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PROLIFERATION OF BONE MARROW AND THYMUS CELLS AND INCREASED OSTEOCLASIA AFTER ANTIGENIC CHALLENGE IN RATS

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Injection of a single dose of an antigenic substance (sheep red blood corpuscles) in young rats results in a significant rise in the number of mitoses in bone marrow (and in thymus) and in addition a significant rise in the number of osteoclasts in the metaphyses of the ribs. This increased osteoclasia appears 1 day after the peak values of the bone marrow and thymus mitoses. This and earlier investigations make it probable that there is a causal relationship between bone marrow stimulation and increased osteoclasia.

Key words: bone marrow; thymus; osteoclasts; antigen; mitosis

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Standardized bleeding, injection of calcium and EDTA, stimulate the rate of mitosis in bone marrow and thymus in rats (Perris & Whitfield 1967a,b, Perris et al. 1971). The common factor in this mechanism is a parathyroid–dependent hypercalcemia. The authors of the present paper have shown that fractures of the femur (Hulth & Johnell 1976a), partial aspiration of bone marrow (Hulth & Johnell 1976b) and soft tissue wounds (Johnell 1977), in rats, also result in stimulation of mitosis of intact bone marrow and of thymus. Whether or not this reaction is also governed by PTH is not clear, but hypercalcemia occurs at fractures. In addition, fracture of the femur and bleeding in rats result in proliferation of bone resorbing osteoclasts in rib metaphyses (Johnell & Hulth 1977). Perris & Morgan (1976) have related in a review article an unpublished investigation showing that injection of an antigen, red blood corpuscles of sheep (S–RBC), in rats, also causes an increased mitosis rate 3 days after the injection. The question is does increased mitosis rate in bone marrow *per se* result in increased osteoclasia. We have therefore studied the effect of antigen injection on the mitosis rate in bone marrow and on the number of osteoclasts.

MATERIAL AND METHODS

Three groups of 40, 42, and 30 rats, respectively, weighing 120–130 g were used, each group including a control group of 7 rats. The first two groups were used for osteoclast counting, the third for mitosis counting. All the experimental animals were given intravenously in a tail vein 0.5 ml of S–RBC; the first group, a pilot study, 10 per cent S–RBC in saline = 2.1×10^{12} RBC/liter, the other two groups 20 per cent S–RBC = 4.0×10^{12} RBC/liter. The animals were sacrificed in groups of 6–7 rats after 1, 2, 3 and 5 days and in the two first groups also after 7 days. The rats seemed to be in good condition and were not visibly influenced by the antigen.

Osteoclast study

Blood was collected to determine Ca^s, albumin^s and hematocrit. In all rats the right fourth and fifth ribs were taken out. The bone was de-

calcified for approximately 20 hours in a 10 per cent 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 10μ thick being made. The sections were cut at 30μ intervals in order to minimize the appearance of the same osteoclast in different sections. The sections were stained for succinic dehydrogenase activity by the method of Pearse 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, periosteally and endosteally. The length was determined by a rule engraved on the eye-piece of the microscope. The very few osteoclasts occurring in the marrow cavity were also counted.

Serum albumin was determined by the bromcresol green method, serum calcium being also analyzed by flame photometry. Hematocrit was taken in all animals.

Mitosis study

The rats were given two injections of colchicine intraperitoneally, the first 6 hours (0.2 mg/100 g animal) and the second 3 hours (0.2 mg/100 g animal) before the rats were sacrificed by ether. The choice of two injections of colchicine was made to prevent the escape of cells in metaphase from the initial block. All animals were given the injections at the same time, the first injection between 8–8.30 a.m., in order to avoid the circadian fluctuations in mitotic activity of bone marrow and thymus cells (Hunt & Perris 1974).

The thymus gland and femoral bones were removed. 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, 120mM 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 suspension of bone marrow cells, the ends of each femur 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 centrifuged.

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

RESULTS

Injection of 20 per cent S-RBC resulted in a significant increase ($P < 0.001$) in the number of osteoclasts on the 3rd day with the level remaining high–until the 5th day after injection (Figure 1). Ca was normal for the first few days but showed a slight, though not definitely significant, increase on the 5th day. Other changes noted were significant increases in albumin[s] on the 2nd day and in hematocrit on the 3rd day after injection. After injection of the smaller dose, 10 per cent S–RBC, there was also a significant increase ($0.01 > P > 0.001$) in osteoclasts on the 3rd day after injection. In these animals there was no increase in Ca^s, which was also true for the hematocrit.

The results of the mitosis counting of bone marrow cells and thymus cells are shown in

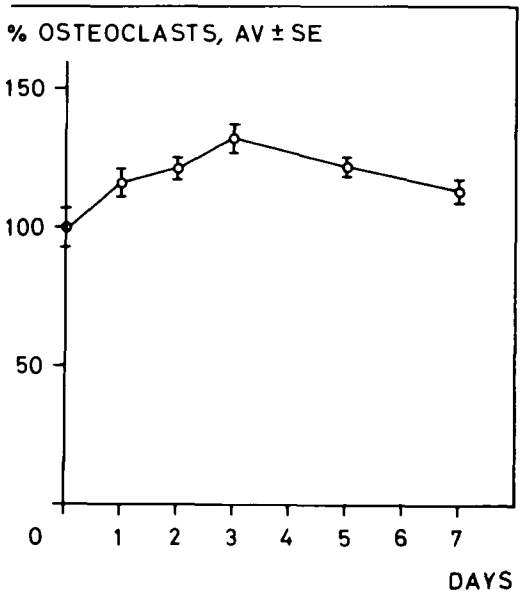

Figure 1. Percentage changes in osteoclast number in rib metaphysis after injection of a single dose of 20 per cent S–RBC. The osteoclast number in the control animals is expressed as 100 per cent.

Figure 2a and b. In control animals 6 hours after the first colchicine injection 16.8 per cent of the bone marrow population was arrested in metaphase. Similar figures for the thymocytes arrested in metaphase were 6.7 per cent. The increase in bone marrow cells in metaphase was significant after 1 day and highly significant after 2 days. Thereafter there was a return towards normal on the 5th day.

The increase in mitosis among thymocytes was highly significant on the 1st and 2nd day after injection of antigen. The course after the 2nd day is impossible to determine because the values were too widely spread.

DISCUSSION

In the experiments performed we have shown that injection of an antigenic substance (S–RBC) results in a significant increase in the number of mitoses in bone marrow (and also in thymus). In addition the antigen resulted in a significant rise in the number of osteoclasts in rib metaphyses in rat. We were not able to perform the osteoclast counting and mitosis counting in the same animals because colchicine is known to depress the amount of osteoclasts in bone marrow (Raisz et al. 1973). Our experiments have shown that the maximal effect on mitosis of the bone marrow cells occurs on the 2nd day, whereas the maximal osteoclast effect is on the 3rd day. In experiments with fractures in rats performed earlier, the increases in both mitosis and osteoclasts reached their peak values after 1 day (Hulth & Johnell 1976, Johnell & Hulth 1977).

An immune response and trauma have an effect in common, viz., an increased rate of mitosis of bone marrow cells. The bone marrow stimulation after bleeding is due to a parathyroid-dependent hypercalcemia. Hypercalcemia also occurs after bone trauma. In the present experiments there was only an insignificant increase in serum calcium after antigenic challenge. The insignificant increase in serum calcium after antigen suggests another mechanism for osteoclasia. Several substances producing osteoclastic resorption of bone have been investigated during the last few years. In addition to PTH and

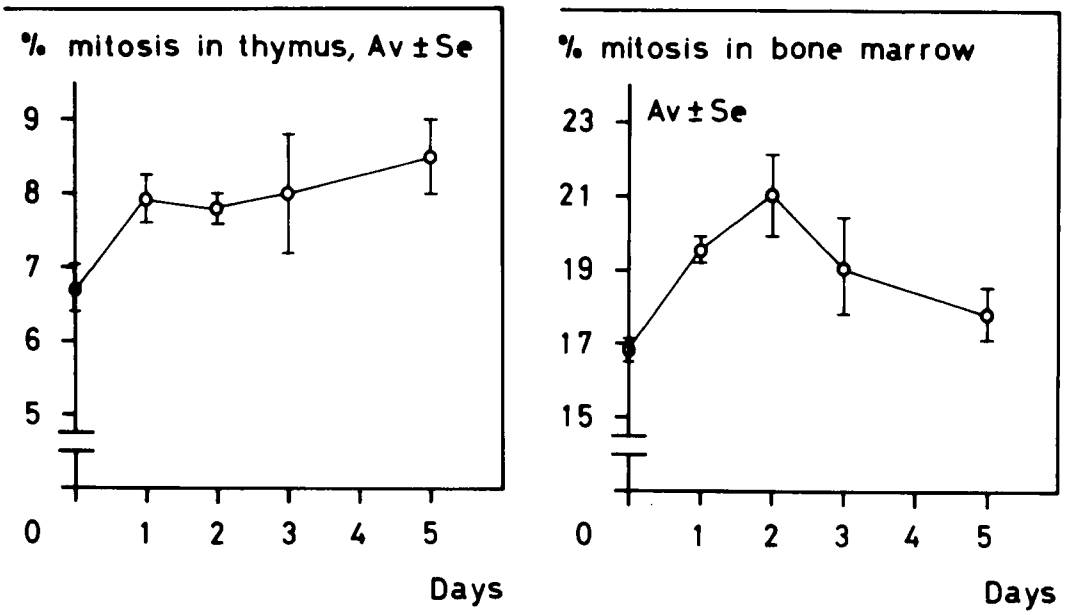

Figure 2a and b. Increase in the number of mitoses in thymus (2a) and in bone marrow (2b), after injection of a single dose of 20 per cent S – RBC.

D–vitamin metabolites, osteoclast activating factor (OAF) produced *in vitro* by mitogenic stimulation of lymphocytes (Horton et al. 1972), and prostaglandins (Deitrich & Raisz 1975) have also been the subject of study. In connection with immune response, prostaglandins appear to mediate a complement dependent effect of serum on osteoclastic bone resorption (Dietrich & Raisz 1975). It is possible therefore that the increased osteoclasia in this experiment depends on prostaglandins.

Irrespective of the substance which mediates the increased osteoclastic activity there appears to be a mutually dependent relationship between increased mitotic activity of bone marrow cells and increased osteoclastic activity. This provides an interesting insight into the function of bone as an organ in which its two parts are intimately dependent on each other. This aspect, though not new, is often overlooked. It is impossible however, at this stage of the research, to apply the experimental findings to human diseases, especially to different types of osteoporoses.

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