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Proliferation of Osteoclasts in Rat Bone Following Bleeding and Femoral Fractures

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Summary. There is a significant transient increase in the number of osteoclasts in intact bone after bleedings and fractures in rats. This rise in the osteoclast population might be due to an increased parathyroid activity released by the trauma, but other factors may be involved. Both bleedings and fractures in rats are followed by hypercalcemia.

Key words: Bleeding — Fractures — Osteoclasts — Hypercalcemia — Bone marrow.

Introduction

Standardized bleedings, injection of calcium and EDTA, and administration of parathyroid hormone stimulate the mitotic activity in rat bone marrow and thymus (Perris and Whitfield, 1967a and b; Perris et al., 1971; Perris and Morgan, 1976). The common factor in this mechanism is a parathyroid-dependent hypercalcemia. Bone marrow mitosis is proportional to plasma calcium concentration (Perris and Morgan, 1976).

Hulth and Johnell (1976a and b) have found that femoral fractures and aspiration of bone marrow also stimulate the mitotic activity of bone marrow cells of the opposite bone and thymus cells. The same is also valid for soft tissue incision (Johnell, 1977).

It is possible that the increase in mitotic activity of thymus and bone marrow cells after fractures is also dependent on the parathyroids. If so, it would be a hypercalcemic response and—what is more important—one could expect an increase in the number of osteoclasts in intact bone marrow both after bleedings and after fractures.

Material and Methods

I. Hemorrhage. A control group of 10 rats and a hemorrhage group of 31 rats were selected from rats weighing 140–160 g. The hemorrhage was induced by cardiac puncture of 1.5 ml blood during ether anesthesia. These animals were killed in groups of 5–10 after 1, 2, 3, 5, and 7 days. The control animals were only anesthetized.

II. Fracture. A control group (15 rats) and a fracture group of 30 rats weighing 100–120 g were used. During ether anesthesia the right femur was fractured by mid-diaphyseal digital pressure, all fractures being diaphyseal. These rats were killed in groups of 5–10 after 4 and 8 h, 1, 2, and 3 days. The control animals were only anesthetized.

In all rats, the right fourth and fifth ribs were taken out; in the hemorrhage experiment the skull bone was also taken out. The bone was decalcified for approx. 20 h in a 10% solution of EDTA containing 0.1 M Tris buffer. The bone was then washed in cold saline, quickly frozen in liquid nitrogen and then cut in a cryostate, 5–6 sections 10 μ thick being made. The sections were cut at 30 μ intervals in order to minimize the appearance of the same osteoclasts in different sections. The sections were stained for succinic dehydrogenase activity by the method of Pearse (1960) with nitroblue tetrazolium salt as the H-acceptor (Tatevossian, 1973).

The osteoclast count was carried out (a) in the trabecular bone of the metaphysis of the rib and (b) along a predetermined length of the cortex of the metaphysis, peri- and endosteally. The length was determined by a rule engraved on the eyepiece of the microscope. The very few osteoclasts occurring in the marrow cavity were also counted.

In the fracture group, serum albumin was determined by the bromcresolgreen method, serum calcium being also analyzed by flame photometry. Hematocrit was measured in all animals.

Results

The microphotographs 1a and b show osteoclasts stained for succinic dehydrogenase. The dark brown staining of the osteoclasts makes them easy to count at a magnification of 60. In the hemorrhage group, the increase in the osteoclast count was statistically significant ($P < 0.001$), having risen on the third day and

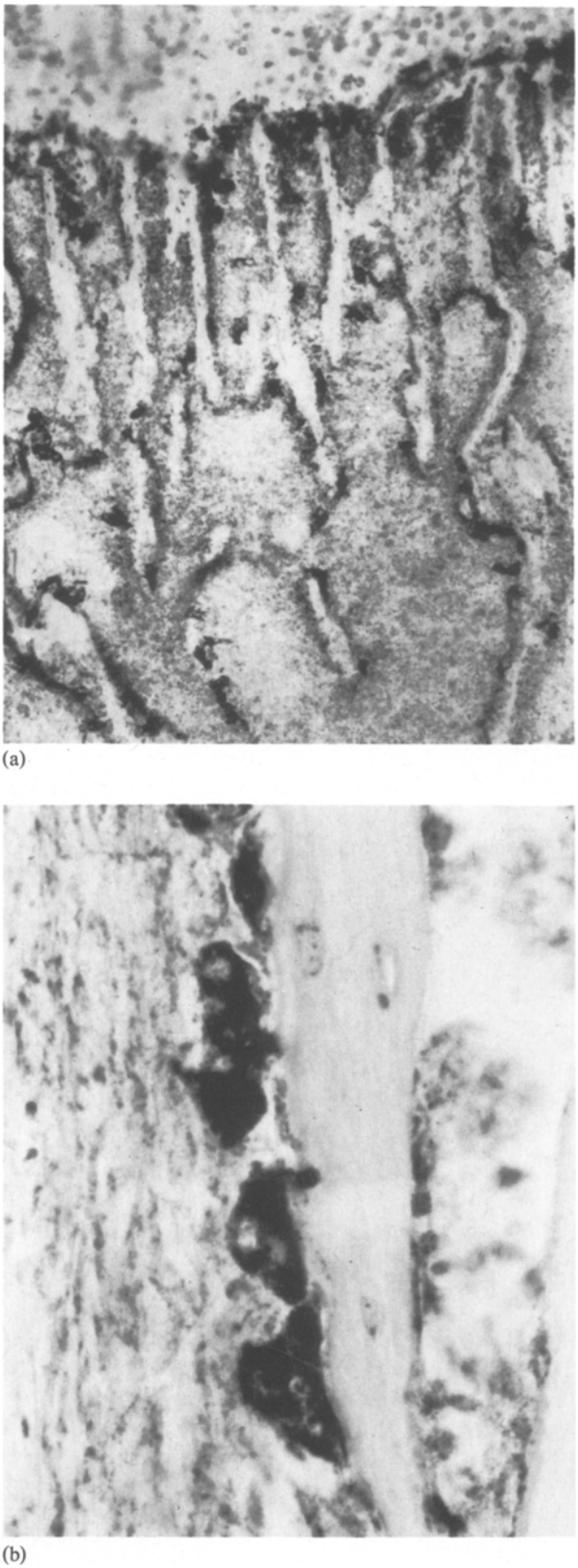

Fig. 1. Microphotograph of rib metaphysis showing osteoclasts stained with succinic dehydrogenase (a) objective magnification ×5,85; (b) objective magnification ×43,80

returned to normal on the seventh day (Fig. 2); osteoclasts in the skull bone were counted the first 3 days in this group, these counts following the same pattern as with the ribs. The hematocrit was also lowered during the first 3 days (Fig. 5).

In the fracture group, the experiment showed a statistically significant ($P < 0.001$) rise of osteoclasts after 1 day (Fig. 3). This corresponds to the increase in serum calcium after 1 day (Fig. 4). After 1 day, the serum albumin was also lowered (Fig. 4). In this fracture experiment, the hematocrit was significantly lowered after 1 day but returned towards normal on the second day (Fig. 6).

Discussion

Different types of trauma, bleedings (Perris et al., 1971), fractures (Hulth and Johnell, 1976a), bone marrow aspiration (Hulth and Johnell, 1976b) and soft tissue trauma (Johnell 1977), result in an increased mitotic activity of the cell population in intact bone marrow and in thymus. It is shown by Perris et al. (1971), that the mitotic rise after bleedings is due to a parathyroid-dependent hypercalcemia. We have shown that even fractures cause hypercalcemia. The hypercalcemia on the day after the fracture was accompanied by a fall in the serum albumin. This is a well known post-traumatic event, but since about 40% of serum calcium is bound to the albumin, this might indicate a still higher increase in ionized calcium after the fracture.

In the present experiments, the femoral fractures were followed by a small but significant decrease in the hematocrit, indicating bleeding. In an earlier report (Hulth and Johnell, 1976a), we did not find a similar decrease in the hematocrit after femoral fracture, but we did observe an increase in the mitotic activity of the bone marrow cells 24 h after the trauma. It is possible that the small accompanying hemorrhage is partly responsible for the increased osteoclasis. The fall in hematocrit was, however, of short duration and therefore the increased mitotic marrow activity from fractures per se might result in an increased number of osteoclasts either via PTH or some other factor. Other factors causing osteoclastic resorption have been studied during the last few years, vitamin D3 metabolites, prostaglandins (Dietrich and Raisz, 1975), mitogenic kinins (Rixon et al., 1971), and osteoclast activating factor (OAF). The latter is a recently identified product of activated lymphocytes (Horton et al., 1972). In another paper (Hulth and Johnell, 1977) we have shown that a single injection of antigen (sheep red blood corpuscles) in rats also results in an increased number of osteoclasts on the third day, at the same

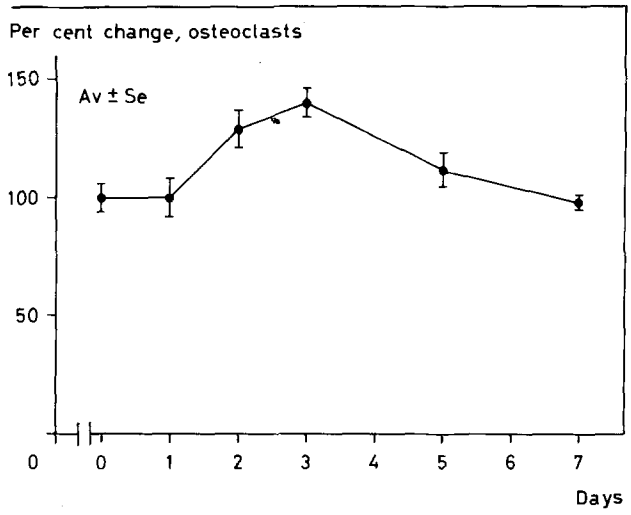

Fig. 2. Percentage changes in osteoclast number in rib metaphysis after bleeding. The osteoclast number in the control animals (53.9 ± 10.7 per rib metaphysis) is expressed as 100%.

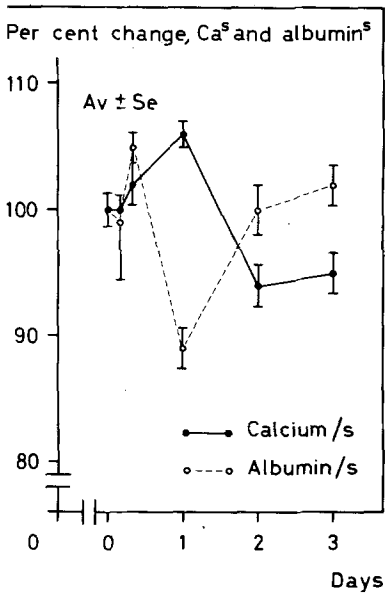

Fig. 4. Percentage changes of in serum Ca and albumin after femur fractures (mean ± SE). Ca in the control group = 2.51 ± 0.13. Albumin in the control group = 31.8 ± 6.3

time as there occurs, according to Perris and Morgan (1976), an increased mitotic activity of the bone marrow. This osteoclasia was followed by only a moderate hypercalcemia and no fall at all in the hematocrit.

The completely new finding in this paper is that both bleedings and fractures produce significant rises in the number of osteoclasts in the rib metaphyses. We identified and counted the osteoclasts using the succinic dehydrogenase activity in these cells (Tatevossian, 1973), in accordance with Radden and Fulmer (1969).

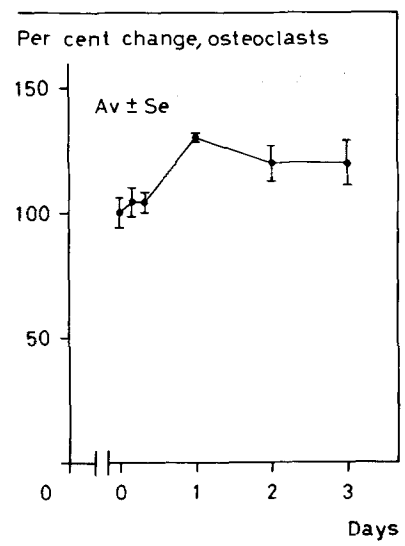

Fig. 3. Percentage changes in osteoclast number in rib metaphysis after femur fracture. The osteoclast number in the control animals (45.5 ± 8.5 per rib metaphysis) is expressed as 100%

The maximum increase in osteoclasts occurred 24 h after the fractures but 3 days after the bleeding. The delay in the reaction in the bleeding group is possibly due to these animals being in less good condition. We had no reason to watch the rise in the mitoses and serum calcium in the bled rats as this is already shown by the Perris group.

The result of the experiment confirms that the osteoclasts are cells which appear and disappear quickly, a finding which is also in agreement with the opinion of Hancox (1972). Tatevossian, in his investigation, found that the osteoclasts increased during the

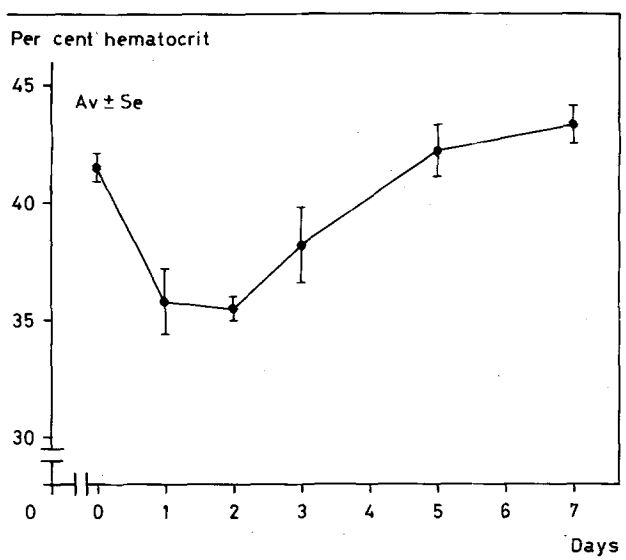

Fig. 5. Changes in hematocrit values after bleeding

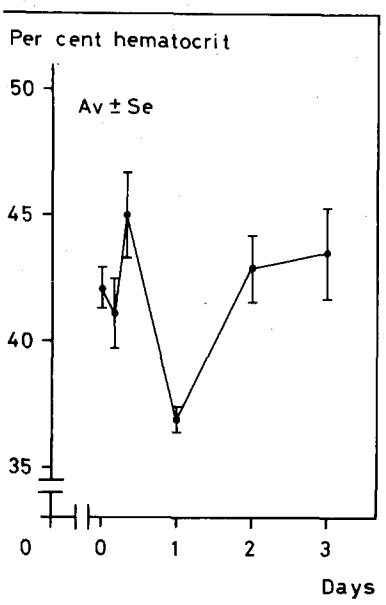

Fig. 6. Changes in hematocrit values after femur fracture

first few hours after injection of parathyroid extract in mice.

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