COMPARATIVE EVALUATION OF BONE HOMOGRAFTS CONSERVED BY DIFFERENT METHODS

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Most authorities consider that the best method of conservation of bone tissue is by freezing [2-4, 10, 11], but others regard lyophilization as a better method [13, 14] or consider that the two are equal in merit [1]. It has also been claimed that even boiled and macerated bone tissue may be used in osteoplastic surgery [5, 12, 15]. The opinion is expressed that bone tissue, if conserved in balanced salt solutions, is superior to frozen bone tissue [3], but its storage life is much more limited.

The author has shown that bone tissue, when conserved in hypertonic salt solutions, undergoes absorption after homotransplantation into a prepared bed in bone, and is replaced by the bone tissue of the bed [6-9].

The object of the present investigation was to make a comparative evaluation of bone tissue conserved by different methods and grafted into a prepared bed in bone.

EXPERIMENTAL METHOD

Experiments were conducted on 88 adult rabbits weighing from 3.5 to 4 kg. Fragments measuring 10-12 mm were taken from the radius subperiosteally by means of a saw. The defect was filled with a bone graft conserved for 1-3 months. The tissues around the wound were infiltrated with penicillin. Immobilization was not used in the postoperative period, and the role of a splint was assumed by the ulna on the side of the operation. The following types of homografts were used: 1) frozen at −70° for 24 h and stored at −25°; 2) lyophilized; 3) boiled and macerated (os purum); and 4) conserved in balanced salt solutions of different concentrations (in 0.85% solution at 4°, in 10 and 20% solution at 4 and 18°). After transplantation roentgenograms of the affected limb were taken every month. At the end of the experiment the region of the graft was studied histologically, starting 2 weeks after operation. Celloidin sections, 10-12 μ in thickness, were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

The study of the roentgenograms of bone homografts conserved by freezing showed that 1 month after transplantation the grafts had united with the bed by means of periosteal callus. Two months after grafting the structure of the graft was indistinguishable, after 3 months homogenization of the graft was complete, and 4 months after the operation a medullary canal had begun to form. Two weeks after transplantation the grafts were intimately connected to the soft tissues of the bed. One month after the operation young bone tissue had formed on the surface of the grafts along the walls of some dilated vascular canals. After 3 months the graft had the appearance of large islets of bone tissue, without osteocytes, among the recipient's bone tissue, and a medullary canal had begun to form. After 4 months the grafts were largely replaced by young bone tissue. In some preparations remnants of the graft could be seen in the form of small, osteocyte-free areas, and the formation of the medullary canal continued.

One month after homotransplantation of lyophilized bone tissue periosteal callus could be seen on the roentgenograms. During the 2nd and 3rd months partial homogenization of the graft took place, and this was complete by 4 months. In the 5th month a medullary canal began to form. Two weeks after transplantation the homografts were joined to the soft tissues of the bed. After 1 month they were firmly joined to the bed by young bone tissue, formed on the surface of the grafts. After 3 months the outlines of the grafts became indistinguishable, and young bone tissue was in process of formation in the vascular canals and on the surface of the grafts. After 5 months the

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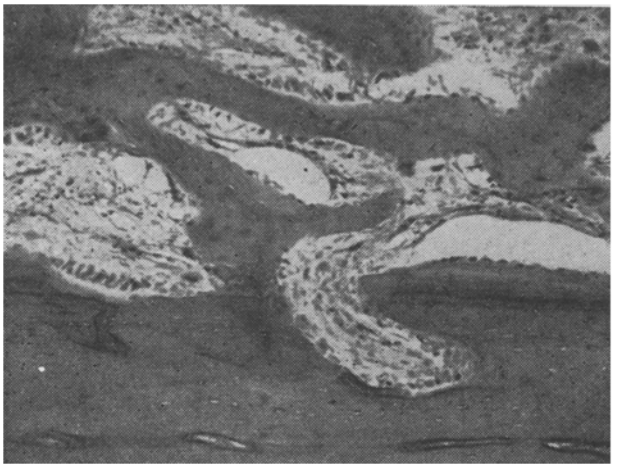

Fig. 1. Homograft conserved in 20% hypertonic salt solution 1 month after transplantation. Objective 15, ocular 10.

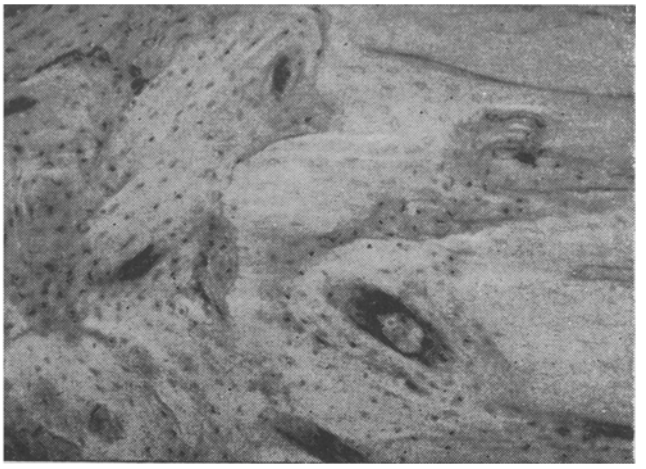

Fig. 2. Homograft conserved in 20% hypertonic salt solution, 5 months after transplantation. Objective 15, ocular 10.

grafts were split up into large fragments by the young bone tissue replacing them, and the formation of a medullary canal had begun. After 6 months the lyophilized grafts were largely replaced by the recipitent's bone tissue, and they contained small osteocyte-free fragments.

On the roentgenograms of the boiled and macerated bone homografts taken 1-2 months after transplantation a weak periosteal callus could be seen. By 4-5 months the outlines of the grafts had become less distinct. Histological examination 2 weeks after grafting showed that no connection was present between the graft and the bed. From 3 to 6 months after transplantation the outlines of the grafts were distinct, and fissures and fibrous connective tissue were encountered between the graft and the bed. On the surface of some grafts narrow bands of bone tissue could be seen, but in other cases the grafts had absorbed without replacement by bone tissue.

One month after homotransplantation of bone tissue conserved in 0.85% salt solution the roentgenograms showed dense periosteal callus. After 2 months the grafts were partly homogenized, and after 3 months homogenization was complete and a medullary canal had begun to form. This was seen more clearly after 4 months. Histological examination 2 weeks after transplantation showed that the grafts were joined to the soft tissues of the bed over a wide area. After 1 month the grafts were intimately connected with the bony bed, young bone tissue had formed on the surface of the grafts and in the vascular canals, and the borders of the grafts were ill defined. After 3 months discrete osteocyte-free areas of different shapes and sizes were observed among the young bone tissue replacing the graft, and a medullary canal had been formed. After 5 months small osteocyte-free fragments were seen among the bone tissue replacing the graft, and the newly formed medullary canal contained fatty marrow.

One month after homotransplantation of the bone tissue conserved in 10% salt solution the roentgenograms showed dense periosteal callus. After 2 months the grafts were partially homogenized, after 3 months homogenization of the grafts was complete, and after 4 months a medullary canal had begun to form. After 5 months the outlines of the medullary canal were more distinct. Histological examination 2 weeks after transplantation showed that the grafts were joined to the soft tissues of the bed. After 1 month the surface of the grafts was intimately connected to the bony bed, and young bone tissue had formed along the walls of the dilated vascular canals and on the surface of the grafts. After 3 months the grafts had the appearance of separate fragments of different sizes among the young bone tissue, and a medullary canal had been formed. After 5 months isolated fragments of the graft could be seen among the newly formed bone tissue. The medullary canal contained fatty marrow. After 8-12 months occasional, small osteocyte-free fragments could still be distinguished among the young bone tissue.

One month after homotransplantation of bone tissue conserved in 20% salt solutions dense periosteal callus was visible on the roentgenograms. After 2 months partial homogenization of the grafts was observed, and after 3 months this was complete. After 4 months a medullary canal began to form, and this process continued during the 5th and 6th months. Histological examination 2 weeks after the operation showed that the grafts were intimately connected with the soft tissues of the bed. After 1 month the grafts were firmly united with the soft tissues of the bed and young bone tissue had formed on the surface of the grafts (Fig. 1). After 1.5-2 months this process was also

observed in the vascular canals of the graft. After 3 months the graft had the appearance of separate, large osteocyte-free fragments amonth the young bone tissue, and a medullary canal had begun to form. After 5 months the fragments of the graft had become smaller (Fig. 2) and the formation of the medullary canal continued. After 8-12 months small, osteocyte-free fragments were occasionally seen among the young bone tissue. The newly formed medullary canal contained fatty marrow.

Hence the fastest replacement of the grafts by the bone tissue of the bed took place after transplantation of bone tissue conserved by freezing and in 0.85-10% salt solutions. The lyophilized bone tissue and the bone tissue conserved in 20% salt solution were replaced more slowly. Young bone tissue formed later in the vascular canals of these grafts, large fragments of the graft persisted longer, and the medullary canal was formed later. However, this difference did not exceed 1.5-2 months, and replacement of the grafts conserved in 20% salt solution took place slightly more intensively than in the case of the lyophilized grafts.

Replacement of all the grafts took place on the side of the bony bed only. Characteristically, the formation of bony callus was earlier in the proximal portion of the graft. If contact between graft and bed was poor and if the graft moved, its replacement was considerably delayed. With firm fixation and good contact between graft and bed replacement took place more intensively, without the formation of periosteal callus. The replacement of boiled and macerated grafts was much slower. At times when the frozen and lyophilized grafts and the grafts conserved in salt solutions were almost completely replaced, the boiled and macerated grafts still remained largely unreplaced. These grafts also behaved differently depending on the depth and the degree of modification of the natural properties of the bone tissue. For instance, grafts boiled for 10 and 40 min formed contact differently with the bony bed. Yet even with considerable changes in the biological properties of the bone tissue, it behaved differently during transplantation from foreign bodies (metals, plastics). These grafts combined the special properties of bone tissue with those of foreign bodies (on the one hand, a tendency toward absorption and replacement, on the other hand a tendency toward the formation of a connective-tissue capsule). An increase in the period of conservation of the bone tissue in balanced physiological saline to 3 months had no significant effect on the process of its replacement after transplantation, and the use of such grafts in conjunction with broad-spectrum antibiotics is evidently perfectly possible. An increase in the concentration of the salt solutions slows the process of replacement of the graft by the bone tissue of the bed, but such solutions have a stronger bactericidal action.

SUMMARY

Experiments on rabbits with homotransplantation into the bony bed of lamellar bone tissue preserved by various methods showed the fastest replacement in frozen bone tissue and bone tissue preserved in saline solutions of 0.85-10% concentration. Lyophilized transplants were displaced slower. With an increase in saline solution concentration the replacement of transplants became slower, but even at a maximum concentration their quality was not inferior to that of lyophilized transplants. An increase in the time of bone tissue preservation in balanced physiological saline solution did not influence the process of replacement in transplantation substantially. Boiled and macerated transplants remained unchanged for a long time and had features characteristic of both bone tissue and foreign bodies.

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