BIOLOGICAL ACTIVITY OF ALLOGENEIC EMBRYONIC BONE MARROW CELLS

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Various methods of stimulating reparative regeneration are used in clinical practice with the aim of activating repair processes taking place in bone tissue [3, 13, 14]. At the present time biological methods of stimulation of osteogenesis are most commonly used [1, 3, 6, 7]. Clinical and experimental investigations have shown that embryonic bones possess marked biological activity in relation to the process of reparative regeneration [2, 4].

The use of embryonic tissues and of a suspension of bone marrow cells, which have a stimulating effect on different aspects of reparative regeneration, has not allowed the factors accelerating the repair process to be definitely identified, or for it to be proved that if union took place after plastic repair with certain biological substrates, it resulted from their use. A contribution to the solution of this problem can be made by the study of osteogenesis *in vivo* under extraskeletal conditions, when the morphogenesis and tissue conversions in the process of development of bone tissue can be investigated, so that new phenomena connected with the function of osteogenic precursor cells can be discovered. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

The action of allogeneic embryonic bone marrow cells on reparative osteogenesis was studied in 134 male chinchilla rabbits.

An embryonic cell suspension was obtained from the bones of 3-week-old rabbit embryos. The bones were shredded, treated with 0.25% trypsin solution, and filtered through Kapron tissue. The cell suspension was resuspended in medium 199 and made up to a concentration of $2.5 \cdot 10^6$. In experiments to study optimization of the conditions of reparative regeneration of long and flat bones, 10 million cells were injected into the defects formed; in other experiments 10 million cells were introduced into diffusion chambers with a pore diameter of 0.45 and 0.9 nm. The sealed diffusion chambers were implanted into the peritoneal cavity of the experimental animals and fixed against the inner surface of the parietal peritoneum with silk threads, which prevented the chambers from adhering to one another. The diffusion chambers were removed from the peritoneal cavity after 7, 10, 14, 21, and 30 days.

Millipore filters with cell regenerators were fixed in cold (4°C) alcohol, stained with hematoxylin and eosin, and mounted in balsam.

Other millipore filters with regenerating cells were fixed in cold alcohol and in Zenker's and Carnoy's fluid and embedded in paraffin wax. Histological sections were stained with hematoxylin and eosin. Neutral mucopolysaccharides were revealed by the methods of McManus and Hotchkiss, RNA by Brachet's method, and alkaline phosphatase activity was determined by Gomori's method and in frozen sections by the standard azo-coupling method with Fast red TP. In the course of the experiment and at the time of sacrifice the animals were under hexobarbital anesthesia. The effect of allogeneic embryonic bone marrow cells on reparative regeneration of bones was estimated from the results of roentgenologic and histological investigations.

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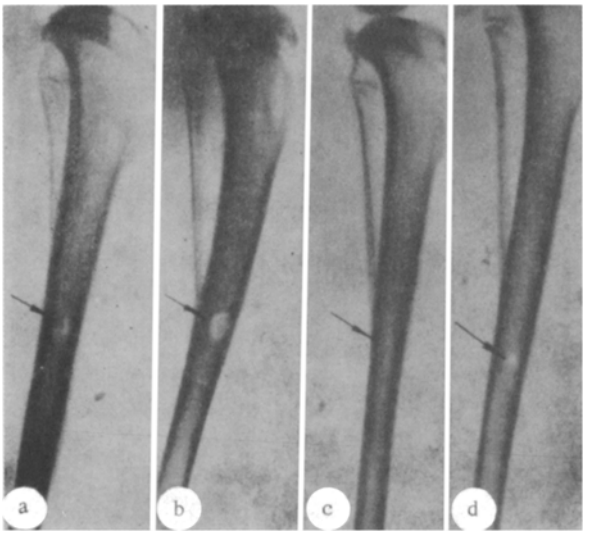

Fig. 1. Intensity of formation of regenerating bone in defect of rabbit tibia (roentgenogram): a) 21st day of experiment. State of bone defect after injection of suspension of allogeneic embryonic bone marrow cells; b) 21st day, control; c) 45th day of experiment: character of replacement of bone defect after injection of suspension of allogeneic embryonic bone marrow cells; d) 45th day, control.

TABLE 1. Morphological Characteristics of Tissue Structures in Diffusion Chambers

Pore diameter of diffusion chambers, nm	Time of observation	No. of chambers studied	Parameters for evaluation of results (No. of chambers)					
			presence of					
			growth of cells	cartilage	bone	alkaline phosphatase activity	neural mucopolysaccharides	RNA
0,9	7	7	7	5	4			
	10	5	3	4	3	—	—	—
	14	4	3	3	3	—	—	—
	21	3	3	3	3	—	—	—
	30	4	4	4	4	—	—	—
0,45	10	6	6	5	5	2	—	—
	20	4	4	4	4	2	—	—
	30	6	6	5	5	4	4	4
Total		39	36	33	31	8	4	4

EXPERIMENTAL RESULTS

The experimental model on rabbits with injection of a suspension of allogeneic embryonic bone marrow cells into defects in long and flat bones showed that filling of the defect with newly formed bone tissue took place more rapidly in animals of the control group (Fig. 1).

The beneficial effect of injection of the suspension of allogeneic embryonic bone marrow cells on osteogenesis in the bone defect can be explained by biological activation of bone marrow mechanocytes, and this is confirmed by observations made by other workers [5, 8-12].

Investigation of total preparations and histological sections of regenerating tissues cultured in diffusion chambers showed that they contained cells and cartilage and bone tissue (Table 1).

On the 7th day after transplantation disconnected rare or dense accumulations of cells, oriented parallel to their surface, could be seen mainly at the periphery of the millipore

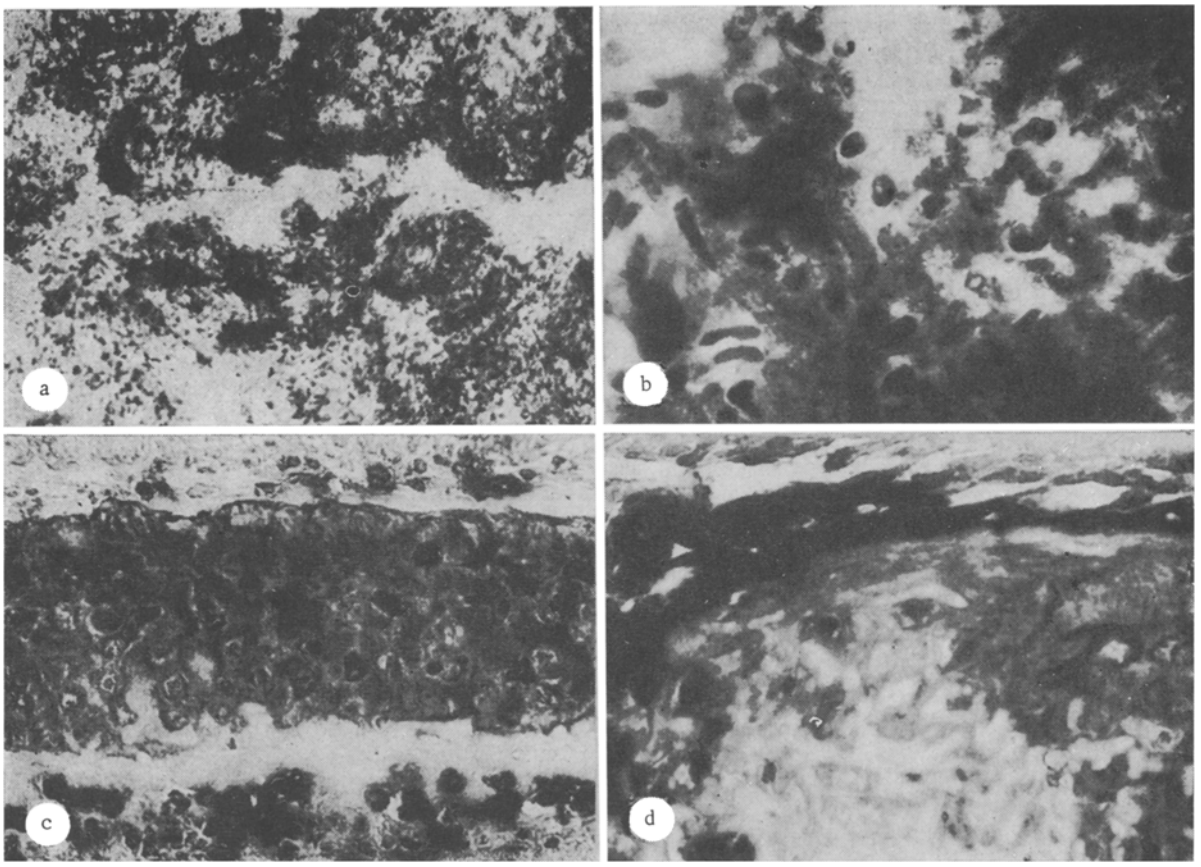

Fig. 2. Formation of bone and cartilage tissue from embryonic bone marrow cells in diffusion chambers: a) formation of bone tissue on surface of millipore filter on 10th day of culture, 70×. Hematoxylin and eosin; b) bony trabeculae covered with osteoblasts on 10th day of culture, 280×. Hematoxylin and eosin; c) regenerating bone between millipore filters of diffusion chamber on 30th day of culture. Azo-coupling method with Fast red TP for alkaline phosphatase, 280×; d) high alkaline phosphatase activity in zone of bone tissue formation on 30th day of culture. Frozen section. Azo-coupling method with Fast red TP for alkaline phosphatase, 280×.

filters. Most were formed by hematopoietic cells of the myeloid series, and mitoses were found in some of them. There were far fewer stromal cells, from 1 to 3% in number.

The fibroblast-like cells were large, round or oval in shape, with an eccentric nucleus and weakly basophilic cytoplasm. Some stromal cells had cytoplasmic outgrowths which formed anastomoses with other cells. On the 10th day the diffusion chambers were filled mainly with fibroblast-like cells, arranged in foci on the filters. The formation of structures of osteogenic territories composed of isolated (dissociated) cells was regularly observed in all chambers at this time of observation (Fig. 2a).

Bony trabeculae, covered with osteoblasts, could be seen in the total preparations at this time (Fig. 2b).

After the 14th day of culture foci of commencing osteogenesis were present among the stromal cells and cells of polyblast type, colonizing the filters. In these areas the stromal cells lay close to each other and the formation of young bony trabeculae was beginning. Osteoblasts were distributed around the bony trabeculae which had already formed. At this time of the investigation the predominant picture in the chambers was one of commencing osteogenesis.

On the 21st and, in particular, on the 30th day of incubation in diffusion chambers an increase in the intensity of osteogenesis was observed. Areas of regenerating bone and cartilage could be identified between the filters (Fig. 2c).

Besides well-formed bony trabeculae with calcified ground substance, areas of cellular proliferation as a manifestation of osteogenesis also were encountered. Besides bony formations, cartilaginous structures also were observed in the grafts. In the deep layers of the primitive bony trabeculae signs of calcification appeared, but around the osteoblasts, bone ground substance continued to be formed. Foci consisting of more mature compact bone tissue, containing osteocytes and chains of osteoblasts, surrounding more mature bony structures, also were seen at these times. These foci had a high content of RNA and neutral mucopolysaccharides. Cells distributed around the periphery of the newly formed bone tissue and lying adjacent to the inner surface of the millipore filters gave a strongly positive reaction for alkaline phosphatase (Fig. 2d).

Allogeneic embryonic bone and bone marrow cells thus preserve their functional biological activity for 30 days of an experiment in diffusion chambers implanted into the peritoneal cavity. The transplanted cell suspension contains stromal cells with osteogenic properties. Determined osteogenic precursor cells can give rise to the formation not only of new cell populations in diffusion chambers, but also of cartilage and bone tissue. The histogenesis of growth of a culture of allogeneic bone marrow embryonic cells in diffusion chambers is evidence that the filling of a bone defect in experimental animals is stimulated by osteogenic precursor cells.

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