestingly seen not only at the fracture site, but also throughout the periosteum of the fractured bone and its growth plate. However no increased activity was seen in the adjacent femur and in the uninjured control leg. These findings confirm that the mitogenic stimulant that activates the structural genes of the dormant cells in the periosteum after a fracture also, and to the same degree, activates the germinal and proliferation zone cells of the growth plate, but only in the injured bone.

The overgrowth of long bones after fractures at present is thought to be due to an increased blood supply to the epiphyses and metaphyses after diaphyseal fractures. However, the human growth plate is essentially devoid of vascular channels, and mere presence of blood supply does not cause mitotic activity. Although some general factors such as food supply and hormones affect growth, local factors far outweigh them in potency and undoubtedly in importance. Recent advances in molecular biology clearly demonstrate that epidermal growth factor (EGF) stimulates DNA synthesis and cell replication in cultures of fetal rat calvariae (Canalis & Raisz 1979). Further experimental studies with fibroblast growth factor (Canalis & Raisz 1980) and platelet-derived growth factor (Canalis 1980) have shown the stimulation of DNA synthesis and collagen synthesis in cultured calvariae. Farley et al. (1982) have demonstrated that human bone contains a factor named "skeletal growth factor" that stimulates bone protein and DNA synthesis in vitro. Finally, Heldin et al. (1983) have shown the growth factor binding to cell receptors, but the mechanism by which it stimulates cell proliferation has remained obscure.

Over the years a variety of techniques have been tried to stimulate the growth mechanism, these included installation of foreign bodies and metallic devices in the bone, arteriovenous fistulae, short-wave diathermy, and lumbar sympathectomy (Campbell 1959, Wilson 1979, Castle 1971). All of these procedures were aimed at increasing the blood supply of the growth plate, but they gave unsatisfactory results and were therefore eliminated from clin-

ical practice. These reports also confirm that the cause of overgrowth is not hyperemia around the epiphyses.

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