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FREE MICROVASCULAR EPIPHYSEAL TRANSPLANTATION: AN EXPERIMENTAL STUDY IN DOGS

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In 1967, Buncke *et al.* studied the joints following replantation of the thumb-index complex in monkeys and showed that they survive completely with preservation of the normal cellular architecture and function. This has been confirmed on many occasions in clinical replantation surgery. The successful experimental transfer of small joints (sometimes including epiphyses) on their vascular pedicle has been reported from this unit (O'Brien, 1977; Hurwitz, 1979) and this has since been applied clinically.

Similar results would be expected of free microvascular epiphyseal transplants, provided an adequate vascular supply to the epiphyseal plate is ensured.

Microvascular transfer of growing ulnas in puppy forelegs has been reported by Donski *et al.* (1979). With the inclusion of the nutrient artery into the vascular pedicle, periosteal and endosteal blood supply to the growth plate was secured. Final growth obtained by transfer with microvascular anastomoses averaged 63 per cent of that in the non-operated control ulna.

Since some data suggested that a vascular pedicle based on periosteal vessels only might achieve the same results, an experiment was designed to investigate this assumption.

METHODS

First experiment. Thirteen puppies (mongrel dogs), approximately 3 to 5 months old, underwent epiphyseal transplantation. The transplant, 4 cm in length, included the growth plate and adjacent metaphyseal and epiphyseal bone.

In the first group of 8 dogs bilateral *heterotopic* transplants were performed by exchanging the distal epiphysis and adjacent bone from one leg with the opposite one. In one leg the artery supplying the periosteum and a concomitant vein were anastomosed to the recipient limb vessels, while the opposite transplant as inset without vascular anastomoses (Fig. 1).

In the second group of 5 dogs a unilateral *orthotopic* excision and replacement of the distal epiphysis and adjacent bone with vascular anastomoses was performed. The transplant was completely detached and then brought back to its original place and its vessels were reanastomosed. The opposite ulna was left undisturbed except for insertion of a metallic marker proximal to the epiphysis to assess growth (Fig. 2).

Operative technique. Puppies weighing 15 to 25 lb were induced with Nembutal and maintained on a mixture of Halothane ½ to 1 per cent oxygen and nitrous oxide.

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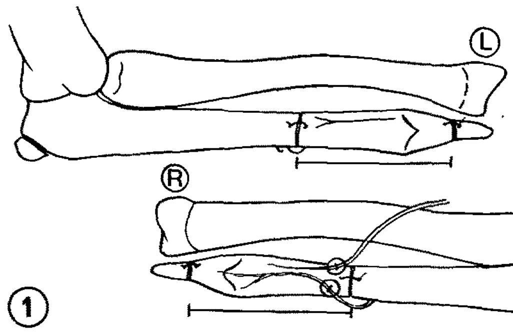

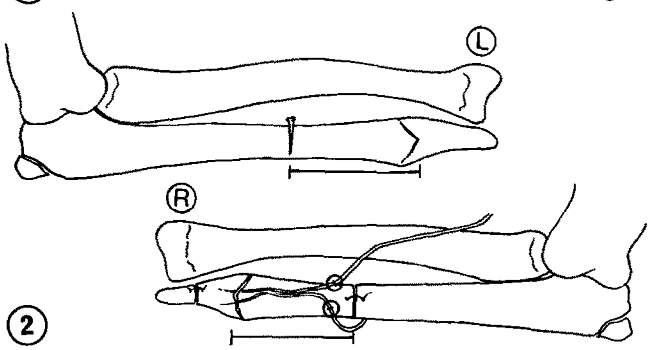

FIG. 1. Heterotopic epiphyseal transfer. The vascular anastomoses are marked in the lower diagram.

FIG. 2. Orthotopic epiphyseal transfer. The vascular anastomoses are marked in the lower diagram. The metallic marker is indicated in the upper diagram.

Both forelegs were shaved and prepared with an antiseptic solution (2·5 per cent tincture of iodine). After exsanguination of the limb with a tourniquet an incision was made along the lateral ulna from wrist to elbow. The distal ulnar epiphysis was isolated as a 4 cm long segment. Osteotomies were performed with a Gigli saw, 2½ cm proximal and 1½ cm distal to the epiphyseal line, leaving the distal end of the epiphysis in situ. The muscle and the periosteum were incised anteriorly and posteriorly along the border of the radius. Thus a segment of tissue containing the palmar interosseous artery and its accompanying veins could be safely dissected out with the transplant. Since the nutrient artery to the ulna enters the bone more proximally, this vessel was excluded from the transplant. After releasing the tourniquet, adequate blood flow was visible by immediate brisk bleeding from the periosteal vessels and from the bone marrow. On the opposite leg the radial artery and the big dorsal vein were dissected over sufficient length to allow easy handling while performing the vascular anastomoses in end to end manner under the operating microscope using a metallised 19 micron nylon microsuture. The arterial diameters averages 1·0 to 1·25 mm and the veins 1·0 to 2·5 mm. The transplant was fixed into place with 24 steel interosseous wire to the proximal ulna and the remaining part of the distal epiphysis. Ischaemia time of the revascularised transplant did not exceed 1 to 1½ hours. The skin was sutured and the limb immobilised in a padded plaster, then a light-cast for a total of 6 weeks. Penrose drains were removed after 48 hours and antibiotics given for the first week.

Follow-up. Serial X-rays were obtained at 3 and 6 weeks, 3, 4, 5 and 6 months. Linear bone growth was determined by measuring the distance between wires (Group 1) or from the proximal wire and proximal metallic marker, respectively, to the tip of the

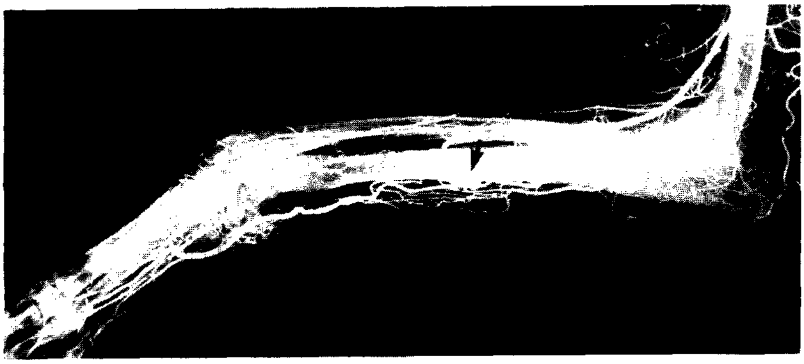

FIG. 3. Arteriogram of heterotopic revascularised transplant at 6 months. The anastomosis is marked with an arrow.

growth plate (Group 2). After a follow-up of 6 months arteriograms were performed in a number of dogs by injection of coloured diluted barium sulphate (Fig. 3).

Second Experiment

In congenital deformities shortening of the upper extremity may cause functional impairment. Lengthening of the ulna in radial agenesis was reported by Dick *et al.* (1977).

The following experiment was designed to examine the effect on growth of an additional epiphysis in an extremity.

Operative technique. In 6 puppies (of mixed breed and age) the left ulnar distal epiphysis was transplanted proximal to the right ulnar distal epiphysis, leaving the vascular supply to the latter undisturbed. This resulted in 2 vascularised epiphyses on the same leg (Fig. 4). The operation was basically performed in the same way as in the first experiment, but the defect on the right side that had to accommodate the left transplanted epiphysis was created 2 to 3 cm proximal to the distal epiphysis. The palmar interosseous vessels on this side were not disturbed. The puppies were followed up for a period of 4 months.

Results of First Experiment

In the first group (heterotopic transplants) one dog had bilateral massive infection; another showed massive infection of the non-vascularised transplant. In the second group (orthotopic transplants) 2 dogs died postoperatively due to anaesthetic compli-

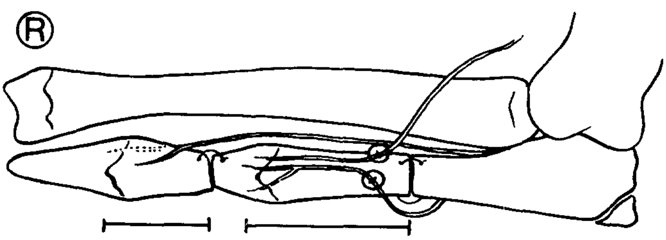

FIG. 4. Heterotopic epiphyseal transfer to the ulna, which now has 2 epiphyses. The microvascular anastomoses are indicated.

cations and one dog showed vascular failure at 3 weeks. This reduced the first group to 7 dogs and the second to 2 dogs and all were followed up for 6 months after their operation.

X-RAYS

A. Changes in the revascularised transfer. At 3 weeks normal healing was in progress at the osteotomy sites with some marginal resorption and callus formation, originating from both the host bone and transplant. The transplant showed radiologically normal architecture and had grown several millimetres.

At 6 weeks bone healing was well advanced and was already complete in half of the dogs (Fig. 5).

At 3 months all the osteotomy sites were well healed in all the animals. Growth was progressing and in 3 dogs the proximal epiphysis of the ulna was closing.

At 4 months the picture was similar. One animal showed a fracture in the distal one-third of the radius due to trauma or stress.

At 5 months the epiphyses were closing in 2 of the dogs.

At 6 months most dogs had achieved the maximum of growth, most epiphyses had closed or were closing at this stage (Fig. 6A). In 2 dogs the transplant had thinned, but had displayed satisfactory growth to this time. In 2 other dogs the distal radius was slightly bent, one dog having had a fracture of the radius.

B. Changes in the non-revascularised transfers. At 3 weeks some marginal bone resorption and discrete callus formation was seen in the host ulna. Marked changes were appearing in the non-vascularised transplant; a gap of 3 to 5 mm at the site of the growth plate separated metaphyseal and epiphyseal parts of the transplant. The bone structure showed signs of patchy density and rarefaction, indicating necrosis (Fig. 7).

At 6 weeks the signs of destruction of the transplants were more marked. The changes in structure had been accentuated. One graft was fractured and the others were partially dissolved.

A 3 months some transplants had almost completely dissolved, while in 2 dogs recalcification of the 2 fragments of the transplant was evident. One dog showed destruction of the distal radial epiphysis, possibly by infection.

At 4 and 5 months the appearance was similar. The 2 dogs with recalcifying transplants showed a gradual closing of the gap between the 2 bone fragments.

At 6 months the 2 above mentioned transplants had recalcified and healed in a relatively shortened position. All the other transplants showed a varying degree of

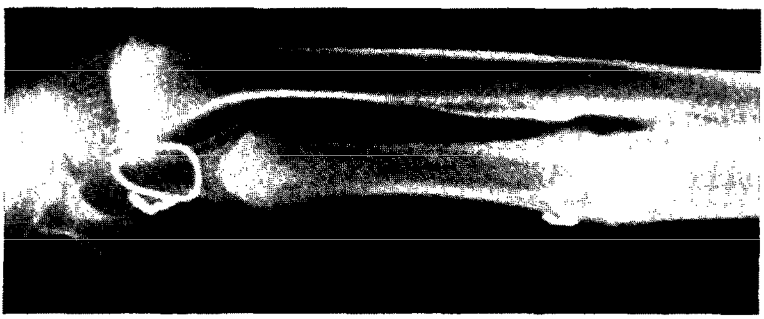

FIG. 5. Heterotopic epiphyseal transplant at 6 weeks. Good union has occurred.

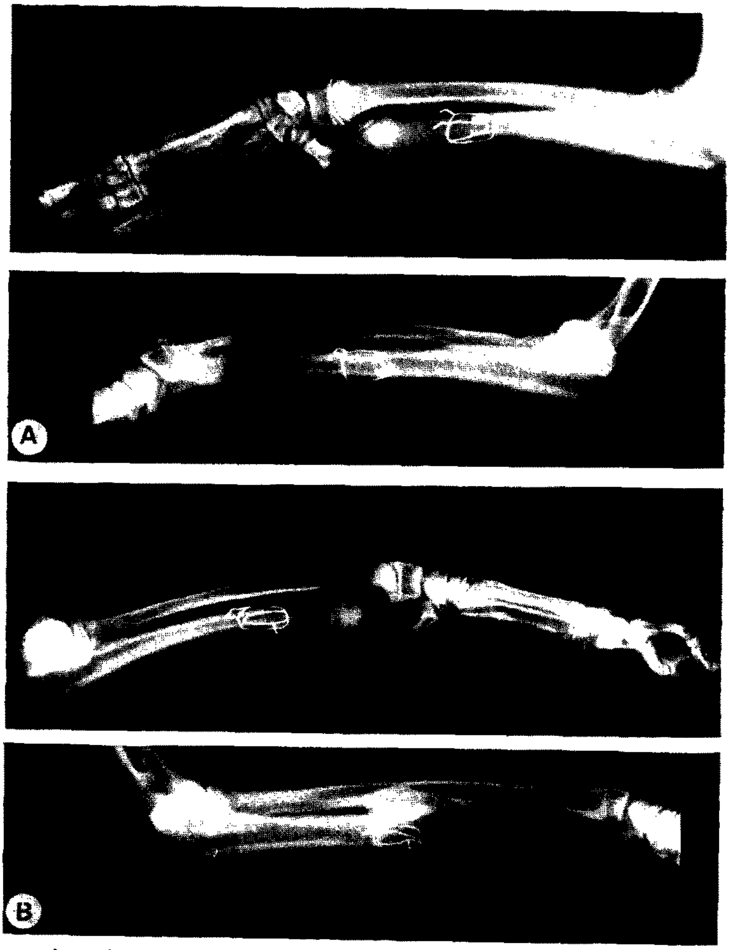

FIG. 6. Heterotopic epiphyseal transfer: X-rays at operation (above) and at 6 months (below). A. Revascularised transplant. B. Non-vascularised control.

FIG. 7. Non-vascularised epiphyseal transplant at 3 weeks.

destruction with gaps of several centimetres (Fig. 6B). Two dogs had a strong bend at the distal end of the radius, but the legs of the other dogs remained straight.

Measurements. Heterotopic epiphyseal grafts without microvascular anastomoses showed in most instances no growth, due to necrosis of the transplant. There was only an increase in length in the 2 cases in which there was fusion and recalcification of the previously separated parts of the transplants. The resulting average increase in length of the ulna was thus 0·13 cm or 0·9 per cent of the initial length of the ulnar bone at operation. (In the following, the percentage of growth will always be based on the initial length of the mentioned bone as measured at operation). Revascularised heterotopic epiphyseal transplants showed an average growth of 2·3 cm or an increase in length of 18·1 per cent (Tables I and II).

The number of *orthotopic* epiphyseal grafts (2 dogs) is too small to be conclusive, but seems to confirm the findings of a previous experimental series (Donski *et al.*, 1979). Growth of orthotopic revascularised transplants averaged an increase in length of 26·1 per cent while the non-operated control ulnas averaged an increase in length of 26·6 per cent (Fig. 8).

TABLE I

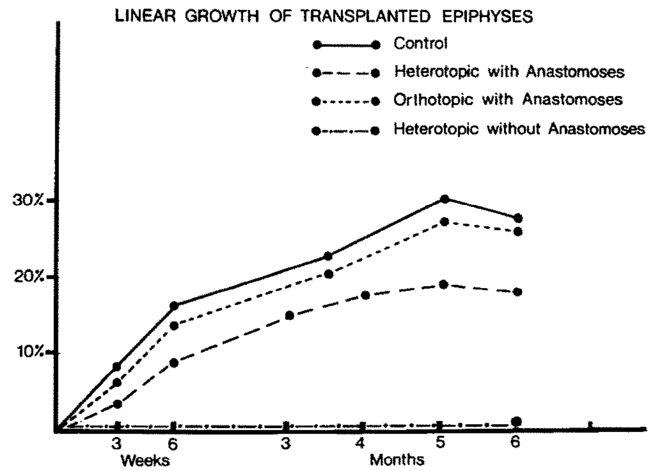

TABLE II

Linear growth of heterotopic epiphyseal tranpslants in 6 months

Dog No.	Transplants with anastomoses: Growth[1]	%	Transplants without anastomoses: Growth	%
48	2·5 cm	19·2	0·6 cm	4·5
51	1·9 cm	11·9	± dissolved	—
59	2·7 cm	23·5	part dissolved	—
65	2·8 cm	21·2	± dissolved	—
76	2·5 cm	19·5	± dissolved	—
87	2·6 cm	19·4	0·3 cm	2·2
91	1·3 cm	12·3	± dissolved	—
Average	2·3 cm	18·1	0·13 cm	0·9

[1] Total increase in length of the ulna within 6 months

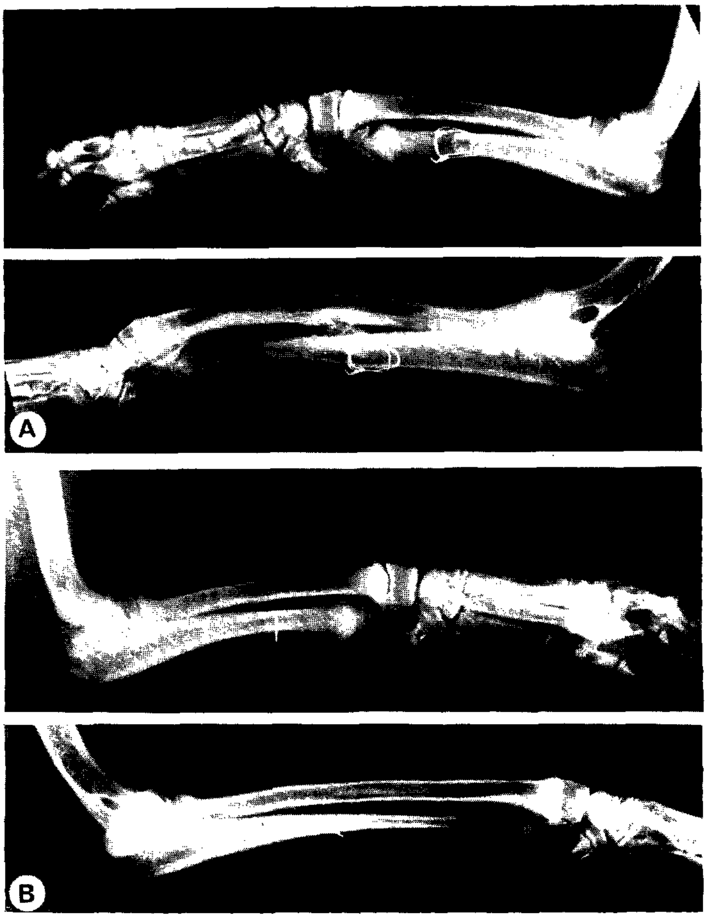

FIG. 8. Orthotopic epiphyseal transfer: X-rays at operation (above) and at 6 months (below). A. Revascularised transplant. B. Non-operated control ulna with wire marker.

The proximal ulnar epiphysis contributed an average increase in length of 1.9 per cent (Range 0 to 4·5 per cent).

The radial bone showed on the vascularised side an average increase in length of 24 per cent; on the side with the non-vascularised transplant an increase of 20·7 per cent.

Results of Second Experiment

Two dogs proved to be older than expected, and their epiphyses closed a short time after the operative procedure. In the remaining 4 dogs growth of the epiphyses was compared with each other, to the overall length of the ulna and to the length of the radial bone.

Sequential X-rays showed normal structure of the transplants and continued growth. Because the operated ulna seemed to grow more than the radius, deviation from a straight line occurred (Fig. 9). One dog had a fracture of the distal one-third of the radius 20 months after the operation. In 2 dogs the distal radius in the left leg was curved.

FIG. 9. Heterotopic epiphyseal transfer: X-rays of the 2 vascularised epiphyses on one leg, at operation (above) and at 4 months (below).

The operated ulna increased in 4 months an average of 37 mm in length (or 21·2 per cent) compared to its size at operation. The transplanted epiphysis contributed to this 18·7 mm (11 per cent), the normal epiphysis 16·3 mm (9·6 per cent) and an average of 2·3 mm (1·3 per cent) was contributed by the proximal ulnar epiphysis. Growth of the right radial bone averaged 31·3 mm (or 22·3 per cent), compared to the size of the radial bone at operation, a difference of 5·7 mm compared with the growth of the ulnar bone. The *left* radial bone grew at the same time an average of 23·7 mm (17·6 per cent) (Table III).

TABLE III

Linear growth in legs with two vascularised epiphyses

	Average growth in 4 months
Ulna (total length)	21·2% (37 mm)
Ulnar Epiphysis	9·6% (16·3 mm)
Transplanted Epiphysis	11·0% (18·7 mm)
Proximal Ulnar Epiphysis	1·3% (2·3 mm)
Radius	22·3% (31·3 mm)
Contralateral Radius	17·6% (23·7 mm)

DISCUSSION

Over the years a number of attempts at experimental and clinical transplantation of epiphyses as free grafts have been reported. Consistently successful transplantations have not been accomplished and the results have mostly been disappointing. Ring (1955) transferred the ulnar epiphyseal plate in rabbits from one side to the other, The thin graft included only a small sliver of metaphyseal and epiphyseal bone. Normal growth, based on growth predicted after a control series, occurred in 5 of 18 transpositions, while in the others growth ceased at some stage of the observation time.

Harris *et al.* (1965) reported results of a similar series in the rabbit: 11 of 25 orthotopic transplantations and 7 of 13 heterotopic transplantations to the opposite side were successful, with normal histological appearance and achieving 80 per cent of anticipated normal growth.

In 1972 Hoffman *et al.* performed a transplant of the proximal epiphysis of the fibula in dogs, using a tubed pedicle to carry the new blood supply. A skin tube from the thigh was placed over the osteotomised proximal fibula which was left attached and nourished by the epiphyseal blood vessels. At a second stage the tubed flap, containing the epiphysis, was detached from the leg and migrated to the abdominal wall. There was growth initially, but at maturity of the dogs the transferred bone was always smaller than that on the normal side.

In order to assess the clinical situation, Freeman (1965) sent a questionnaire to plastic surgeons and received the following replies: 77 autogenous free epiphyseal bone grafts had been performed by 29 surgeons. Of these, 32 were felt to have shown evidence of growth over periods of 8 months to 10 years which represented 42 per cent of the performed operations. The successful transplants were taken mostly from the toes and the metatarsal bones. Better growth was reported with composite grafts and joints transferred by a pedicle, but all eventually showed evidence of joint destruction and developed arthrodesis at a later stage.

Wilson (1966) published a series of 11 cases of autogenous epiphyseal transplants to the hand and forearm. Interphalangeal joints of toes, metatarsal bones and the proximal fibular epiphyses were transplanted as free grafts. Only one case showed any significant evidence of growth.

Recently Rank (1978) reported long-term results of epiphyseal transplants in congenital deformities of the hand. Epiphyseal transplants from discarded finger rudiments or from toes were performed as free grafts or in staged composite flaps. Growth of these elements added to the function of these hands.

Epiphyseal blood supply has been studied in detail in animals and humans by Trueta and Morgan (1960). Blood reaches the epiphyseal plate (growth zone) from 3 sources: the epiphysis, the metaphysis and the circumference of the plate.

(i) Several arteries enter the epiphysis and their terminal capillary loops supply the epiphyseal surface of the growth zone.
(ii) Ramifications of the nutrient artery provide the metaphyseal supply. These vessels are evenly distributed over the central part of the growth plate.
(iii) The outer fringe of the plate is supplied from a system of perforating metaphyseal arteries and these periosteal vessels act as minute nutrient arteries. These vessels have connections to both metaphyseal and epiphyseal circulations.

In the present study the nutrient artery was excluded from the transplant. Blood reaches the metaphyseal side of the growth zone through a multitude of channels in the cortex which form the connection between the periosteum and the bone marrow. The epiphyseal side is equally supplied by the periosteal vessels. How far additional blood supply through a specific epiphyseal vessel would improve the results will only be shown by further investigation.

Microvascular transfers of growing ulnas in puppies, based on a vascular pedicle that included the nutrient artery, were studied in a previous series (Donski *et al.*, 1979). Growth averaged 63 per cent of the growth achieved by the non-operated control ulnas. The present series confirms these results.

Growth of hererotopic epiphyseal transplants averaged 69 per cent of the growth achieved by the non-operated control ulnas. The average increase in length of the ulna in orthotopic epiphyseal transplants is felt to be too high because of the small number (2 dogs) in this series. In the previous series, using the nutrient artery, orthotopic epiphyseal transplants equalled the results of heterotopic transplants.

If we assume that in the second experiment growth of the left radial bone was undisturbed, growth of the right radius was increased by 26·7 per cent, compared with the growth rate of the left side. Normal epiphysis and transplanted epiphysis seemed to contribute equally to the achieved growth although this is not as high as expected. Osteotomy of the radial bone might have allowed unrestricted growth of the operated ulna.

A more refined study of the effects of an accessory epiphysis would be possible by performing the operation on the humerus. This would also leave an undisturbed ulnar epiphysis as a control.

Conclusions

Survival and subsequent growth occurs in a free, non-vascularised transplant only if the transplant is very small. Large transplants need immediate revascularisation for successful transfer. In our experimental model, a vascular pedicle through periosteal vessels achieved the same result as previously obtained using a vascular pedicle that included the nutrient artery. Non-vascularised transplants undergo, as a rule, a dissolution-substitution phase or are destroyed by necrosis and infection, reducing the growth potential or eliminating it completely.

Revascularised epiphyseal transplants showed consistent survival with normal bony architecture and continued growth. Although growth in some cases was almost normal, the average in our series was two-thirds of the normal control side.

It might be possible to achieve better results with additional blood supply to the epiphyseal side of the transplant.

Summary

Orthotopic and heterotopic microvascular epiphyseal transplants based on periosteal blood supply were performed in 2 experiments. Heterotopic transplants averaged 69 per cent of growth of non-operated control bones. Orthotopic transplants achieved a better result but the number of dogs available for evaluation was too small to be conclusive. The question is raised whether additional blood supply to the epiphyseal side of the transplant could improve the results.

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