EPIPHYSIAL GROWTH AFTER FREE FIBULAR TRANSFER WITH AND WITHOUT MICROVASCULAR ANASTOMOSIS

EXPERIMENTAL STUDY IN THE DOG

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The proximal fibular epiphysis was transferred in young puppies using microvascular techniques. The study demonstrated, as have previous investigators, that free epiphysial transfer without vascular anastomosis results in death of the chondrocytes of the growth plate. Histologically, the chondrocytes do not take up labelled proline, indicating diminished metabolic activity; do not take up radioactive thymidine, indicating that they are not dividing; and there is eventual disruption of the normal histological picture. In contrast, where the microvascular anastomoses re-established the blood supply to the growth plate, the epiphyses demonstrated normal histological appearance, uptake of radioactive proline and thymidine and continued to grow but at a slightly diminished rate. It is concluded that continued growth can occur after free vascularised epiphysial transfer in the dog.

The treatment of children with abnormalities of growth due to the absence or loss of an epiphysial plate is difficult. The resulting deformity and discrepancy in limb length is progressive and often results in serious functional handicaps for the affected child. Since the turn of the century (Helferich 1899) surgeons have been attempting to restore the growth potential of bones by transplantation of the epiphysial plate. Some of the reports in the literature have claimed success (Straub 1929; Wenger 1945; Barr 1954; Freeman 1965; Whitesides 1977), while others have been failures (Haas 1931; Eades and Peacock 1966; Wilson 1966; Spira and Farin 1967; Rank 1978). One of the problems has related to the assessment of viability and growth after transplantation. Measurement of growth by radiography is often unreliable because of the problem of dissociating the growth from the physes at the opposite end of the bone. In addition, the presence of an open epiphysial plate does not necessarily mean that it is capable of growth (Spira and Farin 1967). Even in those cases in which growth reportedly continues, the segment rarely grows normally and at maturity the transplanted side is usually smaller than the contralateral side.

There have been many experimental studies of transplantation using conventional techniques (Bisgard 1939; Ring 1955a,b; Heikel 1960a,b; Spira, Farin and Hashomer 1964; Harris, Martin and Tile 1965; Heikel 1965; Hoffman, Sifert and Simon 1972; Calderwood 1974). The best results have followed transplantation with only a thin piece of attached bone which allows for rapid incorporation. For survival during this 7 to 10 day avascular period, the growth plate must depend upon the diffusion of tissue fluids. This problem severely limits the size of the growth plate which can be transplanted and accounts for the variable and unpredictable results of transplantation using conventional methods.

Advances in surgery make it theoretically possible to preserve the viability of a transplanted growth plate by microvascular anastomoses of its critical blood supply (Zaleske et al. 1982). With the success of microsurgical techniques for free tissue transfers of all types, it is only natural that the problems of transplanting an epiphysial plate should be re-addressed. Isolated clinical reports of toe-to-thumb transfer in children have indicated that continued growth can occur after free vascularised growth-plate transfer (Mathes, Buchanan and Weeks 1980; Snowdy, Omer and Sherman 1980), but as yet there have been no well-controlled experimental studies reported using a model which would be readily applicable to human disease. Our objectives were to develop an animal model for the study of free vascularised growth-
plate transfers, to determine if growth continues after such transplantation, and to measure the degree to which normal growth occurs.

MATERIAL AND METHODS

Experimental model. The fibula of the dog has already been established as a model for the study of bone grafts (Enneking et al. 1975). We determined the vascular anatomy of the hindlimb by injecting latex into the femoral artery. The organisation of the vascular tree is similar to that of humans. The popliteal vessels branch into a posterior tibial and larger anterior tibial branch. The peroneal vessels arise from the posterior tibial vessels shortly after the bifurcation. To study the intraosseous blood supply, barium micro-angiograms were prepared and the bone was cleared using the Spalteholz technique after injecting liquid plastic into the vessels. The nutrient artery is a branch of the peroneal artery which enters the fibula medially in its middle third. The epiphysial, metaphysial and perichondrial blood supplies have been reported as being essential for the normal growth of the epiphysial plate (Trueta and Amato 1960; Brashear 1963; Spira and Farin 1967). In order to preserve the nutrient artery as the main metaphysial supply of the fibula, subperiosteal dissection was carried out along the tibia and the upper two-thirds of the fibula was removed. By preserving a thick muscle cuff around the fibula, the perichondrial blood supply to the physis was preserved, thus helping to protect the nutrient artery which lay in the narrow space between the tibia and fibula. The lateral inferior genicular artery and vein were preserved as the main epiphysial supply. The popliteal vessels were used for the vascular pedicle to preserve all of the sources of blood supply to the growth plate while only having to perform one venous and one arterial anastomosis (Fig. 1). The average diameter of these vessels was 1.8 millimetres (1.0 to 2.3 millimetres), thus ensuring a high patency rate.

Groups. Forty-eight puppies of different ages and breeds were divided into three groups (Fig. 2). One limb in each puppy was used as an internal control. The operative procedure was performed under general endotracheal anaesthesia and aseptic conditions with prophylactic antibiotics. With the animal prone, a posterolateral approach was made to the fibula. The operative time per dog averaged eight hours.

In Group A (six puppies) the fibula and its surrounding muscular envelope was completely removed from its bed as previously described, but the transplant remained attached by the popliteal vessels. In Group B (orthotopic transplantation—21 puppies) the same operative procedure was carried out except that the popliteal artery and vein were transected and the transplant was removed. It was then replaced in its bed and microvascular anastomoses were performed. The average duration of ischaemia was 48 minutes. A sham operation in which the contralateral fibula was cut distally was performed on the control limbs of Groups A and B. This was a more valid control than a non-operated
leg since cutting the bone stimulated growth, thus making histological comparisons more accurate. With both legs injured, the dog resumed weight-bearing on both legs simultaneously. In Group C (heterotopic transplantation—21 puppies) the fibulae in both legs were removed in the same manner as in Group B. They were then transferred to the opposite limb where one side had restoration of its blood supply by microvascular anastomosis. The vascular pedicle of the other transplant remained ligated to serve as an avascular control. The duration of ischaemia averaged 80 minutes in this group.

In all animals, the fibula was fixed to the distal tibia with very small ("mini") plates and screws. This eliminated any possible contribution to longitudinal growth by the growth plate of the distal fibula and the radio-opaque screws served as useful markers for the radiographic measurement of growth. After operation, both hindlimbs were placed in plaster casts for two days to prevent soiling of the wound. All dogs in the study resumed full weight-bearing within the first three or four days. Angiograms were done on selected dogs in each group to study the effects of operation on the vascularity of the hindlimb. The caudal femoral artery was able to maintain the viability of the hindlimb when the popliteal artery was transected (Figs 3 and 4).

The 48 dogs were killed at 1, 2, 4, 8, 12, 16 and 26 weeks. In Groups B and C, there were three animals in each time period. To label new bone formation, two doses of tetracycline (50 milligrams of Achromycin per kilogram of body weight) were given intravenously several days apart. At death the limbs were re-explored to find the anastomoses, patency tests were done and the vessels were opened to check for any thrombus.

Specimens. Two small pieces of growth plate were cut from the transplant and used to prepare autoradiographs. The rest of the samples were fixed in formalin, but left undecalcified to preserve the tetracycline labels. The undecalcified specimens were embedded in methylmethacrylate, cut on a Jung microtome, and polished into 5 and 15 micrometre sections. The five micrometre sections were stained with toluidine blue and Goldner’s trichrome to enable the study of morphology. The 15 micrometre sections were mounted unstained for examination by fluorescent microscopy. The thin freshly harvested specimens of growth plate were placed into 0.5 millilitres of Eagles’ medium at 37 degrees Celsius. To one specimen 0.05 millilitres of H-thymidine (50k) was added, and to the other specimen 0.05 millilitres of H-proline (50k). Both solutions were then incubated in a Dubnoff shaker for four hours. The samples were then incubated with a cold chase and placed into buffered formalin. After fixation the specimens were cut and mounted on glass slides. Some were used to prepare autoradiographs by the dipping technique while others were stained with Safranin 0 and haematoxylin and eosin for morphological study.

Assessment of viability. An attempt was made to use technetium bone scans to monitor the vascularity of the transplants after operation. The tibial diaphysis showed considerable uptake of isotope which, combined with the very dense isotope uptake in the epiphysis, tended to obscure the uptake in the fibula. Therefore, a valid interpretation of transplant vascularity was not possible, in spite of the use of a pin-hole collimator to improve resolution.

Morphology. On the histological slides, the number of chondrocytes and the structure of the columns of cartilage, the number of blood vessels in the metaphysis and epiphysis, and the proliferative activity of the marrow elements were all studied. Bone viability was determined by the condition of the osteocytes in the lacunae and the amount of new bone formation. This was determined by observing the number of trabeculae which were covered by plump and cuboidal (metabolically active) osteoblasts. We did not consider the trabeculae that were covered by osteoid, since this matrix can remain intact even when the bone is dead.

Autoradiography. Uptake of 3H-proline is a measure of the metabolic activity of the chondrocytes. While incubating in a Dubnoff shaker, viable chondrocytes take up the 3H-proline and incorporate it into collagen and other proteins. To determine if there was any change in the proliferative activity of the transplanted growth plate, another piece of growth cartilage was incubated with 3H-thymidine, which is a DNA precursor only taken up by cells which are about to divide.

Fluorescent microscopy. Only viable bone is capable of taking up the dual tetracycline label. The tetracycline is incorporated into the hydroxyapatite crystal during bone formation. By measuring the areas covered by a clear dual tetracycline label, one can determine quantitatively bone formation activity in the transplants. To determine the calcification rate, the mean distance between the midpoints of the two fluorescent labels was divided by the interval in days between the two labellings. These measurements were made using a semi-automatic image analyser (Zeiss Mop 3), which was coupled to a microscope. A comparison was made of all the mean values of epiphysial and metaphysial calcification rates in the vascularised transplants and the control limb to see if there were any differences.

Radiographs were taken immediately after operation and each
month thereafter to measure the growth of the transplant. On each radiograph the fibula was measured from the physis proximally to the internal fixation device distally; the previous measurement was subtracted from this length to determine the growth which had taken place in the month. The two sides were then compared. These values were tabulated with the measurements of other dogs made at the same time interval after operation. The control and study slides were tabulated separately. All animals from one month to six months after operation were included, but the number of animals decreased progressively as they were killed. Thus for the calculation of the growth during the sixth month only three animals in Group B and three in Group C were alive for measurement, whereas all animals were included for the first month.

The mean and standard deviation of all the measurements in each month was determined. This value was used as the average growth during that month. We were thus able to compare the pattern of growth of the free vascularised transplant to the sham-operated control fibula.

RESULTS

Viability

Group A. In this group there were six puppies whose purpose was to show that the growth plate of the proximal fibula could survive and continue to grow when nourished by the selected pedicle. Since the transplant always

A gross photograph taken two weeks after operation shows the sham-operated control graft on the left and vascularised transplant on the right (Group B).

Comparison of the histological appearance of the epiphyses shown in Figure 5, the photographs on the right being from the free vascularised transplant. Figures 6 and 7—Safranin O stain showing normal morphological appearance (×100). Figures 8 and 9—Proline autoradiograph demonstrating protein synthesis on both sides. (Fig. 8 × 100; Fig. 9 × 120.) Figures 10 and 11—Thymidine autoradiograph showing the actively dividing chondrocytes (×100). Figures 12 and 13—Tetracycline labelling of new bone formation in both specimens (×100).
remained attached by this pedicle, the growth plate was never ischaemic. It was therefore expected that survival and growth would be the best in this group. By all methods of assessment, there was no measurable difference between this group and Group B, so they will be considered together. **Group B.** There were 21 animals available for study. There was one failure of anastomosis. This was in an animal killed at one week after operation when a thrombus was found in the venous anastomosis. Histological study of this specimen showed it to be completely dead and identical to the avascular transplant in the three dogs of Group C who were killed at one week. In three other dogs the anastomoses could not be found due to extensive scar formation, but the findings on histological study of the animals were the same as the other members of their group.

There was only one infection in Groups A and B. This was a superficial abscess in the sham-operated control leg of a dog in Group B. This did not appear to influence the growth in this leg.

After transplantation the gross appearances of the vascularised fibula and the sham-operated control were initially identical. The only difference was seen in the muscle cuff surrounding the transplanted fibula (Fig. 5). Morphological assessment of the growth plate showed no great differences: there were a comparable number of blood vessels in the epiphysis and metaphysis, the marrow appeared healthy and was proliferating, and the number of chondrocytes in the physis was equal (Figs 6 to 13). The proline autoradiographs perhaps showed a slight decrease in the intensity of uptake on the vascularised side during the first week in some specimens. Thymidine uptake was noted in chondrocytes from both sides, indicating cell division. In the animals killed after one month there was no evidence on gross examination of remodelling of the epiphysis in some specimens; but even at six months the morphologic aspects of the control and vascularised growth plates were very similar (Figs 14 to 18). We measured by fluorescent microscopy the trabecular surface exhibiting a clear dual tetracycline label and calculated the calcification rates in the epiphysis and metaphysis (Table 1). These values showed marked variability from animal to animal in the same time period. This was a reflection of the differences in age at

<table>
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<tr>
<th>Group</th>
<th>Epiphysis</th>
<th>Metaphysis</th>
<th>Experimental</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
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<tr>
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<tr>
<td></td>
<td>4.55 ± 0.43</td>
<td>4.20 ± 0.41</td>
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</tr>
<tr>
<td>Group B</td>
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<td>3.98 ± 0.84</td>
<td>3.98 ± 0.84</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>4.81 ± 0.94</td>
<td>4.75 ± 1.06</td>
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<td>NS</td>
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<tr>
<td>Group C</td>
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<td>4.00 ± 1.05</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4.97 ± 1.09</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant
the time of operation and the variety of breeds used in our experiments. In individual animals, the difference between the vascularised fibula and the sham-operated control, in Groups A and B, was not statistically significant. This emphasised the importance of using each animal as its own control. The difference in calcification rates in the pedicled fibula in Group A and the free vascularised fibular transplant in Group B was not significant. Therefore, there appeared to be no measurable effect due to ischaemia.

**Group C.** In contrast to the results in Groups A and B, there were marked differences between the free vascularised growth plate and the avascular control. The only difference in the operative technique between the two transplants was that one had re-anastomosis of its blood supply while the vascular pedicle of the other fibula remained ligated. At one week after operation the avascular physis showed pale staining with Safranin 0, a cationic dye which is bound stoichiometrically to the glycosaminoglycans in the cartilage. Thus the density of uptake of the dye is an indication of the proteoglycan content of the matrix and is therefore a reflection of the

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**Figures 19 and 20**—Safranin 0 gives very pale staining on the right and most of the lacunae are devoid of cells (x 125). Figures 21 and 22—There is no proline uptake on the non-vascularised right side (x 125).

**Figures 23 and 24**—There has been no tetracycline uptake on the right side (x 60).

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**Fig. 25**—Gross appearance of the fibulae in a Group C dog six months after operation. The specimen on the right is avascular and at two months was seen to be largely resorbed. Figures 26 and 27—Histological appearance of the Group C transplant seen in Figure 25, stained with Safranin 0 (x 100). The right side has become partially revascularised, but there is no evidence of the growth-plate structure.
health of the cartilage. There were no blood vessels seen in the epiphysis and metaphysis and almost none of the marrow cells appeared to be alive. Morphologically, the chondrocytes were distended, many of the cellular membranes had ruptured, and most of the nuclei were pyknotic or had disappeared (Figs 19 to 24). In contrast, the vascularised fibula appeared healthy, grossly and histologically. The cartilage showed normal Safranin O staining. The cartilage columns were arranged in an orderly fashion and the zonal structure of the growth plate was well maintained. The most striking differences were observed in the autoradiographs. There was no uptake of tritiated proline or thymidine in the avascular growth plate. This is the strongest evidence that the growth plate was dead. The avascular fibula did not take up tetracycline. At two weeks the changes were even more dramatic. Grossly, the marrow appeared white, the physis was undergoing more autolysis and did not take up any Safranin O stain. At this time, the structure of the fibula was more or less intact. At one month the avascular transplant began to show some resorption, which was most evident in the proximal aspect where there was more cancellous bone. The two-month specimens showed increased resorption, but there was some evidence of complete. Usually, only a small spicule of diaphysis remained with an area of fibrocartilage which was the remnant of the growth plate (Figs 25, 26 and 27).

At all study intervals the transplant with the vascular anastomosis appeared normal. In three Group C animals we were unable to find the anastomoses but in all three the transplant was viable by all criteria. There were five infections in this group: all involved the limb with the avascular transplant and all cleared with antibiotic therapy. This infection may have contributed to the resorption of the graft in the affected animals. There was never any infection of a vascularised fibular transplant in any animal in Groups B or C.

Assessment of growth (Table II)

Group A. The average monthly growth of the pedicled fibula was 3.3 ± 3.0 millimetres per month, compared with 4.3 ± 2.8 millimetres for the control fibula. This difference was not significant.

Group B. All of the fibulae grew after the free vascularised growth-plate transfer, except in a dog killed at three months in whom the anastomosis could not be found and in whom technical difficulties had been encountered. The absolute growth was usually less than that of the control fibula at all study intervals. For example, in six months the fibulae with the anastomoses grew a total of 37 millimetres (average of three animals) while the sham-operated fibulae grew 41 millimetres during this same time period (Figs 28 and 29). The average monthly growth of the orthotopically transplanted fibula with vascular anastomoses was 6.2 ± 2.8 millimetres, compared with the control sham-operated fibula which

revascularisation in some specimens, particularly distally, where the muscle cuff was thinner. At three months there was more consistent revascularisation of bone. In some aspects of the diaphysis there was a clear dual label indicating new bone formation. The physical mechanism was completely disrupted and there was increased bony resorption. In the four-month and six-month specimens resorption decreased as revascularisation became more

![Fig. 28](image1)

Radiographs showing a typical Group B animal. Figure 28—Immediately after operation. Figure 29—Six months later growth (measured in millimetres) is seen on both sides, but with a diminished rate on the anastomosed side.
Table II. Comparison of monthly growth in the length of the fibula of control and experimental animals measured from the radiographs (means and standard deviations in millimetres)

<table>
<thead>
<tr>
<th>Control</th>
<th>Experimental</th>
<th>P</th>
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<tr>
<td>Group A</td>
<td>4.3 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Group B</td>
<td>7.4 ± 2.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Group C</td>
<td>0</td>
<td>&lt; 0.001</td>
</tr>
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</table>

NS, not significant

averaged 7.4 ± 2.8 millimetres per month. This difference was significant (P < 0.005). Although the absolute growth attained and the average monthly growth were less in the study limb, the pattern of growth was similar in the two limbs. As the animals approached maturity the growth rate decreased in both limbs, but the animals were not followed long enough to observe closure of the physis. Therefore it is not known if premature closure would occur in the fibula with the vascular anastomoses. When the growth of the pedicled fibula in Group A was compared with the orthotopically transplanted fibula in Group B, no significant difference was found.

**Group C.** There was never any measurable growth of any fibula without a vascular anastomosis, and, in fact, each of the avascular transplants became smaller due to resorption. This usually reached a maximum two or three months after operation (Figs 30 to 32). After this time, revascularisation of the transplant from the surrounding soft-tissue bed had usually begun. The graft disappeared completely in four dogs. In most animals the final fibular size was 25 to 30 per cent of its size at operation (Figs 33 and 34). This resorption was probably caused by the thick cuff of avascular muscle which impeded revascularisation and may have been promoted by the non-stressed position of the transplanted fibula. The average monthly growth of the free vascularised transplant was 5.5 ± 2.8 millimetres per month. There was no significant difference between this growth and that of the vascularised Group B transplants.

**DISCUSSION**

This study shows that long-term survival and useful growth is possible in transplanted growth plates which have had their blood supply re-established by microvascular anastomoses. The absolute growth is slightly less than normal. However, the growth of the control fibula may have been stimulated by osteotomy which could account for the small discrepancy between the two sides. There was no difference between the rate of bone formation in the metaphysis or epiphysis in the control or anastomosed fibulae.

It is recognised that the statistical significance of pooled samples is not as great as would have been true had we been able to compare growth rates and bone formation rates within each animal. However, the small increments of growth at the beginning and the end of the study, as well as the species and size difference of animals, precluded this approach.

The resorption that occurred in the avascular fibulae in Group C appeared to be caused by the thick cuff of dead muscle which may have served as a nidus for infection and was undoubtedly a barrier to revascularisation of the bone graft. These findings support clinical experience. After anastomotic failure in free vascularised bone grafts the bone does not behave as a conventional...
bone graft but often becomes resorbed or infected. No fibular transplant with microvascular anastomoses became infected, which suggests that vascularised free bone transplants may be more resistant to infection. This might be due to a better opportunity for prophylactic antibiotic penetration into the vascularised bone.

This report confirms previous studies (Wray et al. 1981; Zaleske et al. 1982) which indicated growth-plate function after transfer of a whole joint. Unlike previous studies, it shows that the fibula is therefore a suitable free bone for further experimentation in order to determine the response of this bone to heterotopic transplantation with altered mechanical demands.

In summary, all fibulae in which a vascular anastomosis had been carried out appeared to grow. This growth appeared to be normal in pattern, but the total increase in length of those fibulae in which an anastomosis had been carried out was somewhat less than was present in the sham-operated bones. In addition, there were no deep infections involving fibulae in which a microvascular anastomosis had been carried out while there were infections of the avascular bones. This final point does also seem worthy of emphasis.

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REFERENCES