# BONE GRAFTING: A COMPARATIVE ANALYSIS OF VASCULARIZED AND NON VASCULARIZED AUTOGRAFTS

A Thesis

by

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ABSTRACT

Vascular perfusion studies, histology, tetracycline labelling, and technitium$^{99}$ scintigraphy were used to compare conventional rib grafts with and without periosteum to rib grafts vascularized by vascular bundle implantation, by dermis island flaps, and by microvascular anastomosis of graft nutrient vessels in dogs. Results indicated that: 1)The vascular pattern of posterior microvascularized rib grafts is identical to that of normal ribs. 2)Implanted vascular bundles remain patent and revascularize bone grafts. 3)Circulation in rib grafts and the patency of anastomoses of microvascularized rib grafts are accurately reflected in technitium$^{99}$ bone scans. 4)Tetracycline labelling is not a reliable indicator of the patency of a bone graft's pedicle blood supply. 5)The vascular invasion of failed microvascularized rib grafts is slower than that of conventional rib grafts. 6)The presence or absence of periosteum has no observable effect on graft revascularization. 7)Dermis island flaps did not enhance graft vascularization.

RESUME

Nous avons mené des études de perfusion vasculaire, de marquage à la tétracycline, de scintigraphie osseuse au technitium$^{99}$ et d'histologie pour comparer les greffes costales conventionelles, avec ou sans périoste, aux greffes costales nourries par implantation du paquet vasculaire, par ilôt dermique et par anastomose microvasculaire des vaisseaux nourriciers du greffon dans le chien. Nos résultats indiquent que: 1)La vasculature des greffons costaux postérieurs microvascularisés est identique à celle des côtes normales. 2)Les implants du paquet vasculaire demeurent perméables et revascularisent les greffes osseuses. 3)La scintigraphie au technitium$^{99}$ est un reflet précis de la circulation dans les greffons osseux et de la perméabilité des anastomoses dans les greffons microvascularisés. 4)Le marquage à la tétracycline n'est pas un indicateur fiable de la perméabilité des vaisseaux sanguins nourriciers de greffons osseux. 5)L'invasion vasculaire d'un greffon costal microvascularisé non-perméable est plus lente que celle observée dans un greffon costal conventionel. 6)La présence ou l'absence de périoste n'a pas d'effet observable sur la revascularisation du greffon. 7)Les ilôts dermiques n'ont pas accéléré la vascularisation des greffons.

# INTRODUCTION

## 1)The Structure, Circulation, and Physiology of Normal Bone

Bone is a remarkable tissue. It is light, yet has strength and durability. It repairs itself if broken, and alters its structure to accomodate changes in stress. It owes these properties to the functional unit of cortical bone, the osteon.

The osteon is a tubular structure consisting of a central vascular supply intricately connected to surrounding circular sheets, or lamellae, of calcified osteoid. Each lamella has its calcified osteoid oriented in a direction which is different from that of the two lamellae adjacent to it. This permits the osteon to better withstand forces from different directions. In each bone, the large number of osteons branch and intertwine as they course along the long bone's axis, thus adding increased strength to the total structure.

Bone, forever dynamic, is in a constant state of remodelling. An old osteon is gradually resorbed by osteoclasts within its Haversian canal until the excavation cavity is in a good configuration for the next generation osteon. Then, osteoblasts lay down new bone in lamellar spurts of activity and a new osteon is formed, often in a location just slightly different from the last in order to accomodate some altered stress. This process takes an average of 4 to 5 weeks per osteon generation(16).

Throughout the process of osteon excavation and repletion, the blood vessels of the Haversian canal provide the nutrients and cellular elements necessary for the bone remodelling. In addition, there is evidence that the osteoblasts and osteoclasts themselves arise from the endothelial cells of these Haversian blood vessels(149). Obviously, in order to

understand the structure and function of bone, it is necessary to understand its blood supply.

The skeletal system receives approximately 4 to 10% of the cardiac output in man(129). Each long bone receives its arterial blood supply from four major sources; the nutrient artery, the two groups of epiphyseal arteries at either end of the bone, and the periosteal perforating arteries from the muscle surrounding the bone(79,84,116).

It has been estimated that the nutrient artery is responsible for 70% of the diaphyseal circulation, and for 33% of the epiphyseal-metaphyseal circulation in the adult. The remainder of the circulation is made up from the periosteal and epiphyseal arteries respectively(129). It is therefore not surprising that if one occludes the nutrient artery, the medulla and the inner 2/3 of the cortex undergo necrosis. Conversely, if one strips off the periosteum and isolates it and the muscle from the diaphysis, the outer 1/3 of the cortex becomes necrotic(63,66,119,149).

Thus, despite the fact that the four arterial systems of adult bone are connected(118,119,146), and will compensate for each other's loss to a certain extent(9,82), they are not independently capable of perfusing the entire bone.

Although there are methods of measuring blood flow(77,129) and blood volume(20,24) in bone, these are difficult tasks. The multiple sources of bone circulation are not easily accessible, and therefore are difficult to isolate. Despite these difficulties, however, blood flow studies(32) and perfusion studies(119) have been carried out. The results have indicated that different areas within bone have their circulation shut down intermittently, as in other body tissues, so that all areas are not perfused all of the time. Also, it has been shown that

blood flow within bone is under local nervous, humeral, and metabolic control(55,89,148). In addition, it has been determined that there are regional differences in flow rates within a given bone(104).

It was classically thought that blood flow in normal bone was largely centrifugal, proceeding from the nutrient artery in the center of the medulla outward to the periphery(119). However, recent flow studies indicate that although arterial supply may be centrifugal, venous return may be centripedal(9,146). Electron microscopy of Haversian canals has shown the presence of twin vessels which could accommodate this system(149).

Although osteocytes seem isolated in their lacunae, multiple canaliculi connect them intimately to the Haversian systems. These systems, in turn, are connected to each other via Volkman's canals, and to the four major arterial systems and their respective venous drainage routes. The medulla and its woven or cancellous bone is well perfused by the cavernous sinusoidal system, which in turn is fed by the nutrient artery.

Thus we see that bone has a highly sophisticated and specialized circulatory system. This system is not only well designed to nourish the skeleton, but it is highly capable of altering the very structure of bone itself.

2)The Fate and Circulation of Conventional Large Cortical Bone Grafts

Bone is not only capable of healing itself and altering its structure with changes in stress, it is also capable of adapting to a new environment if it is transferred from one area of the body to another.

When a large piece of cortical bone, such as the fibula, is transferred to a bony defect, such as a traumatic loss of a segment of tibia, many things happen to both the graft and its host bed. Some of the cells of the graft, particularly those of the periphery, will survive via

plasmatic diffusion or early vascular invasion(117). However, most of the cells of the graft are thought to die because of inadequate circulation(1,33). It should be pointed out that this concept, although largely accepted and quoted in the literature(26), is difficult to prove. There are some who feel that osteocytes can survive without circulation for up to two weeks or longer(1,31). There is good evidence that they can survive at least 25 hours(10).

Within a week of transfer, granulation tissue will have bridged the gap between the graft and the host bone. The vascular invasion of a bone graft has veen reported to occur as early as 5 hours after implantation, but it usually takes 6 or 7 days for the first 3mm of vascular penetration(134).

The vascular invasion of a bone graft can occur in two ways. There is clear evidence that end to end anastomoses of graft-host vessels can occur with instantaneous perfusion of a few selected vessels in the graft(4), but this is unusual. The more common mechanism is the slow, gradual ingrowth of new blood vessels from the host bone into the old circulatory cavities of the graft. Vital microscopy has shown that vessels penetrate cancellous bone at a rate of 0.2 to 0.4mmper day, and cortical bone at a rate of 0.15 to 0.3mm per day(3).

Following vascular invasion, the next step in graft adaptation is bone remodelling. This process differs markedly in cortical and cancellous bone grafts. As the blood vessels invade cancellous bone, new bone is initially laid down over the old necrotic bone(3), and the radiodensity of this bone is gradually increased(26).

On the other hand, when the vasculature invades cortical grafted bone, there is an impressive initial resorption of bone along the old

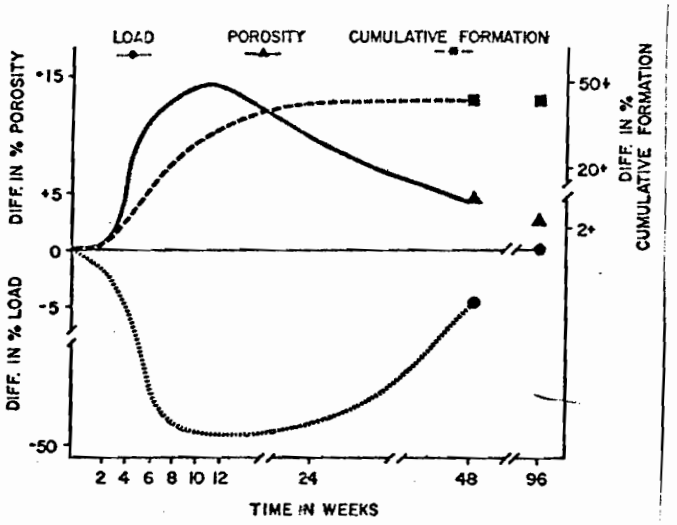



FIGURE 1. The temporal relationships of porosity, cumulative bone formation, and the load required to produce stress fractures in canine fibular autografts. (Reproduced with permission from Burchardt, H., and Enneking, W.F.:Transplantation of Bone. Surg. Clin. North Am. 58;403-427,1978)

osteons. The bone becomes diffusely filled with porous excavation tubes and becomes radiolucent on X ray(3,26). The process of excavation of old cortical bone, followed by apposition of new bone, is known as creeping substitution. This is the english translation of "schleichender ersatz", coined by Barth in 1895, as cited by Berggren(9).

Burchardt et al.(26,51) have shown that the diffuse porosity induced by creeping substitution weakens the bone by up to 40 to 50%, and so results in a high incidence of stress fractures (Fig. 1). Enneking reported stress fractures in 18 of 40 patients (45%) in whom he replaced long bone defects with fibula grafts(52).

In the dog, the porosity and weakness of cortical bone grafts peak at 10 to 12 weeks, and then gradually decrease as the bone apposition phase begins in earnest(26). In man, the process takes longer. The incidence of fatigue fractures in long bone autografts has been reported to peak between 6 and 18 months after grafting(27). It is therefore important to protect these grafts for up to two years after grafting.

Because they have no inherent circulation, large conventional cortical autografts are also prone to a high incidence of non union (33% in Enneking's (52) series), and infection(28). For the same reason, these grafts take poorly in inadequately vascularized beds, such as those damaged by oncological radiotherapy, or those densely scarred as a result of trauma or osteomyelitis. Unfortunately, this is where large cortical autografts are frequently required.

In spite of these difficulties, many large cortical bone grafts do achieve union, resist infection and undergo creeping substitution. If all of the hurdles are overcome, the graft will hypertrophy, alter its structure, and incorporate itself into the recipient bone to take on its new role.

3)The Surgical Vascularization of Bone Grafts

a)The Purpose of the Surgical Vascularization of Bone Grafts

The problems of non union, infection, fatigue fracture, and poor survival in relatively avascular beds with large conventional bone grafts have long stirred the imagination of surgeons to search for a better way to transfer bone. One way which theoretically deletes the above problems from conventional bone grafting is to maintain an intact circulation in a bone graft after transfer.

Imagine a theoretically perfectly vascularized bone graft, such as a segment of fibula, being grafted to a defect in the tibia. Assuming that the circulation of blood in the transferred fibula is able to remain identical to what it was before the transfer, the complications which occur with conventional bone grafting may be avoided. The bone would remain alive, would not undergo creeping substitution, and therefore would not not be prone to stress fracture. The fibular graft would contribute to bony union with the host tibia, and therefore the defect would heal like a fracture. Also, because the grafted fibula has maintained its own circulation, it would be able to survive in a relatively avascular bed and fight infection.

Because of the attractive theoretical advantages of the perfectly vascularized bone transfer, there have been many attempts to vascularize bone grafts in a number of ways. The various methods can be broken down into three major groups:

1)Pedicle bone graft vascularization with intact pedicles of nutrient vessels, skin, muscle, and omentum.

2)Bone grafts transferred with the microanastomosis of nutrient vessels

3)Bone grafts transferred with an artery, vein, or vascular bundle implanted into a hole drilled in the graft.

b)Bone Grafts Vascularized via Pedicles of Nutrient Blood Vessels, Skin, Muscle, and Omentum

Canalis(29) summarized 11 reports of bone grafts vascularized by pedicles of skin or muscle between 1892 and 1938. The first of these was published by Wolfler in 1892 when he used a cervical flap that incorporated a portion of the clavicle for mandibular reconstruction.

Although a small amount of work was done in this field in the first half of the 20th century(158), credit has been given to Snyder et al.(131) for regenerating interest in transferring bone vascularized by intact skin flap pedicles in 1970. In 8 clinical cases of mandibular reconstruction, they transferred the clavicle within a laterally based intact pedicle of skin. Others have used similar techniques(36,81). Conley has described several possible methods of mandibular reconstruction using the clavicle, the scapula, the rib, or the temporal bone vascularized by overlying skin and muscle pedicles(34,35). Bone grafts vascularized by skin flap pedicles have also been used in digit reconstruction(56,91).

Meyers has reported the use of a femoral trochanteric crest bone graft swung on an intact quadratus femoris muscle pedicle in the repair of displaced femoral neck fractures(97,98,99). The graft is used to bridge the gap between the femur and the femoral head. He reports that he has decreased his non union rate from 35% to 5%, and his rate of segmental collapse from 32% to 5% with this technique(99). The technique used by Myers was initially described by Judet(75) to increase the blood supply to the femoral head. Others have used similar muscle pedicle techniques both experimentally(60,71,41,124) and clinically(36,101,135).

Medgyesi(93,94,95) and Baadsgaard(7,8) studied the vascularization of bone via skin and muscle experimentally. Medgyesi concluded that in goats,

a muscle pedicle provided a greater blood supply to bone than did a skin pedicle(95). He reported that torsion of a muscle pedicle is more detrimental to graft circulation than is tension of flexion(94). He also observed new bone formation originating within bone vascularized by muscle pedicles, a true sign of functional viability(93). Canalis observed less resorption of bone in muscle pedicle bone grafts than in conventional bone grafts in dogs and in monkeys(29,30).

Bone has been vascularized experimentally by placing it first in omentum, and then, in a second stage, transferring it on an omental pedicle with microvascular techniques(53). This procedure, however, seems remote from clinical applicability at this time.

In 1971, Strauch et al. described the transfer of an anterior segment of rib and its muscle sleeve on their intact intercostal vascular pedicle in continuity with the internal mammary vessels in the dog(133). In 1974, Ketchum reported a clinical case where such an anterior rib graft on an internal mammary island vascular pedicle was used to replace a mandibular defect(80). In 1977, Taylor reported the reconstruction of a femoral defect with the rotation of the fibula and its muscle sleeve 180 degrees upward on an intact peroneal vessel pedicle(141). Fen reported a similar case in 1980(54).Also in 1980, Bradford(19) reported the use of a posterior rib graft on an island vascular pedicle consisting of the posterior intercostal vessels. The rib was used as an anterior vertebral strut in the management of kyphosis.

In general, a bone graft vascularized by transferring it with its nutrient vascular pedicle uninterrupted is probably ideal. The normal bone circulation remains intact (if the nutrient artery is included), and there are no blood vessel anastomoses to contend with. However, this

FIGURE 2 Experimental Assessment of Microvascularized Bone Grafts

| Principle Investigator and Year of Study | Number of Animals | Areas of Investigation |
|---|---|---|
| McKee(92)1968 | 13 | viability, survival in irradiated beds |
| McCullogh(90)1973 | ? | viability |
| Ostrup(106-109)1974 | 36 | viability, bone formation rate, survival in irradiated beds |
| Adelaar(2)1974 | 16 | viability, union |
| Tschopp(150)1976 | 13 | viability |
| Doi(47)1977 | 42 | viability, union, bone scanning |
| Haw(72)1978 | 23 | union, infection rate, bone scanning |
| Bos(17)1979 | 12 | viability, bone scanning |
| Puckett(113)1979 | 10 | union, stress fractures |
| Donski(48,49)1979,1980 | 31 | growth |
| Berggren(9-14)1981 | over 90 | viability, blood flow, bone scanning, ischemia time, medullary V.S. periosteal cirdulation |

technique is technically difficult and of limited applicability(54,80). On the other hand, there are several body sites where bone grafts vascularized by skin and muscle pedicles can be used(34,56). However, it should be noted that these bone grafts are usually only fed by the perforating periosteal arteries eminating from the skin or muscle which is in contact with the bone; the nutrient artery is excluded. As previously noted, periosteal perforating arteries are normally only responsible for an estimated 30% of diaphyseal circulation.

There is some debate that periosteal circulation alone may be insufficient to provide adequate perfusion to a bone graft. Without the medullary circulation provided by the nutrient artery, a large part of the graft may die(6,38,111). However, there is no doubt that periosteal circulation is able to feed a bone graft at least partially, and certainly enough for it to contribute to union(12). The question of how much circulation is necessary for bone graft survival and function remains controversial.

c)Microvascularized Bone Grafts

A microvascularized bone graft is a composite graft of bone with a sleeve of surrounding soft tissue. The vessels that feed the soft tissue, the periosteal circulation of the bone, and usually the nutrient circulation of the bone as well, are transferred with the composite graft and anastomosed to the vessels in the recipient bed. Thus the bone graft resumes its circulation and is vascularized. The vessel anastomosis is usually performed with a microscope, hence the term "microvascular".

The advent of microsurgery in the last two decades has made this type of bone grafting a reality. Following is a summary of the experimental knowledge (Fig.2), and clinical experience with microvascularized

bone grafts.

I. Experimental Work in Microvascularized Bone Grafting

The first experimental work on microvascular free bone transfers (other than in limb replantation) was probably done by McKee in 1968. Although this work was presented at the American Society of Plastic and Reconstructive Surgery meeting in Toronto in 1971, it was not published until 1978(92). In 17 dogs, 9 of which had received preoperative irradiation, the anterior portion of the 6th rib based on the internal mammary vessels was transferred to the mandible as an onlay graft. The 13 surviving animals were assessed with arteriograms, histology, and radiology from 3 months to 1 year post transfer. McKee reported that bone cells were viable at 3 and 9 months after transplant, that there was no diminution of the size of the bone transplant, and that preoperative irradiation did not appear to have any effect upon the survival of the bone grafts.

The first publication of experimental work on microvascularized bone grafts was by McCullough and Friedrickson in 1973(90). In an unspecified number of dogs, the posterior rib and its intercostal vessels were transferred to the neck. The grafts were assessed with histology and tetracycline labelling. They reported that the vascularized grafts were labelled with tetracycline 3 weeks post transfer, that they were viable histologically, and that their controls showed no evidence of histological viability at 3 weeks.

In 1974, Adelaar et al.(2) published a series of 16 dogs in which a 3cm segment of rib was transferred to a complete defect of the radius, anastomosing the intercostal vessels both proximally and distally to the radial vessels. A control non vascularized rib graft was placed in a

contralateral radial defect. The grafts were assessed from 2 weeks to 3 months with radiology, histology, arteriograms, tetracycline labelling, silicone rubber perfusion, and gross examination of clinical union. In their 9 cases with successfully patent microvascular anastomoses, they reported no difference in clinical union, osteocyte survival, or tetracycline labelling between vascularized and non vascularized bone grafts.

Ostrup transferred posterior rib grafts to mandibular defects in 36 dogs, 10 of which received preoperative irradiation. From this work, 3 papers were published in 1974 and 1975(106,107,108), in addition to his monograph(109). He assessed the grafts with angiography, radiology, histology, clinical union, silicone rubber perfusion, and triple fluorochrome labelling. Histological assessment and fluorochrome labelling revealed that successfully microvascularized bone grafts remained viable(106). The bone formation rate was the same in microvascularized rib grafts placed in irradiated beds as it was in those placed in non irradiated beds(107). In addition, the bone formation rate in vascularized grafts was the same as that in normal bone in the rest of the animal(108). He reported that most of his conventional control grafts were not viable, but he only had 6 of these, and 4 of them were infected(106).

In 1976, Tschopp(150) published work similar to that of Ostrup and reached comparable conclusions. However, his control non vascularized rib grafts picked up fluorochrome labelling as early as 4 weeks post transfer, indicating graft viability.

In 1977, Doi et al.(47) transferred the posterior rib on its intercostal vessels to femoral defects. The results from 42 dogs were presented, 16 of which contained only control non vascularized bone grafts. The grafts were assessed with histology, angiography, technitium$^{99}$($Tc^{99}$) scintigraphy,

and by examination of clinical union from 1 day to 12 weeks post grafting. They concluded that bony union occurs earlier in microvascularized (average 8 weeks) than in non vascularized (average 12 weeks) grafts. They stated that creeping substitution does not occur in vascularized bone grafts. Their bone scanning data was difficult to interpret as they had trouble separating graft scintigraphy from that of the surrounding bone in their model. They did find, however, a higher $Tc^{99}$ uptake by vascularized grafts than by non vascularized grafts in the 8 bone graft specimens that they removed from the animals and measured in a gamma counter.

In 1978, Haw, O'Brien, and Kanata(72) reported work in 15 dogs with microvascularized tibial grafts. They removed segments of tibia along with their nutrient cranial tibial vessels, and then replaced the bone and anastomosed the previously divided vessels. They also had 8 control dogs with no vascular anastomoses. The grafts were assessed with radiology, angiography, $Tc^{99}$ scintigraphy, histology, and estimation of clinical union at 2 to 16 weeks. They demonstrated a decreased infection rate as well as earlier and more frequent bony union in the microvascularized tibial segments. Angiography was unsatisfactory in demonstrating the patency of anastomoses because overlying vessels obscured the small microanastomosed vessels in question. Bone scanning accurately differentiated success and failure of the anastomoses in the 5 cases in which it was used.

In 1979, Puckett et al.(113) reported a study in which lateral segments of rib were placed into bilateral fibula defects in 10 dogs. Each dog acted as his own control as one rib was vascularized by anastomosis of the intercostal vessels, and the rib in the contralateral leg was not microvascularized. All of the animals were sacrificed at 4 months and the grafts were evaluated with radiology, histology, examination of bony union,

and by the measurement of graft stress tolerance. It was observed that the incidence of bony union was the same in both the microvascularized and non vascularized rib grafts. It was also reported that stress tolerance to fracture was 90.5% of normal bone in microvascularized grafts, and 68% of normal bone in non vascularized grafts. However, this difference was not statistically significant.

In a study published by Bos in 1979(17), 12 dogs had a fibula removed and replaced with microanastomosis of the previously divided nutrient posterior tibial vessels. In 5 of the dogs, the contralateral fibula was removed, stripped of periosteum, and then replaced as a control conventional bone graft. The fibulae were assessed at 1 to 20 weeks with $Tc^{99}$ scintigraphy, radiology, histology, and fluorochrome labelling. Bos observed that a positive bone scan one week post operatively was consistent with patent anastomosis of the nutrient vessels of the bone. He also observed that unsuccessfully microvascularized bone grafts with occluded anastomosed had less callus formation and picked up less $Tc^{99}$ than conventional bone grafts. He felt that the sleeve of dead muscle tissue surrounding the graft impaired its periosteal revascularization.

Also in 1979, Donski and O'Brien(48) reported a series of 12 puppy experiments. In 8 of the puppies, they switched the ulnae and their intact epiphyses, anastomosing the palmar interosseus nutrient vessels on one side, and leaving the other side non vascularized as a control. In the other four puppies, the ulna was removed and then replaced on the same side with microanastomosis of its nutrient vessels. Its growth was then compared to that of the contralateral unoperated ulna. Ulnar growth was assessed in all of the puppies with periodic X rays until closure of the epiphyses. They observed that the growth rate in the non vascularized

ulnae was significantly less than in the microvascularized ulnae (8% growth V.S. 17% growth). However, microvascularized ulnae grew significantly less than normal unoperated ulnae (17% growth V.S. 26% growth). They concluded that although microvascularized bone grafts grew better than conventional grafts, they did not grow as well as normal bone. They attributed the latter observation to the fact that anastomosis of the nutrient artery does not reestablish epiphyseal blood flow in developing bone(23,84).

In a subsequent study(49), Donski et al. performed a similar experiment. However, this time, the growth in conventional grafts of ulnae in puppies was compared to that in microvascularized ulnae based on periosteal circulation alone, excluding the medullary nutrient artery circulation. Again, they showed that microvascularized grafts grew better than conventional control grafts, even with the vascularized grafts based on periosteal circulation alone. The number of their experiments was too small to compare medullary artery based versus periosteally based microvascularized bone grafts with respect to growth.

In 1981, Berggren produced a monograph(9) summarizing work which was subsequently published in several papers(10-14,157). More than 90 dogs were used in a variety of experiments in which microvascularized and non vascularized rib grafts were assessed with radiology, microangiography, fluorochrome labelling, histology, and $Tc^{99}$ scintigraphy.

Berggren et al. showed that rib grafts survive microvascular bone transfer, even after 25 hours of ischemia time(10). They found that microvascularized bone grafts which include the medullary nutrient artery have higher blood flow rates(157), and a higher rate of cortical resorption within the grafts than in microvascularized grafts based on periosteal circulation alone(12). They also found that the latter grafts receive enough circulation

for the cortex to survive and contribute to callus formation in spite of the fact that the marrow not uncommonly becomes necrotic(10). They observed that $Tc^{99}$ scintigraphy within a week of microvascular bone grafting is a reliable indicator of the patency of microvascular anastomoses(13).

In summary, the major findings of the experimental assessment of microvascularized bone grafts to date have presented some evidence favoring the following statements:

1)Microvascularized bone grafts remain viable with a normal bone formation rate, even in previously irradiated beds(9,90,92,106-108).

2)Some workers have observed that bony union occurs more quickly and more often in microvascularized bone grafts than in conventional bone grafts(47,72). Others have not found this to be true, and have observed no difference in union rates(2,113). Note that the grafts in the first two experiments(47,72) contained an intact nutrient artery, while those of the last two studies(2,113) were based on periosteal circulation only.

3)Microvascularized bone grafts contribute circulation and callus formation to bony union(12).

4)Some investigators have stated that creeping substitution does not occur in microvascularized bone grafts(47). Others(12) have observed significant cortical resorption and porosity in these grafts.

5)Microvascularized bone grafts may have a greater tolerance to stress than non vascularized conventional grafts(113).

6)There may be a decreased rate of infection in microvascularized bone grafts compared to conventional grafts(72).

7)Failed microvascularized bone grafts with occluded anastomoses pick up $Tc^{99}$ diphosphonates later and less intensely than conventional bone grafts(13,17).

FIGURE 3. Summary of 122 Cases of Microvascularized Bone Grafting Reported in 1975 to 1980.

| Author | Donor Bone | Recipient Bone | Number of Cases |
|---|---|---|---|
| Taylor(140)1975 | fibula | tibia | 2 |
| Buncke(25)1977 | rib | tibia | 3 |
| | rib | mandible | 2 |
| Serafin(125)1977 | rib | mandible | 1 |
| Watari(153)1977 | fibula | femur | 2 |
| | fibula | tibia | 1 |
| | fibula | humerus | 1 |
| Weiland(77)1977 | same cases published in Weiland(79)1979 | | |
| Ariyan(6)1978 | rib | mandible | 1 |
| Daniel(39)1978 | rib | mandible | 3 |
| | ilium | mandible | 3 |
| | ilium | tibia | 1 |
| Harashina(69)1978 | rib | mandible | 2 |
| Judet(76)1978 | fibula | femur | 1 |
| | fibula | tibia | 2 |
| | ilium | tibia | 1 |
| McKee(92)1978 | rib | mandible | 9 |
| O'Brien(102)1979 | ilium | femur | 1 |
| | ilium | tibia | 1 |
| | ilium | calcaneus | 1 |
| | 2nd metatarsal | mandible | 4 |
| Ohmori(103)1979 | 2nd metatarsal | nose | 1 |
| Pho(112)1979 | fibula | radius | 1 |
| Rosen(120)1979 | 2nd metatarsal | mandible | 9 |
| | ilium | mandible | 1 |
| Sanders(123)1979 | ilium | tibia | 1 |
| Serafin(127)1979 | same cases published in Serafin(128)1980 | | |
| Tamai(138)1979 | ilium | calcaneus | 1 |
| Taylor(143)1979 | ilium | tibia | 10 |
| | ilium | tarsus | 1 |
| | ilium | hemipelvis | 1 |
| | ilium | mandible | 2 |
| Weiland(155)1979 | fibula | tibia | 4 |
| | ilium | tibia | 2 |
| | fibula | femur | 1 |
| | fibula | radius | 1 |

(Figure 3 is continued opposite p.20)

8)Microvascularized bone grafts with epiphyses grow better than non vascularized grafts(48,49).

9)Microvascularized bone grafts can survive when perfused with only an intact periosteal circulation, but they have lower blood flow rates and a higher incidence of marrow necrosis than grafts in which the medullary nutrient artery is included. However, the latter have a higher incidence of interior cortical resorption, possibly related to the higher blood flow rates(12,157).

10)In the dog, microvascularized bone grafts can survive up to at least 25 hours of ischemia time before total marrow and osteocyte necrosis occurs(10)

11)Positive $Tc^{99}$ scintigraphy appears to correlate well with the patency of the vascular anastomosis in microvascularized bone grafts in the first week after the transplant, and it is safer than angiography(13,17, 72,109).

II. Clinical Work in Microvascularized Bone Grafting

The first clinical use of a microvascularized bone graft (other than in limb replantation) was probably by McKee in 1970, although this was not published until 1978(92). McKee used a lateral rib graft, based on the intercostal vessels, which he used to replace a traumatic mandibular defect in a young woman. He presented this first case at the American Society of Plastic and Reconstructive Surgery meeting in Toronto in 1971.

The first published clinical use of a microvascularized bone graft was that of Taylor, Miller, and Ham in 1975(140). They presented 2 cases of tibial defects reconstructed with microvascularized fibula grafts. Since then, there have been many published reports of microvascular free bone transfers. Figure 3 summarizes 122 cases reported in 28 papers in the

FIGURE 3(cont'd). Summary of 122 Cases of Microvascularized Bone Grafting Reported in 1975 to 1980.

| Author | Donor Bone | Recipient Bone | Number of Cases |
|---|---|---|---|
| Weiland(156)1979 | fibula | radius | 3 |
| | fibula | humerus | 1 |
| | Fibula | ulna and metarcapal | 1 |
| Ariyan(6)1980 | rib | mandible | 2 |
| Bitter(15)1980 | ilium | mandible | 1 |
| Fen(54)1980 | fibula | radius | 1 |
| | fibula | femur | 2 |
| Fogdestam(57)1980 | ilium | tibia | 1 |
| Franklin(61)1980 | ilium | mandible | 6 |
| Qintan(115)1980 | fibula | humerus | 2 |
| | fibula | radius | 3 |
| | fibula | femur | 2 |
| Serafin(128)1980 | rib | mandible | 11 |
| | rib | maxilla | 3 |
| Salibian(122)1980 | ilium | mandible | 4 |
| | 2nd metatarsal | mandible | 1 |

FIGURE 4. Indications for 122 Cases of Microvascularized Bone Grafting Reported in 1975 to 1980.

| Indications | Number of Cases |
|---|---|
| defect after neoplasm resection | 58 |
| post traumatic defect | 33 |
| non union (after trauma or infection) | 9 |
| replacement of lost epiphysis | 2 |
| total thinoplasty | 1 |
| radionecrosis | 1 |
| not reported | 18 |
| | 122 |

literature from 1975 to 1980. The indications for these grafts are presented in Figure 4 (opposite p.20), and in Figure 5 (opposite p.21). The types of donor and recipient bones of these cases are summarized in Figure 6 (opposite p.21).

The posterior rib, fibula, 2nd metatarsal, and ilium (based on the deep circumflex iliac vessels) microvascularized bone grafts are all endowed with both periosteal and nutrient artery circulations. The anterior and lateral rib grafts, as well as the ilium (based on the superficial circumflex iliac vessels) are perfused with periosteal circulation only. The technical details of harvesting and transferring these grafts are outlined in the references given in Figure 7 (opposite p.22).

Microvascularized bone grafts appear to be particularly indicated in irradiated or scarred tissue beds with inadequate circulation where conventional bone grafts generally do poorly. The vascularized grafts are also useful if there is a skin or mucosal defect as well as a bone defect, as skin can be taken with the ilium(143), the 2nd metatarsal(120), or the rib(128) as a composite graft. Microvascularized fibula and 2nd metatarsal grafts may also contain viable epiphyses if growth is desired(48,156). Microvascularized fibula grafts are used more and more frequently for long bone defects where structural support is required(65). The theoretical advantages of a decreased incidence of non union, infection, and stress fractures in microvascularized bone grafts are attractive, but as yet remain unproven in man.

The main disadvantages of microvascularized bone grafts are their technical difficulty, the prolonged operative time, the sacrifice of major limb vessels, and the potential donor site complications(140). Also, if the anastomosis fails, it has been suggested that the dead muscle

FIGURE 5. Types of Noeplasm Resected in 58 Cases in which the Defect Created was Replaced with a Microvascularised Bone Graft (Patient Age Range 7 to 70 Years)

| Type of Neoplasm Resected | Number of Cases | Types of Bone from which Neoplasm was Resected |
|---|---|---|
| intraoral squamous cell carcinoma | 38 | 38 mandibles |
| fibrous dysplasia | 5 | 3 femurs, 1 tibia, 1 humerus |
| giant cell tumor | 4 | 3 radii, 1 humerus |
| osteosarcoma | 3 | 2 mandibles, 1 radius |
| malignant fibrous histiocytoma | 1 | humerus |
| aneurysmal bone cyst | 1 | femur |
| adamantinoma | 1 | tibia + fibula |
| fibrosarcoma | 1 | femur |
| osteoblastoma | 1 | mandible |
| | 58 | |

FIGURE 6. Donor and Recipient Bones in 122 Cases of Microvascularized Bone Grafting Reported in 1975 to 1980.

| Donor Bones | | Recipient Bones | |
|---|---|---|---|
| ilium | 39 | mandible | 62 |
| rib | 37 | tibia | 29 |
| fibula | 31 | femur | 9 |
| 2nd metatarsal | 15 | radius | 9 |
| | 122 | humerus | 4 |
| | | maxilla | 3 |
| | | calcaneus | 2 |
| | | hemipelvis | 1 |
| | | nose | 1 |
| | | ulna + metacarpals | 1 |
| | | tarsus | 1 |
| | | | 122 |

may inhibit the revascularization of the graft, rendering it inferior to a conventional non vascularized bone graft(17,13).

d)Blood Vessel Implantation into Bone

The attempt to vascularize bone grafts and other types of avascular bone has not been limited to pedicles and to the microvascular anastomosis of the nutrient vessels of bone. In 1946, Vineberg reported the patency of the open ended internal mammary artery implanted into cardiac muscle(152). This demonstration of patency in vessels implanted into organs set the stage for bone vessel implants which began in the early sixties. Following is a summary of the experimental knowledge (Fig. 8, opposite p.22) and clinical experience (Fig. 9, opposite p.23) in this field to date.

I.Experimental Work in Blood Vessel Implantation into Bone

In 1962, Woodhouse(158) introduced the concept of blood vessel implantation into bone. In 1963(159), he reported the implantation of 25 open ended brachial arteries into the intact humeri of dogs. He made a single side cut in these vessels to furthur promote blood flow into the humerus and thereby help to keep the vessel open. The animals were sacrificed at 4 hours to 9 months post operatively. He demonstrated his 60% patency rate by observing and measuring the presence of backflow from the implanted vessel in the living animal(10-15 ml/min.).He then showed that the vessels were able to provide circulation to the entire bone by means of injection studies.

Woodhouse noted that kinking of the artery at its entrance into bone led to thrombosis. He also observed tufts of capillaries eminating from patent arteries as early as 1 week after implantation. In the four implants that he performed on puppies sacrificed at 6 and 9 months, no increase in limb growth was noted.

FIGURE 7. Sources of Details of Operative Technique for Microvascularized Bone Grafting

| Microvascularized Bone Grafts | References |
|---|---|
| Posterior rib graft | Ostrup(111), Daniel(39) |
| Lateral rib graft | Harashina(69), Serafin(128) |
| Anterior rib graft | Ariyan(5) |
| Fibula | Taylor(144), Qingtan(115) |
| 2nd metatarsal | Rosen(120), O'Brien(102) |
| Ilium | Taylor(142,143) |
| General reference | Serafin, Buncke(126) |

FIGURE 8. Summary of the Experimental Assessment of Blood Vessel Implantation into Bone

| Author | Experimental Model | Number of Implants | Patency Rate |
|---|---|---|---|
| Woodhouse(159)1963 | brachial a.→intact humerus | 25 | 60% |
| Dickerson(44)1963 | femoral a.→intact femur | 13 | 77% |
| Boyd(18)1965 | femoral a.→intact femoral head | | |
| | intrapelvic route | 7 | 0% |
| | extrapelvic route | 7 | 100% |
| Dickerson(45)1966 | femoral a.→intact femur (puppies) | 16 | 69% |
| Hori(73)1979 | artery with side cuts →intact tibia | 14 | 100% |
| | artery without side cuts →intact tibia | 5 | 100% |
| | artery without side cuts →isolated tibia | 11 | 0% |
| | vein through intact tibia, then anastomosed to artery (A.-V. shunt) | 12 | 91% |
| | vein through isolated tibia, then anastomosed to artery | 22 | 32% |
| | vascular bundle→isolated tibia | 23 | 100% |
| | vascular bundle→tibia stored in 0.5% Hibitane for 7 days | 12 | 83% |
| | vascular bundle→tibial homograft | 6 | 67% |
| | vascular bundle→necrotic femoral head | 27 | 100% |

In 1963, Dickerson and Duthie also reported on artery implantation into intact dog bone(44). In 13 dogs, the femoral artery was implanted into the femur. The end of the implanted artery was maintained open by everting it over a sleeve of polyethylene tubing. The animals were sacrificed at 1 day to 9 weeks after the procedure. The patency of the vessels was assessed with cinefluorography, injection studies, and marrow pressure studies. They observed a 77% patency rate. They noted that intramedullary pressure was the same in both the implanted and the control femurs.

In 1965, Boyd and Ault reported femoral artery implantation into the femoral head of 14 dogs(18). Like Dickerson(44), Boyd everted the end of the artery over a polyethylene sleeve and did not make side cuts in the artery wall. None of the arteries implanted into 7 femoral heads via a pelvic route remained patent. This was felt to be due to vessel obstruction as it passed through the acetabulofemoral joint. On the other hand, they achieved 100% patency in those 7 animals in which the artery was implanted via an extrapelvic approach.

In 1966, Dickerson reported work on femoral artery implantation into the diaphysis of the intact femur in 16 puppies(45). In 5 additional control puppies, drill holes were performed, but no artery was implanted. He found that 69% of the implants remained patent. He noted an increased growth in 8 out of 11 of the successfully implanted femurs as compared to the contralateral unoperated side. He also observed an increase in the cortical width and in the size and number of Haversian canals in the implanted femurs.

In 1968, Dickerson published a picture of a small titanium end piece to keep implanted arteries patent(46).

In 1979, Hori, Tamai et al. published the most extensive experimental

FIGURE 9. Summary of the Clinical Use of Blood Vessel Implantation into Bone.

| Author | Number of Cases | Indications for Vessel Implantation |
|---|---|---|
| Torto(145)1967 | 36 | leg length discrepancy in polio children |
| Hori(73)1979 | 12 | avascular necrosis of the femoral head |
| | 9 | avascular necrosis of the lunate |
| | 2 | avascular necrosis of the talus |
| | 1 | avascular necrosis of the scaphoid |
| | 1 | Perthe's disease |
| | 1 | osteomyelitis of the humerus |
| | 1 | bone graft to tibial defect |

work on vessel implants to date(73). Nine different experiments were performed in 160 dogs. Unlike previous investigators, they did not leave the implanted vessel ends open. The vessels were ligated at the distal end before implantation.

They compared arteries with and without side cuts in their walls implanted into intact bone and found all vessels in both groups patent (Fig. 8, opposite p.22). They concluded that side cuts in the arterial walls were unnecessary for the maintenance of patency.

Hori et al. observed that arteries implanted into isolated avascular bone all thrombosed and therefore felt that the venous return available in intact bone was important to the maintenance of patency. They then demonstrated vascular connections between the artery and vein of an isolated vascular bundle with india ink perfusion. They felt that the intact venous return afforded by the vein present within a vascular bundle would permit the patency of implants, even in isolated bone. They went on to demonstrate this patency of vascular bundles in 23 out of 23 implants in isolated tibial segments.

In other experiments, Hori et al. observed patency in vascular bundles implanted in isolated tibiae which had been stored in 0.5% Hibitane for 7 days, in femoral heads in which they had previously induced avascular necrosis, and in tibial homografts.

Hori et al. found capillary budding off of the implanted vessels as early as 3 days after implantation. They observed the revascularization of an entire 7 cm segment of isolated tibia, and of entirely necrotic femoral heads by the neovasculature arising from implanted vascular bundles. They also noted new bone formation in these isolated necrotic bones and presumed that the responsible osteoblasts and osteoclasts had entered the bone via

the neovasculature eminating from the implanted vascular bundle.

II. Clinical Work in Blood Vessel Implantation into Bone

In 1967, Torto and Zannini(145) published a clinical report of vessel implantation into the femoral epiphyses of polio children with leg lenght discrepancies in order to stimulate bone growth. They operated on 36 children with leg length discrepancies of 2 to 10 cm. They reported on only 24 of these cases. The follow up period was 10 to 20 months.

In 20 of the 24 cases on which they had reported, they had implanted the anastomotica magna artery directly into the distal epiphysis of the femur. They achieved a 0.5 to 2.0 cm decrease in leg length discrepancy in 13 of these 20 cases (65%). In the other 4 of the 24 cases, the distal end of a saphenous vein graft was anastomosed to the femoral artery end to side, and the proximal end of the graft was implanted directly into the distal femoralepiphysis. Two of these 4 cases (50%) achieved a 1 to 2cm decrease in leg length discrepancy. In the 9 of these 24 cases in which the leg length discrepancy was not decreased, they felt that they had halted the increase in the rate of leg length discrepancy in 7 cases. They concluded that arterial implantation into epiphyses was a valid procedure for stimulating bone growth in children. It was felt that growth stimulation was superior if the children were less than 10 years of age.

In 1979, Hori et al. reported on the clinical use of vascular bundle implantation into bone in 27 patients(73). Their indications in each case are summarized in Figure 9 (opposite p.23). Their follow up ranged from 4 months to 3 years. The results of the operations were only presented for their cases of avascular necrosis of the scaphoid and lunate bones.

In their 9 cases of avascular necrosis of the lunate, 8 showed satisfactory results clinically and radiologically. Good results included decreased pain, increased range of motion, and radiological evidence of bony remodelling. In their case of avascular necrosis of the proximal scaphoid, union and remodelling of the bone were radiologically evident at 3 months after operation.

4)Methods of Analysis of Bone Graft Viability

The multiple number of methods of analysis in experiments dealing with bone grafts indicate that no single mode of assessment is satisfactory. This is particularly true of the assessment of bone graft viabilily, a key factor in the evaluation of the vascularization of bone grafts. Following is a review of the capabilities and the shortcomings of the three most commonly used methods of assessment of bone viability; tetracycline labelling, $Tc^{99}$ scintigraphy, and histology.

a)Tetracycline Labelling and Bone Viability

The tetracyclines belong to a family of compounds called polycyclic napthacene carboxamides(130). In addition to its properties as an antibiotic, tetracycline binds to calcium containing crystals and subsequently fluoresces in ultraviolet light.

The fluorescence of tetracycline in undecalcified bone was first reported by Milch in 1957(100). The mechanism of tetracycline binding to actively forming bone remains unclear. Whether osteoblasts concentrate the tetracycline in the new matrix, or whether the tetracycline passively diffuses into calcifying areas in living bone is unknown. It is known that tetracycline is chemically adsorbed to $CaCl_2$ in solution(130), to $CaCO_3$ and hydroxyapatite crystals in vitro(74), and to boiled bone, alcohol-ether extracted bone, or excised dead bone in vitro as well(43,139).

Tetracycline will exhibit fluorescence in all of these situations. Trtracycline labelling also occurs in non osseus soft tissue calcifications in vivo(68).

Because tetracycline is chemically adsorbed to bone and fluoresces in vitro, the fluorescence of tetracycline per se is not an indication of bone viability, although circulation or plasmatic diffusion is necessary for the tetracycline to reach the bone in vivo. However, if two subsequent doses of tetracycline result in two distinct fluorescent rings in a Haversian system, this is taken as evidence that active mineralization has occurred in the interim, an indicator of osteoblast viability(9). Because of this property, most investigators agree that sequential tetracycline ring fluorescence is the sine qua non of bone viability(40).

The rate of bone apposition can be accurately calculated by measuring the distance between sequential tetracycline fluorescent rings in a known amount of time(62). Bone apposition may also be labelled by fluorochromes of other colors. Hematoporphyrin(50) and alizarin(150) produce a red fluorescence, DCAF(50) and calcein(150) produce a green fluorescence, and xylenol orange(150) produces, as expected, an orange fluorescence. More than one color may be used in a given experiment to study bone deposition at different points in time(105).

In active bone turnover, tetracycline is preferentially complexed in the zone of rapid mineralization(136). Histological examination has shown that the tetracycline label does not cross osseus lamellae, cement, or other growth lines(70). Once incorporated into the bony matrix, it remains there until the matrix is remodelled(21,130).

The major shortcoming of tetracycline labelling in the clinical setting is that an invasive bone biopsy must be performed to reveal its

presence. Another problem is that tetracycline is not incorporated into all parts of normal living bone(11). This may be because there are cycles of bony remodelling, with alternating periods of quiescence and activity(137). This may also be due to the local autoregulation of circulation in bone(32,119). As some areas of circulation are intermittently shut down, a given tetracycline dose is not permitted to enter that area.

Because tetracycline is not incorporated into all parts of normal living bone, it should be administered several times, and the bone biopsy should not be too small if serial ring labelling is to be ensured and false negative results are to be avoided. Frost(62) and Puranen(114) have elaborated the details of bone processing and photography for tetracycline labelling.

In summary, sequential tetracycline lamellar ring labelling is the standard of bone viability by which others are measured. When properly performed, this test is accurate and reliable. Its main drawback is that it requires an invasive bone biopsy, which is difficult in the clinical setting.

b)Technitium$^{99}$ Scintigraphy and Bone Viability

Bone scanning with radioactive tracers has revolutionized the investigation of the skeletal system in the last ten years. Wheras conventional X rays will show a change when there has been a 30 to 50% turnover in bony mineral, scintigraphy will show a change with an alteration of only 5 to 15%(86). The evaluation of the viabilily of bone grafts with scintigraphy has received a lot of recent attention, some of it controversial(13,65).

$Tc^{99}$ is a radioactive tracer. It is inexpensive, and delivers a low dosage of radiation to the patient because of its short half life of 6 hours. By itself, it has little affinity to bone(78). However, it forms a stable

complex with several of the family of diphosphonates, which do have a high affinity for bone.

Like tetracycline, $Tc^{99}$ diphosphonates do not bind only to living bone. They will bind to calcium containing crystals in vitro(59), and to non osseus soft tissue calcifications in vivo(67). Like tetracycline, $Tc^{99}$ diphosphonates are deposited primarily at the site of active mineralization in bone. Also, like tetracycline, it is not known whether osteoblasts concentrate the $Tc^{99}$ diphosphonates in the new matrix, or whether the $Tc^{99}$ diphosphonates get to the calcifying areas of bone only by passive diffusion(58). However, it is known that the bony uptake of $Tc^{99}$ diphosphonates in vivo is related to blood flow(83,121), and to bone metabolism(32,58,96).

Unlike tetracycline labelling, which infers bone viability by the bone formation present between the sequentially labelled lamellar rings, $Tc^{99}$ diphosphonates have no such ability to infer bone viability per se, as a bone scan is a single measurement in time. What a positive bone scan does show, however, is that there is bone which is being perfused either either by active circulation or by plasmatic diffusion. Otherwise, the $Tc^{99}$ could not get to the bone and the scan would be cold. As most perfused bone is alive, for practical purposes, $Tc^{99}$ diphosphonates do reflect bone viability. This has been substantiated clinically in situations such as cold scans showing dead bone in frostbite(85). In avascular necrosis of the femoral head, D'Ambrosia(37) has shown a good correlation of $Tc^{99}$ scintigraphy and bone viability as measured by tetracycline labelling.

$Tc^{99}$ has gained popularity in the evaluation of the progress of bone grafts because it is non invasive. Unlike tetracycline labelling and histology, assessment with scintigraphy does not require a bone biopsy.

Experimentally, scintigraphy has been shown to be accurate and more sensitive than X rays in the prediction of bone graft failure and non union(22,88,132,147). In animal studies, if a bone graft is to take successfully, hot areas develop on a bone scan at the graft host junction, usually by 7 to 14 days(147). If this does not occur, it is a sign of impending nonunion(132). With continuing successful take, the hot areas gradually move in from the graft host junctions to the center of the graft(22,132). If the entire graft remains cold, it is a sign of graft failure and impending resorption(132).

Experimentally, bone scanning within a week of the transfer of microvascularized bone grafts has been shown to accurately reflect the patency of the vascular anastomoses(13,17,42,72,88). Scintigraphy is a safer, and perhaps more accurate method than angiography for this assessment(72). The grafted bone shows up as totally hot on the bone scan, beginning immediately post operatively, if the vessels are patent. This stands to reason, as a successfully perfused graft will receive the $Tc^{99}$ diphosphonate label, while one which has a blocked blood supply will not.

The clinical use of bone scanning to assess the patency of the blood supply to microvascularized bone grafts is still largely limited to case reports(87). However, this role may expand. In view of the fact that microvascularized bone grafts can withstand long ischemic periods(10), a cold scan the morning after a microvascular bone transfer may become an indication for reexploration and the revision of the microvascular anastomoses.

In summary, although $Tc^{99}$ scintigraphy does not imply bone viability per se, it does indicate that the bone is being perfused either by active circulation or by plasmatic diffusion. The technique is non invasive. Experimentally, $Tc^{99}$ scintigraphy has permitted the prediction of non union

and graft failure in the conventional bone graft, and the patency of the vascular anastomoses of microvascularized bone grafts.

c)Histology and Bone Viability

The most sensitive histological indicator of bone viability is the appearance of its marrow. The difference between viable and necrotic marrow is easily distinguished. Necrosis in the marrow also leaves an indicator of its presence after marrow revascularization, as a previously necrotic marrow will show fibrosis instead of the active cellular elements seen in normal marrow. However, after circulatory arrest in bone, it can take up to 3 or 4 days for necrotic changes to become evident in the bone marrow.

On the other hand, life and death in the cortex of bone is very difficult, if not impossible, to certify histologically. With bone death, the cortical lacunae usually don't show osteocyte loss until 7 to 14 days, and this is often incomplete until 3 to 4 weeks. Also, there is a normal "physiological" loss of osteocytes in lacunae with aging(31). In addition, the loss of osteocytes in lacunae may be an artifact of histological processing(9). For these reasons, the presence or absence of osteocytes in the lacunae of bone are unreliable signs of bone viability or death.

Special stains, such as von Kossa's stain and toluidine blue will indicate newly formed uncalcified osteoid, which reflects the degree of metabolic activity by living bone(6). However, this technique, like standard histology, does not allow one to affirm or dely the death of cortical bone.

Therefore, although histology can certify the death of the marrow of bone, it cannot do so for the cortex. Because of this drawback, histology is now usually used as an adjunct to the more reliable standard of serial

tetracycline labelling, and to the newer technique of $Tc^{99}$ scintigraphy in most experimental and clinical assessments of bone viability.

5)The Purpose of this Study.

The author has developed a technique which permits the assessment and correlation of tetracycline labelling, $Tc^{99}$ scintigraphy, histology, and the three dimensional vascular perfusion pattern within a single bone graft specimen. This is the first study in which all of these 4 parameters have been correlated within the same bone graft specimens. Armed with this technique, the purpose of this study is to answer the following questions:

1. What is the 3 dimensional appearance of the vascular tree in
   a) conventional non vascularized bone grafts with and without periosteum?
   b) bone grafts vascularized by vascular bundle implantation?
   c) bone grafts vascularized by microvascular anastomosis of the nutrient vessels?
   d) bone grafts vascularized by wrapping them with dermis island flaps?
2. What are the interrelationships of the vascular tree, tetracycline labelling, $Tc^{99}$ scintigraphy, and histology within a bone graft?
3. Is $Tc^{99}$ scintigraphy
   a) an accurate method of assessing the patency of anastomoses in microvascularized bone grafts?
   b) a reliable indicator of the presence of circulation in any bone graft?
   c) an indicator of bone viability in bone grafts as judged by tetracycline labelling?

FIGURE 12

# Vascular Bundle Implant into Rib Graft

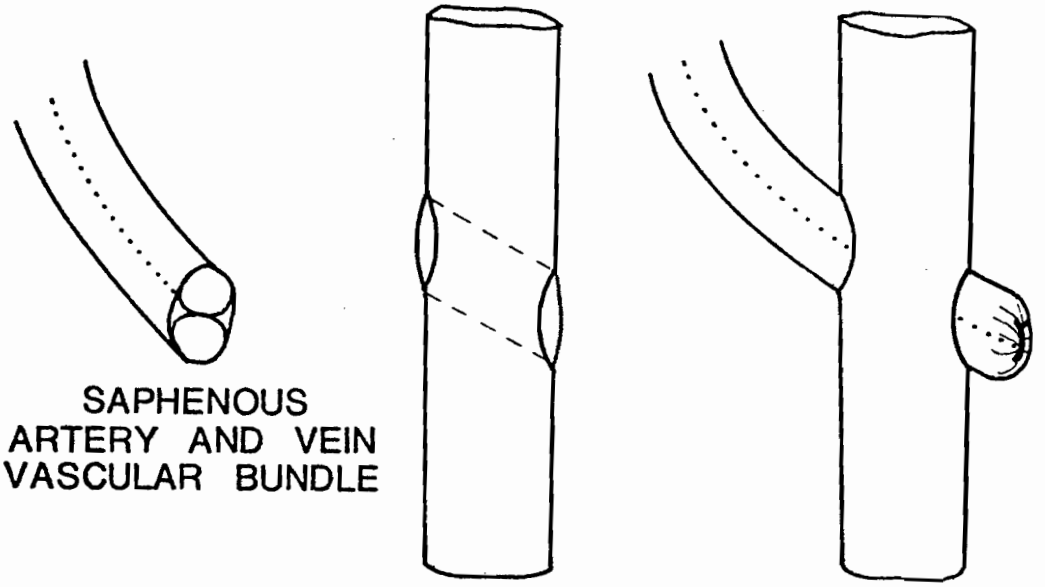
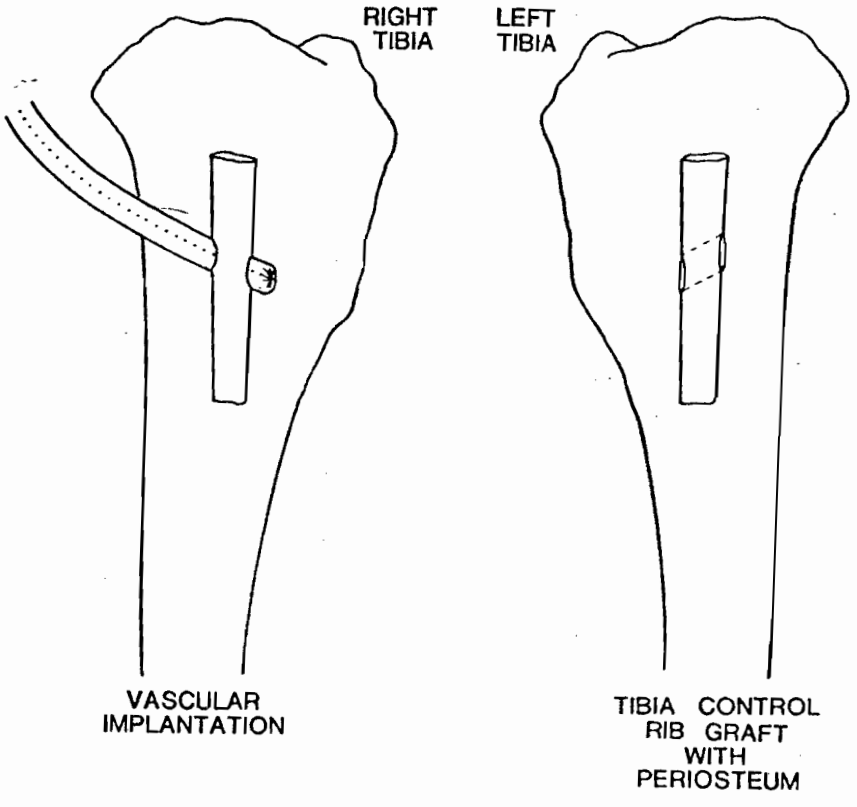



FIGURE 13

## Vascular Bundle Implant into Rib Graft



Experimental Group 1. - Conventional rib grafts with and without periosteum

In this group, 4 rib grafts were harvested and placed in each animal as illustrated in Figure 11. In one leg, a rib graft with periosteum was placed in the tibial window, and a rib graft stripped of periosteum was placed in a subcutaneous tunnel. In the other leg, a rib graft stripped of periosteum was placed in the tibial window, and a rib graft with intact periosteum was placed in a subcutaneous tunnel.

A total of 6 animals were used in this group. One dog was sacrificed one week post operatively, and the other 5 were sacrificed at 4 weeks.

Experimental Group 2. - Vascular bundle implantation into rib grafts

In this group, 2 rib grafts with periosteum were harvested in each animal. A hole with a diameter slightly larger than that of the vascular bundle to be implanted was drilled tangentially in both grafts as illustrated in Figures 12 and 13. In the control leg, no vascular bundle was implanted into the rib graft drill hole. In the experimental leg, the previously isolated peroneal (fibular) vascular bundle was ligated and divided distally. The ligatures were left long and threaded through the eye of a needle. The needle, ligatures, and vascular bundle were then pulled through the hole drilled in the rib graft. The vascular bundle was secured by tying the long end of its ligatures around the graft.

A total of 15 animals were used in this group. Eight of the dogs were sacrificed at 1 week, and the other 7 were sacrificed at 4 weeks.

Experimental Group 3. - Microvascularized rib grafts

In this group, an 8 to 10cm segment of rib was disarticulated from the costovertebral joint as described by Ostrup(106). The pleura, intercostal muscles, and the intercostal artery and vein adjacent to the rib

FIGURE 14

# Microvascular Anastomosis of Rib Graft Nutrient Artery

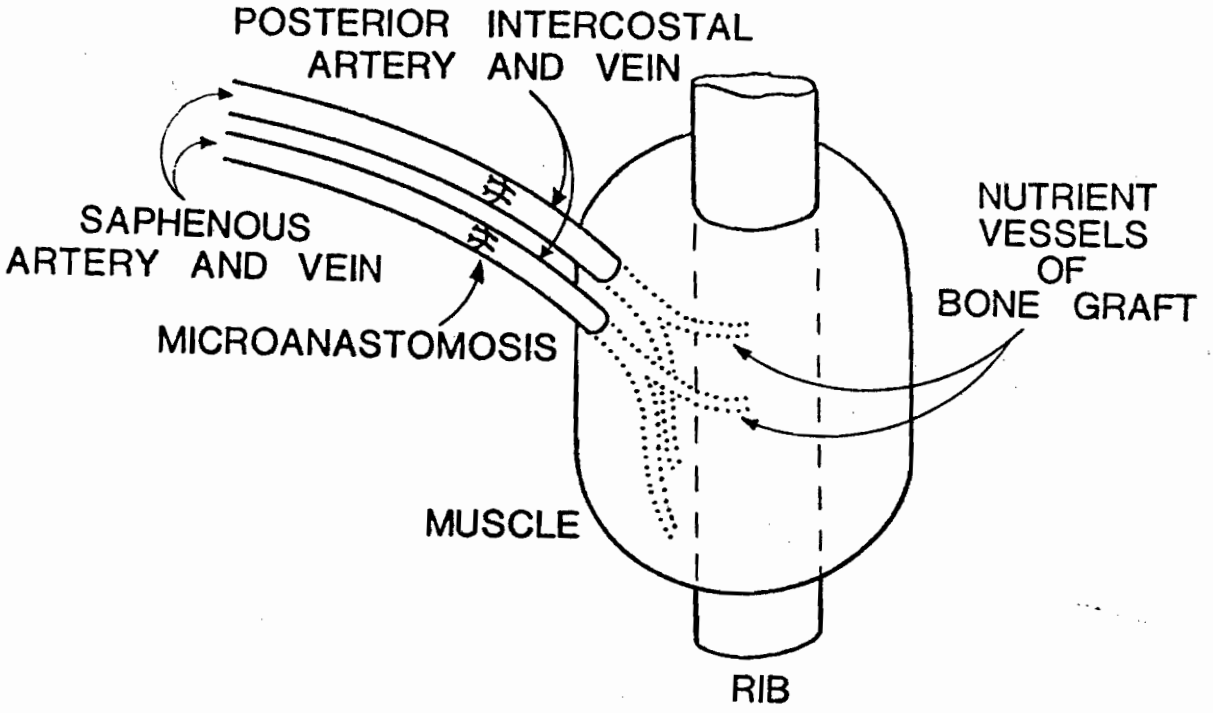
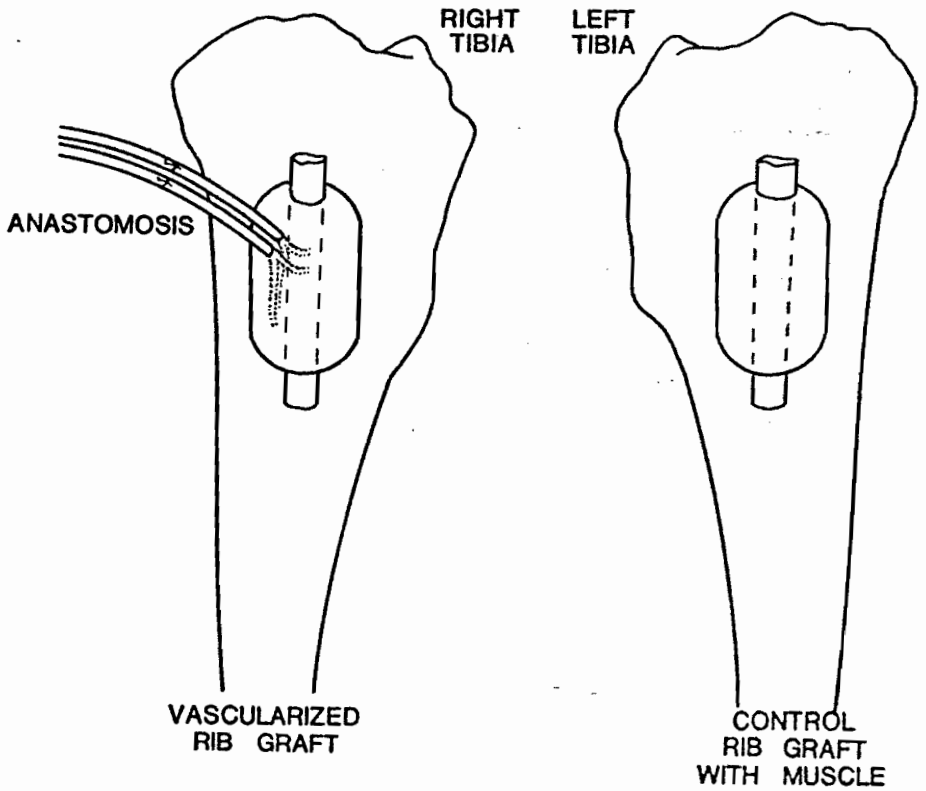



FIGURE 15

## Microvascular Anastomosis of Rib Graft Nutrient Artery



were harvested with it as a composite graft. The proximal portions of the vessels were dissected off of the chest wall as far posteriorly as the azygous vein. At this level, they were ligated and divided, yielding a vascular pedicle measuring a maximum of 1 cm. To elongate this pedicle, the posterior rib was divided between its two vertebral articulation sites. The nutrient artery to the rib remained intact as it entered the bone just anteriorly to the costovertebral joint(110).

The posterior 4 cm of the composite graft was placed in the tibial window of the experimental leg. Its posterior intercostal artery was anastomosed either end to end,or end to side with the previously isolated plantar saphenous artery. The posterior intercostal vein was anastomosed end to end with the previously isolated dorsal saphenous vein. The resulting microvascularized rib graft is illustrated in Figure 14. The only anticoagulation used was the irrigation of the vessel ends with a 1/1000 heparin solution at the time of anastomosis. The anastomoses were performed with 10-0 nylon interrupted sutures (10 V 34) under Zeiss OPMI 8 operating microscope magnification.

The anterior 4 cm of the composite graft was placed in the tibial window of the contralateral control leg without vascular anastomosis, as illustrated in Figure 15.

A total of 19 animals were used in this group. Ten of the dogs were sacrificed at 1 week post operatively, and the remaining 9 dogs were sacrificed at 4 weeks.

Experimental Group 4. - Dermis island vascularization of rib grafts

In each animal of this group, 2 rib grafts with periosteum were harvested. In the control leg, the rib graft was placed in the tibial window without furthur manipulation. In the experimental leg, a 4 x 1 cm

FIGURE 16

# Dermis Island Pedicle

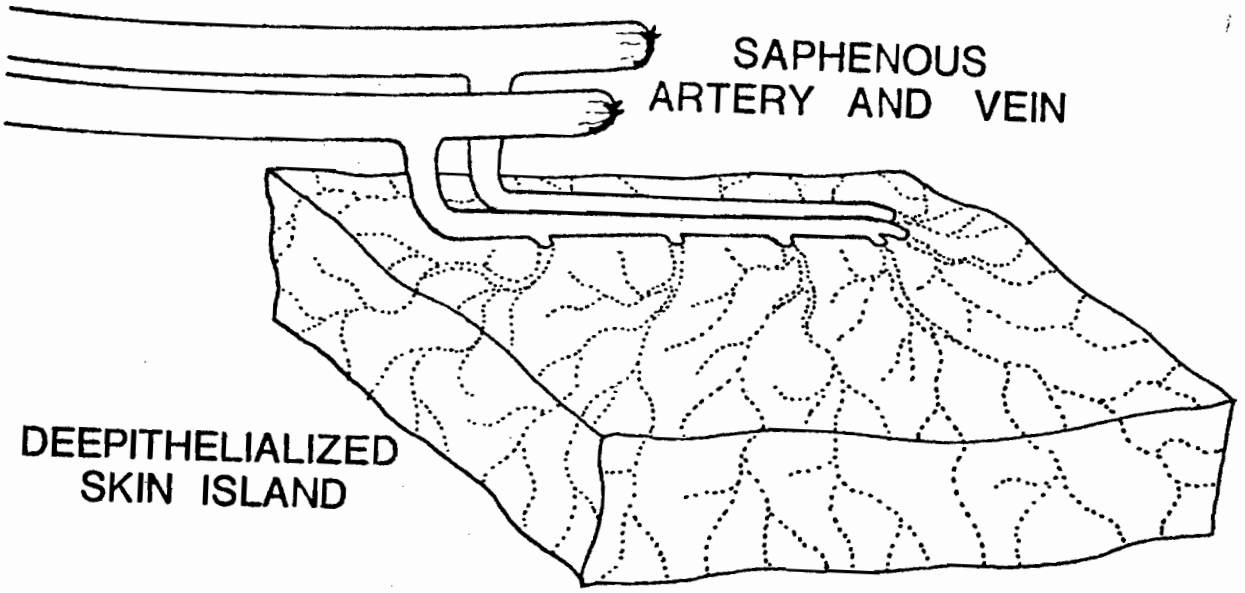
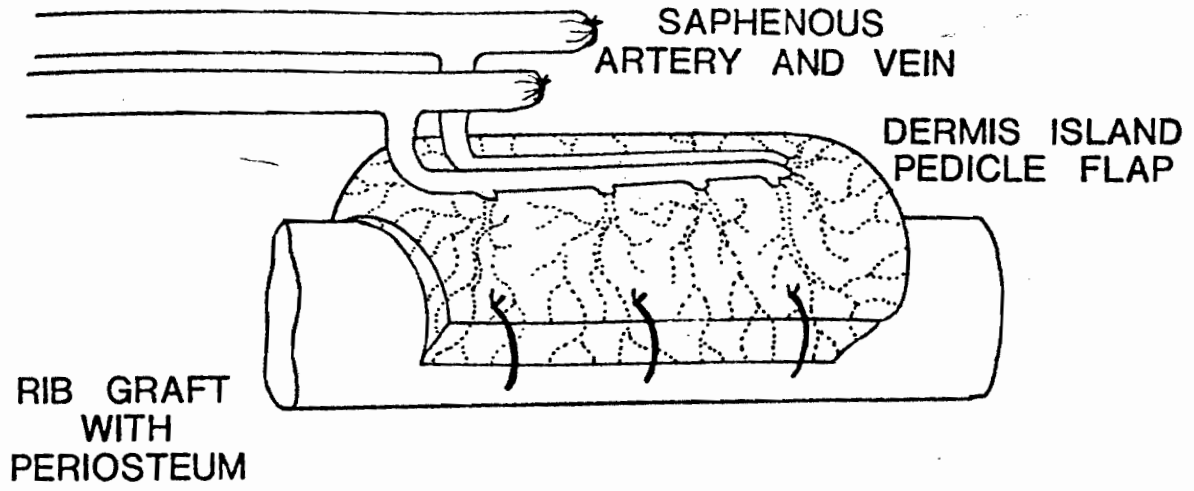



FIGURE 17

# Dermis Island Pedicle Flap Onlay to Rib Graft



skin island was isolated on its vascular pedicle which consisted of the dorsal saphenous artery and vein. The skin was deepithilialized with scalpel dissection (Fig. 16).

Viability of the dermis island was confirmed by the observation of brisk punctate dermal bleeding, and of dermis island fluorescence with a Wood's lamp following intravenous fluorescein injection (250mg/animal).

The dermal surface of the flap was then wrapped around the rib graft as illustrated in Figure 17. Following this, the rib graft was placed in the experimental leg's tibial window as illustrated in Figure 18 (opposite p.36).

Evaluation of the Rib Grafts

Each bone was assessed with tetracycline labelling, $Tc^{99}$ scintigraphy, histology, and a study of its 3 dimensional Microfil perfused vascular tree. The manner in which this was done is summarized in Figure 19 (opposite p.36).

Each of the four days prior to sacrifice, the animals received an intramuscular injection of 250mg of oxytetracycline. Five hours before sacrifice, they were given an intravenous injection of 10mCi of $Tc^{99}$ M.D.P. (methylene diphosphonate).

At the time of sacrifice, the root of the posterior intercostal of the unoperated 8th or 9th rib, as well as both femoral arteries at the groins, were isolated and injected with an orange perfusion compound called Microfil.* The 1 micron particle size of this silicone rubber compound, along with its low viscosity, allows filling of the entire vasculature of an organ(20). In each animal, Microfil was injected with firm manual pressure in the volume of 40cc for the rib, and 190cc per leg. The animals were refrigerated overnight to ensure solidification of the rubber.

*Canton Biomedical Products Inc., Boulder, Colorado 80302

FIGURE 18

Dermis Island Pedicle Flap Onlay to Rib Graft

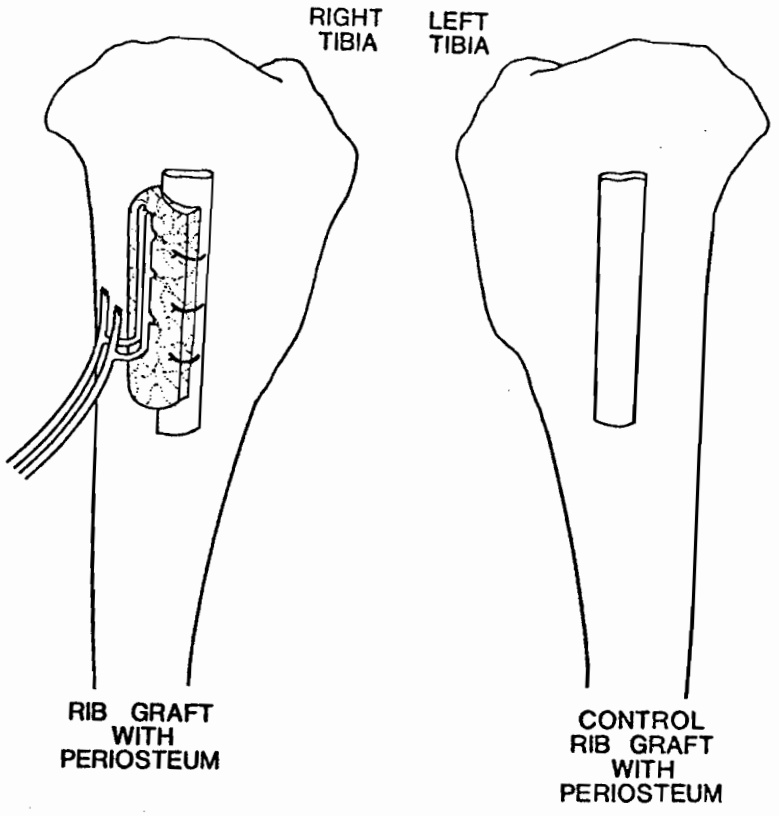



FIGURE 19. Summary of Rib Graft Evaluation Technique

oxytetracycline 250mg I.M./day x 4 days before sacrifice

↓

10mCi of $Tc^{99}$ M.D.P. I.V. 5 hours before sacrifice

↓

sacrifice - femoral arteries and normal 9th rib perfused with Microfil

↓

overnight refrigeration

↓

rib grafts and normal rib removed, stripped of soft tissue, and placed in formalin in test tubes (normal rib cut into 4)

↓

$Tc^{99}$ bone scan of rib grafts and normal rib segments

↓

6 biopsies per graft and 2 biopsies per normal rib
1/2 of the biopsies for histology, 1/2 for tetracycline labelling

↓

bone clearing for evaluation of Microfil filled vascular tree

↓

correlation of vascular tree, $Tc^{99}$ bone scans, histology and tetracycline labelling

The morning after sacrifice, each rib graft and the perfused normal rib were isolated and stripped of soft tissue. The proximal end of each graft was labelled with a fine drill hole. The normal rib was divided into four 4 cm segments. The specimens were placed in test tubes containing 10% formalin.

The rib grafts were placed adjacent to the segments of normal rib under a Searle scintillation gamma camera. The proximal ends of the grafts were labelled with a radioactive marker adjacent to the bones for the future recognition of graft orientation. The qualitative $Tc^{99}$ uptake pattern of the rib grafts was compared to that of the normal rib segments on the resulting bone scan.

The bones were left aside for 4 to 6 days to permit the decay of their radioactivity. Each rib graft was then biopsied at the proximal and distal ends, and at the center, as seen in Figure 20 (opposite p.37). All 3 areas were biopsied twice with a standard 4 inch diamond rotating blade on a Bronwill band saw. In this way, each rib graft yielded 3 biopsies for histology taken adjacent to 3 biopsies for tetracycline labelling.

The tetracycline biopsies, which had an average width of 400 microns, were stored in the dark in 75% ethanol. Each specimen was observed and photographed with incident fluorescence using a Leitz Ortholux fluorescence microscope fitted with a Ploem incident illuminator with dichroic mirror housing filters H2(390-490/515nm) or D(355-425/460nm). The photography was performed with 400 ASA Ektachrome daylight film.

Biopsies for histology were decalcified either in Fotheringham's* solution or in 10% formic acid. They were then submitted for routine histological processing and stained with hematoxylin and eosin.

After the rib graft and normal rib specimens were biopsied, the

*Courtesy of Mr. J. Fotheringham, Dept. of pathology, Montreal Gen. Hosp.

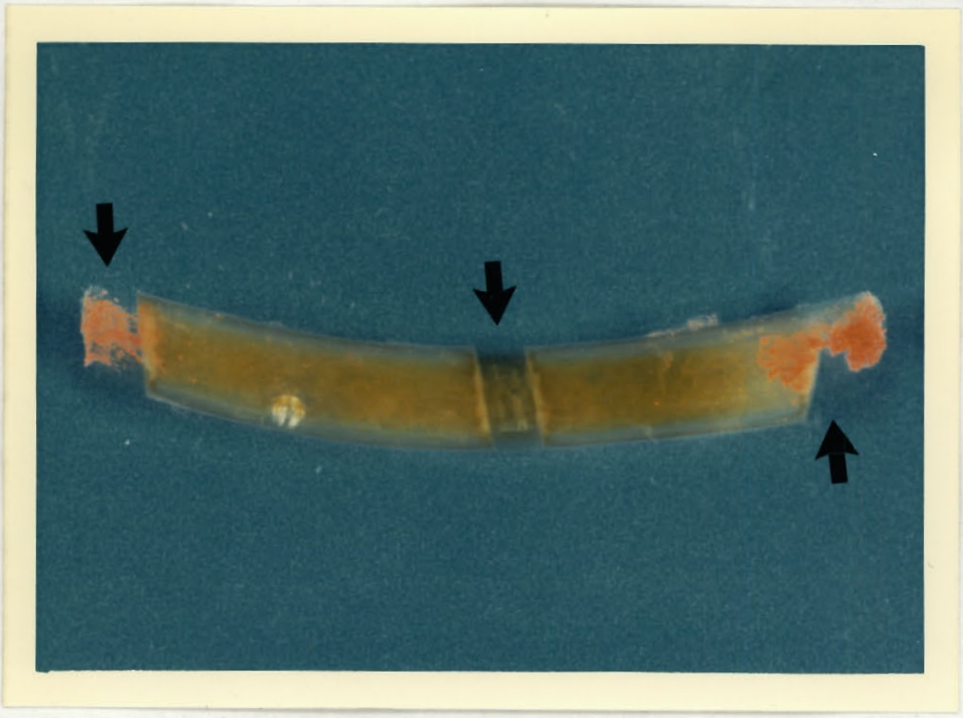


FIGURE 20. Conventional rib graft without periosteum placed in tibial defect. The graft was perfused with Microfil and cleared 1 week after grafting. Note the central avascular area. This graft, as all the others, was biopsied twice at each site pointed out by the arrows to yield 6 biopsies; 3 for histology, and 3 for tetracycline labelling. These were then correlated with the presence or absence of vascularization.

bones were cleared with a technique similar to that of Gelberman(64). They were decalcified either in Fotheringham's solution or in 30% formic acid until soft, usually 5 to 8 days. The bones were then bleached in 15% hydrogen peroxide for the next two days. They were dehydrated in 75% and 95% ethanol for 24 hours each, followed by absolute ethanol for 7 days. The solution was changed three times in that week. The bones were then placed in chloroform for 3 days, and then finally in methyl salicylate until clear, usually 2 days. They were photographed in 50% methyl salicylate and 50% benzyl benzoate.

The three dimensional Microfil filled vascular tree findings were then correlated with the findings at the histology and tetracycline labelling biopsy sites, as well as with the findings of the $Tc^{99}$ bone scans.

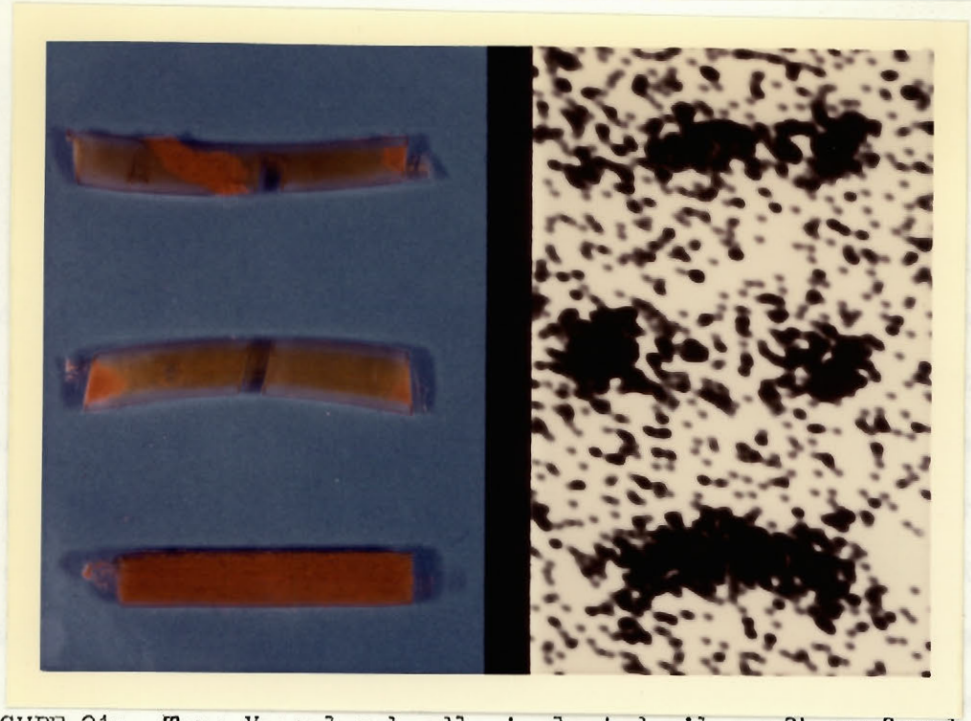


FIGURE 21a. Top: Vascular bundle implanted rib graft perfused with Microfil and cleared 1 week after implantation.
Center: Conventional rib graft perfused with Microfil and cleared 1 week after grafting.
Bottom: Normal rib segment from the same animal as the two bones above it, perfused with Microfil and cleared.
Right: Bone scans of the bones on the left.

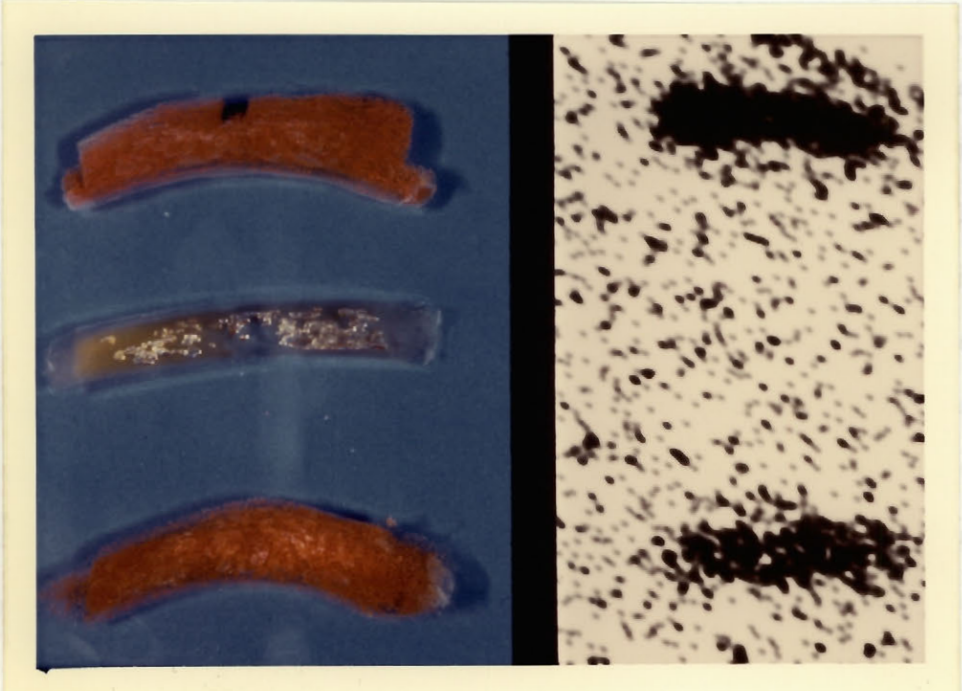


FIGURE 21b. Top: Microvascularized rib graft perfused with Microfil and cleared 1 week after grafting.
Center: Control graft surrounded with muscle but without vascular anastomosis, perfused with Microfil and cleared

The Correlation of the Vascular Perfusion Pattern with Bone Scintigraphy

There were 90 grafts in which both the bone scan and the Microfil perfusion of the vascular tree followed by bone clearing were performed. In 90 out of 90 of these grafts, the bone scans accurately reflected the various vascular patterns observed. Hot areas on a bone scan occurred in the same areas of a graft that Microfil perfusion did, whether this was at one end only, at both ends, at one end and the center, at both ends and the center, or in the entire graft (Fig. 21). If there was no Microfil in the graft, it was cold on the bone scan. Therefore, there was a good correlation of the vascular perfusion and the hot areas on the bone scans in all of the bone grafts.

The Correlation of the Vascular Perfusion Pattern with Histology

The Microfil was consistently observed within the confines of the vascular spaces in the histological specimens. However, not all vascular spaces were completely filled with Microfil, particularly in the larger vascular lakes of the medullary cavities. Nevertheless, the areas seen to contain Microfil in the cleared bone grafts correlated very well with the histological picture of active circulation in the bone marrow. Evidence of active marrow circulation included normal marrow cellular activity, new bone formation, and leukocyte infiltration or fibrosis in previously necrotic marrow.

On the other hand, histology in avascular areas such as seen in the center of the graft illustrated in Figure 20 almost always revealed marrow necrosis without any of the above good histological evidence of active marrow circulation. Therefore, there was a good correlation of Microfil vascular perfusion and histology.

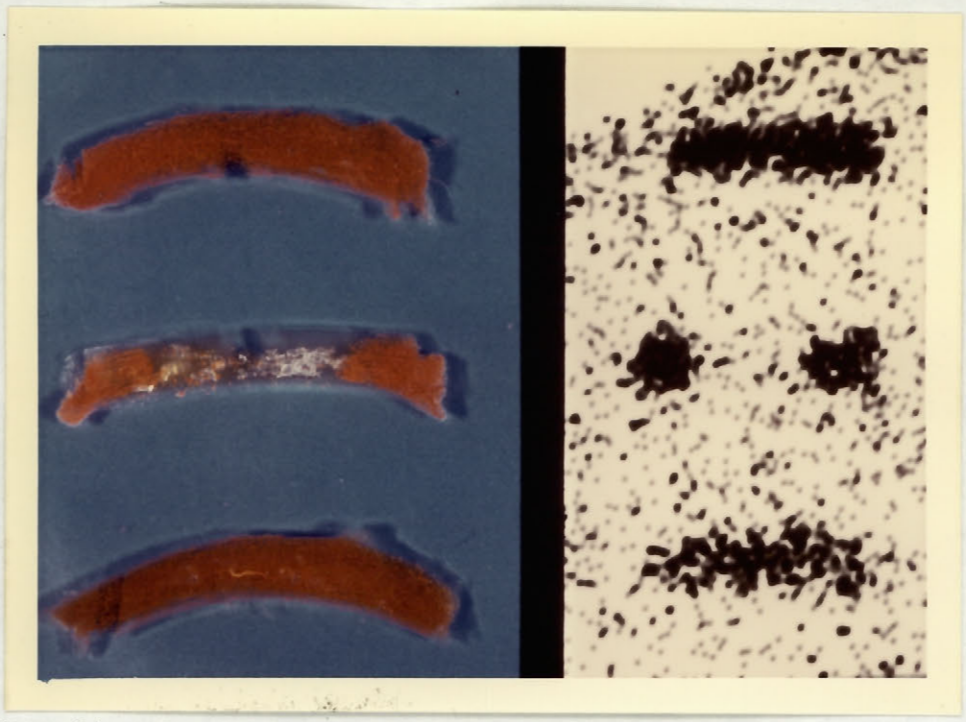


FIGURE 21c. Top: Microvascularized rib graft perfused with Microfil and cleared 1 month after grafting.
Center: Control graft surrounded with muscle but without vascular anastomosis, perfused with Microfil and cleared 1 month after grafting.
Bottom: Normal rib segment from the same animal as the two bones above it, perfused with Microfil and cleared.
Right: Bone scans of the bones on the left.

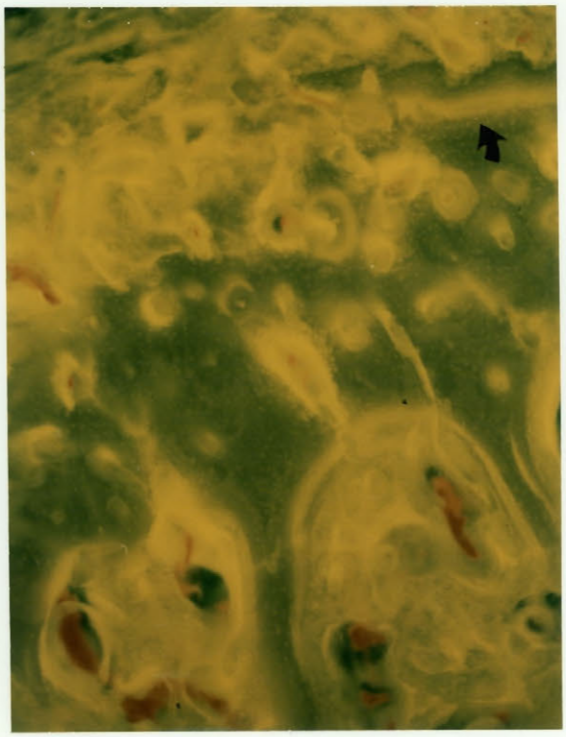


FIGURE 22. Tetracycline labelling biopsy of a conventional graft area perfused with Microfil. The Microfil shows up as red and the tetracycline fluorescence as yellow in the photograph. Recanalizing vasculature in a previously necrotic marrow is seen at the bottom. Periosteum (double line), and periosteal new bone formation is seen at the top.

The Correlation of the Vascular Perfusion Pattern, Tetracycline Labelling, Bone Scintigraphy, and Histology

In the areas of the cleared grafts where Microfil perfusion was evident, there was a good correlation of Microfil perfusion and tetracycline labelling. The greater the amount of Microfil, the more intense was the tetracycline labelling (Fig. 22). All of the areas of the grafts perfused with Microfil were also hot on the bone scans and showed good histological evidence of bone circulation and viability in the marrow.

However, there were several biopsies of areas within grafts where Microfil was absent. These avascular areas were commonly found in failed or control microvascular rib grafts, and in the centers of conventional rib grafts where the Microfil had entered only a few millimeters at either end of the grafts (see central avascular area in graft in Fig. 20 opposite p. 37). There was positive tetracycline labelling in 10 out of 14 biopsies of such central avascular areas where the biopsies were at least 10 mm from the nearest Microfil perfusion, which showed no good evidence of histological viability, and which were taken from areas shown as cold on the bone scans. In these 10 biopsies, double tetracycline rings were observed in 5, while only single rings were seen in the other 5.

In summary, Microfil vascular tree perfusion, bone scintigraphy, and histology correlated well with each other in both perfused and avascular areas of the rib grafts. They also correlated well with tetracycline labelling in perfused areas of the grafts. However, in avascular segments of bone grafts, tetracycline labelling was frequently positive wheras bone scintigraphy was cold and histology revealed marrow necrosis with no good evidence of bone circulation and viability.

FIGURE 23. Conventional Rib Grafts - Experimental Group 1.

| Rib Graft Type | Time after Grafting | Number of Grafts |
|---|---|---|
| conventional with periosteum placed in tibial window | 1 week | 6 |
| | 4 weeks | 9 |
| conventional without periosteum placed in tibial window | 1 week | 1 |
| | 4 weeks | 5 |
| conventional with periosteum placed subcutaneously | 1 week | 1 |
| | 4 weeks | 4 |
| conventional without periosteum placed subcutaneously | 1 week | 1 |
| | 4 weeks | 4 |
| total number of conventional grafts | | 31 |

FIGURE 24. The Depth of Medullary Vascular Penetration in Conventional Rib Grafts at 1 Week after Grafting.

| Conventional Graft Type | Graft Number | Proximal Invasion(mm) | Distal Invasion(mm) |
|---|---|---|---|
| with periosteum→tibia | 2 | 14 | 0 |
| | 8 | 8 | 6 |
| | 16 | 3 | 4 |
| | 22 | 3 | 28 |
| | 28 | 6 | 3 |
| | 136 | 3 | 9 |
| no periosteum→tibia | 13 | 4 | 7 |
| with periosteum→subcutaneous | 14 | 6 | 1 |
| no periosteum→subcutaneous | 15 | 3 | 5 |

For the above conventional rib grafts placed in tibial defects, the mean medullary vascular penetration is 7 mm, and the median is 4 mm.

Conventional Rib Grafts

As can be seen in Figure 23, 31 conventional bone grafts were studied. By one week after grafting, all of the grafts were penetrated by vessels invading the medulla at the graft host junctions (see Fig.20, opposite p.37, and Fig. 21a center, opposite p.39). The depth of vascular medullary penetration in the 7 conventional grafts placed in the tibial defects ranged from 0 to 28 mm with an average of 7 mm and a median of 4 mm as seen in Figure 24. The large central portion of these grafts was still avascular at this time.

Only 3 of the 9 grafts examined at one week revealed periosteal vascular invasion. The vascular contribution of these periosteal perforators was far less important than that of the medullary vascular penetration at this time.

In the 22 conventional grafts examined at 4 weeks, the vascular tree was usually seen coursing throughout the entire lenght of the graft, as seen in Figure 25 (opposite p. 42). However, when compared to perfused cleared segments of normal rib, the vessel distribution in these grafts appeared sparse with relatively large areas still avascular. The vessels eminated from both medullary and periosteal sources in all of the grafts. Periosteal perforators contributed a good deal more to circulation at four weeks than they did at one week.

There was no observable difference in the extent or pattern of vascular invasion among the four groups of conventional rib grafts. The conservation or stripping of periosteum did not appear to alter vascularization, nor did the location of the bone graft, be it within a tibial defect or placed subcutaneously. The vascular tree seen in different conventional bone grafts within the same animal were similar, but there were notable differences among the grafts of different animals. In some

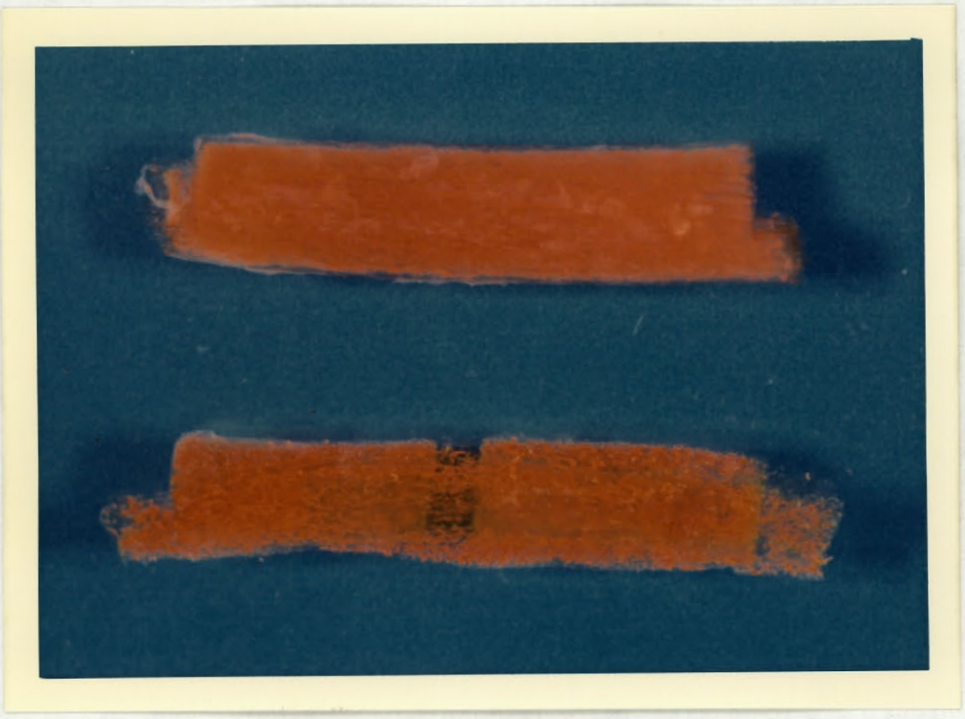


FIGURE 25. Top: Conventional rib graft placed in tibial defect, then perfused with Microfil and cleared at 4 weeks after grafting.
Bottom: Normal rib segment perfused with Microfil and cleared.
(see text p.41 for details)

animals, graft vascular invasion was twice as advanced as that seen in other animals with the same types of grafts in the same periods of time.

The bone scintigraphy accurately reflected the vascular penetration in each individual conventional graft. As a general pattern, the bone scans were usually hot at both ends and cold in the middle, as seen in the center of Figure 21a, opposite p. 39. At one month, they were usually entirely hot. Both of these patterns reflected the usual graft vascular penetration of the conventional grafts. Interestingly, the scans of the conventional grafts assessed at one month were usually hotter than those of normal rib segments of the same animals, despite the fact that the grafts were more sparsely vascularized than the normal rib segments.

The histological picture observed in the medulla was similar in all 4 groups of conventional bone grafts. Where there was no Microfil vascular perfusion, the marrow contained acellular necrotic tissue. In areas adjacent to neovascular invasion, populations of white cells were clearing up the debris of necrotic material. In areas of recent vascular invasion, fibrosis had occurred with fibrous tissue filling in the spaces in the marrow. In areas of well established vascularization, new bone formation was seen over the old necrotic bone in the medulla. In the most mature areas examined at 1 month, this new woven bone was gradually filling in the old marrow cavities and beginning to take on the appearance of cortical lamellar bone.

Histological changes in the cortex parallelled those of the medulla, but the progression was slower. At one week, little change could be detected except for the necrosis of tissue seen in the larger Haversian canals. Neovascular invasion of the canals was only rarely seen at this time, but was quite common at one month. When present, the predominant activity in neovascularized cortex appeared to be osteoclastic, with the widening of

FIGURE 26. Vascular Bundle Implantation. - Experimental Group 2.

| Rib Graft Type | Time after Grafting | Number of Grafts |
|---|---|---|
| with vascular bundle implantation | 1 week | 7 |
| | 4 weeks | 7 |
| drill hole without vascular bundle implantation | 1 week | 4 |
| | 4 weeks | 6 |
| total number of grafts | | 24 |

perfused Haversian canals. A less important degree of osteoblastic activity was also observed. The findings were compatible with early creeping substitution of the cortex at one month after grafting.

An exception to the above histological pattern observed in conventional bone grafts occurred in those placed subcutaneously. In these grafts, the progressive picture of necrosis, leukocyte invasion, fibrosis, and new bone formation with advancing vascularization were observed as well. However, by one month, a large portion of the cortical bone and both the new and old medullary bone were being resorbed and replaced with fibrous tissue.

There was a poor correlation of cortical and medullary lacunar osteocyte loss with other histological criteria of bone death. In general, there were fewer osteocytes in the lacunae of avascular areas of conventional grafts when compared to vascularized normal rib histology. However, even at one month, the number of osteocytes present in normal rib lacunae was no different than that seen in many biopsies of avascular graft areas that otherwise showed only necrosis of medullary and Haversian canal elements.

As mentioned previously, tetracycline labelling was intense in perfused graft areas which were hot on the bone scans and which showed good evidence of histological viability such as leukocyte invasion, fibrosis, and bone remodelling. In the central avascular portions of conventional bone grafts, where there was no good histological evidence of viability, and where there were cold areas in bone scintigraphy, tetracycline labelling occurred almost every time.

Vascular Bundle Implantation into Rib Grafts

As seen in Figure 26, 24 grafts were assessed in this group. In 12 of the 14 grafts into which were implanted vascular bundles, the vessels

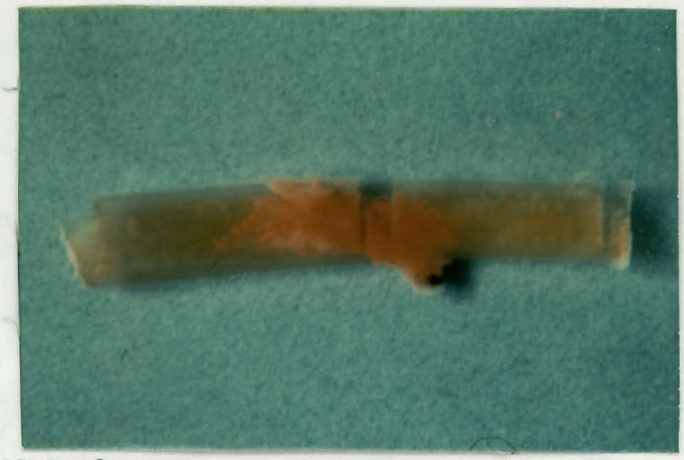


FIGURE 27a. Neovasculature eminating from a vascular bundle at 9 days after implantation.

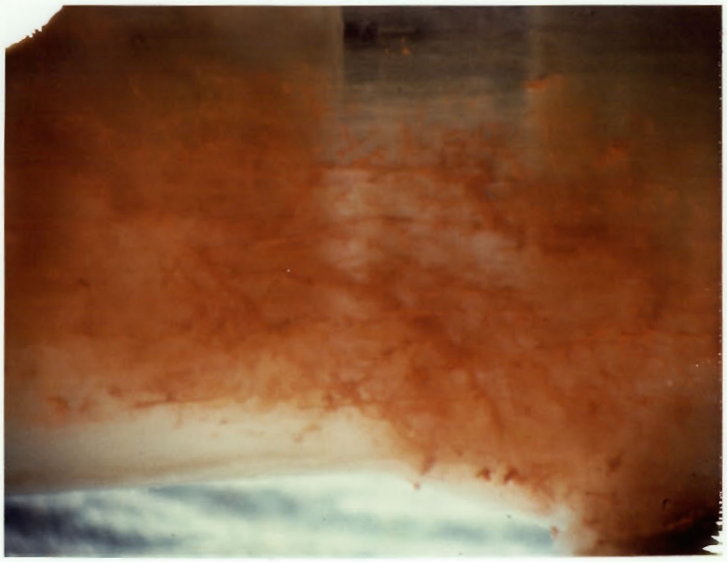


FIGURE 27b. Close up view of the neovasculature seen in Figure 27a.

remained patent. Of the 2 non patent vascular bundles, one was in arterial spasm at the time of implantation, and the other one was kinked at its entry site into the graft as a reault of excessive tension on the vascular bundle at the time of operation. As these 2 operations are considered technically unsuccessful, 12 out of 12 of the successfully implanted vascular bundles remained patent.

One of the animals in which an implant was performed was not perfused with Microfil. Histology of the graft specimen from this dog revealed that both the artery and the vein of the implanted portion of the vascular bundle contained freely flowing blood 8 days after implantation.

All but 1 of the 12 successful implants had begun to invade the grafts with neovasculature at the time of assessment, as shown in Figure 27, and in Figure 21a, opposite p.39. The one exception had only been implanted 4 days prior to assessment. The distances of graft vascular invasion eminating from implanted vascular bundles are outlined in Figure 28, opposite p.45. Although easily obtainable at 1 week, these measurements were more difficult to estimate at 4 weeks after implantation. The vessels eminating from the vascular bundles had usually met those arriving from the graft host junctions by this time. Nevertheless, the average distance of graft vascular penetration by implanted vascular bundles was estimated to be 6.8 mm at one week, and 22.5 mm at 4 weeks.

Vascular penetration also occurred via granulation tissue entering the drill holes of the control grafts without vascular bundle implantation, particularly at 4 weeks. However, the graft vascular penetration by the implanted vascular bundles was consistently greater.

At 1 week after implantation, bone scintigraphy accurately predicted the patency of all of the implanted vascular bundles which had begun to invade the rib grafts with neovasculature. In such a graft, the implant

FIGURE 28. Distances of Graft Neovascular Invasion Eminating from Implanted Vascular Bundles.

| Graft Number | Patency of implant | Time of Sacrifice | Width of Neovascular Spread from Implant | Comment |
|---|---|---|---|---|
| 258 | patent | 4 days | 0 mm | |
| 135 | patent | 4 days | 8 mm | |
| 21 | non patent | 5 days | - | arterial spasm at operation |
| 93 | patent | 6 days | 3 mm | |
| 27 | patent | 8 days | 7 mm | |
| 264 | patent | 8 days | - | no Microfil perfusion |
| 87 | patent | 9 days | 16 mm | |
| 81 | patent | 27 days | 8 mm | |
| 208 | patent | 28 days | 24 mm | |
| 252 | patent | 28 days | 29 mm | |
| 57 | patent | 29 days | 26 mm | |
| 123 | patent | 29 days | 24 mm | |
| 39 | non patent | 30 days | - | vessel kinked at graft entry at operation |
| 226 | patent | 32 days | 24 mm | |

FIGURE 29. Microvascularized Rib Grafts - Experimental Group 3.

| Rib Graft Type | Time after Grafting | Number of Grafts |
|---|---|---|
| Successfully microvascularized | 1 week | 5 |
| | 4 weeks | 5 |
| Unsuccessfully microvascularized | 1 week | 5 |
| | 4 weeks | 4 |
| Control, surrounded by muscle sleeve, but without vascular anastomoses | 1 week | 10 |
| | 4 weeks | 7 |
| Total number of grafts | | 36 |

site was represented by a hot area in the center of the bone scan, as seen in Figure 21a, opposite p.39. The thrombosed implant, and the patent implant which had not yet begun to invade the graft by 4 days, were cold on the bone scan. Bone scintigraphy was not as helpful in determining implant patency at 4 weeks because of interference from vascularity eminating from the graft host junctions.

The histology and tetracycline labelling findings in the grafts of this group were the same as they were in the conventional bone grafts, except that there were fewer biopsies of avascular areas.

Microvascularized Rib Grafts

Of the 19 microvascularized rib grafts (Fig. 29), 10 were successful and 9 were failures as judged by the presence or absence of Microfil perfusion of the intercostal vessels and muscles at autopsy. One failure was due to a post operative fracture of the tibia, another animal developed a wound infection, and a third chewed his wound open. The other 6 failures were due to microvascular anastomosis thrombosis or disruption.

Microfil perfusion of the 10 successfully microvascularized rib grafts revealed a vascular pattern identical to that of the normal rib in all but one of the grafts (Fig. 30, opposite p.46). In the latter graft, there was adequate perfusion of the cortex by periosteal perforators, but the nutrient artery was not perfused and the medulla was largely avascular. Presumably, in spite of the patent anastomoses and perfused intercostal vessels, the nutrient artery to this graft was either thrombosed or traumatized perioperatively. This graft was also cooler on the bone scan than the other 4 successfully microvascularized grafts examined at 1 week.

The vascular perfusion patterns of the 17 control microvascular grafts surrounded by dead muscle tissue were compared to those observed

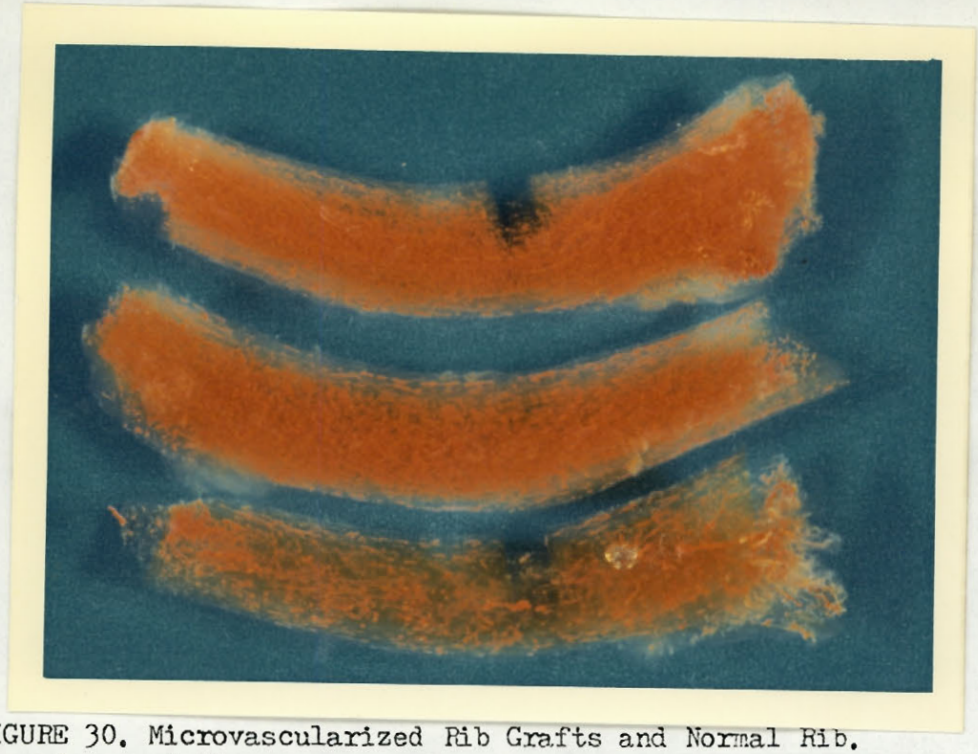


FIGURE 30. Microvascularized Rib Grafts and Normal Rib.
Top: Successfully microvascularized posterior rib graft containing a patent nutrient artery, perfused with Microfil and cleared 1 week after grafting.
Center: Normal rib segment from same animal as bone above it. Perfused with Microfil and cleared.
Bottom: Successfully microvascularized posterior rib graft with thrombosed nutrient artery (perfused with periosteal circulation alone). Perfused with Microfil and cleared 1 week after grafting.

FIGURE 31. The Depth of Medullary Vascular Penetration in Control Microvascular Rib Grafts at 1 Week after Grafting.

| Graft Number | Proximal Invasion | Distal Invasion |
|---|---|---|
| 46 | 4mm | 0mm |
| 52 | 0mm | 0mm |
| 106 | 0mm | 0mm |
| 112 | 3mm | 2mm |
| 130 | 0mm | 8mm |
| 142 | 4mm | 6mm |
| 203 | 0mm | 0mm |
| 215 | 0mm | 0mm |
| 221 | 0mm | 0mm |
| 239 | 0mm | 8mm |

in the 21 conventional rib grafts placed in tibial defects. At one week, an average of 3.5 mm with a median of 0 mm of medullary vascular penetration occurred at the graft host junctions in the control microvascular grafts(Fig. 31). An average of 7 mm with a median of 4 mm of graft host junction vascular invasion occurred in the comparable 7 conventional bone grafts (Fig. 24, opposite p. 41). The distance of vascular penetration at the graft host junction was statistically significantly less in the microvascular control grafts than in the conventional grafts (Student's unpaired T test with a P value of .02). Two out of 7 conventional grafts placed in tibial defects had periosteal perforators at one week while there were none in the 10 control microvascular grafts.

At 4 weeks, there was a striking difference in the vascular patterns of the conventional rib grafts and the control microvascular rib grafts, as seen in Figure 32, opposite p.47. In the latter, very small periosteal perforators could be seen in only 3 of the 7 grafts. Vascular penetration was largely restricted to graft host junction medullary invasion and there was a central avascular area in all of the grafts. On the other hand, all 14 of the conventional grafts placed in tibial defects had abundant periosteal perforators, and therefore, no large central avascular area.

Bone scintigraphy accurately predicted graft perfusion in the 10 successfully microvascularized rib grafts, both at 1 and at 4 weeks. They appeared as diffusely hot bones on the bone scans (see Fig 21b,c, opposite p.39,40). They were generally hotter than the normal rib segments from the same animal. In the 9 failed microvascular grafts and in the 19 control microvascular grafts, the scans were cold except for hot areas representing vascularity entering at either end of the graft from the graft host junctions.

Histologically, microvascularized grafts appeared more like normal

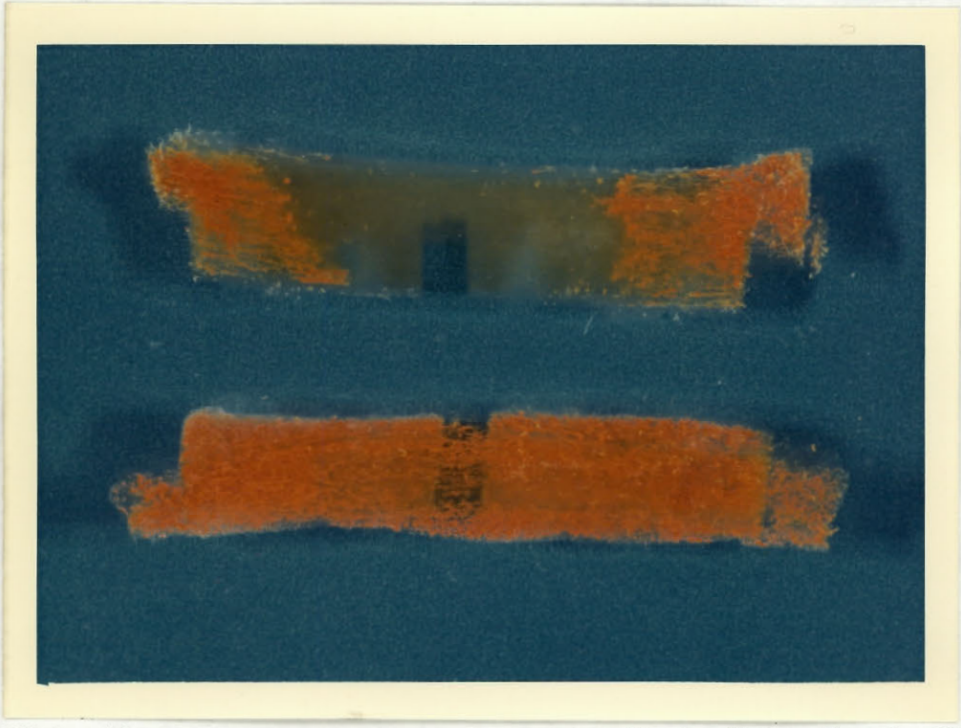


FIGURE 32. Top: Control microvascular rib graft perfused with Microfil and cleared 4 weeks after grafting.
Bottom: Conventional rib graft perfused with Microfil and cleared 4 weeks after grafting.
See text p.46 for details.

bone than like bone grafts because they had no areas of avascular necrosis in the marrow. The only exception was the graft perfused only by its periosteal blood supply. This graft showed an area of necrosis in one of its 3 biopsies, and fibrosis in all 3 of them. Smaller areas of fibrosis were also seen in the marrow of 2 of the 9 other successfully microvascularized grafts.

At one week, new bone formation over the original woven bone in the medulla of microvascularized grafts was quite advanced in many sections. However, bone remodelling of the cortex was not prominent at this time.

At one month, advanced new bone formation could be seen in the marrow. Some areas were beginning to look like Haversian systems with lamellar bone. Bone remodelling of the cortex at one month was still not prominent in most biopsies of microvascularized rib grafts. However, a few of these biopsies revealed extensive remodelling with large cortical spaces cored out by osteoclasts.

In both the microvascularized rib grafts and their controls, tetracycline labelling revealed the same patterns seen in the other types of grafts. In areas of perfusion and remodelling, there was intensive labelling. In the non perfused areas of the control grafts, tetracycline labellingwas still always present. In all of the animals with successfully microvascularized rib grafts, the tetracycline labelling biopsies of these grafts were indistinguishable from those of the control non vascularized grafts in the contralateral legs. All of the latter biopsies were positive with double tetracycline labelling both at one week and at one month.

Dermis Island Vascularization of Rib Grafts

A total of 4 rib grafts wrapped with dermis island flaps were examined; 2 at 1 week, and 2 at 4 weeks after grafting. The vascular pattern, bone scintigraphy, tetracycline labelling and histology were

no different in these grafts than in the four controls without dermis island flap wrapping in the contralateral legs of the same animals. In particular, there was no increase in the periosteal vascular invasion of the grafts from the dermis islands, despite adequate vascularization of all 4 flaps, as demonstrated by Microfil perfusion at autopsy.

## DISCUSSION

### The Correlation of the Vascular Perfusion Pattern, Bone Scintigraphy, Tetracycline Labelling, and Histology

In every one of the 90 grafts in which both Microfil perfusion and bone scintigraphy were performed, an excellent correlation was observed. This indicates that if a segment of bone is being actively perfused with blood, it will show up as a hot area on a bone scan. Berggren et al.(13) have shown that the reverse is not necessarily true. They demonstrated that a rib graft can be hot on a bone scan with only a thin periosteal sleeve showing evidence of viability, as judged by fluorochrome labelling.

It would appear, therefore, that a cold scan of a bone graft would indicate no circulation within the graft. However, a hot scan does not necessarily indicate that circulation is present within a graft, but may represent circulation on its surface. Thus, although bone scintigraphy does not necessarily reflect the viability status of a bone graft, as pointed out by Bos(17), it does furnish us with clues about its circulatory status.

The observation that a conventional bone graft is hotter than a normal rib of the same animal when scanned at one month is worthy of note because perfusion studies indicate that those same grafts are vascularized over a smaller total area than the normal ribs. This supports the concept that $Tc^{99}$ diphosphonate uptake is not only related to blood flow, but to some other factor such as bone metabolism as well(32,58). The fact that a microvascularized rib graft containing the nutrient artery is hotter than a normal rib from the same animal could more easily be a flow phenomenon. These relatively small grafts receive their blood under relatively high pressures in comparison to the normal rib.

It has been assumed that tetracycline labelling does not occur in avascular areas of bone(11). However, in this first bone graft study in which the correlation of vascular perfusion and tetracycline labelling was examined, there is evidence that this concept is not valid. The frequent observation of positive tetracycline labelling in avascular areas of bone grafts in this study indicates that the tetracycline must have arrived there by diffusion through extracellular fluid. Tetracycline has been shown capable of this in vitro(43,139). That these avascular areas were cold on the bone scans indicates that $Tc^{99}$ diphosphonates do not diffuse as readily as tetracycline in extracellular fluid, a factor which may help to explain discordant findings in the two techniques. $Tc^{99}$ scintigraphy would therefore appear to reflect vascular perfusion in bone grafts more accurately than tetracycline labelling.

The observation of double tetracycline rings in central avascular areas of bone grafts examined at 4 to 8 days after grafting would appear to indicate that osteoblasts can survive and produce bone for at least 5 days with only passive diffusion of nutrients through extracellular. These results also indicate that although serial tetracycline labelling may represent bone viability, it does not necessarily represent bone graft vascular perfusion.

The inability to distinguish the tetracycline labelling biopsies of the successfully microvascularized grafts from those of the control non vascularized grafts of the same animals both at 1 week and at 1 month is furthur evidence that tetracycline labelling is not a reliable indicator of the patency of a bone graft's pedicle blood supply. It produces false positive results in non vascularized bone grafts. This brings into question the clinical use of tetracycline labelling and bone biopsy to assess bone graft perfusion(6,36,40,131).

The observation of the persistent presence of osteocytes in lacunae in spite of avascularity supports the findings of Catto(31) in her studies of human avascular necrosis of the femoral head. However, the concurrent finding, in this study, of positive serial tetracycline labelling in these avascular areas lends credence to the possibility that these osteocytes survive longer than is generally thought.

Conventional Bone Grafts

The observation of the initial bone graft vascularization occurring via the medulla of the graft host junction, followed later by periosteal vascularization agrees with the findings of others(134). Although the preservation of the periosteum of a bone graft has been shown to increase its callus formation(151), it did not appear to have a significant effect on graft vascularization in this model. These results concur with those of Abbott(1).

The vascular perfusion patterns seen in different conventional bone grafts within the same animal were similar, but there were notable differences of vascular invasion rates in similar types of grafts placed in different animals. In other words, the ability of neovasculature to invade a bone graft may differ markedly from one animal to another. This observation stresses the importance of having a control bone graft within the same animal as an experimental bone graft when studying techniques of graft vascularization. Unfortunately, many experiments assessing vascularized bone grafts have been designed with controls in different animals. than those in which were placed the experimental grafts(47,72,90,109,150).

Albrektsson(3) recorded that graft medullary vascular invasion proceeded at a rate of 0.2 to 0.4 mm/day in the rabbit. Stringa(134) observed 3 mm of penetration in 7 days in the same animal. In the present study, the median vascular penetration in the conventional grafts placed in tibial

defects was found to be 4 mm at 1 week. However, in one of the grafts, a long continuous vessel was seen extending from the graft host junction to a point 28 mm within the graft. This event may well have been the result of a spontaneous graft host end to end vessel anastomosis such as described by Albrektsson(4).

By one month, early creeping substitution was noted in the cortex of well vascularized segments of conventional bone grafts. Although some osteoblastic activity was present, the osteoclastic carving of spaces in the cortex was far more predominant. These findings are in agreement with those of Burchardt and Enneking(26) who reported that the porosity of cortical bone grafts peaked from 10 to 12 weeks in the dog.

With $Tc^{99}$ scintigraphy, the temporal progression of hot areas at the graft host junctions moving to the center of a bone graft has been reported (132,147). The present study indicates that the advancing hot areas seen on the bone scan reflect the advancing vascular invasion of the bone graft. Conversely, the lack of a hot area at the graft host junction signifies the failure of vascular medullary invasion of the graft. In this way, bone scintigraphy provides us with a non invasive technique of following bone graft vascularization.

Vascular Bundle Implantation into Rib Grafts

The maintenance of patency in 12 out of 12 successfully implanted vascular bundles into rib grafts supports the findings of Hori et al.(73). The fact that neovasculature arises from these vascular bundles and invades isolated bone also supports their observations. In this study, the rate of this vascular invasion was measured to be an average of 6.8 mm at 1 week, and 22.5 mm at 4 weeks.

The observation that graft vascular penetration does occur via granulation tissue entering the drill holes of the control grafts without

vascular bundle implantation is different from the findings of Hori et al.(73), who observed little change. However, in this study, graft vascular invasion by the implanted vascular bundles was consistently greater than in the controls.

Vascular bundle implanted grafts were clearly inferior to microvascularized rib grafts in that the latter largely remained vascularized and did not undergo marrow necrosis. Vascular bundle implanted grafts did undergo marrow necrosis after transplantation, and had to be revascularized like conventional bone grafts. However, the implant added the equivalent of at least one extra graft host junction to the center of a conventional graft, because neovasculature eminating from the vascular bundle migrated both proximally and distally to meet oncoming vasculature from either end of the graft. In this way, the vascularization of the implanted graft was greatly accelerated when compared to the conventional graft.

In spite of the above major disadvantage, vascular bundle implanted grafts do have several advantages over microvascularized bone grafts: 1)The average operating time for the lone surgeon in this study was 7.6 hours for the microvascularized graft animals, and 4.2 hours for the vascular bundle implanted graft animals. Vascular bundle implantation could be an attractive alternative to microsurgery in a patient who is unfit to withstand a long anesthetic. 2)If the microvascularized graft fails, it is not revascularized as readily as a conventional graft. Vascular bundle implanted grafts do not have this problem as they are not surrounded by a dead muscle sleeve. 3)Microsurgical technology is not required for vascular bundle implantation. The technique is therefore available to a greater number of surgeons operating in smaller centers.

Vascular bundle implanted grafts, like microvascularized grafts, are not necessary if a conventional graft would do as well in an adequately

vascularized recipient bone gap. However, in poorly vascularized recipient beds resulting from radiation, trauma, or osteomyelitis, vascular bundle implantation into conventional bone grafts would increase their chances of successful vascularization and subsequent incorporation. Also, in long bone defects, a vascular bundle implantation into a conventional bone graft would increase its rate of vascularization, thereby increasing its rate of creeping substitution, and decreasing its time of porosity and weakness.

The progress of vascular bundle implanted grafts can be readily followed with non invasive $Tc^{99}$ bone scintigraphy. The ideal time to scan an implanted graft for the assessment of its vascular bundle patency is probably 2 to 3 weeks after grafting. This would ensure enough time for the neovascularization eminating from the vascular bundle to have begun its invasion of the bone graft in earnest, thereby showing up as a hot area on the bone scan.

Microvascularized Bone Grafts

The observation that two of the microvascularized rib grafts were able to remain perfused in spite of wounds which had been chewed open is interesting. The vascular bundle leading to the microanastomoses and the perfused muscle around the graft were able to support early granulation tissue in one wound which was opened at least 28 hours before sacrifice. This illustrates a distinct advantage of successfully microvascularized grafts over conventional grafts.

All except one of the successfully microvascularized posterior rib grafts revealed vascular patterns identical to the equivalent unoperated ribs in the same animals. In the exception, in spite of the patent microvascular anastomoses of the posterior intercostal vessels, the nutrient artery to the rib graft was thrombosed and the bone was perfused with

periosteal circulation only. It is interesting to note that this graft was not as hot on the bone scan as the rest of the microvascularized grafts which contained a patent nutrient artery.

The clinical importance of the above observation may be found in the microvascularized fibular graft, which relies more heavily on its nutrient artery and less on its periosteal circulation than does the rib. A warm (as opposed to hot) fibular bone scan the day after a microvascularized fibula transfer may not necessarily reflect a thrombosed peroneal vessel microanastomosis, but may be the result of the perioperative thrombosis of the nutrient artery to the fibula. The first problem is surgically correctable, but the second is not.

$Tc^{99}$ scintigraphy accurately differentiated the 10 successful microvascularized bone grafts from the 9 which failed and from the 17 control grafts without anastomosis. These results concur with those of Haw et al. (72), Bos(17), and Berggren et al.(13). However, the last authors stress that bone scintigraphy will differentiate patent from non patent microanastomoses only in the first week after grafting. Their reason is that a failed microvascularized graft can produce a hot bone scan in the second post operative week. However, the observations in this study indicate that the pattern of the bone scan can give a clue to the patency of microanastomoses even at one month post operatively (see Fig. 21c, opposite p.40). At this time, a successfully microvascularized graft will continue to be diffusely hot, as it is wholly vascularized. On the other hand, a failed microvascularized graft will be undergoing medullary vascularization from either end of the graft, with very little periosteal vascular invasion because of the dead muscle sleeve. Its bone scan pattern will therefore tend to be cold in the middle and hot at both ends.

Control microvascular bone grafts surrounded by a dead muscle sleeve

were found to be vascularized more slowly than conventional bone grafts in two ways. First, at one week after grafting, the medullary vascular invasion of control microvascular bone grafts was found to be statistically significantly less than that seen in conventional bone grafts. One possible explanation for this finding is the extracorporeal exposure time of the grafts. All of the grafts were preserved in saline soaked gauze between harvesting and grafting. However, this time period averaged 3 hours for the microvascularized grafts and their controls, while it was only 1 hour for the conventional grafts. Puranen (114) has shown a decreased osteogenic capacity in bone grafts with increased air exposure. Perhaps this factor also decreases a graft's receptiveness to vascular invasion.

The second way in which the vascular invasion of control microvascular grafts was slower than that of conventional grafts was by reduced periosteal vascular penetration. At one month, the periosteal vascular invasion of conventional grafts was strikingly greater than that of both the control microvascular grafts and the failed microvascularized grafts. The likely cause of this effect is the barrier created by the dead muscle sleeve in the latter grafts (see Fig. 32, opposite p. 47).

These observations shed new light on the concept proposed by Taylor (140) that failed microvascularized bone grafts will act as conventional bone grafts. The findings of this study confirm the suspicions of Bos(17) and Berggren(13) that failed microvascularized bone grafts do not become vascularized as quickly as conventional bone grafts.

Doi et al.(47) stated that creeping substitution does not occur in microvascularized rib grafts. However, Berggren et al.(12) observed a remarkable enlargement of cortical vascular canals and a 25 to 60% cortical resorption in all posterior microvascularized rib grafts. They attributed this effect to the presence of hyperemia and hyperoxia in

these bones. A marked cortical Haversian canal enlargement in some of the microvascularized grafts examined at 4 weeks was also observed in this study. It is possible that part of this effect is also due to a remodelling of the rib osteons to accommodate tibial stress patterns.

Regardless of the reason for which it occurs, the increased cortical porosity observed in microvascularized bone grafts renders them prone to stress fractures, just as creeping substitution does in conventional bone grafts. Whether the effect is less important of lasts for a shorter time in microvascularized bone grafts remains to be determined.

Dermis Island Vascularization of Rib Grafts

Only 4 animals were used to study the effect of wrapping dermis island flaps over rib grafts. This group was discontinued when it was observed that the dermis island flaps did not enhance graft vascularization over controls without the flap. However, the grafts in this model were placed in an ideal recipient bed with ample vascularization. It seems possible that a vascularized dermis island flap would contribute more significantly to bone graft circulation if the graft was placed in a more ischemic recipient site.

## CONCLUSIONS

This is the first experimental investigation in which silicone rubber vascular perfusion with bone clearing, histology, tetracycline labelling, and $Tc^{99}$ bone scanning have been used concurrently for the assessment of vascularized bone grafts. This technique has permitted the study of the relationship of $Tc^{99}$ scintigraphy and tetracycline labelling to the vascular tree. The technique appears to be a valuable tool in the study of vascularized bone grafts.

Bone scintigraphy proved to be reliable in the assessment of the patency of anastomoses of microvascularized rib grafts both at 1 week and at 1 month after grafting. The patency and vascular invasion of vascular bundles implanted into conventional rib grafts may also be accurately followed with bone scanning. The progress of the vascular invasion of conventional bone grafts is also accurately reflected with scintigraphy. All of the above are possible because of the simple basic principle that bone actively perfused with blood shows up as hot on a bone scan.

On the other hand, tetracycline labelling was found to be an unreliable indicator of the vascular perfusion of rib grafts. Positive tetracycline labelling was repeatedly observed in avascular areas of conventional bone grafts. It was also repeatedly observed in the non vascularized controls of microvascularized bone grafts both at 1 week and 1 month after grafting. It seems likely that tetracycline is readily diffusible in extravascular fluid. Although tetracycline labelling may be an accurate indicator of bone viability, it is not a reliable indicator of the vascular perfusion of rib grafts.

Successfully microvascularized posterior rib grafts containing the nutrient artery were found to have a circulatory pattern identical to that seen in the normal rib. On the other hand, failed microvascularized rib grafts were found to be revascularized more slowly than conventional rib grafts in both the medullary and periosteal vascular invasion of the grafts.

Vascular bundle implants into rib grafts were found to remain patent. The neovasculature arising from the vascular bundles was found to invade the rib grafts at a rate comparable to that of the neovasculature penetrating the grafts at the graft host junctions. Thus, implanted vascular bundles are capable of accelerating the process of creeping substitution in conventional bone grafts.

Neither the preservation of periosteum, nor the wrapping of rib grafts with dermis island flaps increased the rate of vascular invasion observed in conventional bone grafts.

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