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Experimental study on growth of epiphysial plate: free graft in rabbits

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SUMMARY. **The growth potential of a free graft of an epiphysial plate was investigated in rabbits. Two epiphysial plate grafts were harvested from each iliac crest. One was grafted to the head (onto bone) and the other to the ear (onto cartilage). Both of the epiphysial plates enlarged to a maximum height of 1.4 cm and became similar to iliac crests. Enchondral ossification was observed up till approximately 28 weeks of age. We conclude that an epiphysial plate has growth potential after free heterotopic transplantation.**

Transplantation of an epiphysial plate to ensure bone lengthening by maintaining growth has been a challenging subject since it was first performed by Helferich in 1899.[1] There have been several clinical and experimental studies reporting results of the growth potential of a free non-vascularised graft of an epiphysial plate. These reports have been conflicting.[2–14] At present, it is commonly believed that terminal bone grafts in the digits of children inevitably resorb and, even in the short term, lacking epiphysial activity, they fail to grow.[14] Advances in surgery have made it possible to preserve the viability of a transplanted epiphysial plate by microvascular anastomoses of its critical blood supply.[15–23] Several clinical reports indicate that continued growth could occur after vascularised epiphysial plate transfer.[24,25] In past experimental and clinical studies, the fibula, the ulna, or whole joint was used with ununited epiphyses. Survival was unlikely unless microvascular anastomoses were performed. Therefore, in those studies, the growth potential of the epiphysial plate itself was not investigated. It remains unknown whether a free non-vascularised epiphysial plate has its own growth potential. The aim of this study was to investigate the growth potential of the epiphysial plate after free heterotopic transplantation in rabbits.

Materials and methods

Thirty female Japanese white rabbits, 12 weeks old, weighing between 1800 and 2000 g were used for this study. An anaesthetic was administered, consisting of halothane, nitrous oxide, and oxygen.

An incision was made along the iliac crest and a spindle-shaped graft of epiphysial plate measuring approximately $0.1 \times 0.5 \times 2.0$ cm was harvested using an elevator (Figs 1, 2). Almost the whole epiphysial plate was taken from the iliac crest. Two identically sized grafts were taken, one from the left and one from the right iliac crest. The plates consisted of cartilage and overlying fibrous tissue (Fig. 3). No bone tissue was included. After removing the surrounding soft tissue, each graft was weighed. One of the grafts was autografted to the head of each of the 30 rabbits in a subperiosteal pocket, with the epiphysial plates placed with the cartilaginous surface immediately against the recipient bone. The second graft was transferred to the left ear into a subperichondrial pocket with good cartilage-to-cartilage contact (Fig. 4). No fixation was used to immobilise the grafts.

The rabbits were divided into 6 groups; these were killed at 4, 8, 12, 16, 20, and 24 weeks, respectively, and the grafts harvested. They were found to be incorporated into the adjacent cartilage or bone, but separated easily from that tissue. They were stripped of all soft tissue and the weight and height of each was measured. After roentgenograms of these specimens were taken, the specimens were divided in half. One-half was decalcified and embedded in paraffin. Sections 10 μm thick were prepared and stained with haematoxylin and eosin and examined under a light microscope. The second half was placed in 70% ethanol and subsequently embedded in methacrylate. Transverse sections were cut with a milling machine. Specimens were ground to 60 μm for contact microradiography (CMR).

Results

Four rabbits died following surgery due to post-anaesthesia complications. These 4 rabbits were replaced and the procedure repeated.

Epiphysial plates grafted to the head and ear showed similar patterns of growth at 4, 8, 12 and 16 weeks. At each of these intervals, they demonstrated significant growth in both recipient sites (Figs 5–7). The mean weight of the epiphysial plates at the time of grafting was $0.24\ g \pm 0.03$ g. The weights of the specimens taken from the head at 4, 8, 12, and 16 weeks were $0.44\ g \pm 0.12$ g; $0.78\ g \pm 0.20$ g; $0.91\ g \pm 0.18$ g; and $1.04\ g \pm 0.26$ g, respectively. Those of specimens taken from the ear were $0.41\ g \pm 0.07$ g; $0.73\ g \pm 0.24$ g; $0.88\ g \pm 0.19$ g; and $1.06\ g \pm 0.35$ g, respectively (Fig. 8). The height also gradually increased up to the 16th

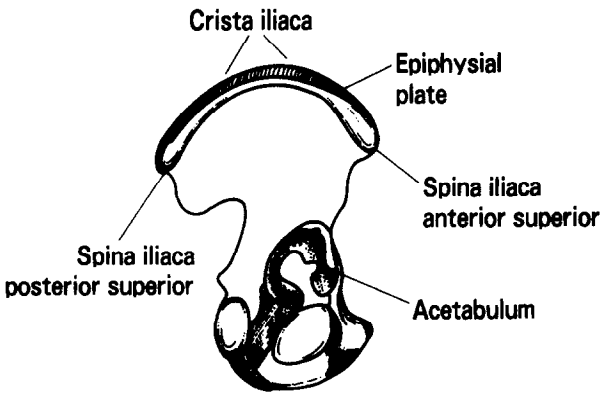

Fig. 1

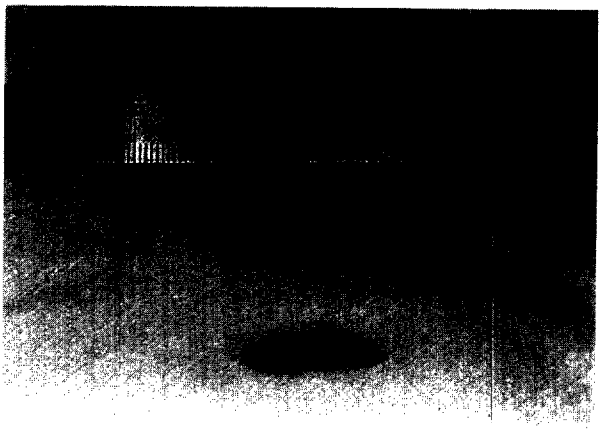

Fig. 2

Fig. 3

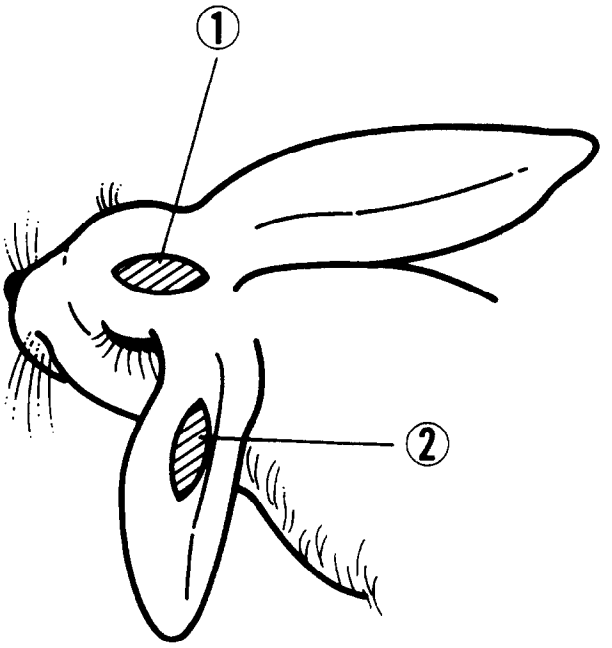

Fig. 4

Figure 1—Anatomy of the hip bone: an epiphysial plate (cross hatched) is placed along the upper part of the iliac crest. Almost the whole plate is harvested as a graft. **Figure 2**—Similarly sized epiphysial plates, one from the left and the other from right iliac crest. **Figure 3**—Histology of the harvested epiphysial plate: note the cartilaginous zone and the absence of bony tissue. The arrow indicates the side of the graft which lay against the bone or cartilage recipient site (H & E, × 100). **Figure 4**—The recipient sites: ① a subperiosteal pocket; ② a subperichondrial pocket.

postoperative week (Fig. 9). The epiphysial plates in both recipient sites grew to a maximum of 1.4 cm and became similar to the iliac crest in shape. The overall increase in both weight and height demonstrated no significant difference between the bony and cartilaginous recipient sites. Decalcified histologic sections before 16 postoperative weeks demonstrated a regular pattern of parallel columns of cartilage cells and the formation of bone tissue (Fig. 10). CMR showed the cartilaginous zone and newly-formed bone (Fig. 11).

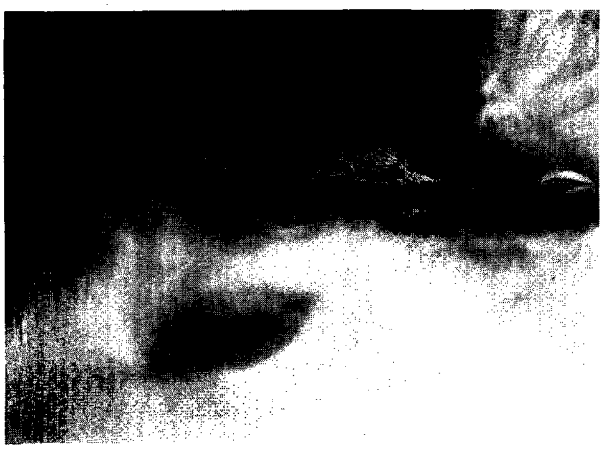

Fig. 5

Fig. 6

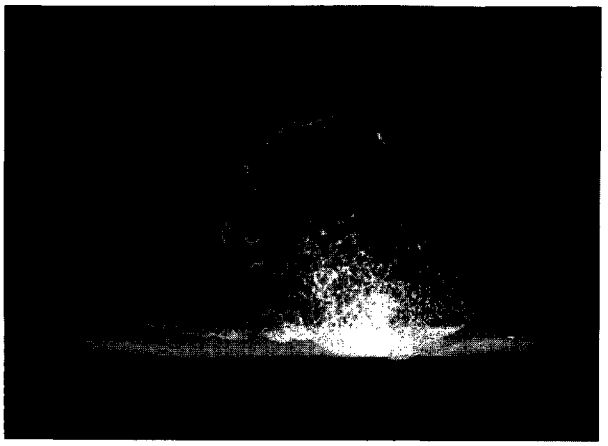

Fig. 7

Figure 5—Photograph of a rabbit 16 weeks after an epiphysial plate was grafted to its head. The graft reached 1.3 cm in height. **Figure 6**—Photograph of a rabbit ear 16 weeks after an epiphysial plate was grafted to it. The graft reached 1.4 cm in height. **Figure 7**—Soft X-ray of the specimen taken from the ear after 16 postoperative weeks.

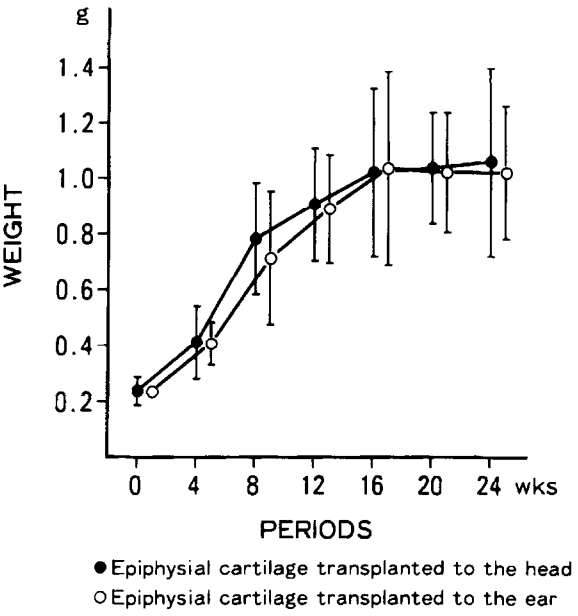

Fig. 8

Figure 8—Graph of weight measurements of the specimens.

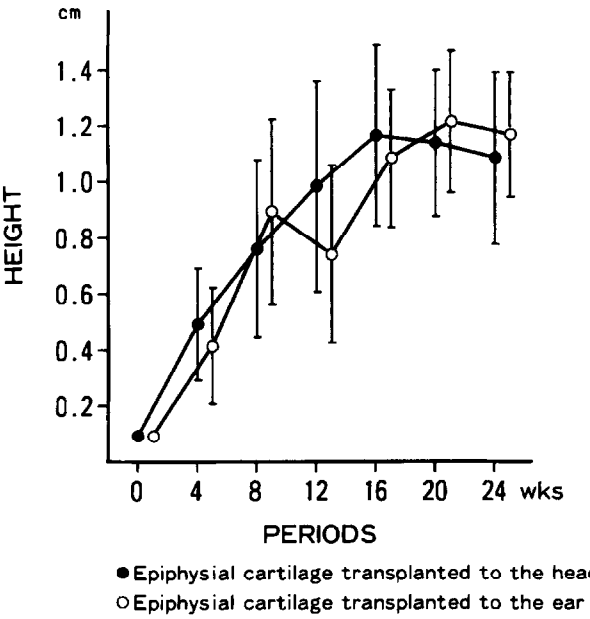

Fig. 9

Figure 9—Graph of height measurements of the specimens.

At 20 and 24 weeks, grafts taken from the head weighed 1.04 g ± 0.17 g and 1.09 g ± 0.33 g; grafts taken from the ear weighed 1.02 g ± 0.22 g and 1.04 g ± 0.20 g, respectively (Fig. 8). No significant increase in both weight and height was found after 16 postoperative weeks (Fig. 9). Decalcified histologic sections after 20 postoperative weeks showed that the formation of bone tissue continued and the columns of cartilaginous cells disappeared entirely (Fig. 12).

Discussion

This experiment, using epiphysial cartilage of the iliac crest, showed reproducible bone growth irrespective of the recipient site (whether adjacent to bone or cartilage). In past experiments, consistently successful transplantation of an epiphysial plate has not been accomplished. Epiphyses have been transplanted as free grafts, but the results have been disappointing in that growth was usually not maintained.[19,26] A poss-

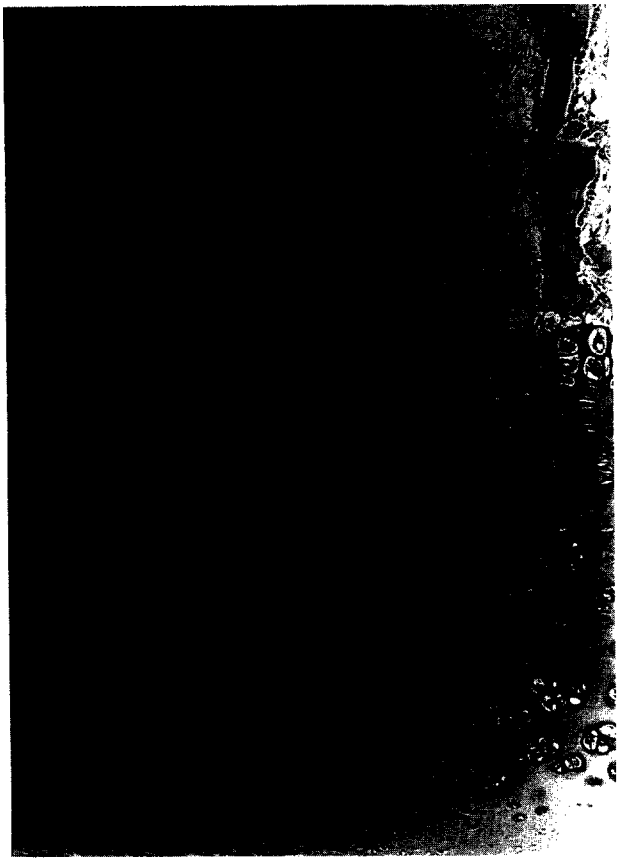

Fig. 10

Figure 10—Histology at 8 weeks post transplantation in the ear: note the regular pattern of parallel columns of cartilage cells and the formation of bone tissue (H & E. × 100).

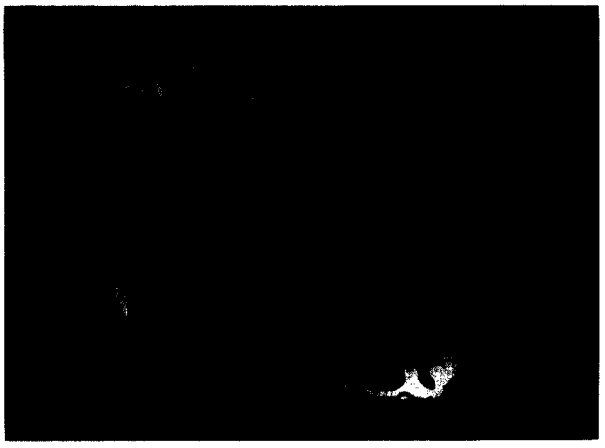

Fig. 11

Figure 11—Contact microcardiogram after 12 postoperative weeks: note the cartilaginous zone (arrows) and the woven pattern of new bone.

ible cause of our positive results is that the growth potential or the osteogenic capacity of the epiphysial plate of the iliac crest is superior to that of the proximal fibular epiphysis or the distal ulnar epiphysis used in previous studies. A second possibility is that the grafts in the previous experiments used total, massive segments with thick layers of adjacent bone from the epiphysis and diaphyses being included with

Fig. 12

Figure 12—Histology at 20 weeks post transplantation in the ear: central development of a marrow cavity can be observed in the compact bone. A small area of cartilaginous tissue (arrow) is observed but no columns are seen (H & E. × 20).

the cartilage. This may be unfavourable to the survival of the transplanted epiphysial cartilage. In our study, we used a thin sheet of epiphysial cartilage without the adjacent bone tissue. Freeman reported that actively growing tissue required more oxygen than mature tissue and that the degree of vitality of a graft depended on the maintenance of a high degree of oxygenation.[9] Siffert reported that nutrients to the growth plate were transported from the prevascular spaces by diffusion through the highly hydrated cartilage matrix.[27] It was concluded that after transplantation the epiphysis and plate usually failed to grow because of an interruption of the vascular supply to the epiphysis and therefore to the growth zone of the plate.[27,28] However, it has not been made clear whether it is the epiphysial cartilage itself which requires rich nutrition in order to generate bone. We postulate that the epiphysial cartilage does not depend on the vascularity of the underlying bed for bone generation. Although not proven, we also postulate that contact with the recipient bone or cartilage is an important factor for bone generation. The increase of the weight and height of the transplanted epiphysial cartilage was surprisingly similar between corresponding grafts from each recipient site. The columns of cartilaginous cells were observed before approximately 28 weeks of age (16 weeks postoperatively), and growth stopped at this time. The epiphysial plate normally closes at this age (approximately 6 or 7 months) in rabbits used in this study. The regulation of growth remains unchanged even after heterotopic transplantation. Further investigations to determine how a transplanted epiphysial plate reacts when transferred to a heterotopic anatomical site with altered stress loads and absence of normal nerve supply are necessary.

The free graft of epiphysial cartilage might be useful in the treatment of children with congenital or acquired skeletal deformities due to the absence or loss of an epiphysial plate. The application of this technique to provide a growing strut to the short columella in patients with cleft lip and palate might result in a

gradual lengthening. The cartilaginous epiphysial iliac crest could be exposed and a slice of cartilage harvested from the outer or the inner table using # 11 blade. Although not proven, it is postulated that harvesting a thin slice of epiphysial cartilage from the iliac crest will not adversely affect the further growth of the donor bone because the epiphysial plate has inherently strong potential for bone regeneration.

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