Cell Proliferation in the Bone Marrow and Thymus Following Partial Bone Marrow Aspiration

A. Hulth and O. Johnell

Department of Orthopedic Surgery, Malmö General Hospital, University of Lund, S-214 01 Malmö (Sweden), 18 May 1976.

Summary. Aspiration of the one femoral bone marrow caused a significant rise of the cell population, arrested in colchicine-metaphase both of the bone marrow from the other femur and of the thymus.

In an earlier report[1], the authors of the present paper have shown that tibia and femoral fractures in growing rats stimulate bone marrow and thymus to increased mitotic activity during the first 2 or 3 days after the fracture. The bone consists, however, of two different cohering tissues, the bone tissue itself and the bone marrow. It would be of interest to know whether the mitotic activity is increased solely by damage to the bone marrow. It is possible to evacuate most of the bone marrow from the femoral medullary canal via a small hole in the intercondylar groove, which must be considered a quite insignificant bone trauma.

Material and methods. Inbred rats of Sprague Dawley strain weighing between 100–120 g were used, divided into 2 groups: one control (5 rats) and one experimental group. On the animals belonging to the experimental group, a pin-point arthrotomy was made of the right knee joint. A needle was inserted through the top of the femoral intercondylar groove, and the femoral bone marrow was aspirated. The procedure was performed under ether anesthesia, which also was given to the animals of the control group. The rats were then kept in cages with food ad libitum.

In the experimental group the animals were killed after 1, 2, 4 and 7 days (5 rats at each time). The rats were given i. p. injections of colchicine each (0.2 mg/100 g animal), the first 6 h and the last 3 h before the rats were killed by ether. The reason for 2 injections of colchicine was 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 08.00–8.30 h in order to avoid the circadian fluctuations in mitotic activity of bone marrow and thymus[2].

Groups of normal rats and rats with bone marrow aspirations were used for hematocrit determinations. The thymus gland and the left femur were removed. Thymocyte and the bone marrow cell suspensions were prepared in a balanced glucose salts medium (5.5 m*M* glucose, 5.0 m*M* KCl, 0.63 m*M* $CaCl_2$, 1.0 mM $MgSO_4$, 5.0 m*M* Na_2HPO_4, 120 m*M* NaCl, 5.0 m*M* *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 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 centrifugated.

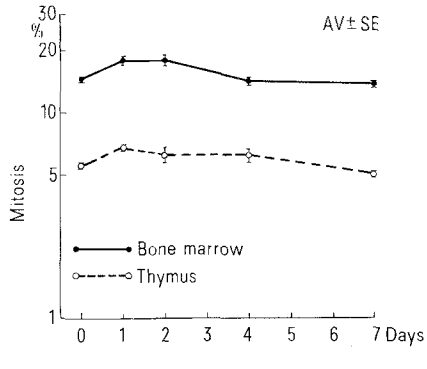
Samples of the cell suspensions were placed on slides, immediately fixed in alcohol, and stained in hematoxylin eosin. The slides were scored for the percentage of the total cell population in metaphase. Each preparation had 2 slides and on each at least 500 cells were counted (a total of at least 1000 cells were counted). During the counting procedure the slides were labelled in code.

Result. The mitotic index in the control group was in bone marrow 14,7% and in thymus 5,4%. In the bone marrow aspirated group mitotic activity was increased in bone marrow cell suspension from the left femur, both 1 and 2 days after the aspiration compared with the control group ($p<0.001$), then returned to slightly under the normal value on the 4th day and remained there on the 7th day. The thymus cells, arrested in metaphase, increased after 1 day with 25% ($0.01>p>0.001$) and then slowly decreased towards the normal value on the 7th day (see Figure).

The hematocrite in the control animal groups was 49.7%, and, after 1 and 2 days, 47.3 and 48.1% (no statistical difference).

Discussion. The present investigation shows that damage to bone marrow in young rats causes an increase of the mitotic activity of both thymus and bone marrow of an intact bone. The bone marrow was evacuated via an insignificant hole in the intercondylar groove in the knee joint after a pin-point arthrotomy. The result is of the same type but quantitatively a little less than after fractures of tibia or femur in rats[1]. We considered the in-increased mitotic activity after fractures to be due to release of mitogenic kinins from the trauma site[3,4]. It is possible that the same mechanism appears in this experiment with pure marrow trauma.

There are, however, other things to consider when discussing the stimulation of mitoses in bone marrow and thymus after damage to bone marrow. A great amount of research has been carried out by Canadian groups on the effect of hypercalcemia, especially increase of ionized calcium. Injections of calcium, parathyroid hormone,



Semilogarithmic diagram showing the percentage of total cells in colchicine-metaphase in intact bone marrow and thymus after aspiration of bone marrow from the other femur.

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EDTA[5-7] and standardized bleedings[8], give about the same effect of raised mitose frequency of bone marrow and thymus. In the two last-mentioned experiments the hypercalcemia is secondary to an initial hypocalcemia. The Canadian scientists make it evident that the parathyroid glands are invested with the superior controlling function of the mitotic level of thymus and bone marrow. In our experiments, however, the bleeding caused by the bone marrow aspiration was insignificant.

The concept of chalones, on which a major literature exists[9], might also yield an explanation of the mitotic stimulation. Chalones are mitotic inhibitors normally fixed to the cell membranes, but which are thought to fall off, e.g. at traumatic incidents.

This report is merely preliminary, seeing that without further research it is impossible to say in which way the stimulation of mitoses is mediated.

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A Transmission and Scanning Electron Microscope Study of Primary Neural Induction

MARJORIE A. ENGLAND[1] and S. VIVIENNE COWPER[2,3]

Department of Anatomy, University of Leicester, Medical School, University Road, Leicester LE1 7RH (England); and Stereoscan Unit, Bedford College, Regent's Park, London N. W.1 (England), 8 June 1976.

Summary. Normal primary neural induction has been further studied by TEM and SEM. A single mesoderm cell is usually in contact with several ectoderm cells. The mesoderm cells are also contacting other mesoderm cells. It is suggested that ectoderm cells are induced in groups and that induction is synchronized by these contacts. At the points of contact between mesoderm and ectoderm cells cytoplasmic changes are present in the induced tissue.

Primary neural induction has been shown to occur in the very early stage-5 chick embryo[4,5]. At this point in development the ectoderm layer immediately anterior to Hensen's node has thickened. The presumptive notochord mesoderm cells are also present as a mass ventral to this area of thickened ectoderm and recent studies by scanning electron microscopy have demonstrated that the mesoderm and ectoderm cells are apposed at the time of induction[6].

The ectoderm and mesoderm cells have now been examined at the time of neural induction using both the SEM and TEM to obtain a more detailed assessment of the ultrastructural features. Particular attention has been paid to the cellular contacts and the changes in the cytoplasm at the junctional regions.

Materials and methods. White leghorn chick embryos were incubated at 37.5 °C until stage –5[7]. The eggs were then opened and the embryos cut off the yolk and mounted by New Culture[8]. The specimens were immediately placed in KARNOVSKY's[9] fixative for 12 h. They were then buffered in Cacodylate buffer[10] for 12 h.

SEM preparation. A narrow fracture was directed across the area pellucida immediately anterior to Hensen's node. This exposed the ectoderm, mesoderm, and endoderm in the region to be studied. In two specimens the mesoderm layer was sheared off slightly caudad to the ectoderm

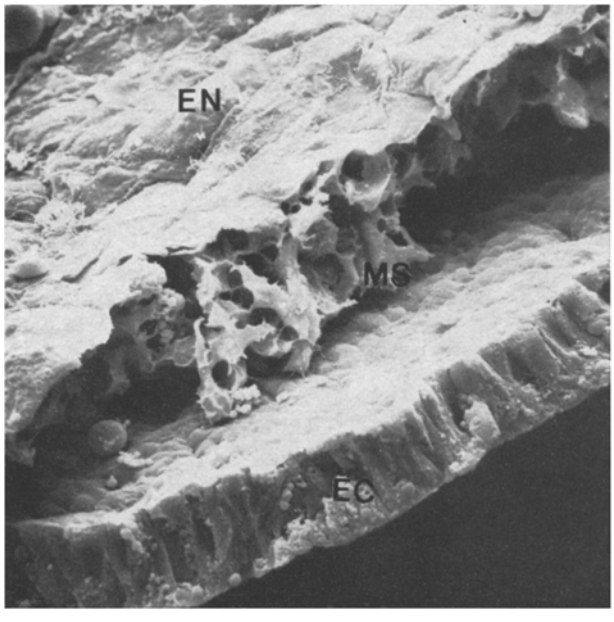

Fig. 1. The three embryonic layers anterior to Hensen's node. EC, ectoderm; MS, mesoderm; En, endoderm. ×219.

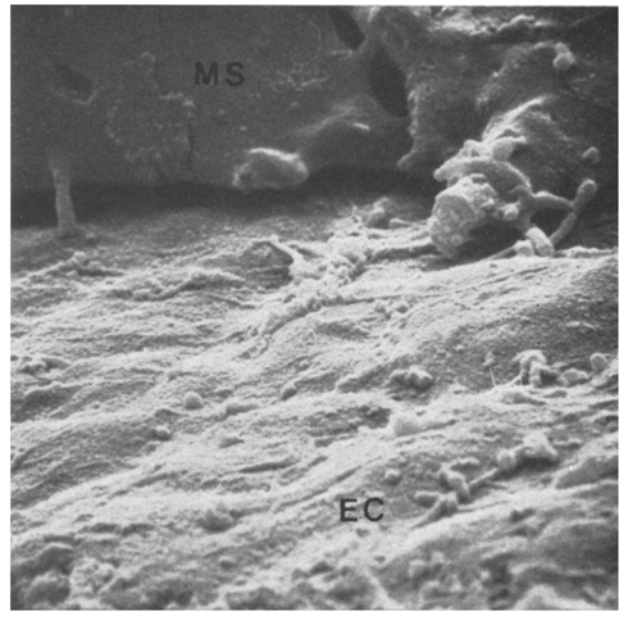

Fig. 2. The fibrils and filopodia on the ventral ectoderm layer. EC, ectoderm; MS, mesoderm. × 11,000.