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THE MITOTIC ACTIVITY OF BONE MARROW AND THYMUS AFTER COMBINED ANTIGENIC CHALLENGE AND TRAUMA

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A significant increase in the mitotic rate of cells in thymus and bone marrow occurs 2–3 days after the infliction of various traumas or the injection of antigenic erythrocytes. This cell response probably occurs in order to produce the cells which are needed for the defence of the body after injury. The present investigation shows that the cell response after a fracture is abolished if the rats are fractured 3 days after injection of antigenic erythrocytes.

Key words: antigen; bone marrow; mitosis; thymus

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Perris and co-workers found, in 1971, an increased mitosis in the cells of bone marrow and thymus after standardized bleedings. Hulth & Johnell (1976a) found the same phenomenon after fractures, soft tissue trauma (Johnell 1977), bone marrow aspiration (Hulth & Johnell 1976b) and injections of antigenic erythrocytes (Hulth & Johnell 1978). In 1979, Johnell & Hulth showed that when two traumatic injuries take place with an interval of only 2 days, the cell response to the second trauma is completely abolished. With an interval of 10 days, however, the cellular proliferation returns, although it is delayed.

In this paper we have studied the combination of an antigenic injury, followed on the third day by a fracture.

MATERIAL AND METHODS

Three groups of 25, 23 and 10 rats, respectively, weighing 120–130 g were used. In addition there was a control group of 15 rats. The first group was given intravenously in a tail vein 0.5 ml of sheep red blood corpuscles (S–RBC), 20 per cent S–RBC in saline = 4.0×10^{12} RBC/litre. The animals were kept in cages and supplied with water and food *ad libitum*. They were killed in groups of five at various times from 1 to 8 days after the injection. The second group was also given S–RBC but after 3 days the left femur was broken, the animals being killed in groups of five to six, 1 to 4 days after the fracture. The third group of ten animals was fractured and killed after 1 and 2 days. The control group was killed without prior intervention. The rats were given two injections of colchicine intraperitoneally, the first 6 hours (0.2 mg/100 g animal) and the second 3 hours (same dose) before the animals were sacrificed using ether. All animals were given the injections of colchicine at the same time, the first injection being between 8.00 and 8.30 a.m., in order to avoid possible circadian variations.

The thymus gland and the right femur were removed. Thymocyte and bone marrow cell suspensions were prepared in a balanced glucose salt 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 Trisbuffer (pH 7.2)). The thymocyte suspension was prepared by mincing

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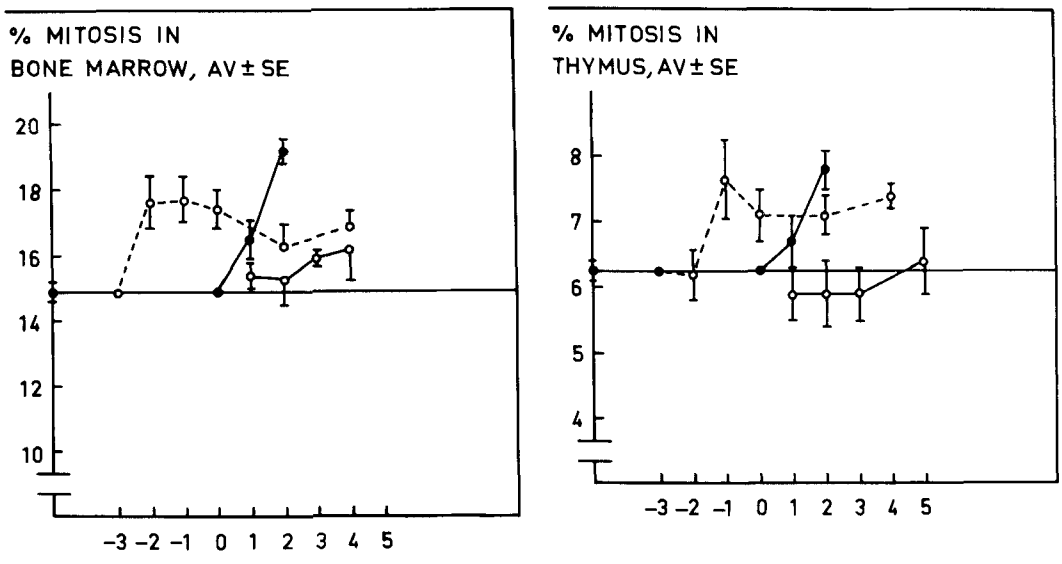

Figure 1 A and B. Effect on mitosis in bone marrow (A) and thymus (B) after 0.5 ml 20 per cent S–RBC on day 3 (○---○); 0.5 ml 20 per cent S–RBC on day 3, fracture on day 0 (○——○); or fracture on day 0 (●——●).

S–RBC causes a significant increase in the mitosis rate of bone marrow and thymus, after 24 hours and 48 hours, respectively. Fracture of the femur results in a significant increase in the mitosis rate of bone marrow and thymus after 48 hours. Fractures 3 days after injection of S–RBC are without effect on the mitosis rate of bone marrow and thymus.

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 left femurs were removed, the core of marrow was washed out with 1.5 ml of the medium and then they were 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, i.e. a total of at least 1000 cells were counted.

RESULTS

The results are shown in Figure 1 A and B. The mitotic rate of bone marrow cells increased significantly 24 hours after the S–RBC injections and remained high for 5 days. Non-injected animals with fractures showed significant increases in the mitotic rate of the bone marrow cells, thus reacting in the same way as in earlier experiments (Hulth & Johnell 1976 a). The animals, that 3 days before had received an injection of S–RBC, did not, however, react at all after the fracture.

The changes in the mitotic rate of thymus cells followed the same pattern except that the reaction in the S–RBC animals was delayed – the significant increase in mitosis appeared first 48 hours after the antigenic injection.

DISCUSSION

Various types of trauma and also an antigenic challenge bring about increases in the mitotic rate of cells of bone marrow and thymus in young rats. At the same time there occurs a significant increase in the number of osteoclasts in the ribs (Hulth & Johnell 1978, Johnell & Hulth 1977).

This investigation shows that the increase in the mitotic activity of bone marrow and thymus after traumatic injuries does not occur when the trauma comes a short interval after injections of antigenic red blood corpuscles. In an earlier investigation on the effect of double trauma, we have shown that the organism behaves in a refractory manner during the first days after a trauma. If the interval is 10 days, there is a mitotic response of the usual strength but it is delayed for 24 hours. It is probable that the mitotic reactions are inflammatory responses by which leucocytes and macrophages, needed for the defence of the organism, are produced. It is possible, on the one hand, that the capacity to produce new cells is limited and therefore the organism cannot mobilize an adequate cell response when two injuries come too close to each other or, on the other hand, that new production of cells is suppressed for a limited period after an injury.

The mitotic activation is perhaps caused by some biochemical mediator, released from the injury. As to what kind of mediator is in action is still purely a matter of conjecture. Mitogenic activity of macrophages has, however, been demonstrated in cell-mediated immune reactions and also in various types of non-immunological inflammation, the factor having been classified as one of the lympokines (Pelletier et al. 1978).

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