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CELL PROLIFERATION IN BONE MARROW AND THYMUS FOLLOWING SOFT TISSUE DAMAGE

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Earlier experiments by the author have shown that an increase in mitosis in bone marrow and thymus occurs after fractures and bone marrow aspiration. In this paper it is shown that soft tissue damage causes a statistically certain increase in mitosis both in bone marrow and thymus after 1 day. A possible explanation for this is liberation of a mitogenic kinin.

Key words: mitoses; bone marrow; thymus; wound; cell proliferation

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Mitotic stimulation in rat bone marrow and thymus has been reported following standardized bleedings (Perris et al. 1971), administration of parathyroid hormone (Perris et al. 1967, Perris & Morgan 1976), and injection of calcium (Perris et al. 1967), and kinins (Rixon et al. 1971).

In factures, autoradiographic studies of the proliferative response of osteogenic cells in mice have shown a mitogenic effect of the osteoblasts in the fractured bone and to a certain degree even in the opposite bone (Tonna & Cronkite 1961, Hyldebrant et al. 1974). In an earlier report by the author of this paper, it was shown that after femoral and tibial fracture in growing rats the mitotic activity increases in the first 2 or 3 days in the opposite femoral bone marrow and in the thymus (Hulth & Johnell 1976). In order to look at the reaction from the bone marrow locally, we aspirated bone marrow from right femurs and found an increase of mitotic activity in the bone marrow of the opposite femur and in the thymus, though to a slightly smaller extent than in the fracture group (Hulth & Johnell 1977). In the present study, the mitotic activity in bone marrow is investigated after damage to soft tissue.

MATERIAL AND METHODS

Thirty-one inbred Sprague Dawley rats, weighing 100 and 120 g, were used. They were divided into a control group and a soft tissue damage group. In the latter group, under anaesthesia, a dorsal incision approximately 3 cm in length was made over the lumbar spinal processes cutting through the skin and the muscles, the skin being thereafter sutured. The rats were kept in a cage and supplied with food *ad libitum*. In the experimental group, the animals were killed after 1, 2, 4 and 7 days.

The rats were given two injections of colchicine each (0.2 mg/100 g body wt), intraperitoneally, 6 and 3 hours before they were killed by an overdose of ether. The reason for the two injections of colchicine was to prevent the escape of

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cells in metaphase from the initial block. All animals were given the injections at the same time (the first injection between 8 and 8.30 a.m.) in order to avoid the circadian fluctuations in mitotic activity of bone marrow and thymus (Hunt & Perris 1973).

Groups of normal rats and rats after soft tissue damage were used for haematocrit determinations. The thymus gland and the left femur were removed. The thymocyte and bone marrow cell suspensions were prepared in a balanced glucose salts medium (5.5 mM glucose, 5.0 mM KCL, 0.63 mM $CaCl_2$, 1.0 mM $MgSO_4$, 5.0 mM Na_2HPO_4, 120 mM NaCl, 5.0 mM Tris-buffer (pH 7.2)). The thymocyte suspension was prepared by mincing the gland in the medium with scissors, the resulting suspension being filtered through gauze. To prepare the suspensions of bone marrow cells, the ends of the femurs were removed and the core of marrow was "washed out" with 1.5 ml of the medium, and then dispersed by passing the tissue several times through a syringe with an 18-gauge needle. Then both thymus and bone marrow suspensions were gently centrifugated.

Samples of the cell suspensions were placed on slides, immediately fixed in alcohol, and stained in haematoxylin eosin. The slides were scored for the percentage of the total cell population in metaphase. Each preparation had two slides and at least 500 cells were counted on each (a total of at least 1000 cells were counted). During the counting procedure the slides were labelled in code.

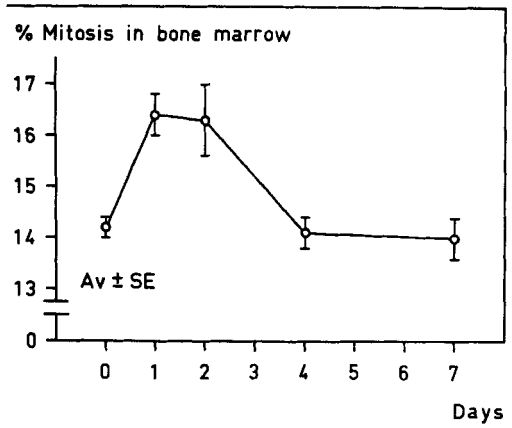

Figure 1. Percentage of total cells in metaphase in bone marrow from the left femur after colchicine injection.

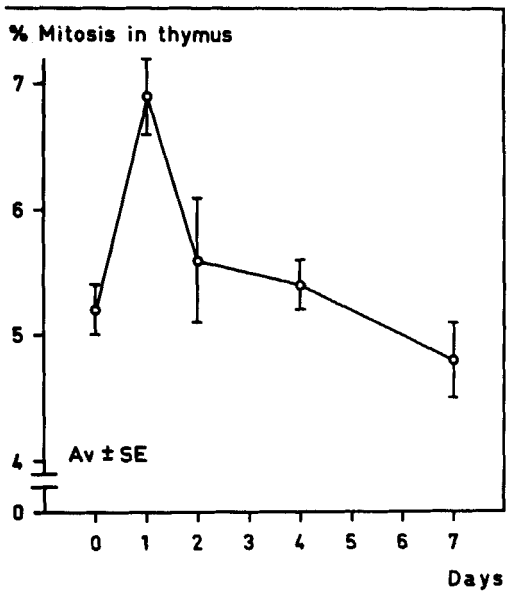

Figure 2. Percentage of total cells in metaphase in thymus after colchicine injection.

RESULTS

In the soft tissue damage group, the mitotic activity was increased in the bone marrow cell suspension from the left femur 1 day after the trauma ($0.1 > P > 0.001$), returning towards the normal value (Figure 2). The haematocrit The thymus cells, arrested in metaphase, increased 1 day after the damage ($P < 0.001$) and then returned to the normal value (Figure 2). The hematocrit did not differ from the control animals in the experimental group.

DISCUSSION

Various types of trauma seem to increase bone marrow and thymic cell mitotic activity. This is valid not only for standardized bleedings (Perris et al. 1971) and fractures (Hulth & Johnell 1976) but also for slight trauma such as bone marrow aspiration (Hulth & Johnell 1977). In this paper it is shown that a soft tissue incision gives the same result. The mitotic activity after bleeding is governed by the parathyroid gland but it is not certain

how bone and soft tissue trauma cause increased mitotic bone marrow activity.

It has previously been shown that injection of bradykinin also causes an increased mitotic activity in bone marrow and thymus, thus supporting the theory of mitogenic kinin. In this experiment, it might be possible that mitogenic kinins are liberated from the trauma site, even though the parathyroid hormone might also be involved.

It is impossible to say, at the present stage of the research, what is the purpose, if any, of the bone marrow cell proliferation after various types of trauma. After haemorrhages, of course, the bone marrow stem has to substitute the lost blood cells. It is perhaps possible that the cellular response of the bone marrow has some important purpose in producing cells for migration into the fracture site or the soft tissue wound.

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