OSTEOGENESIS FOLLOWING TRANSPLANTATION OF MARROW IN DIFFUSION CHAMBERS

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Transformation of bone marrow tissue in a diffusion chamber has been studied. Particular attention was paid to the investigation of the histogenesis of bone, developing in the transplants, and to the formation of hemopoietic elements.

EXPERIMENTAL METHOD

Pieces of tibial marrow were placed in diffusion chambers made from two Millipore filters (HA, thickness 150 μ; pore size 0.45 μ). The chambers were implanted in the peritoneal cavity. Autotransplantation was carried out in 39 mongrel rats and two rabbits, and isotransplantation in 8 mice of the AFB line. On the 3rd-40th day the chambers were extracted, separated from the surrounding tissue, and fixed in alcohol-formol or 96° alcohol in the cold. On the 3rd and 7th day the chambers were disassembled and the filters stained totally with hematoxylin-eosin and by Gomori's method for alkaline phosphatase. At other times series of sections were cut after embedding of the material in paraffin wax. In some cases decalcification was carried out. The sections were stained with hematoxylin-eosin, by Van Gieson's method, by the PAS method and by Gomori's method for alkaline phosphatase. In the last case Gomori's reaction was performed on parallel sections after preliminary treatment with citrate buffer at pH 4.5. Control sections, treated and not treated with citrate buffer, were passed through cobalt nitrate and sulfur water without preliminary incubation in a substrate with glycerophosphate. These preparations also served for the detection of the calcium phosphate of the ground substance of the bond tissue.

EXPERIMENTAL RESULTS

Three days after autotransplantation of the pieces of marrow into the rats the chamber was filled mainly with myeloid cells. Many of these were in a state of degeneration. Fibroblasts and histiocytes were also present. Many cells of intermediate type were observed, possibly in a state of transformation from myeloid into reticulum cells.

Seven days after transplantation a cell syncytium was observed in the chamber, consisting of stellate cells of mesenchymal type, giving off numerous processes. Their cytoplasm often contained vacuoles and was divided into zones of endoplasm and exoplasm, distinguished by their degree of staining. Few mitoses were found among these cells. Against the background of this cell syncytium were groups of much larger cells (Fig. 1, a). Their cytoplasm, with its many processes, was distinguished by its increased basophilia, and their nuclei contained several large nucleoli. These cells were in a state of intensive proliferation (in 100 cells about 10 mitoses were counted, whereas only about 0.2 mitosis was found among 100 stellate cells composing the syncytium). A few collections of normal and degenerating myeloid elements were also found in the chambers.

After 11 days the chambers were filled with connective tissue consisting of elongated fibroblastic elements, between which a fibrous ground substance was formed. Among the cells lying next to the filters areas could be distinguished consisting of large cells with basophilic cytoplasm. Mitoses were frequently seen in these cells, and there was intensive deposition of a PAS-positive, homogeneous ground substance. Solitary myeloid cells could be seen in the chambers. In some areas fragments of dead bone were present, devoid of cells. No cell reaction could be seen around them.

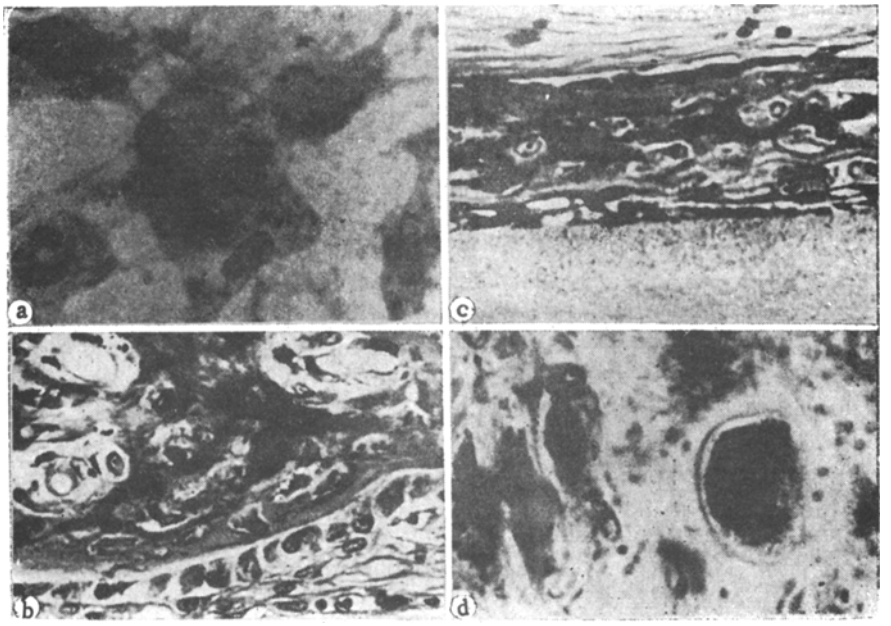

Fig. 1. Contents of diffusion chambers with transplanted bone marrow. a) after 7 days. Total preparation. Alcohol-formol, hematoxylin, objective 24 ×; b) after 18 days. Section. Alcohol-formol; PAS-hematoxylin; objective 24 ×; c) after 24 days. Section. Alcohol-formol; PAS-hematoxylin; objective 24 ×; d) after 30 days. Section. Alcohol-formol; PAS-hematoxylin; objective 40 ×.

After 18 days intensive osteogenesis could be seen in the chambers (Fig. 1, b). The osteogenic tissue consisted of bands of typical osteoblasts, lying on the surface of the bone trabeculae. In some cases bone tissue occupied the greater part of the chamber; in other chambers isolated foci of osteogenesis were situated among the connective tissue. Histogenesis of bone took the form of development of lamellar bone. The ground substance of the trabeculae gave a PAS-reaction of varied intensity. Often the trabeculae showed an irregular, granular staining on account of

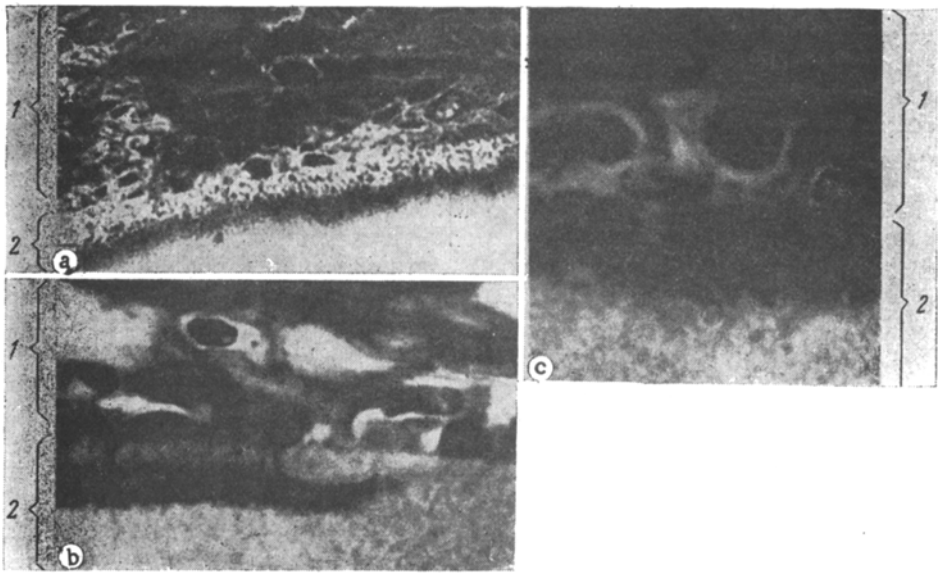

Fig. 2. Deposition of ground substance of bone in the filter. 1) contents of chamber; 2) filter: a) after 18 days. Section. Alcohol; Gomori; objective 24 ×; b) after 18 days. Section. Alcohol-formol; PAS-hematoxylin; objective 24 ×; c) after 18 days. Section. Alcohol-formol; PAS-hematoxylin; objective 90 ×.

the presence of polysaccharides unfermented by amylase, superimposed on the background of the ordinary homogenous, PAS-positive ground substance of the bone matrix. Granules of glycogen were seen in the osteocytes of the young bone tissue and in the ground substance of the bone.

The osteoblasts and ground substance of the trabeculae gave a positive Gomori reaction (Fig. 2, a). Deposition of calcium phosphate was seen in the ground substance of the bone (Fig. 2, a).

Characteristic pictures were observed in some cases in places where the bone trabeculae were in contact with the surface of the filter forming the wall of the diffusion chamber. The PAS-reaction (Fig. 2,c) revealed the deposition of polysaccharides, unfermented by amylase, in the substance of the filter. This zone of the filter, and this alone, was stained red by Van Gieson's method and gave a positive Gomori reaction (Fig. 2, b), and in a narrower zone it gave a reaction for calcium phosphate. These deposits had a finely granular structure; they were found only in areas in which the osteoblasts lay directly on the surface of the filter, and even there they were not present along the whole line of contact between the osteoblastic layer and the filter, but only near the osteoblasts with large, clear nuclei. The depth of these deposits in the substance of the filter was remarkably constant, so that a uniform band, or fragments of a band of uniform thickness (about 14 μ) were formed in the filter.

After 24 and 30 days the chambers were filled with a large amount of bone tissue, the structure of which differed in the various parts of the chamber. On the surface of the filters compact lamellae of bone were observed, resembling the cortical layers of the bone organ (Fig. 1, c). The central part of the chamber was filled with bone trabeculae, forming a cancellous structure. The surface of the trabeculae was covered by a layer of typical osteoblasts, and the spaces between the trabeculae with cells of reticulum type. In some chambers the whole of the bone formed a compact structure. Variations in the intensity of staining by the PAS method indicated differences in the maturity of the individual zones of bone tissue in these cases.

In some chambers cartilage tissue was found along with the bone. Foci of cartilage with chondrocytes, cartilage capsules, and a basophilic ground substance were intimately connected with the bone tissue. At the border between cartilage and bone tissue intermediate in structure was situated (Fig. 1, d). In it, cartilage capsules could be distinguished around chondrocytes (some of which showed signs of degeneration); meanwhile PAS-positive granules and fibrils could be seen in the ground substance, such as are found in developed bone tissue. In such places the basophilia of the ground substance of the cartilage was much less intensive. No endochondral destruction of cartilage was observed. As at the preceding period, in the region of the osteoblasts lying on the surface of the filters deposition of ground substance in the interior of the filters was observed, indistinguishable from bone matrix. After 40 days the bone tissue in most cases possessed a compact structure. The osteoblasts were fewer in number and no deposition of ground substance of bone in the interior of the filter was observed.

Development of myeloid tissue was observed in only 2 of the 29 chambers fixed after the 18th day. In both cases the integrity of the chambers was disturbed, and capillaries penetrated into their interior. Foci of hemopoietic tissue were situated near the bone trabeculae and in contact with the capillary walls. The results of the experiments on mice and rabbits were similar to those obtained on rats.

Our findings, and also those of Rosin and co-workers [4], show that during transplantation of bone marrow in a diffusion chamber, on the whole the same stages of osteogenesis occur as during free transplantation of marrow (without a diffusion chamber) subcutaneously [3]. In the first 2 weeks after transplantation the marrow tissue in the chamber is replaced by a reticular syncytium, while the hemopoietic cells disappear practically completely. In the course of the 3rd week, in all the 29 chambers fixed after the 18th day, osteogenesis developed against the background of the reticular tissue. It may be that bone formation does not take place in mice after transplantation of a suspension of marrow cells, and for this reason osteogenesis was not observed in these animals [2].

The source of osteogenesis was the reticulum cells or osteoblasts of the endosteum inevitably included in the transplant. Evidently foci of osteogenesis develop in the chambers from the large, stellate, basophilic cells distinguishable against the background of the reticular syncytium 7 days after transplantation. It may be noted that on the 7th day the reaction for alkaline phosphatase in these cells, as in the remainder of the reticular tissue, was negative.

The fact that when rat's spleen is transplanted in a chamber bone does not develop [4] does not rule out the possibility that bone may develop from the reticulum cells of the marrow. The reticular tissues of the rat's spleen, where hemopoiesis is not usually a prominent feature, and of the bone marrow may differ substantially from each other. Both bone and cartilage tissue may be formed in the chambers, which demonstrates the biopotential nature of

the osteogenic cells of the marrow. Because of the absence of blood vessels in the chamber, endochondral processes do not take place; transformation of cartilage tissue into bone is observed without preliminary resorption of the cartilage matrix (metachondral ossification). Similar transformations have been described in the case of normal histogenesis of certain lamellar bones of the visceral skeleton, where nodules of so-called secondary cartilage are formed [1].

During the differentiation of bone, deposition of the ground substance of bone in the material of the filter could be observed inside the diffusion chamber. This ground substance had all the characteristic signs of the bone matrix: it contained polysaccharides, possessed alkaline phosphatase activity, became calcified, and was formed in a zone at a distance of about 14 μ from the layer of active osteoblasts. A distinct impression was created that the layer of osteoblasts was working on one side only.

During autotransplantation of bone marrow subcutaneously and into the anterior chamber of the eye, after the formation of bone in the transplants hemopoiesis is restored [3, 5].

It was found, however, that hemopoiesis is not restored in the bone formed in a diffusion chamber. It is interesting that in the two cases in which we observed foci of hemopoiesis in the chambers, the filters were torn and capillaries had penetrated into the chambers. In Rosin's investigation [4], hemopoiesis was observed in the chamber in only one of 39 cases, and judging by the photomicrograph accompanying his paper, the chamber here, too, was damaged. The most probable explanation is that the source of hemopoiesis in the bone marrow transplants was formed by repopulating hemopoietic cells, and that they appeared only when the conditions made repopulation possible. It seems that the hemopoietic cells (possible lymphocytes), having left the blood stream and entered the chamber filled with living bone tissue, found that the conditions there were suitable for proliferation and differentiation, in contrast to the hemopoietic cells which entered the chamber at the time of transplantation. If this is the right explanation, the question arises of the nature of the influence by means of which the bone maintains differentiation of the hemopoietic tissue.

SUMMARY

Bone marrow forms in diffusion chambers following the transplantation of rat's bone marrow. No hemopoiesis occurs in the bone marrow within these chambers. The bone ground substance is deposited into the filter tissue at a distance of about 14 μ from the osteoblasts.

LITERATURE CITED

1. A. Ya. Fridenshtein, Doklady Akad. Nauk SSSR, 68, 5, 931 (1949).
2. I. Berman and H. Kaplan, Blood, 14, 1040 (1959).
3. A. Danis, Etude de l'ossification dans les greffes de moelle osseuse. Bruxelles (1956).
4. A. Rosin et al., Exp. Cell. Res., 29, 176 (1963).
5. M. Urist and F. McLean, J. Bone Jt. Surg., 34-A, 443 (1952).
