CONDITIONS AFFECTING INDUCTION OF OSTEOGENESIS AFTER IMPLANTATION OF BONE MATRIX INTO MICE

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Induction of osteogenesis was observed in 90-100% of cases of implantation of decalcified bone matrix intramuscularly into mice, beneath the kidney capsule, beneath the kidney capsule after ligation of the blood vessels, and intramuscularly into animals after curettage of the medullary canal of the femur. No morphological differences were found under these circumstances. Both implants with large bone lamellae filled with medullary cells and implants with small areas of osteogenic tissues were found in each series of experiments. It is concluded that the induction process is independent both of the degree of vascularization of the implantation site and of the degree of calcification of the surrounding tissue, and it is evidently determined by the presence of inducible cells susceptible to the induction stimulus.

KEY WORDS: bone matrix; induction of bone; inducible cells.

After intramuscular implantation of decalcified bone matrix, bone with bone marrow is induced [2-9]. The inducing system in this case is the bone matrix, and the reacting system the recipient's connective tissue. The quantity of new bone and, correspondingly of bone marrow thus formed differs both in different animals and after implantation of the matrix in different parts of the body of the same animal [4, 8, 9]. Since the inducing system in each case is an identically prepared matrix, these changes in the induction process must evidently be associated with the state of the recipient's reacting system.

In order to study conditions affecting the intensity of osteogenesis following induction, in the present investigation decalcified matrix was implanted intramuscularly, beneath the capsule of the kidney, either of the intact organ or after ligation of its blood vessel, and intramuscularly into animals after curettage of the medullary cavity of one femur in mice.

EXPERIMENTAL METHOD

Decalcified bone matrix was prepared by Urist's scheme [5]. The tibias were removed from (CBA × C57BL)F_1 mice weighing 20-25 g, defatted with a mixture of methanol and chloroform (1:1) for 1 h at 25°C, rinsed with cold water, and decalcified in 0.6 N HCl for 2 h at 2°C, after which they were placed in 2 M $CaCl_2$ for 4 h at 2°C, in 0.5 M EDTA (pH 7.4) for 1 h at 2°C, in 8 M LiCl for 4 h at 2°C, and in distilled water for 1 h at 55°C. After being thus prepared, the bone cylinders were implanted into syngeneic recipients into the muscle of the anterior abdominal wall or beneath the capsule of the left kidney. The artery and vein of the kidney of some recipients were ligated at the time of implantation. The control for this group consisted of animals with ligation of the kidney and with bone matrix implanted into the anterior abdominal wall. Simultaneously with implantation of the bone matrix into the anterior abdominal wall, the right femur of some of the recipients was curetted. All the implants were removed after 1-1.5 months, fixed with alcohol-formol, decalcified, and examined histologically.

EXPERIMENTAL RESULTS

Lamellar bone with a well-defined osteoblastic layer and a cavity filled with bone marrow cells was formed inside the medullary cavity of the implanted matrix in all groups of recipients after 1-1.5 months (Figs. 1 and 2). The quantity of induced bone and marrow varied in different animals (Fig. 3). As a rule the intensity of osteogenesis correlated with the degree of resorption of the implanted matrix: When intensive

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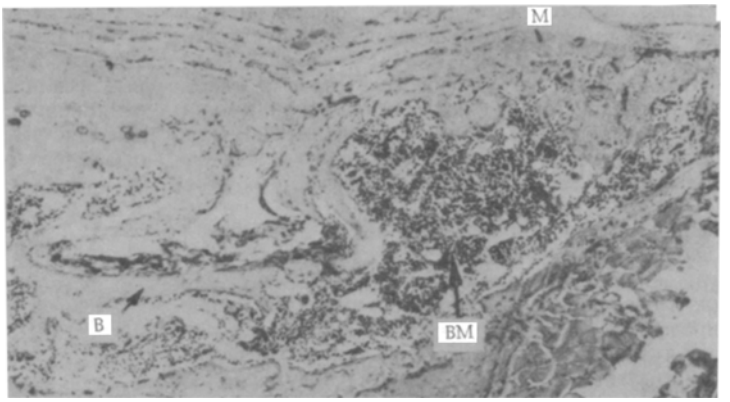

Fig. 1. Induction of bone with bone marrow one month after implantation of bone matrix intramuscularly. M) Bone matrix; B) induced bone; BM) induced bone marrow. Hematoxylin-eosin, 25×.

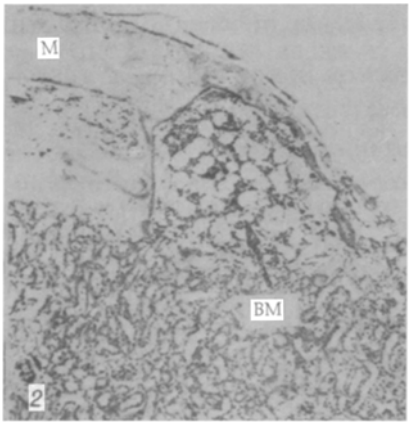

Fig. 2. Induction of bone with bone marrow beneath kidney capsule one month after implantation of matrix. Legend as in Fig. 1. Here and in Fig. 3, stained with hematoxylin-eosin, 2.5 times.

resorption took place, the largest quantity of bone and bone marrow was observed, whereas in implants with weak resorption only small islets of osteogenic tissue could be seen. Necrosis of the cortex and of a large part of the medulla of the kidney was observed in recipients with ligated renal vessels, the kidney was shrunken, and the implant could be clearly distinguished on its surface. As in all the other cases, lamellar bone with a cavity filled with marrow cells was formed inside the implant.

Although the model of induction of osteogenesis with the aid of decalcified bone matrix has been known for a long time the mechanisms of induction still remain unexplained. The existence of an osteogenesis factor (bone morphogenetic protein), secreted by the matrix and causing differentiation in the direction of osteogenesis, has been suggested [7]. The intensity of this process differed in different recipients, and the inducer evidently only triggers the mechanism of osteogenesis, and the rate of this process, the size of the bone and marrow foci induced are determined by the recipient into which the matrix is implanted.

When possible influences of the recipient on the course of the induction process were studied in this investigation it was found that after implantation of bone matrix intramuscularly, beneath the kidney capsule, beneath the kidney capsule after ligation of the vessel, and into animals subjected to curettage of the medullary cavity, induction of osteogenesis took place in 90-100% of cases. Morphologically the induction process did not differ in different series of experiments. By 1.5 months bone with marrow cells was induced inside the matrix. By contrast with rats [4], in which the induction of osteogenesis by means of bone matrix takes place

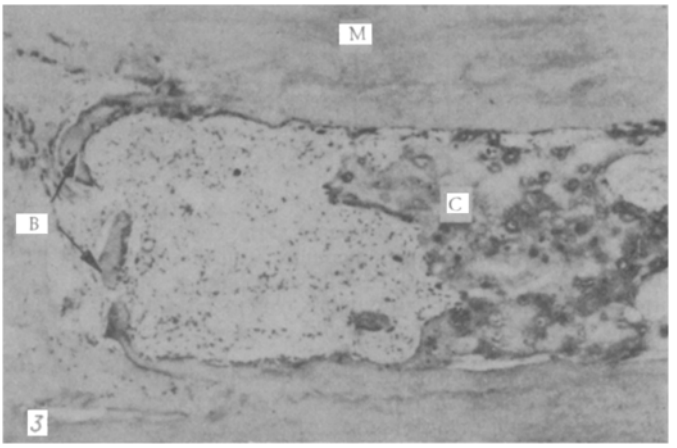

Fig. 3. Bone induction in animal after curettage of medullary canal of femur one month after implantation of matrix: matrix poorly resorbed, no induction of bone marrow can be seen. C) Chondrogenesis; remainder of legend as in Fig. 1.

TABLE 1. Induction of Osteogenesis after Implantation of Matrix into Mice

Implantation of matrix	Time of fixation, days	Number of implants	
		total	with induction of bone
Intramuscularly	30—45	30	30
Under kidney capsule	30	20	18
Under capsule of ligated kidney	30	14	14
Intramuscular control	30—45	25	25
Intramuscularly* into curetted animal	30	5	3

*In two cases complete resorption of the implanted material was observed.

much less intensively under the kidney capsule than under muscle fascia, in mice osteogenesis under the influence of matrix takes place just as intensively beneath the kidney capsule as after intramuscular implantation. It is interesting to note that the kidney shrinks and becomes calcified if matrix is implanted beneath its capsule and if the renal vessels are ligated, and bone with a medullary cavity and with hematopoietic cells is induced after 1-1.5 months inside the implanted matrix. The results show that the induction process is independent both of the degree of vascularization of the site of implantation of the inducer and of the degree of calcification of the surrounding tissue, and it is evidently determined by the presence of inducible cells susceptible to the induction stimulus [2, 3]. Inducible cells differ from determined precursor cells of native bone marrow, for which the best site of heterotropic transplantation is the kidney capsule, and the number of which increases after curettage [1, 2]. The response of the recipient to the induction stimulus may evidently be determined not only by the presence of inducible cells, but also by the ability of the implanted matrix to undergo resorption. It is not yet clear what is the mechanism of this process or which cells participate in it.

LITERATURE CITED

1. Yu. V. Gerasimov and R. K. Chailakhyan, Byull. Éksp. Biol. Med., No. 9, 362 (1978).
2. A. Ya. Fridenshtein and K. S. Lalykina, Induction of Bone Tissue and Osteogenic Precursor Cells [in Russian], Moscow (1973).
3. C. Huggins, S. Wiseman and A. Reddi, J. Exp. Med., 132, 1250 (1970).
4. G. J. Linden, J. Anat., 119, 359 (1975).
5. M. R. Urist, Clin. Orthop., 91, 210 (1973).
6. M. R. Urist, N. Nakata, J. M. Felsen, et al., Clin. Orthop., 125, 251 (1977).
7. M. R. Urist et al., Clin. Orthop., 53, 267 (1967).

8. K. Wlodarski, N. M. Hancox, and B. Brooks, J. Bone Jt. Surg., 55-B, 595 (1973).
9. K. Wlodarski et al., Folia Biol. (Krakow), 22, 377 (1974).

QUANTITATIVE HISTOCHEMICAL STUDY OF ALKALINE PHOSPHATASE ISOZYMES IN THE UTERUS OF OVARIECTOMIZED GOLDEN HAMSTERS DURING ESTROGENIZATION

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Activity of two isozyme forms of alkaline phosphatase in the uterus of ovariectomized golden hamsters was investigated. The animals of different groups received single daily injections of 10 μg estrogen (benzestrol) for 4 and 16 days. Administration of the estrogen did not affect total alkaline phosphatase activity in the uterine epithelium but reduced the activity of the enzyme in the stroma. It was also shown that with an increase in the duration of estrogenization the relative proportion of the alkaline phosphatase isozyme of intestinal type in the epithelial cells of the uterine cavity increased.

KEY WORDS: estrogenization; uterus; histochemistry.

Investigation of the isozyme spectra of various enzymes is nowadays widely used in oncologic practice [14]. An isozyme of alkaline phosphatase not normally encountered (the placental form of Regan's isozyme) is found in the blood serum of patients with certain forms of cancer and also in the tumor tissue [8]. The distinguishing features of this isozyme are its thermostability, its inhibitability by L-phenylalanine, its resistance to imidazole and levamisole, and its antigenic properties. In normal tissues only the alkaline phosphatase of intestinal epithelium (the intestinal type) resembles the placental type in its properties.

Since prolonged estrogenization is supposed to lead to the development of pretumor and tumor processes in the reproductive organs of women and female experimental animals, an attempt was made to study changes in the activity of two alkaline phosphatase isozymes in the uterus of ovariectomized golden hamsters during estrogenization of varied duration.

EXPERIMENTAL METHOD

Experiments were carried out on 48 ovariectomized golden hamsters, divided into four groups. The animals of group 1 received a single subcutaneous injection of the synthetic estrogen octestrol (benzestrol) in a dose of 10 μg (in 0.2 ml peach oil). The animals of group 2 received injections of 10 μg benzestrol daily for 4 days. The animals of group 3 received 10 μg benzestrol daily for 16 days. Animals of group 4 (control) received no hormones.

The hamsters were killed by decapitation 18-20 h after the last injection of the hormone. The uterine cornua, frozen in liquid nitrogen, were taken for examination. Alkaline phosphatase in frozen sections 10 μ thick was revealed by Burstone's method with naphthol AS-MX and fast blue (Reanal, Hungary). To determine the alkaline phosphatase isozymes, L-levamisole [6], an inhibitor of the isozyme of hepatic type, was added to the incubation medium in concentrations of 0.01, 0.05, and 0.5 mmole/ml medium [4].*

The intensity of the reaction was estimated quantitatively from a cytospectrophotometer with digital print-out (objective 40×, ocular 7×, wavelength 550 nm, probe area 4.9 μ^2. Enzyme activity was expressed in optical density units (o.d.u.). Enzyme activity in the presence of inhibitor was expressed as a percentage of

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