DEVELOPMENT AND DIFFERENTIATION OF BONE MARROW MECHANOCYTES

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KEY WORDS: bone marrow; mechanocytes; trauma.

Stromal cells or mechanocytes, which account for only 4.1% of the total number of cells settling in the medullary spaces, have low proliferative activity and are renewed very slowly. They include endothelial, reticular, and undifferentiated connective-tissue cells. Total removal of bone marrow from the medullary canal is accompanied by destruction of the vascular network, followed by hemorrhage and the formation of a blood clot. During restoration of the medullary contents, besides the development of cells of the hematopoietic series, mechanocytes also develop and form granulation tissue. The latter, as Fridenshtein et al. [2] notes, is formed with the participation of cells migrating from osteons of the cortical lamina. However, it is not always possible to identify the development of foci or mechanocytes among the obviously overwhelming majority of hematopoietic cells. The study of early forms of mechanocytes may therefore be difficult. Because of the difficulties of finding mechanocytes on histological survey sections, special models with removal of bone marrow from the medullary canal, where it can more easily be curetted (compared with its removal from the cancellous bone of the epiphyses), have recently been used. However, complete removal of bone marrow from the medullary canal by curettage is difficult. It leads to masking of the small early foci of mechanocytes in the medullary canal. Other difficulties associated with the study of early forms of mechanocytes are that, with unfavorable conditions in the injured medullary canal, the development of mechanocytes does not always begin on the 2nd day after trauma even if all cells have been removed from it. The exact time of their appearance can thus not be established. An oriented search for these cells is often negative. The circumstances described above have made it impossible to give an exact description of the beginning of mechanocyte development.

The aim of this investigation was to discover early stages of development of these cells, which appear first in the medullary cavity after removal of bone marrow.

EXPERIMENTAL METHOD

The curettage model, described in the literature [3] for the study of bone marrow regeneration, was modified. A special feature of our model [3] was that all bone marrow cells could be removed and tissue fluid drained from the bone, which is necessary for normalization of pressure in the medullary cavity. For this purpose, a burr-hole 0.3-0.5 mm in diameter was drilled in the metaphysis of the rabbit's tibia, and through it all bone marrow cells were removed by means of a strong jet of 0.5% procaine solution, injected into the medullary canal. A small narrow (0.2 mm) transverse incision was then made (taking great care of the periosteum and preserving it at the edges of the bone wound) through the periosteum and the whole thickness of the cortical lamina of the middle part of the medullary canal of the diaphysis. Premedication was used before the operation. The animals were taken from the experiment on the 1st, 2nd, and 3rd days after the operation. The repair process was studied histologically. Sections were stained with hematoxylin and eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

Histological investigation showed that by the end of the 1st day the medullary cavity was filled with plasma-like edema fluid, mixed with erythrocytes in the form of amorphous masses, which filled the canal densely. In the lumen of the absolute majority of vascular canals in the cortical lamina the cell nuclei of the vessels were unstained. The lumen of the vessels,

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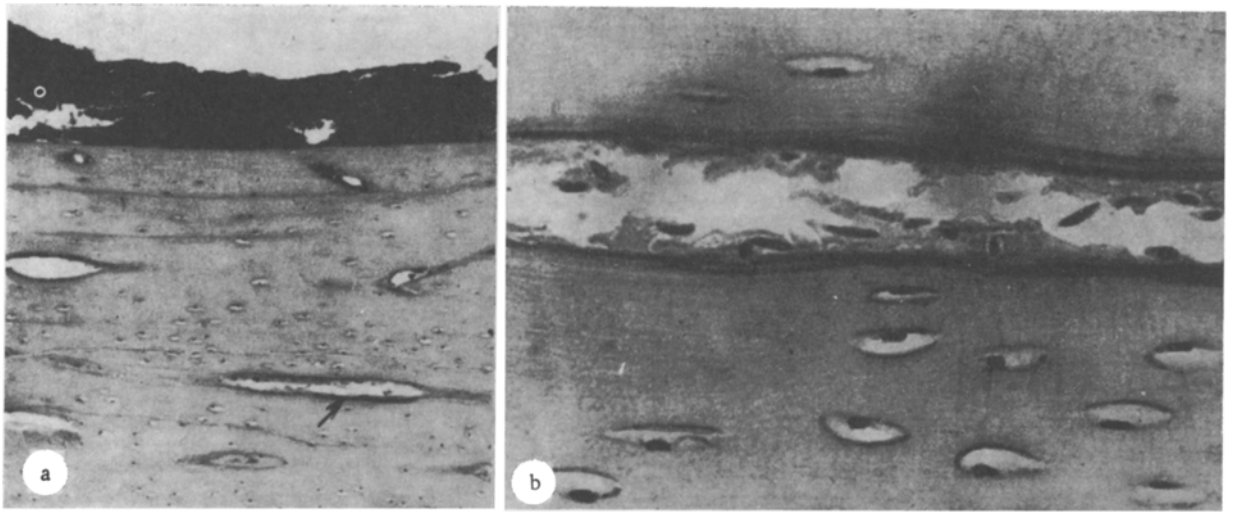

Fig. 1. Diaphyseal cortical lamina with vascular canals. *a*) Single cells can be identified in some of them (arrow), with blood clots in the medullary canal. Stained with hematoxylin and eosin; b) vascular canal (arrow) containing single fibroblast-like cells, 24 h after removal of the bone marrow from the medullary cavity. 500 ×.

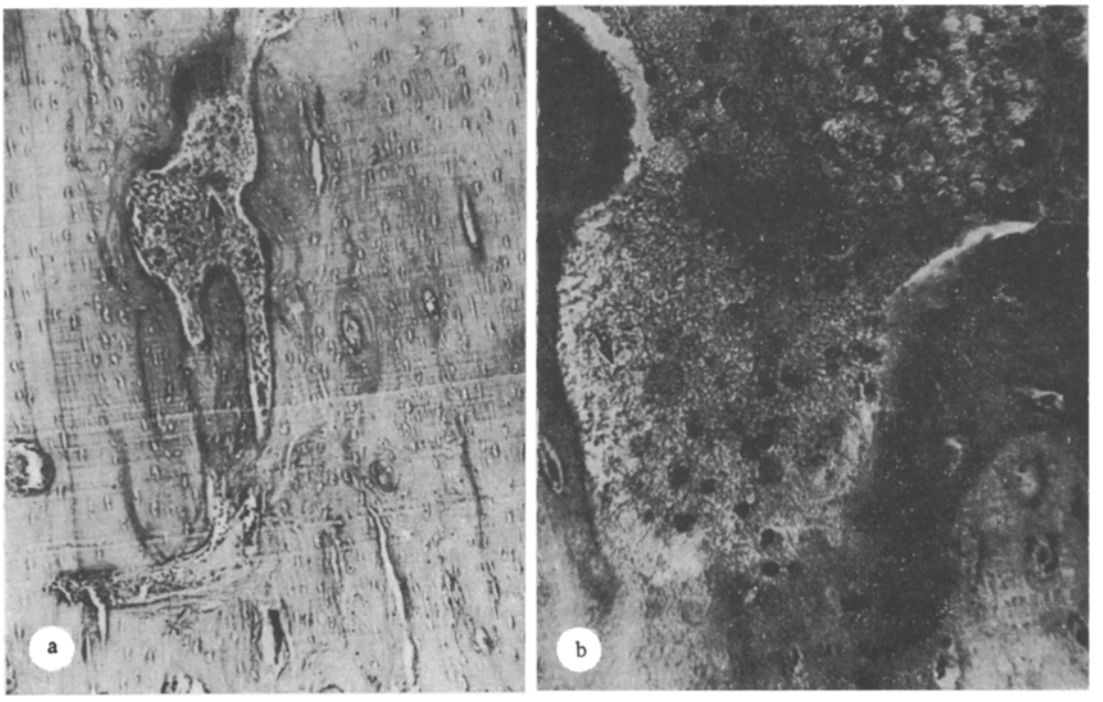

Fig. 2. Diaphyseal cortical lamina. *a*) Dilated vascular canals (arrow) containing fibroblast-like cells. Stained with hematoxylin and eosin. 100 ×; b) Fibroblast-like cells in vascular canal 2 days after removal of bone marrow from medullary cavity. 400 ×.

constituting the intraosseous vascular microcirculatory network, were filled with disintegrating blood-stained masses. Only in the lumen of individual vessels could solitary fibroblast-like cells be seen (Fig. 1).

Deposition of sediment, with the presence of plasma territories, lying closer to the cortical lamina of the diaphysis, where single round cavities (cysts) were present, was observed 2 days after the operation in the amorphous blood stained masses in the medullary canal with disintegrating erythrocytes. Cell-free masses were visible in the lumen of the overwhelming majority of vascular canals. Round or oval cells with a large, dense nucleus (Fig. 2), which could be identified as pericytes, were discovered only in some of the Volkmann's perfor-

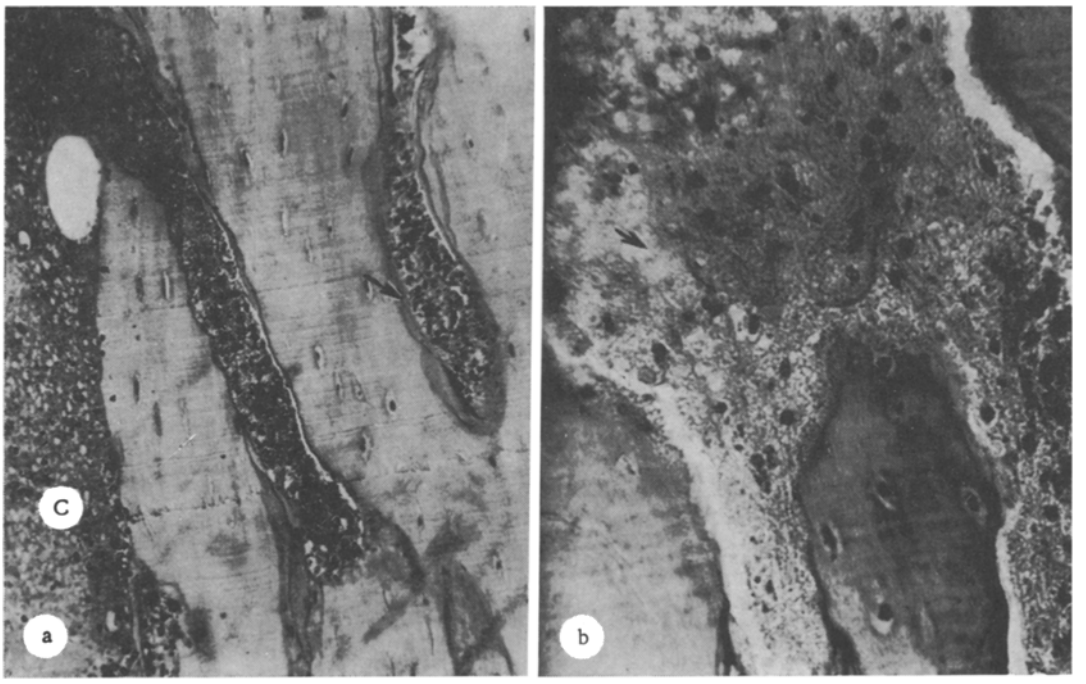

Fig. 3. Inner layers of diaphyseal cortical lamina. Hematoxylin and eosin. *a*) Dilated vascular canal (arrow), emptying into medullary cavity (C) and containing fibroblast-like cells. 200 ×; b) Fibroblast-like cells in dilated perforating vascular canal, 3 days after removal of bone marrow from medullary cavity. 400 ×.

ating canals, located nearer to the diaphyseal medullary cavity. Cells of the same character were found in the lumen of the somewhat dilated Volkmann's canals emptying into the medullary cavity. These cells, surrounded by fibrin threads, emerged in some places on the inner surface of the cortical lamina. On the 3rd day of regeneration the vascular canals of the cortical lamina were more numerous, and the same cells could be seen there as 2 days after the operation (Fig. 3). Only in zones nearer to the periosteum, were cell-free masses visible in the lumen of the canals as before. More cells migrating into the medullary cavity could be seen near the inner surface of the cortical lamina. In the medullary cavity itself, the masses of disintegrating erythrocytes were becoming much smaller, and numerous round cystic cavities appeared among them (their walls, as electron-microscopic study showed, consisted of collagen fibrils), filled with liquid resembling plasma.

The experimental results thus show that whereas most cells of the vessel walls of the microcirculatory bed, located in all vascular canals of the cortical lamina without exception (10 cases), were unstained, the circulation of the blood was disturbed. These observations can be fully explained by the character of the blood supply to the diaphyseal cortical lamina, which receives its main blood supply (two-thirds of its thickness) from the medullary vascular network, anastomosing with the periosteal network. Removal of the medullary contents leads inevitably to disturbances of the microcirculation in the bone during the first days. In experiments in which a small (0.2 mm) transperiosteal transverse incision was made in the middle part of the cortical lamina, through its whole thickness, numerous cysts appeared on the 2nd day in the medullary canal, where they were formed closer to its inner surface; They appeared later if no such incision was made in this zone. Disintegrating masses of erythrocytes predominated in the middle of the medullary canal. The presence of cysts among the extrusive disintegrating masses of blood in the medullary canal 48 h after removal of the bone marrow was interpreted as an adaptive manifestation in the microcirculatory system, aiding restoration of the disturbed blood supply to the bone of the diaphyseal cortical lamina and cells of the vessel walls located in it. These circumstances may explain the recovery of the staining properties of some of the vessel wall cells in the Volkmann's and Haversian canals, which we discovered. The presence of fibrin threads in the gap of the incision near its outflow into the medullary canal can also be interpreted as an adaptive reaction, for undifferentiated connective-tissue cells were seen among them.

This morphological picture indicates the development of intraosseous homeostasis and preservation of viability in cells of the vessel walls during restoration of the circulation in the damaged bone under favorable conditions. Restoration of the circulation of tissue fluid, it must be assumed, was facilitated by making an incision through the whole thickness of the cortical lamina in the middle of the diaphysis, for without it no such restoration of the staining properties of the vessel wall cells took place. Preservation of viability of the vascular cells was manifested as recovery not only of the staining properties, but also of their biological properties — their ability to proliferate. Recovery of these properties is probably attributable to the pericytes of the vessels forming the microcirculatory network of the vascular canals. As Rusakov [1] pointed out, the long-term viability of the vascular network in bone organs is formed in the process of phylogeny, in the struggle for existence and survival of the organism. Thus we were able to identify the beginning of accumulation of fibroblast-like cells near the cortical lamina, which was postulated by Fridenshtein et al. [2]. The model which we used enabled the beginning of mechanocyte development to be clearly traced. This was facilitated by normalization of the intraosseous circulation in the form of the appearance of tissue cysts in the medullary canal. We regard them as analogs of lymphatic microcirculatory vessels in other tissues.

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REPARATIVE CHANGES IN LATERAL HYPOTHALAMIC NEURONS DURING FOOD DEPRIVATION

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KEY WORDS: reparative changes; food deprivation; hypothalamus; ultrastructure of the neuron.

During food deprivation polymorphic ultrastructural changes arise in neurons in the lateral hypothalamic region (LHR) of the rat brain, and the dynamics of these changes depends on the stage of the experiment [1, 2]. In various pathological states not only destructive changes, but also processes of a reparative character are observed [4, 5, 7].

Reparative changes in neurons of different parts of LHR were studied in rats during food deprivation.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats weighing initially 190-200 g. Ten animals, receiving water and food *ad lib.* served as the control group. The experimental group consisted of 20 rats, which were killed 1, 3, 5, and 7 days after the beginning of starvation (5 animals at each time). The animals were anesthetized with ether and the brain perfused through the aorta with a solution of 2.5% glutaraldehyde and 2% paraformaldehyde in phosphate buffer, pH 7.2-7.4. The anterior, middle, and posterior parts of LHR were excised and the material was treated with a 2% buffered solution of osmic acid. Pieces of brain were dehydrated and embedded in a mixture of Araldite and Epon-812. The sections were examined and photographed with the HU-600 electron microscope (Hitachi, Japan).

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