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INFLUENCE OF GROWTH AND TRAUMA ON BONE MASS AND MINERAL TURNOVER IN RATS

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Loss of bone mineral in fractured limbs of rats has been demonstrated by *Bohr & Sörensen* (1950), *Bauer* (1954), *Bohr* (1955) and others.

This loss was not confined to the fractured bone but occurred also in the neighbouring bones. The loss of bone mineral was associated with an increased uptake of bone-seeking radionuclides which was believed to reflect an increased bone formation rate that compensates for the resorption caused by the fracture. In preliminary experiments in this laboratory, it was not possible to obtain any measurable change in the mineral content either of the femur following fracture of the tibia or in the tibia following fracture of the femur in rats. When comparing our data with those of other investigators, it was found that the body weights of the animals in our experiments were much greater than those of the previous investigators' so that our rats had reached a weight where growth was slow while previous investigators had used animals around 150 g, when growth is rapid.

The purpose of the present study was to investigate the effect of growth on post-traumatic osteopenia in the rat.

MATERIAL AND METHODS

One tibia (left or right at random) was fractured under aether anesthesia in twenty-nine male, Sprague Dawley rats of the Charles River strain. At the time of fracture, the animals' ages ranged from 26 to 78 days (approximate weight range 70–300 g). Thirteen days following fracture they were injected subcutaneously with about two microcuries of Strontium-85 and twenty-four hours later, two weeks following fracture, they were killed. The femora from the fractured and the control limbs were cleaned of soft tissue and the fresh bone was weighed suspended from a fine copper wire in distilled water at 4° C, mopped dry and re-weighed in air. The volume was calculated as the difference between these two weights. The bones were then ashed at 600° C for forty-eight hours and the ash weighed. Radioactivity was measured in a well scintillation detector to a precision of 1 per cent and expressed as a per cent of the injected dose.

RESULTS

The ash weight of the femora on the fractured side was less than that of the control femora (Figure 1). Moreover, the specific ash weight (g ash/cc bone) was less on the fractured side (Figure 2). The volume of the femur on the fractured side was also less than that on the control side (Figure 3). When ash weight on the control and the fractured sides was plotted as a function of the bone volume there was no significant difference between control and fractured (Figure 4). Thus, even though the femora on the fractured side had less total ash than the controls, the ash content for any given volume was the same in both groups.

The total uptake of the isotope was less in the femora on the fractured side than in the femora of the control side (Figure 5) but the specific activity was higher on the fractured side (Figure 6). Specific activity (Figure 7) was found to be a negative function of specific ash weight (density) in the femora on the fractured side as well as the

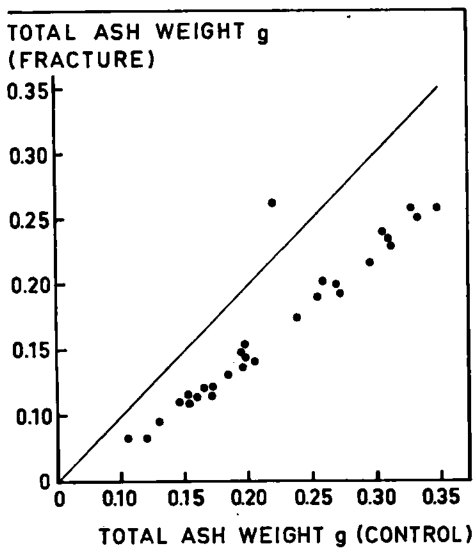
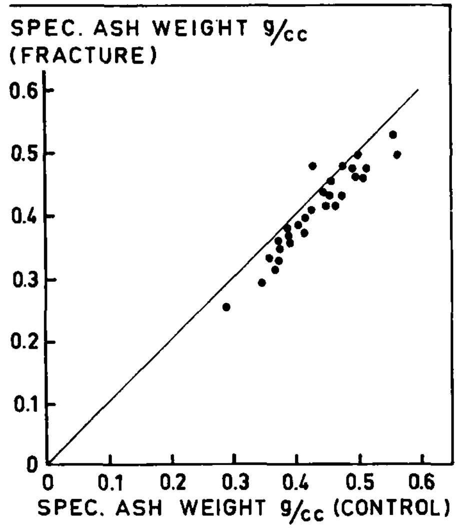


Figure 1. Total ash content of the femora on the fractured side is plotted against total ash content of the femora on the control side. All except one point fall below the horizontal line indicating that the ash content of the control femora is greater than that on the fractured side.

Figure 2. Specific ash weight in the fractured femur is plotted against specific ash weight in the control femur. All except one point fall below the oblique line indicating that the specific ash weight on the control side is greater than that on the fractured side.



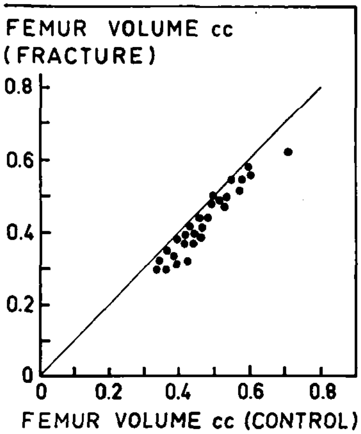


Figure 3. Femur volume on the fractured side is plotted against femur volume on the control side. All points fall below the oblique line indicating that femur volume was always greater on the control side.

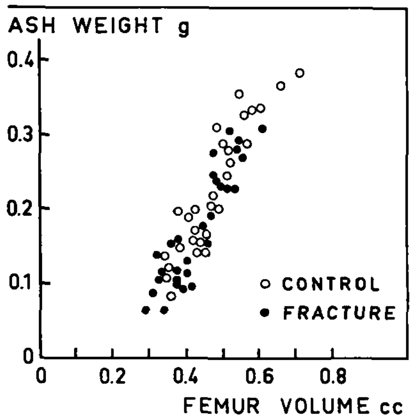


Figure 4. Ash weight of the femora is plotted against femur volume for the control and the fractured sides. There is clearly no significant differences between the two sides.

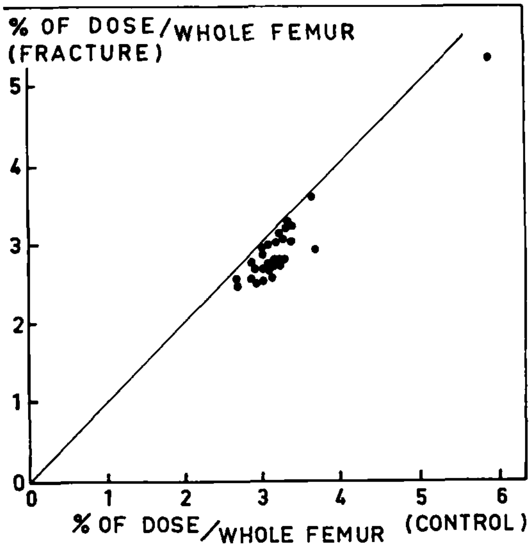


Figure 5. Total radioactivity in the femur of the fractured side is plotted against total radioactivity on the control side. All points fall below the oblique line indicating that radioactive uptake is always greater in the control femora.

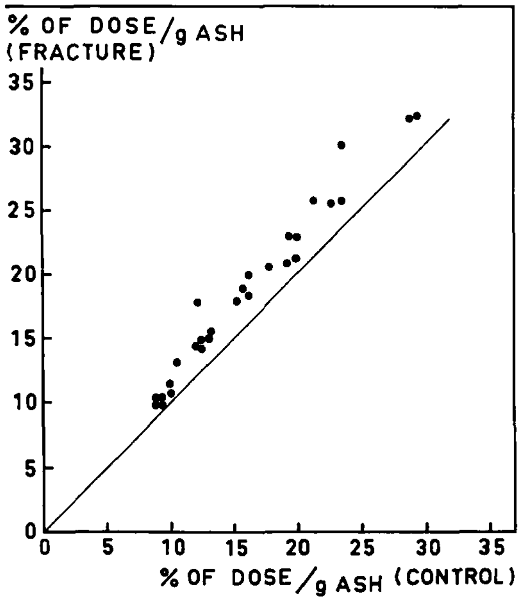


Figure 6. Specific activity in the fractured side was plotted against specific activity in the femora on the control side. In this case, all the points fall above the oblique line indicating that the specific activity is always greater in the femora on the fractured side.

control side. Analysis of covariance revealed no significant difference between these two regressions so that for any given specific ash weight the specific activity was the same on the fractured side as on the control side. Actually the specific activity tended to be higher in the control.

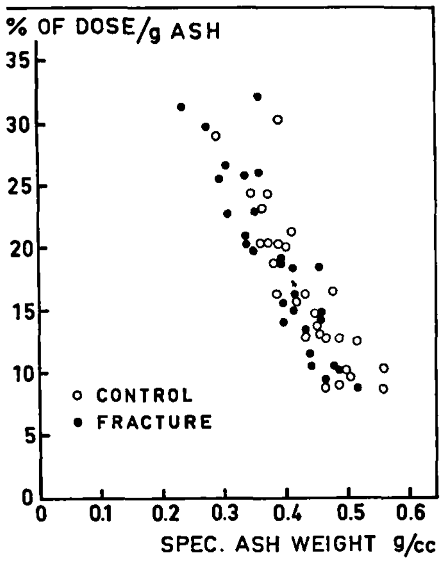


Figure 7. Specific activity in the control and fractured femora is plotted against specific ash weight. The negative linear regressions so formed are calculated by the method of least squares and compared by analysis of covariance; there is no significant slope or intercept difference ($0.05 < P < 0.1$).

DISCUSSION

When the above investigators demonstrated that the tibia ipsilateral to a fractured femur had less ash than the control tibia as well as a higher specific activity, they assumed that this was evidence of post-traumatic osteopenia with a compensatory increase in bone formation rate. However, it has subsequently been shown that the femur density increases linearly with body weight in the rat and also with femur volume (*Saville & Smith* 1966, *Smith & Saville* 1966). We have shown that the femur ipsilateral to the fractured tibia is smaller than the control femur but has the appropiate ash content and isotope uptake for its volume. Moreover, the specific activity is also appropiate for bones of their volume and density. In short, what seems to have happened is that following fracture, growth has slowed down in the femur ipsilateral to the fracture but not in the contralateral femur. The apparent changes in mineral content and uptake of isotope are nothing more than the difference between smaller and less dense bones, compared with larger and denser ones. All the bones were normal with respect to both density and isotope uptake for bones of their volume.

Therefore, it may be concluded that the twenty-four hour uptake of Strontium-85 in rat bone is not merely a function of bone formation rate but also reflects bone density or variables related to bone density.

This phenomenon may be explained by an exchange process which occurs more rapidly in the smaller and less dense bones.

SUMMARY

The ash content, the volume and the twenty-four hour Strontium-85 uptake were measured in the femora of rats two weeks after fracture of one tibial shaft. The decreased mineral content of the femur ipsilateral to the fractured tibia was possible to explain by unilateral cessation of growth in the femur of the fractured limb. The ash content and twenty-four hour isotope uptake were the same on the two sides when bone volume and density were held constant.

In this experiment specific activity twenty-four hours following injection of Strontium-85 was best correlated with femur density and may not be regarded entirely as a parameter of bone formation rate.

RESUME

La teneur en cehdres, le volume et l'absorption pendant 24 heures de Strontium 85 ont été mesurés dans le fémur de rats deux semaines après une fracture d'un tibia. La diminution de la teneur en minéral du fémur du même côté que le tibia fracturé fournit peut-être l'explication de la cessation unilatérale de la croissance du fémur dans le membre fracturé. La teneur en cendres et l'absorption d'isotope pendant 24 heures sont identiques des deux côtés lorsque le volume et la densité de l'os sont maintenus constants.

Dans cette expérience, l'activité spécifique dans les 24 heures qui suivent l'injection de Strontium 85 est plutôt en relation avec la densité du fémur et ne doit pas être considérée entièrement comme le paramètre du taux de la formation osseuse.

ZUSAMMENFASSUNG

Der Aschengehalt, das Volumen und die vierunzwanzigstündige Strontium-85 Aufnahme wurden in Femuren von Ratten zwei Wochen nach Bruch eines Tibiaschaftes gemessen. Der herabgesetzte Mineralgehalt des Femurs ipsilateral zur gebrochenen Tibia konnte durch das einseitige Aufhören des Wachstums am Femur des gebrochenen Gliedes erklärt werden. Der Aschengehalt und die vierundzwanzigstündige Aufnahme von Isotopen waren die gleichen auf beiden Seiten, wenn Knochenvolumen und -dichtigkeit konstant gehalten wurden.

In diesem Versuch war spezifische Aktivitet, die 24 Stunden nach der Injektion von Strontium-85 auftrat, am besten mit der Dichtigkeit des Femurs verbunden und sollte nicht vollständig als ein Massstab der Knochenbildungsgeschwindigkeit angesehen werden.

REFERENCES

Bauer, G. C. H. (1954) Rate of bone salt formation in a healing fracture determined in rats by means of radiocalcium. *Acta orthop. scand.* **23**, 169.

Bohr, H. (1955) Studies on fracture healing. *J. Bone Jt Surg.* **37** A, 327.

Bohr, H. & Sørensen, A. H. (1950) Study of fracture healing by means of radioactive tracers. *J. Bone Jt Surg.* **32** A, 567.

Saville, P. D. & Smith, R. E. (1966) Bone density, breaking force and leg muscle mass as functions of body weight in bipedal rats. *Amer. J. Psysiol. Anthropol.* **25**, 35.

Smith, R. E. & Saville, P. D. (1966) Bone breaking stress as a function of weight-bearing in bipedal rats. *Amer. J. Physiol. Anthropol.* **25**, 159.