A comparison of freeze-dried and fresh cortical allografts in the canine femur

by

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PROLOGUE

"Clinical medicine entered the scientific era on the rock of anatomy. We are still working the seam of physiology. The gold of the future lies in cellular biology."

R. G. Burwell
The Scientific Basis of Bone
Homo-Transplantation
1968

INTRODUCTION

Historically man has been called to the aid of his fellow man in many different situations. Some of the most unselfish and heroic acts have involved instances where tissues were donated directly or indirectly through a tissue bank to prevent suffering, permanent deformity, or death, of a close relative, friend or total stranger. Never is the need of such tissue quite so great as in time of severe disaster or world war.

Through the decades we have learned that tissue banks can and do serve a useful and positive function (2,42,90,92,117, 175,193). However, with the possible exception of stored blood and plasma, the use of preserved tissue has never been widely accepted in clinical work. Much of the early literature on tissue banks has been concerned, in particular, with bone transplantation. Experiments involving the use of such bone have indicated a decided preference by most surgeons for the use of the patient's own bone, i.e., autogenous cortical and/or cancellous bone, when possible (1,2,3,4,46,69,90,109, 117,139,140,141). Allografts and especially xenografts of bone have been shown histologically to have a delayed and indefinite rate of accretion as well as to incite different degrees of hypersensitivity within the recipient. Other problems encountered, particularly in the pre 1935 era, were infection with graft resorption and lack of proper fracture immobilization which usually lead to a delayed union or nonunion at the graft-host interface.

Although autogenous bone is preferred over any other type of bone graft, problems have also been encountered from its use. Certainly no guarantee can be made that autogenous bone will be one hundred percent effective in the role for which it is desired. Also, and probably more importantly, the procurement of autogenous bone usually requires that an additional surgical procedure be performed. The profound effects this can have on the patient due to increased surgery time, additional pain and blood loss, and possible contamination are obvious (152).

In theory, these problems can be avoided by the utilization of sterile, preserved bone from a tissue bank. Certain treatments of bank bone which may reduce its antigenicity to nearly that of autogenous bone include freezing and freeze-drying procedures. These two methods generally reduce the immunogenicity of bone, permitting it to be used in many clinical instances (89,90,111). A 1974 survey of the Association of Bone and Joint Surgeons indicated that if a freeze-dry bone bank was established on a national basis, 69% of the practicing orthopedic surgeons would use it (51). The Tissue Bank of the Naval Medical School in Bethesda, Maryland, introduced the concept and is probably the largest proponent for the use of freeze-dried tissues (89,90). Their motto Ex Morte Vita - from death, life - has answered the pleas of needy victims all over the country (193).

The use of stored bank bone is not unique to human medicine alone. Its use is presently enjoying a revitalized interest in veterinary orthopedic surgery as well (26,188). Veterinary surgeons have learned from human orthopedists the value of segmental bone or whole joint replacement in instances of incurable disease or severe trauma (111,113,175,189,197). However, the preferred use of autogenous bone to preserved tissue also exists in veterinary medicine (82,126,185).

Next to immunologic studies on grafts and transplants, the most important area of research is that concerned with time sequence studies of the eventual fate of the graft. Most of the studies performed thus far have involved the use of laboratory rodents over a short span of time. These studies have yielded informative data concerning the immune response and graft revascularization and replacement. Veterinary surgeons have also benefited from clinical studies performed on man, but for obvious reasons, these grafted segments cannot be removed en bloc and evaluated histologically at the end of a stated time interval. Instead, periodic radiographs and biopsies have had to suffice, and many patients have been subsequently lost for follow-up investigation.

The purpose of this investigation was to evaluate and compare histologically and radiographically freeze-dried and fresh cortical allografts in the canine femur over a one year time interval following surgery. These were also compared to control dogs on which a sham procedure was performed.

It is hoped that this study, and others like it now being performed by both veterinary and human orthopedists, will be a greater impetus for the use of preserved bank bone in veterinary orthopedic surgery.

TERMINOLOGY

Since its inception the science of transplantation has included the usage of a special terminology to denote the origin of the graft material utilized. A brief review of this terminology is essential as a prelude to the material to follow. A major revision in this terminology has evolved in recent years. Ray has commented that the leaders in immunology are responsible for introducing this change and, along with it, a considerable amount of confusion (147).

Grafts may be classified according to their origin, their location in bone, and the type of bone obtained (22). The following is a generic classification of grafting terminology (11,22,23,24,85,90,138,147):

Autograft:	tissues taken from one operative site and transplanted to another in the same individual; the host is the donor and the recipient
Allograft:	formerly homograft; tissues taken from one individual and transplanted to another individual of the same species; the donor is unrelated to the recipient
Isograft:	tissue transfer between individuals with identical genetic backgrounds, i.e., inbred strains

Syngenesiografts:	tissue grafts taken from blood-related relatives
Xenograft:	formerly heterograft; tissues taken from one individual and transplanted to another individual of a different species
Orthotopic graft:	denotes tissue that has been surgically transferred to a location that is normally occupied by tissue of the same type
Nonorthotopic (Heterotopic graft:	denotes the surgical deposition of tissues to a site not normally occupied by that tissue

In its strictest sense, the term transplant or graft implies that the donor tissue contains living cells that are capable of surviving and growing at the new location (25,178). An implant, on the other hand, involves the transfer of non-living tissue (11,25). Urist further defines an implant as a, "nonviable, coagulated, frozen or denatured tissue that does not contain cells having the capacity to proliferate and produce new bone" (178). In addition, Urist has proposed the term "derivative of bone" to describe bone from which mineral, water, ground substance, protein, or lipid has been removed (178).

Except during the historical review when reference to early scientists and the evolution of grafting are made, the material to follow will utilize the most recent terminology. Although the fresh allografts utilized in this study are, by definition, transplants or grafts because they contain living cells, the freeze-dried allografts are, in fact, implants of derivatives of bone, because they are devoid of living cells. To simplify matters, however, both will be referred to as grafts or transplants.

LITERATURE REVIEW

Historical Review

Although it is not the purpose of this review to cover the history of transplantation in its entirety, certain salient areas will be covered in preparation for the material to follow. Chase and Herndon, Bassett, Weinstein, Hyatt, and others have compiled excellent historical reviews, and much of the initial information is cited from their articles.

The exact date on which the first bone transplantation was performed is speculative at best. Several Renaissance paintings reportedly depict Saints Cosmas and Damian transplanting an entire leg from a dead black man to replace a cancerous leg in a white man (162). Archaeologists have uncovered prehistoric skulls that had been trephined and also have found evidence that teeth were transplanted in ancient Egypt, Greece, pre-Columbian North and South America, Rome and China (162).

It is interesting to note that religion may have once been involved in medicine as well as in governmental affairs. In 1668, the surgeon Van Meek'ren repaired a defect in a Russian soldier's cranium utilizing a bone graft from a dog's skull. The patient was subsequently excommunicated from

the Church for this act, only to be reinstated some two years later by having the graft removed (147).

Hutchison cites Merrem as giving an account of the first successful bone graft surgery in animals in 1810 (86). Perhaps this was the guiding light for other surgeons of the day because ten years later Philipp von Walther pioneered the use of the autogenous bone transfer in man (49). In 1878, almost sixty years later, Macewan removed the entire diaphysis of the humerus of a three year old boy with osteomyelitis and replaced it with allografts of bone devoid of periosteum that were harvested from other patients (49,150, 190).

In the meantime, many scientists began to study the normal processes of osteogenesis, bone growth, and fracture repair. It was apparent that these fundamentals had to be learned before the eventual fate of a bone graft could be understood. Unfortunately, a number of obstacles stood in the way of any rapid progress. Infection was one of the major reasons for the subsequent failure of a number of experimental and clinical studies. A dramatic improvement occurred with the advent of Lister's principals of asepsis in the era 1860-1870, but infection remained the Achille's heel of surgery until the introduction of antibiotics in 1935. Furthermore, there was a general lack of sophisticated equipment to aid these scientists in the histologic evaluation of tissue trauma

and healing. Some of the theories advanced at the turn of the century, however, remain fundamental to much of the orthopedic surgery performed today.

Three different concepts of bone healing evolved from the work of these early scientists (190):

1. Bone growth occurs from the periosteum
2. Bone growth occurs from the adjacent host bone
3. If grafts are utilized, bone growth occurs from osteoblasts within the graft.

In 1739, Duhamel performed an experiment in which he inserted silver wires subperiosteally and found them to be covered by new bone several weeks later (12,49). He became convinced that the primary role of the periosteum was that of osteogenesis (12). Duhamel's ideas prevailed and went without serious challenge until 1763. At that time, Von Haller theorized that the periosteum served primarily as a support for blood vessels which were the real agents for osteogenesis in fracture healing. He believed, in fact, that osteogenesis was due to an exudation from arteries and that the periosteum had no osteogenic capacity (12,49). As a result of these opposing viewpoints, two schools of thought arose.

One of Von Haller's students was John Hunter. In a series of classic experiments, Hunter supported the validity of his professor's beliefs by demonstrating that bone actually grew from a region called the epiphyseal plate (49).

In spite of these findings, the controversy remained unsettled for the next eighty years. In 1836, Heine performed total subperiosteal rib resections experimentally and observed that the bone eventually grew back (49). However, it was not until Flourens published similar data in 1842 that general acceptance was established for the theory that the periosteum was osteogenic and the chief agent involved in the healing of bone defects (12,49).

One of the first scientific studies on bone transplantation and the fate of the bone graft began with the work of Ollier in 1867. Ollier believed that true transplantation of bone could only be successfully accomplished by using living, autogenous, periosteum-covered bone (10,150). By leaving the periosteum, or as he termed it "maternal membrane of bone", intact, the graft would receive the nutrition it required to survive (145). He was the first to distinguish between autografts, homografts and heterografts and recommended that heterografts never be used (145). In an indirect fashion, Ollier may have been the first to advocate the use of stored bank bone when he observed that at temperatures below -16^{o} C putrefaction of bone was delayed (91).

From the clinical and experimental work of Macewan, which spanned the period from 1878-1912, another theory of osteogenesis evolved. Macewan theorized that bone formation was the result of specific cells which resided within bone. These

cells were named osteoblasts by Gegenbaur in 1864 (12). Chase, Herndon and Phemister remarked that Macewan's rejection of the osteogenic capacity of the periosteum was due primarily to his failure to recognize the cambium layer as part of the periosteum (49,139). It is also interesting to note that Marchand, in 1901, postulated that osteoblasts were of mesenchymal cell origin (12). Marchand's suggestion may have added some skepticism to the osteoblast theory of osteogenesis at that time, but directly applies to the present theories of osteoinduction.

To add further confusion to the theory of osteogenesis, a third opinion soon surfaced through the work of Barth. His experiments, from 1893-1898, indicated that all the elements of transplanted bone die and are slowly replaced by elements from the adjacent host bone and the surrounding osteogenic tissues of the graft bed (10,45,49,190). He also believed that the periosteum perished, and there was no significant differences between different types of bone grafts (145).

Because of the existence of these different schools of thought, scientists continued both clinical and experimental studies in hopes of finding answers to their questions. In 1889, Senn reported the successful use of decalcified ox bone chips in a patient with an infected bone cavity (66). That same year, Morpurgo observed new bone formation following the experimental transplantation of cadaver periosteum that had been preserved at 15 degrees centigrade (49,90). Ten years

later, Grohé repeated the experiments of Morpurgo with similar results (49). In 1900, Saltykow preserved rat periosteum in gelatin for two weeks and found that it still had the capacity to proliferate at the end of that time (49).

It was not until the work of Georg Axhausen was published in 1907 and 1909 that periosteum-covered bone was widely accepted for clinical use as graft material. Not only did Axhausen confirm Ollier's data by showing that the periosteum survived transplantation and produced new bone, but he also condemned the clinical use of heterogenous bone (10). In his experiments with homogenous bone transplants, Axhausen reported that osteogenesis did occur but that it was in a reduced amount compared to autogenous transfers. He also reported the growth of new bone following the experimental use of homografts taken from animals that had been dead for up to thirty hours (28).

In 1908 Lexer became the first to perform a whole-joint transplantation in a human (49). During the following year, he reported the successful regeneration of a bone defect in a patient by using pieces of bone taken from freshly amputated limbs of other patients (28).

In 1912, Baschkirzew and Petrow experimentally transplanted bone fragments devoid of periosteum and marrow into soft tissues. They noted the appearance of new bone formation and theorized that the reorganization of bone

transplants occurred from the surrounding tissues (145). In doing so, they echoed the views of Barth concerning the fate of transplanted bone stated some twenty years before them.

At this point in medical history, it appeared that at least autogenous bone transfers were well accepted. Chase and Herndon, in their historical review of bone grafting, indicate that 45 documented clinical reports of autogenous bone transplantations and 10 involving homogenous transfers occurred during the decade immediately preceding the twentieth century. They go on to say that in the first decade of the twentieth century there were 162 autogenous and 29 homogenous bone transfers with the number in both categories increasing markedly in the second decade (49).

F. H. Albee and D. B. Phemister were prominent human orthopedists of that era. In 1914, Phemister reported that homogenous grafts in animals behaved similarly but with somewhat diminished powers as compared to autogenous grafts (139). He also theorized that the body had three main functions following bone transplantation (139):

1. The preservation of nutrition and reestablishment of circulation of the transplant
2. The union of the ends of the transplant with the ends of the fragments
3. The transformation of the transplant into a duplicate of the normal bone whose place it fills.

He believed that perfect coaptation and perfect immobilization were of absolute necessity to achieve the optimum results.

Through studies on dogs, Phemister discounted Axhausen's theory that osteogenesis would not occur in transplants devoid of periosteum and endosteum. He agreed, however, that if the periosteum and endosteum were left intact a greater number of living cells would be transferred and, hence, a faster union would occur (139).

F. H. Albee, one of the most innovative surgeons of his day, in 1909, became the first to use a bone graft for hip arthrodesis. In 1911 he established the technique of transferring large grafts of bone from a patient's leg to the spine in cases of spinal tuberculosis (1,4,16). He also was one of the first orthopedists to advocate the use of power tools for bone grafting procedures (2). In 1923, Albee recommended that, for the best possible results, bone grafts should be autogenous in nature and consist of all four bone layers: periosteum, cortex, endosteum and marrow. He believed that the graft should act as the internal fixation agent, and advocated avoiding any type of metal internal fixation device (2). Both Albee and Phemister were of the opinion that stressing the graft-host interface was of absolute necessity to stimulate osteogenesis and recommended external fixation as the only means of immobilization. It is interesting to note that Albee alone reported over 1600 successful autogenous transplantations in 1919 (49).

Albee's convictions concerning the desirability for avoidance of internal fixation devices were probably well founded. In the years following Hausmann's introduction of the first bone plate for internal fixation in 1886, there was a

high incidence of wound sepsis and mechanical dysfunction of the plate (44,192). These continued to be major problems for quite some time and probably were the main reasons for their disfavor among many surgeons of that era.

In 1920 an experimental study on autogenous and homogenous bone grafts in dogs was performed by Brooks and Hudson. Their results revealed that homogenous transplants of bone were successful 76.8% of the time compared with an 84.8% success rate using autogenous grafts. They concluded by saying that tissue incompatibility of different individuals, as opposed to the age of the donor or recipient, was the most important factor in determining the success or failure of the homogenous transplant (28).

Further support for Ollier's theory came from the work of Lexer in 1924. Lexer believed that under optimal stimulation distinctive cells within the periosteum, bone marrow, and Haversian canals of the graft bone would become osteogenic. He termed this optimal stimulation as being trauma and inflammation (10).

In 1935, bone transplantation, and surgery in general, began a new and more successful era. That year marked the advent of the use of antibiotics starting with the introduction of sulfonamides and penicillin soon thereafter (16). The incidence of postoperative infection and graft failure were subsequently dramatically reduced.

By 1944, it was well established by the many surgeons studying transplantation that autogenous, cancellous bone was the most highly osteogenic and the most preferred grafting material to use. Lipscomb cites the work of Abbott and his coauthors published in 1947 as declaring the definitive reasons for the use of cancellous or cortical bone grafts. Cortical grafts were recommended for conditions requiring strength, as in ununited fractures of the shafts of long bones. Cancellous grafts, on the other hand, were recommended for ununited fractures of the ends of long bones, for defects in bones caused by tumors or infection, and for joint arthrodesis. They also followed Albee's suggestions in emphasizing that as little metal as possible be used for internal fixation. Instead, one of Abbott's coworkers, Horn, advocated the use of double or triple cortical grafts packed with cancellous bone in cases of severe segmental defects (109).

By the year 1945, bone grafting, utilizing autogenous cancellous or cortical bone, had become an established surgical procedure with wide acceptance. Although the use of preserved bone had been experimented with for many years it still lacked acceptance for general clinical use. It was and still is considered to be a second choice to autogenous bone (90,194). As previously emphasized, many of the earlier experiments failed due to sepsis and lack of proper graft immobilization. Another reason for graft failure was a foreign protein reaction which was not very well

understood and feared by many surgeons. However, the idea of using preserved bone in place of autografts remained entertaining. Theoretically preserved bone would help reduce surgery time and eliminate the post-operative pain often experienced from the harvest site. Also, in cases of severe bone loss or in certain debilitated patients, sufficient autogenous bone probably would not be available to satisfactorily meet the amount required. This point was further emphasized by a letter written in 1945 by Dr. William Von Lackum of the New York Orthopedic Hospital, emphasizing the need of bank bone, especially for patients with severe scoliosis (42).

Several questions concerning the use of preserved bone were now seriously considered. Should bone be harvested from a living being, or could it be taken from a cadaver, and in either case, do any cellular elements survive? What type of foreign protein reaction can be expected? What is the optimum method of storing bone, and how long can it be preserved? In view of these questions, it is appropriate to emphasize a statement made by Hyatt in 1960 concerning the use of preserved bone. "The ideal bone bank resides in the ilium and tibia of the afflicted patient" (90).

Although Albee recommended the use of autogenous bone in preference to anything else, he was the first to make specifications concerning the proper methods of bone preservation. He recommended that bone be immersed into sterile

petrolatum or that it be wrapped in petrolatum gauze and kept at 4° C for 24 - 48 hours (90).

The first bone bank was probably created by Inclan in 1942. He reported the successful use of homologous preserved bone in patients in which there was significant operative risk and in patients from whom fresh autogenous bone was not available. The bone to be preserved was obtained aseptically, kept covered and immersed in citrated blood of the patient or donor, refrigerated between 37-40° F, and bacteriologically controlled during the period of preservation (92).

Bush followed in 1944 with a report on the use of homogenous bone grafts in 67 operations with only four complications; he felt that neither blood type nor Rh factor influenced the results of bone transplantation. He also believed that bone could be safely stored in sealed containers at 2-5° C for periods of up to three weeks and could be kept indefinitely if preserved at -25° C (41).

Many methods of bone preservation were subsequently experimented with in both clinical and laboratory situations with varying degrees of success. An excellent historical review of bone preservation in chronological sequence has been compiled by Hyatt (90). Methods of bone preservation and storage have included: refrigeration, boiling, chilling and boiling, freezing, freeze-drying, dehydrating in glycerol followed by slow freezing, and immersion in alcohol, merthiolate, penicillin-streptomycin, plasma and citrated

blood (10,42,49,90,174,190). In addition, Peltier reported the successful use of sterile plaster of paris for segmental bone replacement in 1959 (136). In 1966, Ottolenghii clinically replaced an entire femur using a femur that was harvested from a cadaver (130). The use of the composite homograft-autograft (homograft cortical bone with autograft cancellous bone) was advocated by Burwell in 1964 (35).

In 1972, Ray utilized glycerol to dehydrate bone followed by slow freezing to minus 78^{o} C. He found that bone preserved in this manner could subsequently be grown in tissue culture (147). Mankin reported in 1976 on 19 massive resections and transplantations utilizing cadaver bone that had been similarly processed. These allografts were utilized to replace a variety of malignant or aggressive bone tumors, and, except for two grafts that became infected, no recurrence or metastasis had occurred. Mankin concluded that allograft replacement may play a significant future role in certain types of neoplastic conditions of bones and joints (111).

The future of bone transplantation holds many exciting possibilities. Of tremendous interest is the work done by Ostrup and Doi (57,129). Ostrup described the auto-transfer of a section of rib to a defect created in a mandible using microvascular anastomoses. Doi and his coworkers utilized a similar technique to transfer rib segments as

inlay grafts in the femur of dogs. With this approach, the surgeon theoretically is able to maintain the viability of the entire graft by preventing the death of the osteocytes contained therein.

Of further interest is a study performed by Simmons (157,158). Apparently, the time of day that a bone graft surgery is performed has a great influence on the eventual outcome. Simmons reported that bone union was more prevalent in osteotomy implants made at 0400-0600 hours than at 1600-1800 hours regardless of the graft type. Clinical nonunions increased by 50-60% during the day when autogenous or surface decalcified bone allografts were used. Nonunions were also increased by 25% during the day when fresh bone allografts were used. Simmons concluded that circadian shifts in the connective tissue bed surrounding the graft may have been responsible for these differences (157).

Today, bone transplantation is enjoying a revitalized interest in both human and veterinary orthopedic surgery. Ray has estimated that over 200,000 bone grafting operations are performed each year in the United States (148). A report on the clinical use of frozen cortical bone grafts in both dogs and cats has recently received national attention (188). In addition, prosthetic devices for the total replacement of the femur and humerus have recently been reported by human orthopedists (113,197).

In an age where total hip and knee replacement is very commonplace, it is appropriate that a renewed interest in bone transplantation should occur. Perhaps through the work of both human and veterinary orthopedic surgeons, the clinical use of preserved bone will finally achieve a more universal acceptance.

Indications for the Use of Bone Grafts

The bone graft can fulfill one or both of the following depending upon its origin: (1) it can act as a center of osteogenesis, e.g., an autogenous cancellous graft; (2) it can serve as a means of osteofixation, e.g., an inlay or onlay cortical graft; or (3) it can perform both of the preceding, e.g., cortico-cancellous grafts from the wing of the ilium or the medial surface of the tibial crest. Composite grafts consisting of a combination of cortical allograft and cancellous autograft bone are becoming more popular since Burwell proved their efficacy (35).

The primary indications for the use of bone grafts are (77,116,191):

1. To span bone defects in:
   a. fractures with the loss of a segment of bone
   b. <u>en bloc</u> tumor excision
   c. following currettage of a bone cyst
2. As an inlay or onlay graft to facilitate osteogenesis and healing in:

a. delayed union

b. nonunion

c. osteomyelitis

3. Joint arthrodesis

4. Spinal fusion

The mere insertion of donor bone, either autogenous or as an allograft, does not assure complete or even partial success. The surgeon may increase grafting success through proper handling of the graft and careful preparation of the recipient bed. Probably the two most important factors in the final outcome of this type of surgery are the vascular supply of the host bed and the ability to completely immobilize the graft within the bed (3). The existence of infection or an ongoing disease process at the recipient site will dramatically reduce the rate of success. To further emphasize this point, Phemister has recommended waiting for two years after an infected wound has healed before transplanting bone to that site (140).

The surgeon must also fulfill the following basic criteria when inserting the graft (3,8,14,17,41,44,63,77,84,87,101,116, 141,165,178,186):

1. Establish a stable and accurate interface between the host and donor bone with as large an area of contact as possible

2. Achieve rigid immobilization of the graft-host interface

3. Restore the proper anatomical alignment between the graft and host bone
4. Prepare the host bed by:
    a. removing all scar tissue
    b. providing adequate fascial and skin coverage of the graft
    c. avoiding infection

The most common reasons for graft failure include: infection, insufficient coverage of the graft by soft tissues, inadequate blood supply from the graft bed, graft mobility with subsequent resorption, fracture of the graft due to mechanical stress or rarefaction from rapid and uncontrolled osteoclasis, heat necrosis caused by the injudicious use of power equipment, and insertion of the graft into an infected host bed (3,19,22,31,44,46,60, 93 ,108,117,119,132,135,161,168, 175). Murray has emphasized that many of these problems could be avoided by simply not treating these surgeries as a matter of "routine". Instead, careful attention to detail should be given to the patient before, during and after surgery (116).

The Fate of the Bone Graft

In the early 1950's, following several decades of successful clinical trials, scientists began to seriously examine the fate of bone transplants at the cellular level. Up to that time, only isolated studies in laboratory animals

had been performed. Most of the opinions concerning the fate of bone transplants were derived from serial radiographic examinations of patients. Occasionally, biopsies were taken, but, for the most part, radiographic interpretations had to suffice.

Hutchison, in an early study on the fate of bone grafts in rabbits, remarked, "Much of the confusion and contradiction on the subject of bone grafting is simply due to a lack of precise knowledge of the sequence of histological changes which occur in the tissues of the graft and in the graft bed. Too often an opinion is based solely on the clinical results or on an isolated histological observation of an experimental graft, ignoring the earlier and later tissue changes" (86). Since then, many studies have been performed, and the sequential fate of the bone graft at the cellular level is more fully understood today than previously. The areas presently requiring further clarification are those concerning the host immune response to foreign tissue grafts and the principle of bone induction.

The clinical success of a cortical allograft is dependent upon four biodynamic functions of the graft (91):

1. Biological acceptance of the allograft at the operative site
2. Dynamic anatomical function
3. Provision of an anatomical template for ingrowing

host tissues, i.e., the ingrowing host tissues use it as a guide for revascularization and new tissue formation

4. Ultimate complete replacement of the allograft by the host tissues in the anatomical form of the allograft

The final anatomical configuration of a graft following its reduplication is determined by the functional demands of the operative site (90).

The blood supply to the long bones consists of the nutrient artery, the epiphyseal and metaphyseal arteries and the periosteal vessels (95,96,137,171,184). The periosteal vessels anastomose with the nutrient system through the cortex, and with the metaphyseal and epiphyseal systems at either end of the bone (137). The nutrient artery supplies 50-70% of the blood to the diaphyseal shaft of the long bones. It is the main vascular supply to the sinusoidal system of vessels within the bone marrow, and perfuses the inner two-thirds of the cortex as well as the central canals of the osteons (151,171,172,184). The osteons located in the outer one-third of the cortex are supplied by the periosteal vessels (151,184). At the cellular level, the osteocytes, trapped within their lacunae, receive their nutrients through the canaliculi. Typically, osteocytes can be found within .1 mm - .2 mm of a blood vessel (11,73,198). Once bone has been detached from its circulatory supply, the osteocytes

soon perish. This occurs not only with autogenous grafts, but also with allografts. In a study performed by Heslop, it was discovered that osteocytes in both autografts and allografts survived transplantation. These surviving cells, however, had a predilection for the outer surface of the transplant (78). They were probably being maintained by host tissue fluids that perfused through the superficial canaliculi. The osteocytes located deeper within the cortex subsequently died due to lack of nutrients. Concerning the eventual fate of the graft, it is unknown if the remaining viable osteocytes participate actively in the formation of new bone around and within the graft (73,78,128,169,198). It appears rather unlikely that they possess this ability, and they have never been observed to undergo mitosis (78,169). If surviving graft osteocytes are actively involved in osteogenesis, as some scientists suspect, they must first differentiate, then undergo a fibroblastic transformation, and finally re-differentiate into an osteogenic cell (169).

The reparative processes which occur at the graft-host interface have been likened to those seen in fracture repair. When an allograft is utilized, these reparative processes are delayed in onset and usually take longer to complete.

There are at least four phases of bone graft healing (11, 150).:

1. Reactive bone formation
2. Revascularization

3. Perivascular new bone formation

4. Appositional bone replacement

Reactive bone formation

Initially, a fibrin clot develops at each graft-host interface. Within a few days, the clot begins to organize and undergoes a rapid invasion by vessel buds and newly formed vessels from the host bed (167). Host osteoblasts and perivascular connective tissue cells follow the proliferating vascular buds, and callus formation, similar to fracture callus, occurs (76,177). The callus appears within the medullary canal and bridges the gap between the graft and the host bone externally as well. This phase occurs between eight and thirty-five days following grafting (11).

Revascularization

Eventually, vessels and proliferating vascular elements from the host bed and surrounding soft tissues begin to invade the graft peripherally. This process is facilitated by one or both of the following: (1) groups of osteoclasts make cavities within the necrotic graft which are immediately filled by loops of vessels, or (2) bundles of vessels erode the necrotic bone themselves as they advance (127). The medullary canal of the graft is also invaded by a fine fibered, embryonic type of connective tissue filled with blood vessels and cells with phagocytic, osteoclastic, and osteoblastic potentialities. This tissue originates from the cambium layer of the periosteum and the marrow of the

host (9,14,76,103). Also, newly formed vessels within the callus become reoriented parallel to the longitudinal axis of the bone (167). Due to the ongoing resorption within and around the graft, it becomes mechanically weak, and radiographically has a "moth eaten" or radiolucent appearance. The time required for the complete revascularization process is directly dependent upon the size of the bone graft. This process occurs endosteally, periosteally, and at the graft-host interface simultaneously, and is seen between seventy and one hundred twenty days following grafting (11).

Perivascular new bone formation

This phase occurs either simultaneously with or very closely after the phase of revascularization (54). As the Haversian canals of the graft are revascularized they become enlarged due to the resorption of the necrotic bone. Advancing osteogenic cells, including host osteoblasts and pluripotential connective tissue cells that have been stimulated to become osteogenic in nature, begin to deposit bone within the canals. This new osseous material undergoes maturation by becoming enclosed within lacunae, and the process is repeated until the central canal of the osteon approaches a normal diameter (83,99). Soon the graft becomes a conglomeration of living and dead bone (73). These events occur between ninety and one hundred fifty days after graft insertion.

Appositional bone replacement

This final phase of graft replacement goes on indefinitely until all or most of the necrotic bone has been removed. It involves the replacement of that osseous matrix not removed during the initial invasion by host vessels (11). It has been shown that portions of necrotic graft bone remain for many years following surgery (6).

The preceding phases of bone graft healing are inherently designed to architecturally remodel the graft in accordance with local structural requirements. In so doing, the bone graft fulfills its functions, namely, the stimulation of osteogenesis from the host bed, and providing an anatomical template for the invasion of host tissues (15).

Typically, the processes of revascularization, resorption and perivascular new bone formation are delayed when cortical allografts, as opposed to cortical autografts, are utilized (6, 78,86,194). Although bone transplants in rats do not seem to be influenced by the age of the host, Hutchison has reported that young bone is easier to revascularize than older bone in rabbits (75,86). No difference has been found between the newly formed osteons within the graft and those within the host (128).

The importance of firm immobilization with accurate and close approximation of the graft host interface cannot be overemphasized. Fitts and his co-workers have noted that mechanical factors appear to determine the success or failure of grafting more so than whether the graft is an allograft

or autograft (63). In addition, excessively large gaps at the interface allow granulation tissue to form between the graft and host bone which leads to a delay in healing (3). Although Albee and Phemister advocated avoidance of internal fixation for the immobilization of bone grafts, others have since recommended the use of internal fixation devices for this purpose (3,17,141,173). Generally, neither the bone plate nor any other means of internal fixation should be used as a substitute for the proper type of graft (17).

The major complication with cortical allografts is graft fracture due to the delay in revascularization and replacement with new bone (135). Therefore, cortical allografts must be protected for prolonged periods of time, and, in some cases, for many years. The best method of providing optimum internal fixation of a graft would appear to be through the use of a bone plate. Jenny, however, has reported that specially designed intramedullary nails also work very well in dogs (63,153). Plate and screw fixation has a biological advantage over the nail because it does much less damage to the medullary and cortical blood supply, and, if compression is utilized, the rigidity of fixation is increased and the fracture gap is narrowed (7). In theory, capillary buds with their accompanying osteoblasts from host Haversian canals can transverse the fracture gap and directly penetrate the Haversian system of the graft (43). Trueta reported that compression causes increased amounts of bone to be deposited within the compressed area and concluded

that the increased ossification was due to enhanced osteogenic vascularity (171). Bassett later reported that compression favors the specialization of osteoblasts while tension favors the specialization of osteoclasts and fibroblasts (13).

The surgeon should take every precaution to avoid bacterial contamination of the graft and the host bed. The graft should never be allowed to touch the skin and should be handled only by instruments. In 1946, Armstrong reemphasized the "no touch technique" originally advocated by Lane. This technique requires that neither the wound nor anything that is introduced into it is touched by the gloved hand or allowed to come into contact with the skin of the patient (8).

The Principle of Bone Induction

The origin of the bone that forms in association with the replacement of a bone graft is thought to occur from at least two sources: (1) preexisting host osteogenic cells and (2) mesenchymal cells of the graft bed that are induced in some manner by the graft to become osteogenic in nature (182). In addition, the inflammatory reaction created by the transplant gives rise to a number of hematogenous (lymphocytes and monocytes) and histiogenous (lymphoid wandering cells and histiocytes) cells possessing the ability to transform into fibroblasts and become potential precursors of osteogenic cells (54).

Although the principle of induction has been recognized for many years, it is not well understood. It has been defined as the mechanism producing cellular differentiation due to the physicochemical effect of one tissue upon and in contact with another (177,178,182,183). In this case, it is inferred that during the resorption phase the bone graft somehow stimulates the mesenchymal cells of the host bed and the perivascular connective tissue cells to become osteogenic in nature. While Urist contends that close contact between the graft and host tissue is required, other scientists theorize necrosis of the graft must occur first before any substance can be released (72,145,182).

Bone induction was once believed to be a local chemical phenomenon under the influence of an organizing substance (97,106,150). Later modifications of this theory emphasized the existence of a diffusible osteogenic inductor from the graft (68,86,143). Trueta believed that instead of having an effect on host mesenchymal cells, this substance acted directly on the host blood vessels penetrating the graft. Because of the angioblastic effect it had on these vessels, he named the unknown substance "vascular stimulating factor" (VSF) (171). Craven later refuted this theory by showing that muscle mesenchymal cells are the forebearers of osteoprogenitor cells, and blood vascular tissues are a secondary source of cells with osteogenetic competence (52).

One of the latest theories on induction suggests that the surface charge of the transplant directly affects the amount of induced bone formation. In theory, the higher the negative surface charge of the graft, the more suitable it is to produce this effect (61). Demineralized bone has been found to possess a higher negative surface charge than nondemineralized bone (61). It has been proposed, therefore, that surface demineralization of grafts has a more profound inductive effect although it weakens the graft (61, 121,122, 123,179). In contrast to the above, Spence and Nade in separate tests, have reported that surface demineralization of bone was less effective in producing new bone than either cortical or cancellous bone (120,160). Fresh cortical allografts and untreated, freeze-dried allografts produce very minimal amounts of new bone formation by osteoinduction (131, 180,181). In fact, Urist has found that implants of devitalized bone generally do not set up an induction system (178).

In summary, the principle of bone induction as a part of normal fracture healing is recognized; however the significance of this process as a primary means of bone graft replacement in this experiment is questioned.

The Immune Response

The immune system of the recipient of a tissue allograft influences the final disposition of a transplant. Unless the tissue is host derived, a reaction termed

the "immune response" results due to the introduction of a foreign protein into the host. This response has been defined as the process by which the host recipient protects its cellular integrity from the intrusion of foreign graft material which is immunologically unacceptable to the recipient (24). Specifically, the reaction which occurs following the use of allografts has been termed the "allograft (homograft) response or reaction" (11,24). The intensity of this reaction is in direct proportion to the cellular content of the graft (e.g., skin as opposed to bone) and the genetic disparity of the donor and the recipient (11). The term antigenicity is utilized in place of "foreign protein", and is defined as the ability of a foreign material (antigen) to be recognized by the host as "nonself" and to elicit the formation of antibodies and/or immunocompetant cells that can react with that antigen (65). The immune response resulting in the production of these immunocompetant cells are divided into: (1) "humoral" immunity, and (2) delayed or cell-mediated immunity (59,65, 125,134). The host reaction following the introduction of a specific antigen is often a combination of both responses with one usually predominating (59).

The humoral response is mediated through thymus-independent, bone marrow derived B lymphocytes (50,134). It is aroused by the introduction of an antigen which is then conveyed to an immunologically reactive site such as a lymph

node (125). This results in the production of antibodies that are subsequently released into the bloodstream and can be found in the Immunoglobulin (Ig) fraction of the serum (59). Although their most important role is the production of a cytotoxic effect, their role in the rejection of a tissue allograft is uncertain. Elves cites two reasons for this uncertainty. Antibodies appear in the serum only after damage to the graft is well advanced, and are detectable only when the graft and the host are disparate for the major histocompatibility system (59).

The delayed or cell-mediated immune response is thought to be of considerable importance in graft rejection (59,125, 134,144). This response is caused by the reactivity of thymus derived, small T lymphocytes. Following their recognition of antigenic material, the resting small T lymphocytes become biosynthetically active. These activated cells can function directly as killer cells by secreting lymphotoxins while attaching to target cells, or they may influence macrophages and other cells to function as effectors by secreting lymphokines. The sensitized T cell possesses antigen recognition sites that are not present on B cells; a reaction is initiated by a union between a specific antigen and these receptor sites. This system appears adapted to deal with antigens that exist peripherally and do not contact lymphoid tissue (50). It has recently been demonstrated in mice that passenger leukocytes may play a role

in providing a helper stimulus which potentiates the generation of cytotoxic T cells to serologically defined determinants (159).

Allergic or immune reactions have been grouped into four types of hypersensitivity diseases. A Type I reaction is characterized by anaphylaxis and is of no concern here. Type II reactions involve the production of antibody in sera. This group includes the "humoral" immune response. Type III reactions involve the production of immune complexes and are of importance in the inflammatory response. Delayed or cell-mediated immunity is classified as a Type IV reaction (50,59,125).

The most severe reaction between donor tissue and the host occurs when they differ at the major histocompatibility system (MHS) or complex (MHC) (125). These systems have been divided into major and minor systems. In order for the donor tissue to be accepted by the host, there must be a compatibility between the major systems. The major histocompatibility system in man is referred to as HL-A, in mice H-2 and in rats H-1 (or Ag-B) (125).

There is a significant contrast between the inflammatory reaction created by fresh bone allografts as opposed to autografts. Allografts usually incite a cellular reaction which starts around two to four weeks after surgery (18,21,155). This reaction is characterized by the appearance of lymphocytes, monocytes, macrophages, eosinophils,

mast cells and plasma cells (18,21,31,38,78,146). In addition, graft resorption and replacement are delayed, probably due to the local immune response. At least one study has shown this cellular response to create morphologically abnormal collagen, fibroblasts, and blood vessels (78). This allograft reaction is in contrast with autogenous grafts which usually incite only a minimal inflammatory response and are replaced rapidly with new bone (18,21,48,149,150).

Burwell and others have reported the occurrence of a primary immune response in the regional lymph nodes draining an allograft of cancellous bone three days following its implantation (33,38,39,102). When a second-set allograft of cancellous bone was implanted this response occurred in two days. This was manifested by a sectoral distribution of large and medium lymphoid cells in both the cortex and medulla of the lymph nodes. Burwell concluded that the small lymphocytes that are thought to be responsible for graft rejection were probably produced by mitosis from these large lymphoid cells. It was also noted that the involved lymph nodes had a corresponding increase in weight and cortical thickness (33,38,39).

Methods directed toward modifying the immune mechanism to facilitate graft acceptance have included pre-treatment of the graft itself and immunosuppression of the host. Bonfiglio has reported that animals injected with soluble protein extracts of bone in adjuvant prior to grafting

experienced a markedly diminished inflammatory response (18, 20,21). In contrast, a primary graft did not predispose a secondary graft to the same diminished response. Instead, the inflammatory response was increased (18,21). Hutzschenreuter is of the opinion that grafting under the conditions of a second-set reaction is beneficial because the graft is resorbed and replaced at a faster rate (87).

Antilymphocyte globulin (AGL) and azothioprine, while paritally suppressing the host immune response, do not appear to significantly increase the rate of graft acceptance (32,79). Cortisone has been shown to be an effective immunosuppressant in gerbils with detrimental effects to bone and cartilage induction (195).

Burwell and others have reported that the nucleated red cell of the bone marrow is the principal antigenic component of a fresh allograft (34,38,39,120). In theory, the removal of the marrow prior to transplantation will markedly reduce the immune response. While this may be true in rodents, Elves has remarked that this type of transplant is still quite antigenic in dogs. In this species, the red cells do not carry a significant amount of antigens of the major H-antigen system (59). Instead, the major antigenic stimulus in bone may involve each of the following constituents: surviving osteocytes, chondroitin sulfate, mucoproteins, polysaccharides, and possibly the lipid content (124).

Generally the processes of freezing and freeze-drying have been demonstrated to effectively reduce the inflammatory response elicited by the bone allograft. Boiling, irradiation, merthiolate preservation and cultivation in the blood of the recipient have also had the same effect, but the mechanism of how this occurs is unknown (11,21,33,34,37,40,65,94,102). Chalmers and Brooks have reported that freeze-dried bone allografts allow a second-set skin graft to survive for a significant period of time as opposed to fresh allografts (29,48). Chalmers has called this process of increased survival the enhancement effect (48).

Heiple has formulated the following rating scale for bone grafts in terms of their osteogenic properties from the most desirable to the least (76):

1. Fresh autogenous bone
2. Freeze-dried allograft bone
3. Frozen allograft bone
4. Decalcified allograft bone
5. Frozen irradiated allograft bone
6. Freeze-dried irradiated allograft bone
7. Fresh allograft bone
8. Deproteinized xenograft bone
9. Deproteinized allograft bone

In addition, tissue typing to prevent a disparity of the major histocompatibility system (MHS) between the donor and the host is presently receiving more emphasis than previously (70,118).

Burwell has suggested that the various methods used to prepare bone for storage result in a considerable impairment of its transplantation antigenicity. The ultimate reason for the relative inadequacy of homogenous bone as opposed to autogenous bone may be due to an impairment in vascular and cellular invasion with a subsequent impairment of osteogenesis (37).

THE PRESERVATION OF BONE BY FREEZING AND DRYING

History

Although the use of freeze-drying or lyophilization as a means of tissue preservation had its origin almost thirty years ago, it was a scientific curiosity as early as 1813 (91). The concept was utilized to preserve bacteria and viruses at the turn of this century, and subsequently was used to preserve plasma in 1935 (91). Pharmaceutical companies soon followed suit by demonstrating this process to be a valuable means of preserving proteins, enzymes and vitamins (104). The growth of freeze-drying in popularity as a means of food preservation has increased over the years to the point where products of this type are quite commonplace in the local supermarket.

In 1951, Kreuz and his co-workers published the first account of the clinical use of freeze-dried bank bone (104). This publication led to what may now be the largest tissue bank in this country and possibly the world, i.e., the Tissue Bank at the Naval Medical School in Bethesda, Maryland. These workers and their followers perfected the science of freeze-drying as a means of preservation of not only bone, but also skin, cartilage, arteries, veins, nerves, fascia and tendons (47,88). The term science is, indeed, stressed for in this sense it encompasses the disciplines of microbiology and immunology as well as tissue biochemistry and cyropreservation.

Prior to 1950 the two most popular methods of tissue preservation were the frozen tissue banks first advocated by Bush in 1947 and the merthiolate preservation of tissues advanced by Reynolds and Oliver in 1949 (41,149). Although merthiolate preserved bone enjoyed a modest success, it proved to be an unreliable means of tissue storage. Bone preserved in this manner required frequent bacterial culture assays to check for contamination, and the merthiolate residue occasionally elicited a sensitivity reaction in the recipient. Even though the clinical use of frozen bank bone is still very common, bone so preserved is also not without potential problems. Under ideal conditions, bone stored by freezing should achieve molecular immobility of tissue ice crystals (90). The usual sequence of events, however, involves the following: ice crystals begin to form from tissue water at 0^{o} C and at -20^{o} to -30^{o} C tend to enlarge as storage time increases (89,90). Bone preserved at these temperatures, even in doubly sealed glass jars, will gradually undergo an evaporative moisture loss (89). The ice crystals that form consist of pure water (91). Gradually as the bone dehydrates, the tissue salt concentration increases until a hypertonic salt or "brine" solution exists (89,90). Solutions of this nature are not only lethal to cells, but also may have a progressively destructive effect on the bone matrix (89). This process further lowers the freezing point of bone. Protein slowly

begins to denature because of the pH change and the tissues are adversely affected due to thawing at this low temperature (104). Theoretically, a time will be reached when the frozen bone will be entirely unsuitable for transplantation. The most suitable temperature for cryogenic preservation remains unknown, but it is generally believed that the lower the temperature, the better.

The main advantage of freeze-drying is that the treated tissue can be stored under vacuum at room temperature for an indefinite period. Hyatt has reported the successful clinical use of bone stored in this manner with a residual moisture level of 2% - 4% after five years (89). Also, freeze-dried bone can be shipped to any hospital in the country without elaborate precautions of temperature control (64,104,193). Frequent tissue cultures are unnecessary, a minimum of tissue distortion occurs, and the biochemical constituents are better preserved as compared to the usual freezing methods employed by frozen tissue banks (133).

The primary disadvantage of this procedure is the initial cost of equipment and the time required for processing (89,90, 91). Although it was once felt that there was little effect on protein structure of enzyme systems, these are probably affected to some degree (62,64,90,104).

Procedure

The process of tissue procurement for subsequent preservation must meet certain basic criteria in order to maintain the biological value of the tissue as a future transplant. For example, the U.S. Naval Tissue Bank obtains all of its allografts from recently deceased individuals. Following legal consent from the next of kin, tissue harvest is begun anywhere from eight to twenty-four hours after death (89,91). The donor must be free from generalized viral or bacterial contamination, malignancy or transmissible disease, or the administration of long acting isotopes (89,133). All tissue samples are taken under sterile operating room conditions with strict bacteriological controls, i.e., cultures are taken before freeze-drying and again at the time of surgery (47). They are double-sealed in glass jars and frozen to -70° C.

The freeze-drying unit basically consists of three shelves. The upper one is the drying plate while the lower two are the condensing plates. The operating vacuum is kept between 5-10 microns, and the plates are maintained at -45° C. Once the frozen tissues have reached equilibrium at -70° C, they are placed on the drying shelf in glass jars. Usually within twenty-four hours, all but ten percent of the residual moisture has been removed from the bone. At this time, the temperature of the drying plate is gradually elevated to room temperature. A final residual moisture of 1% - 5% as determined by drying

the tissue sample to a constant weight over phosphorus pentoxide at 25° C or 45° C is desired. This process takes approximately fourteen days depending upon the tissue size and density. The final product is vacuum sealed and stored at room temperature (47,64,89,90,91,104,176,193). The ideal conditions of temperature and rate of evacuation during the freeze-drying process are presently unknown (133).

During freeze-drying, tissue ice crystals are vaporized partly through open ended channels previously occupied by ice and partly through any cracks or fissures that were formed during the course of the removal of unfrozen water (110). Although all of the osteocytes are killed during processing, the final graft has the same volume and shape as before processing (91). Precautions should be taken because treated cortical bone becomes very dense and brittle, and cancellous bone is easily crumbled (47,91,100,104).

Rehydration

Because the freeze-drying process removes all of the tissue water to a residual moisture level of 1% - 5%, it is reasonable to assume that bone preserved in this manner must be rehydrated prior to its intended use. As previously indicated, this type of bone is extremely brittle and very difficult with which to work. In a study performed by Klen, it was discovered that freeze-dried bone had tissue elasticity and impact resistance levels that were 30% and

20% of their respective normal values (100). Theoretically, these elastic and physical properties can be regained simply by rehydrating the bone in physiological saline solution prior to surgery (47).

Various techniques for rehydrating freeze-dried grafts have evolved over the last three decades and have included the use of the following solutions and rehydration times: physiological saline solution for twelve to twenty-four hours, physiological saline solution for maintained twenty-four hours at 40^{o} C, physiological saline solution for periods not greater than one hour and saline-penicillin-streptomycin solution for twenty-four to forty-eight hours under refrigeration (47,89,90,91,100,107,196).

A rehydration time of twenty-four hours appears to be used most frequently. Klen cast a serious doubt on the added benefits of rehydrating bone for periods of greater than one hour when he discovered that measurable quantities of potassium, calcium, esterified fatty acids and proteins were washed out of bone that had been rehydrated for periods of twenty-four hours or longer. He concluded that rehydration for one hour was sufficient because at this time the degree of elasticity was returned to half the value of a fresh graft, impact resistance returned to a value of 80%, and there was only a minimum of substances removed from the graft (100).

Freeze-dried cancellous bone can be rehydrated by a slightly different process than cortical bone. Carr has reported that cancellous bone can be packed into the operative area in the dry state because sufficient reconstitution will eventually be achieved through the patient's hematoma (47). However, Pappas recommends that cancellous bone be rehydrated for at least two hours prior to surgery (133).

Maintenance of Graft Sterility

Since its inception, the use of preserved bank bone has raised serious questions concerning the efficacy of this tissue as a substitute for the patient's own bone. It is well-acknowledged that there are inherent risks involved in this procedure, not the least of which is the transplantation of infected graft material. Even though the freeze-drying process involves the sudden exposure of tissue to freezing temperatures and subsequent dessication followed by cell death, it should not be expected to sterilize the tissue. As previously indicated, bacteria and viruses were preserved by freeze-drying at the turn of the century (91). Many bacteria and viruses, including some of the common pathogens, are resistant to freezing and freeze-drying (56). Bone treated by storage in antiseptics and antibiotics, boiling and autoclaving may also continue to harbor bacteria (56).

The concern over the maintenance of sterility of preserved tissue is well-founded. Even bone that has been

harvested under sterile, operating room conditions from healthy donors has been found to be contaminated. In 1952 and 1955, the rejection rates of tissue thus harvested were 16.3% and 8% respectively at the U.S. Naval Tissue Bank (47, 105). DeVries later claimed that tissues cultured negative at the time of harvest and subsequently freeze-dried could possibly yield a positive culture at the time of surgery (56). The need for a stringent bacteriologic control was obvious.

Different methods of maintaining tissue sterility have since been demonstrated to be effective. Flosdorf described a method of incorporating penicillin into the graft prior to the freeze-drying process (64). Hyatt later described a method of harvesting bone under sterile conditions, culturing it, then washing the bone in a solution of penicillin and streptomycin. The bone was sealed in sterile jars, freeze-dried, and cultured at the time of use (91).

Other methods of tissue sterilization have included the use of irradiation and ethylene oxide. The controversy over the efficacy of these two methods remains a strong issue of debate among scientists.

Cobalt^{60} radiation was used by DeVries to sterilize bone transplants. He concluded that the process did not destroy the ability of the transplant to form new bone (56). Tarsoly later concurred that compact bone that was freeze-dried and radiosterilized was suitable for allogenic transplantation (166). Other scientists have disagreed by

claiming that radiation considerably reduces the mechanical properties of the transplant (36,100,170,179). Urist has shown that irradiation in excess of 1.0 million rads has a severe effect on the bone matrix and recommended that irradiation not be used for sterilization of bone for bone banks (179). In addition, irradiated, freeze-dried bone has shown to take twice as long to rehydrate when compared to non-irradiated bone (100).

The use of ethylene oxide for tissue sterilization has been reported to be quite effective (58,107,196). This method theoretically allows tissues to be harvested under clean but nonsterile conditions, sterilized, cultured and subsequently freeze-dried. Egyedi has argued that while ethylene oxide will kill surface bacterial contaminants, it will not affect the bacteria that may be within the bone itself. He has recommended that bone should be rehydrated with an antibiotic solution at the time of intended use (58). One possible side effect of this method of sterilization is a local tissue reaction thought to be caused by ethylene oxide residues remaining in the tissue (133).

A method which remains very popular is the acquisition of tissues by sterile autopsy within twenty-four hours after death (114).

The injudicious transfer of preserved bone to an infected site in the recipient will also invariably result in graft resorption and failure (47,91,112). This

problem has also been encountered in grafts used in maxillo-facial surgery due to the inability of the surgeon to seal off the grafts from contamination by oral or nasal fluids (112).

In summary, at least two basic requirements exist for the successful transplantation of preserved bone: (1) a healthy graft-host tissue bed, and (2) a sterile, biologically acceptable graft.

Clinical Applications for the Use of Freeze-dried Cortical Allografts

The indications for the use of freeze-dried cortical or cancellous bone are many and varied. Preserved allografts, and especially xenografts, are not as satisfactory as are the patient's own tissue for grafting. Nevertheless, their use eliminates the severe pain often encountered from the second operative site, and reduces shock, blood loss and total surgery time. Allografts are also indicated when the surgical demands go beyond the limits of the patient's available tissue, and in those patients whose systemic condition reduces the amount of acceptable bone available (90). These conditions especially hold true for the young, the aged, and the poor surgical risk (89).

Before utilizing an allograft, the surgeon must have a definite understanding of the purpose for which it is intended. It is generally accepted that is osteogenesis is the primary concern, the fresh cancellous autograft is the primary graft

choice. For osteofixation, however, the preserved cortical allograft is of greatest value. The final success of the allograft within the living host depends to a great degree on the graft and host tissue accomplishing the following biodynamic functions: the biological acceptance of the graft, the ability of the graft to withstand the mechanical demands exerted on it, and the ability of the graft to serve as an anatomical template for ingrowing host tissue which will eventually replace the graft (193).

The primary clinical indications for the use of freeze-dried cortical and/or cancellous allografts are the following (47,64,71,85,90,91,98,112,154):

a. Extensive bone replacement
b. Replacement of bone cysts following currettage
c. Replacement of bone tumors following en bloc excision
d. Spinal fusion
e. Joint arthrodesis
f. As an aid in the repair of fractures of long bones afflicted with osteomyelitis or characterized by delayed or nonunion
g. Congenital or acquired bone deformities
h. As an aid in the internal fixation of fractures
i. Peridontal osseous defects

The use of freeze-dried bone in both clinical and experimental situations has enjoyed a fair degree of success.

Carr reported the occurrence of only nine failures in ninety-nine clinical cases, a success rate of over 90% (47). Gresham utilized freeze-dried cortical bone as onlay grafts for fresh and nonunion fractures with a success rate of 85% (71). Marble achieved an 82.4% success rate when using freeze-dried bone to fill in cystic defects of jaws and concluded that it was an excellent substitute for autogenous bone (112). DeFries reported on the successful replacement of an entire mandible of a patient with a freeze-dried mandibular allograft which he hollowed out and filled with the patient's own cancellous bone. At the end of two years, the graft was still undergoing gradual resorption and replacement by host tissue (55).

Reports of failure and graft fracture also exist, and some researchers believe that frozen cortical allografts are accepted more readily than freeze-dried allografts (30,53). In a study involving the segmental replacement of a portion of the dog femur with freeze-dried cortical allografts, Roberts reported a success rate of only 29%. The high rate of failure was believed to be due to the splitting of the grafts caused by the intramedullary nail used for internal fixation (153). Because graft splitting appears to be an inherent problem with freeze-dried allografts, Hollins has recommended the use of small, threaded Kirschner wires as a means of obtaining firm fixation to the host bone and reducing the incidence of splitting (81).

Gresham has summarized the primary reasons for the failure of freeze-dried cortical allografts as follows (71):

a. Poor blood supply of the host bed
b. Poor mechanical fixation of the graft
c. Lack of firm contact with host bone
d. Placing bone screws too close to the fracture site
e. Inadequate rehydration of the graft prior to surgery

MATERIALS AND METHODS

Experimental Animals

Fifteen adult dogs between two and ten years old weighing 17.3 to 36.4 kilograms were randomly chosen without regard to sex or breed and utilized as models in this experiment. Each animal was given a thorough physical examination, vaccinated against distemper-hepatitis-leptospirosis, and wormed when diagnosed positive for the presence of intestinal parasites.

To establish a data base line for post-surgical evaluation of hematology and serum chemistry tests, a hemogram was obtained on each animal which included hemoglobin (Hb). packed cell volume (PCV), total white blood cell count (WBC) and differential, plasma protein, and fibrinogen. Blood-urea-nitrogen (BUN) and alkaline phosphatase levels were also taken. Although a Knott's test indicated that three test animals were positive for heartworm (dogs #4, #725 and #670), no treatment was given them for this condition.

During the entire test period, the animals were kept apart from all other animals not involved in this experiment. Each dog was housed in a stainless steel cage with slatted floors. The cages were cleaned twice daily and steam cleaned once per week.

The diet consisted of a combination of canned meat and dry kibble food which was fed once daily. Water was provided ad libitum.

Daily observations were made to monitor appetite and urinary or digestive problems. Frequently this was followed by a ten to twenty minute exercise period.

Formulation of Test Groups

Three test groups were formulated so that the freeze-dried and fresh cortical allografts could be compared radiographically and histologically with fresh cortical autografts at three different time periods during the course of one year. Three animals were randomly chosen as control grafts. Of the remaining twelve animals, six were randomly selected to receive fresh cortical allografts while the remaining six dogs were to receive freeze-dried cortical allografts.

The six dogs receiving fresh cortical allografts were divided into pairs according to size and without regard to their age. In this manner, each dog would receive a graft from and donate a graft to its partner.

The following test groups and graft-harvest time intervals were formulated:

Group 1 — 120 day observation period

a. 1 control
b. 1 fresh cortical allograft
c. 1 freeze-dried cortical allograft

Group 2 — 240 day observation period

a. 1 control
b. 3 fresh cortical allograft
c. 3 freeze-dried cortical allograft

Group 3 365 day observation period

a. 1 control
b. 2 fresh cortical allograft
c. 2 freeze-dried cortical allograft

Graft Preparation

A graft length of 5 cm. was chosen for use in this experiment. Little could be done to insure absolute uniformity in the diameter between the graft and host bone except to match pairs of dogs according to their gross size, as was done in the fresh cortical allograft series. In the freeze-dried cortical allograft series, several segments of bone were rehydrated at the time of surgery, and the one which best matched the host bone in size was utilized.

Each graft was stripped of its periosteum, and the bone marrow was removed by thorough flushing with sterile saline. The bone which was later freeze-dried had been aseptically harvested from living donors four months earlier, and temporarily stored in sterile containers at -70° C for a period of three months. In a manner similar to that described by Hyatt, the bone was freeze-dried at -45° C to a moisture level of 1-2%, and stored in sterile containers at -70° C until utilized (89,90,91). No attempt was made to vacuum seal this bone for storage at room temperature.

The moisture level achieved by this process is in agreement with Hyatt who recommends a residual moisture content of no greater than 5% (89,90,91). A series of tests performed

just prior to this experiment revealed that bone which had been freeze-dried for 48 hours, weighed, subjected to baking for 24 hours in a moisture-proof oven, and weighed again had a moisture content of less than 1%.

Prior to use the freeze-dried bone segments were thawed and rehydrated in a sterile saline-neomycin[1] solution for a period of two hours.

Microbiology

Introduction

Each test animal was assayed for the presence of bacteria in the following manner.

Fresh cortical autografts (controls) Although no cultures were taken at the time of surgery, aerobic and anaerobic cultures were taken at the time of graft harvest. Sterile swabs were utilized to culture the graft surface underlying the bone plate as well as the intramedullary canal.

Fresh cortical allografts A similar method was employed as that utilized in the control study.

Freeze-dried cortical allografts Prior to the freeze-drying process aerobic and anaerobic cultures were taken with sterile swabs that had been moistened in saline. With the aid of a rongeur, bone fragments were made which were also submitted for culturing. At the time of graft harvest, these specimens were again cultured in a similar manner as that employed in the fresh auto- and allograft series.

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[1] Poly-Mycin, Osborn International Multifoods, Minneapolis, Mn.

Procedure

In each case the culture swabs were inserted into sterile CO_2 tubes and submitted directly to the microbiology laboratory. Within an hour, the following methods were employed for bacterial detection. One milliliter of peptone yeast broth (PYB) was transferred anaerobically to a sterile tube. The culture swab was removed from the CO_2 tube and inserted into the broth solution.

For aerobic bacterial detection, the inoculated PYB was transferred to a blood agar plate and to trypticase soy broth (TSB). Both were incubated at 37° C (80).

For anaerobic bacterial detection, the inoculated PYB was transferred to chopped meat glucose broth, sweet E broth, and a fresh trypticase soy agar (TSA) plate incubated under H_2CO_2. All three were incubated at 37° C (80).

The inoculated PYB was also Gram stained and examined. In addition, a wet mount was prepared and examined using phase microscopy.

The cultures were examined every day for bacterial growth. After ten days, they were Gram stained, streaked on a fresh TSA plate and incubated at 37° C under H_2CO_2. These plates were examined every two days for a total of six days.

Culture results are given in Table 1.

Equipment

Surgical instruments and implants

ASIF[1] instrumentation and methods of internal fixation were utilized in each case. This included the use of the narrow Dynamic Compression Plate (six-, seven- and eight-hole plates), 3.2 mm drill bit, 4.5 mm bone tap, and 4.5 mm cortical bone screws.

A standard oscillating bone saw[2] was utilized to create the segmental femoral defects, and a roto osteotome[3] with a burr bit was used to contour the bone at the graft-host interface. All screw-holes were drilled with a nitrogen powered air drill.

Other instruments utilized were Kerns[4] and Richards[5] bone holding forceps, and a general surgery pack.

The Dynamic Compression Plate (DCP) (5,115)

The ASIF-Dynamic Compression Plate was selected because of its general design which allows for self compression at the fracture interface without requiring the use of a special

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[1] Synthes Ltd., Wayne, Pa.

[2] Stryker Corp., Kalamazoo, Mi.

[3] Stryker Corp., Kalamazoo, Mi.

[4] Orthopedic Equipment Co., Inc., Bourbon, Ind.

[5] Richards Mfg. Co., Inc., Memphis, Tenn.

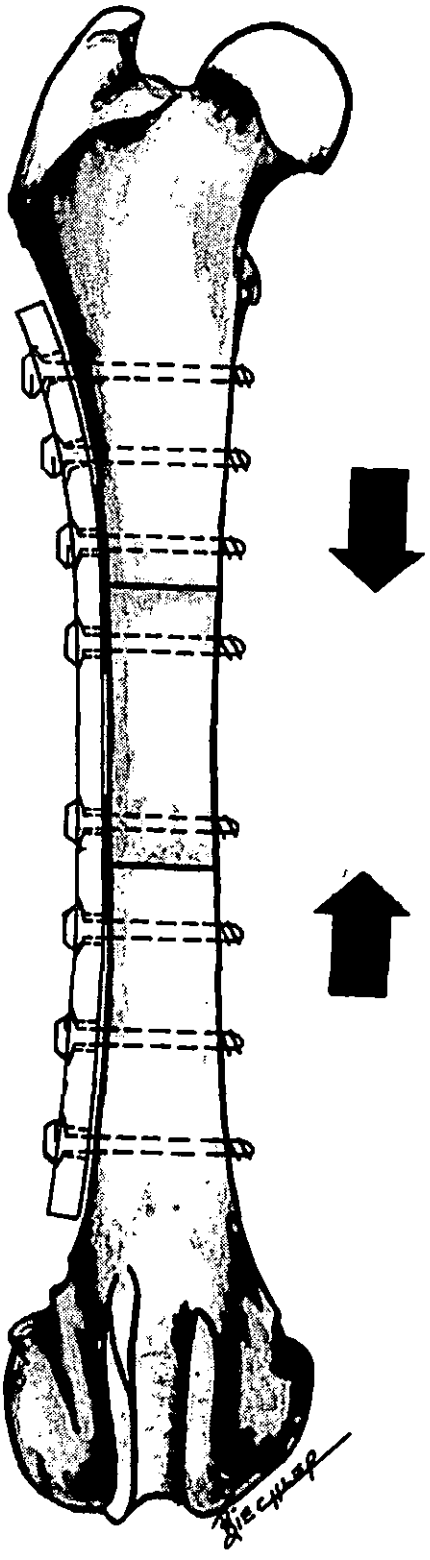
compression apparatus. Due to the large size of the experimental animals in this project, six-, seven-, and eight-hole narrow plates were utilized.

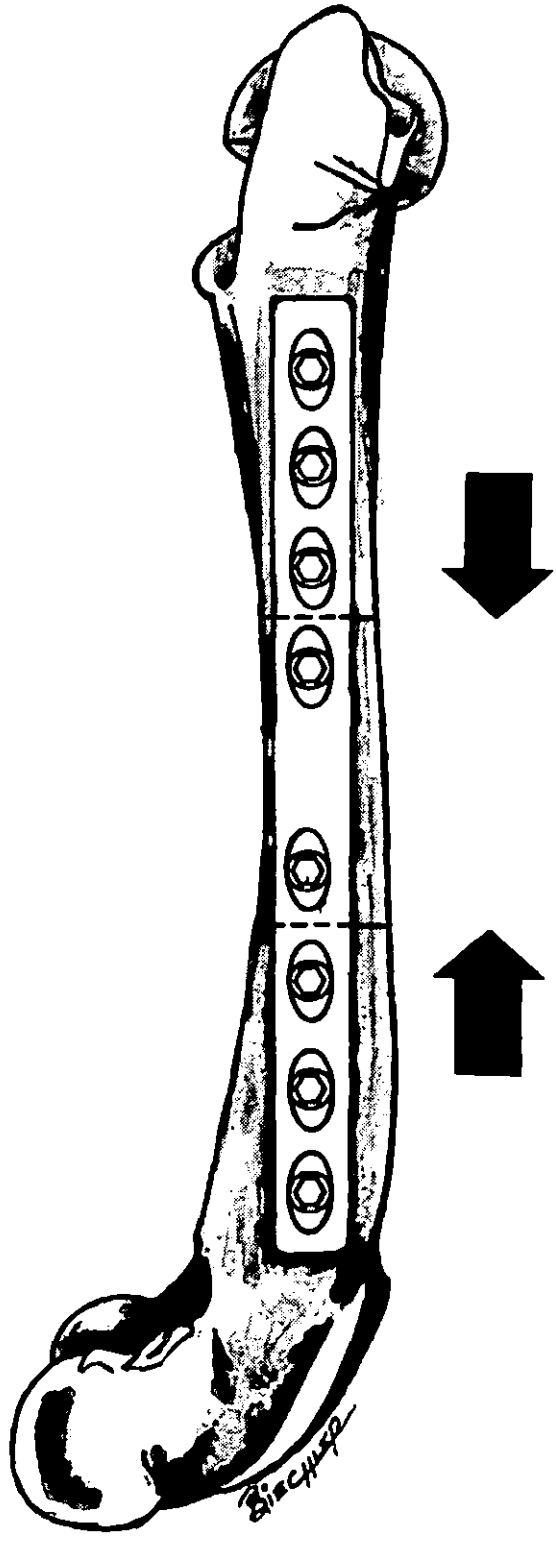
The plate screw holes have been designed to permit a "sliding and compressive movement" at the fracture site. To achieve compression, a specially designed drill guide termed "load guide" must be utilized which allows for a 1 mm eccentric placement of the drill hole. Also provided was a neutral drill guide or "no load guide" which essentially placed the drill hole in the center of the plate screw hole and provides only minimal (0.1 mm) compression at the fracture interface.

To achieve self compression, these procedures should be followed. The bone plate is contoured to the bone surface and secured on one side of the fracture interface by means of a neutrally placed cortical bone screw. On the opposite side of the interface, the load drill guide is utilized to drill the second screw hole. After the drill hole is tapped, a bone screw of the appropriate length is started. As the head of the screw engages the plate the spherical gliding principle of self compression is achieved. The head of the screw glides toward the fracture interface, and a compressive force between the two fracture segments is created (Figure 1).

The preceding technique was utilized in each surgical procedure. The graft segment was neutrally drilled, i.e., under "no load" and both host segments were compressed against it.

Figure 1. The Dynamic Compression Plate (DCP) following application to the antero-lateral surface of the femur and the direction of the "sliding and compressive movement" at each interface.





Anesthesia and Pre-Surgical Preparations

Each animal was fasted for twenty-four hours prior to the surgical procedure.

Approximately thirty minutes prior to anesthetic induction, all animals received 0.04 mg/kg of atropine sulfate[1] subcutaneously. Anesthesia was induced with 5% pentothal sodium[2] administered intravenously at a dosage rate of 11 mg/kg. Following endotracheal intubation, anesthesia was maintained with a combination of Halothane[3], nitrous oxide and oxygen in a semi-closed system.

The right hind limb was prepared for surgery in the following manner. The hair was clipped from the dorsal midline extending cranially over the lumbar fossa and caudally over the base of the tail including the perineal region. The hair was removed from the inguinal and abdominal region as well as on all sides of the limb distally to the hock (Figure 2).

The limb was supported by a sling attached to the paw and surgically scrubbed with betadine solution[4]. A minimum

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[1] D-M Pharmaceuticals, Inc., Rockville, Md.

[2] Pentothal. Abbott Labs., North Chicago, Ill.

[3] Fluothane. Ayerst Labs., Inc., New York, N.Y.

[4] Purdue-Frederick Co., Norwalk, Conn.

of five scrubs were utilized, each one followed by an alcohol rinse. Before transport into the surgery suite, the leg was sprayed with betadine solution.

The animal was positioned in lateral recumbency with the right leg still suspended. The leg itself was covered with a sterile orthopedic stockinette, and the entire area around the limb was draped appropriately (Figure 3).

Lactated Ringers solution[1] was administered as a slow intravenous drip throughout the surgery, not exceeding 20 ml/kg/hour.

Surgical Procedure

A lateral approach to the shaft of the femur, as described by Piermattei, was made (142). Special attention was given to maintenance of hemostasis and sterility throughout the procedure. Following the skin incision, all actively bleeding vessels were ligated with 3-0 chromic catgut[2] (Figure 4). In an effort to prevent surface skin contamination of the graft, sterile huck towels were fastened with towel clamps to the skin edges around the periphery of the operative site (Figure 5).

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[1] Abbott Labs., North Chicago, Ill.

[2] Ethicon, Inc., Somerville, N.J.

Figure 2. Shows the right hind limb following preparation for surgery.

Figure 3. The patient positioned in lateral recumbency with the right hind limb covered by a sterile stockinette. The entire area around the limb was draped in the manner depicted.

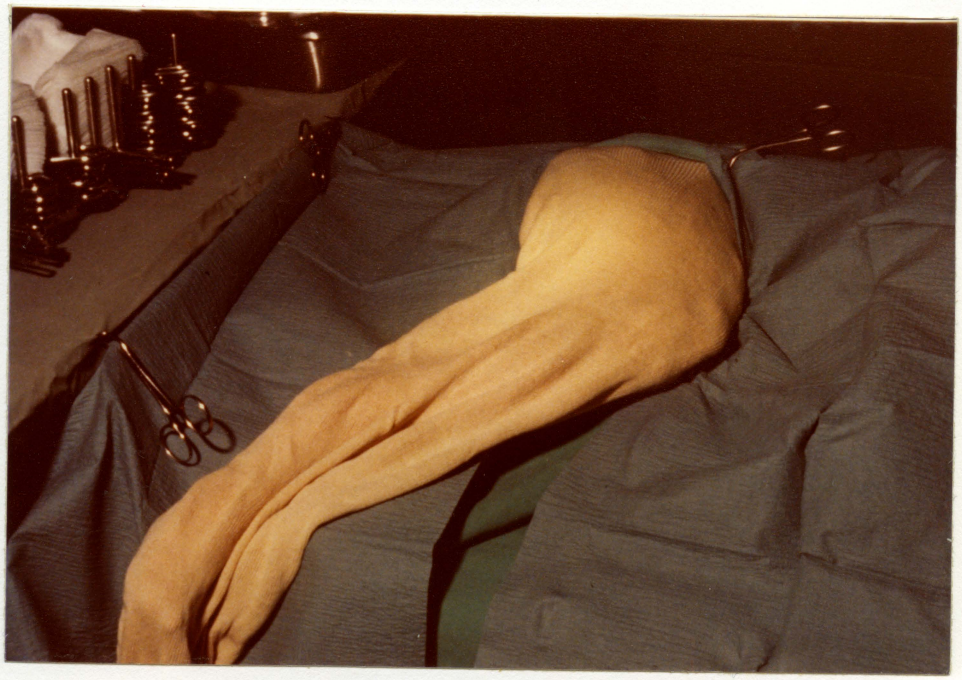


Figure 4. Following a lateral skin incision that extended from just dorsal to the greater trochanter to the lateral condyle of the femur, efforts to insure hemostasis were established.

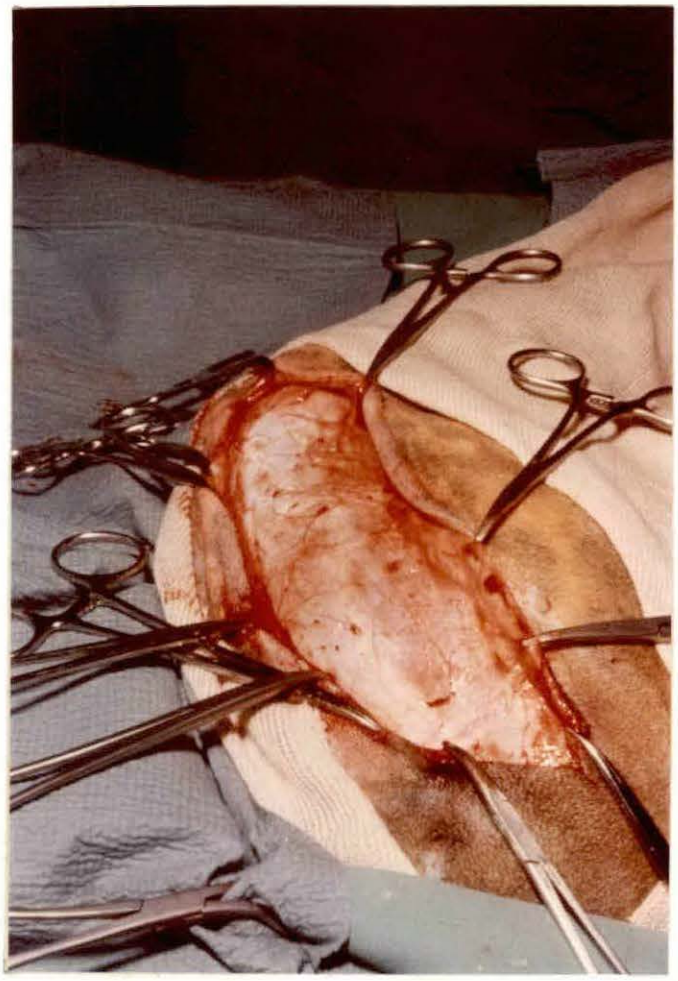
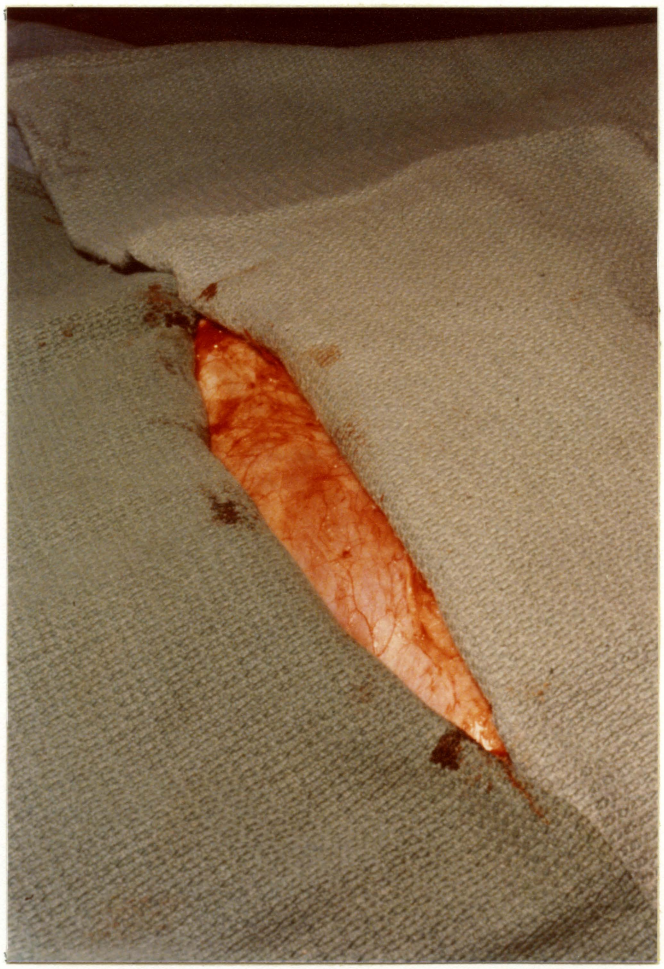


Figure 5. Sterile huck towels were fastened to the skin edges around the periphery of the operative site to prevent surface skin contamination of the bone graft.



A plane of dissection was created along the fibrous aponeurosis between the tensor fascia lata and the biceps femoris beginning at its proximal origin and extending distally to the level of the lateral femoral condyle. The muscular branch of the femoral artery and vein was ligated, and the biceps femoris and tensor fascia lata were retracted with self-retaining retractors.

The periosteum overlying the lateral surface of the femur was elevated and retracted toward the cranial and caudal surfaces of the bone (Figure 6). An aluminum template was placed on the lateral surface of the femur extending from the greater trochanter to the metaphyseal region, and bent to conform to the shape of the bone. A Dynamic Compression Plate (DCP) of appropriate length was then contoured to the shape of the template (Figures 7 and 8).

A 5 cm. section of the mid-diaphyseal region was measured and marked with a scalpel blade (Figures 9 and 10). Under constant saline irrigation, an oscillating bone saw was utilized to cut the measured segment (Figure 11). Close attention was given to the protection of the soft tissue surrounding the femur. The insertion of the adductor magnus muscle on the posterior aspect of the bone segment was removed, but strict precautions were taken to prevent the detachment of this muscle from the remaining host bone segments.

Figure 6. The periosteum overlying the lateral surface of the femur was elevated and retracted.

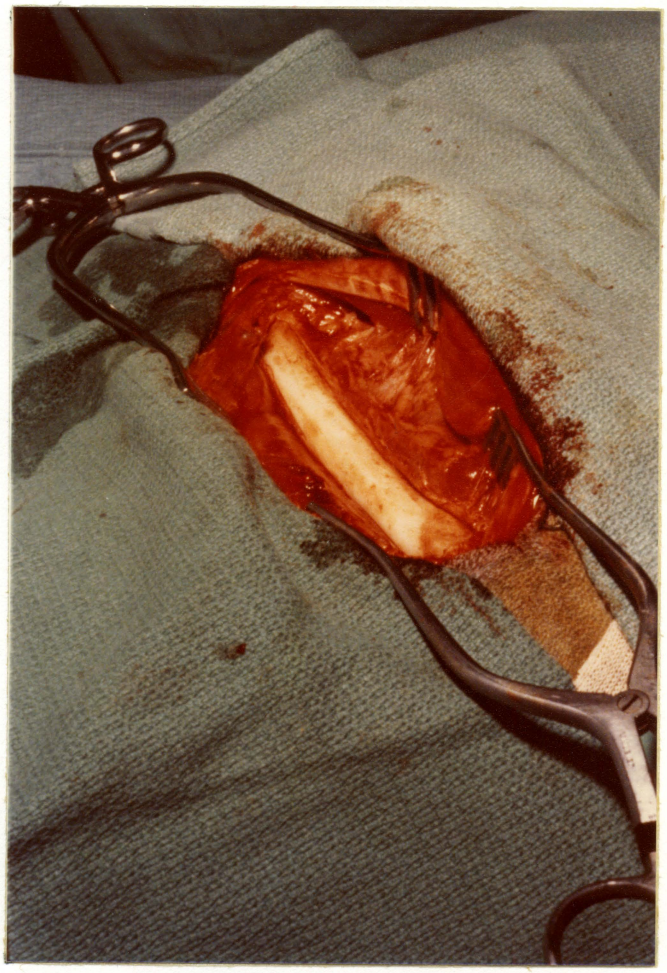


Figure 7. An aluminum template was placed on the lateral surface of the femur and bent to conform to the shape of the bone.

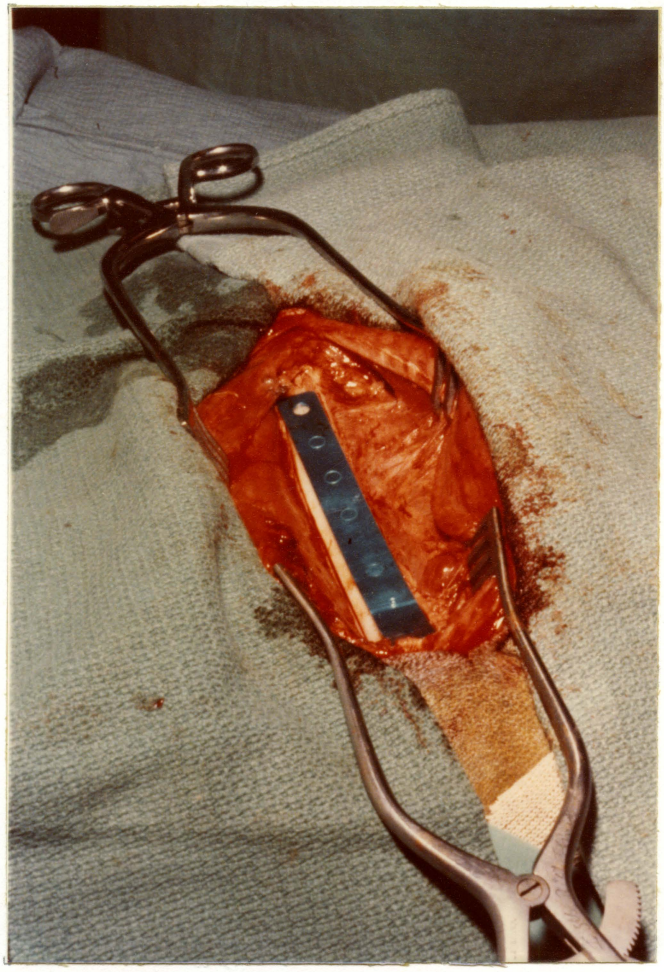


Figure 8. A Dynamic Compression Plate (DCP) was contoured to the shape of the template and placed on the lateral surface of the femur.

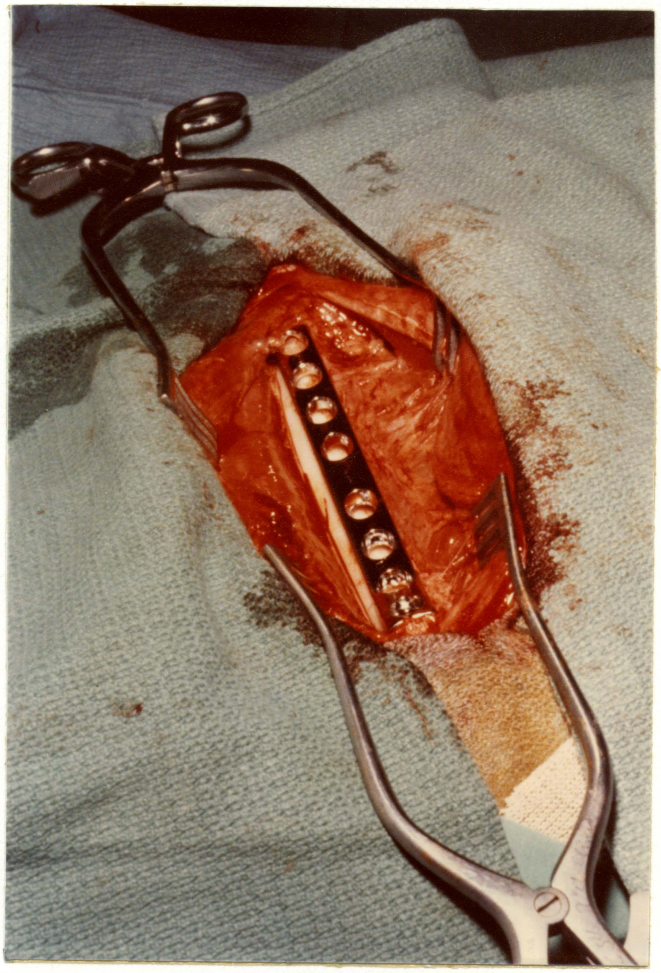


Figure 9. A 5 cm. section of the mid-diaphyseal region of the femur was measured and marked with a scalpel blade.

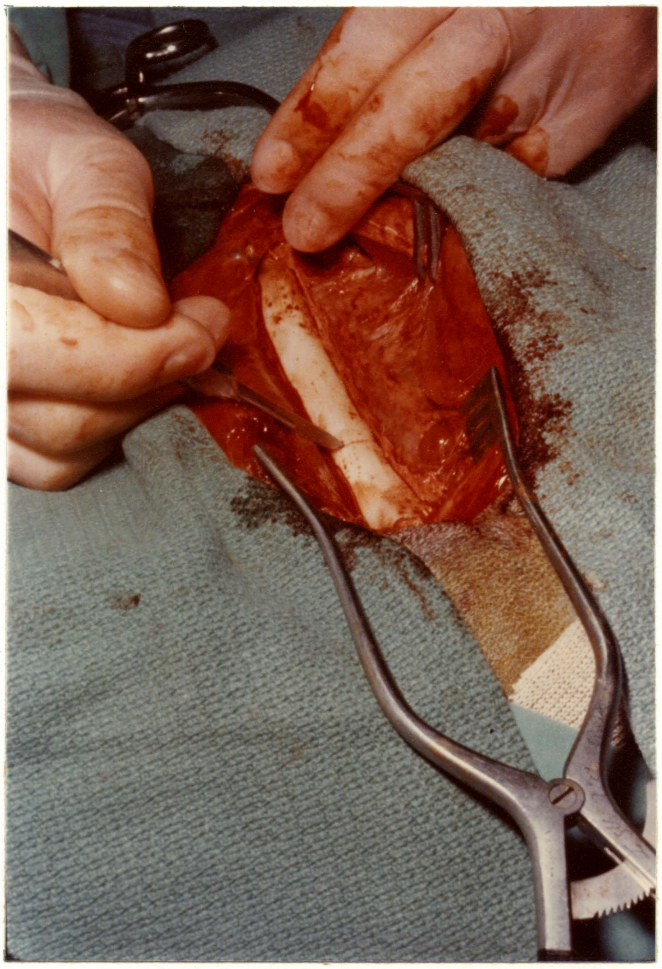


Figure 10. Measurement of the 5 cm. segment.

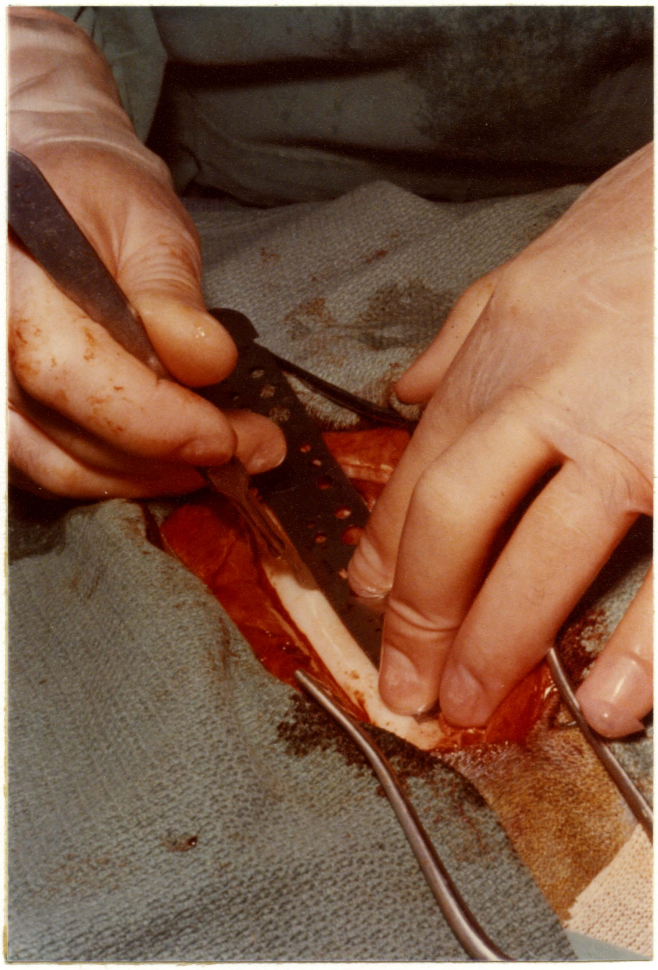
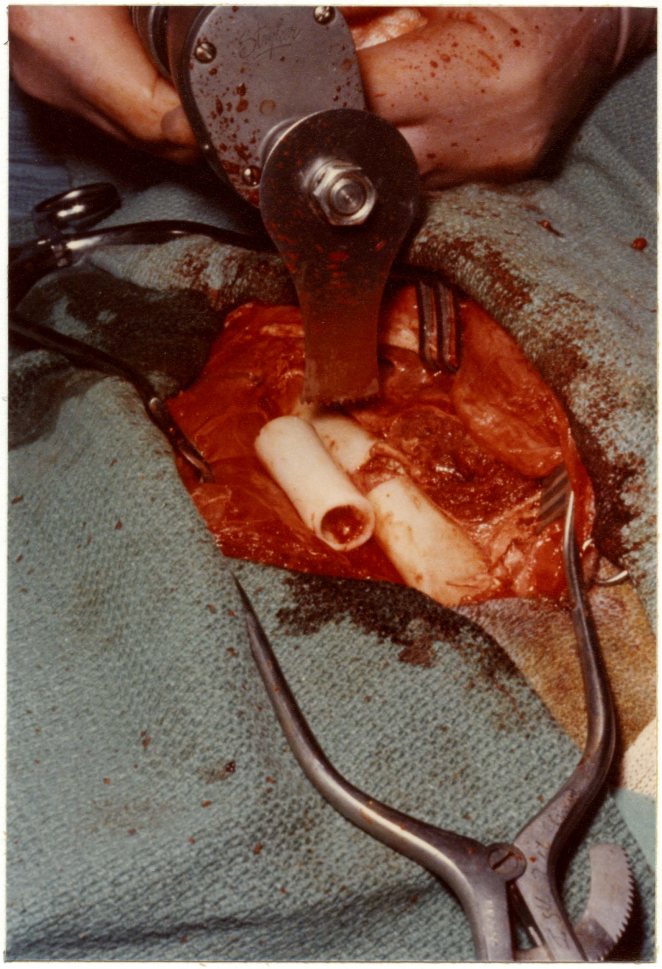


Figure 11. The measured segment was cut with an oscillating bone saw.



In the fresh cortical autograft (control) series, the remaining periosteal and muscle attachments were removed from the segment. The bone marrow was also removed, and the intramedullary canal was thoroughly flushed with saline. The segment was then reinserted at the harvest site.

The fresh allograft series was treated in exactly the same manner with the exception being that two surgical procedures were performed simultaneously by two teams of surgeons. Following the cleaning process, the teams traded bone segments and inserted them into the host segmental defects (Figure 12).

The procedures involving the use of freeze-dried bone were conducted in a slightly different fashion. This bone not only required rehydration in saline but also had to be measured to fit the segmental defect it was to replace. The bone was cut with an oscillating bone saw in a manner similar to that employed on the host bone.

Following their insertion, the bone grafts were adjusted to make accurate conformation with the host bone (Figure 13). A gap of less than 1 mm. was desired at the interface, and special emphasis was placed on establishing homogeneous cortical contact along the medial surface of the femur. Although it was extremely difficult to match the graft and host surfaces exactly, this process was greatly facilitated by the use of a roto-osteotome with a burr attachment. With this instrument,

Figure 12. A diagram depicting the procedure involved in the transfer of the fresh cortical allografts.

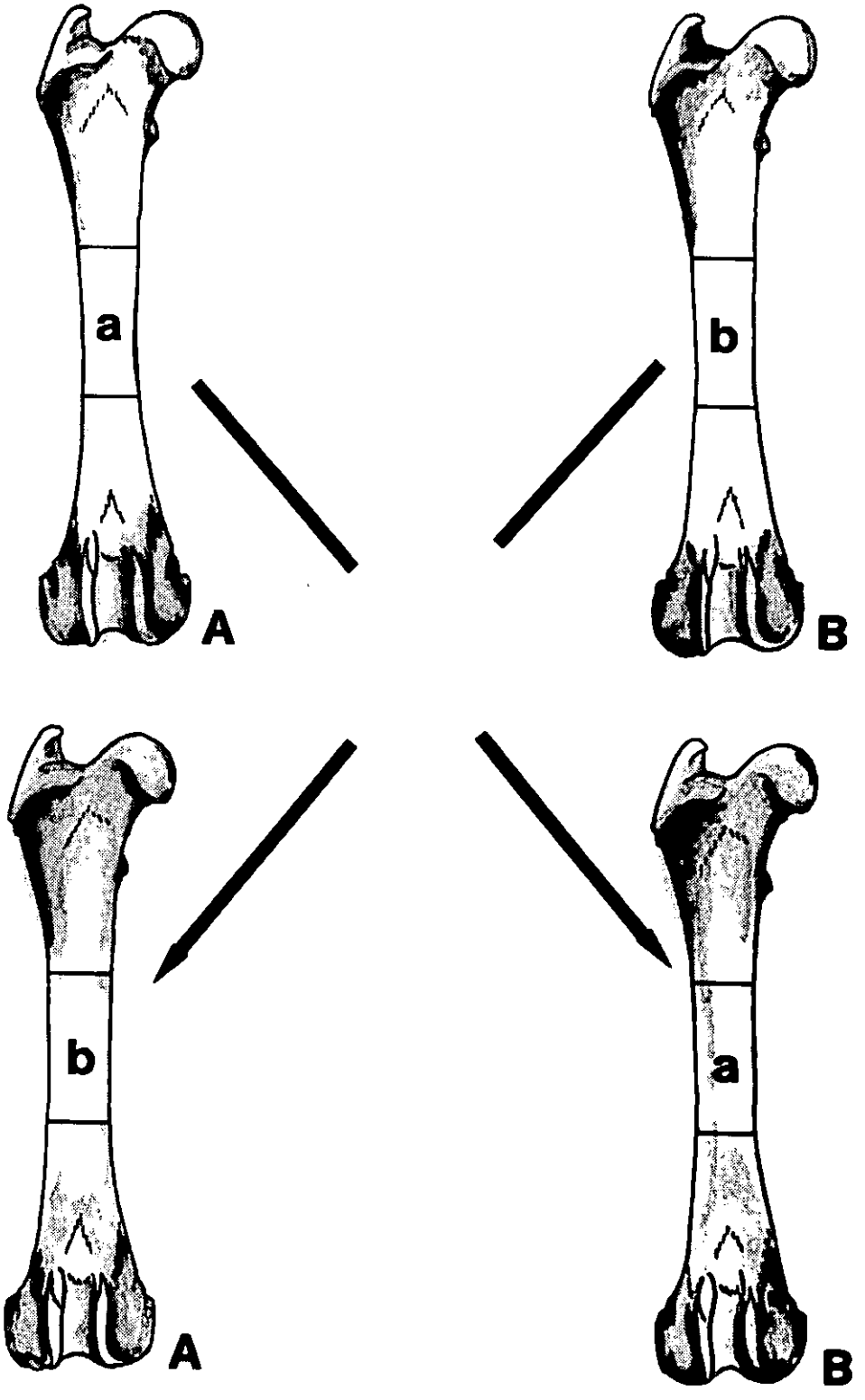

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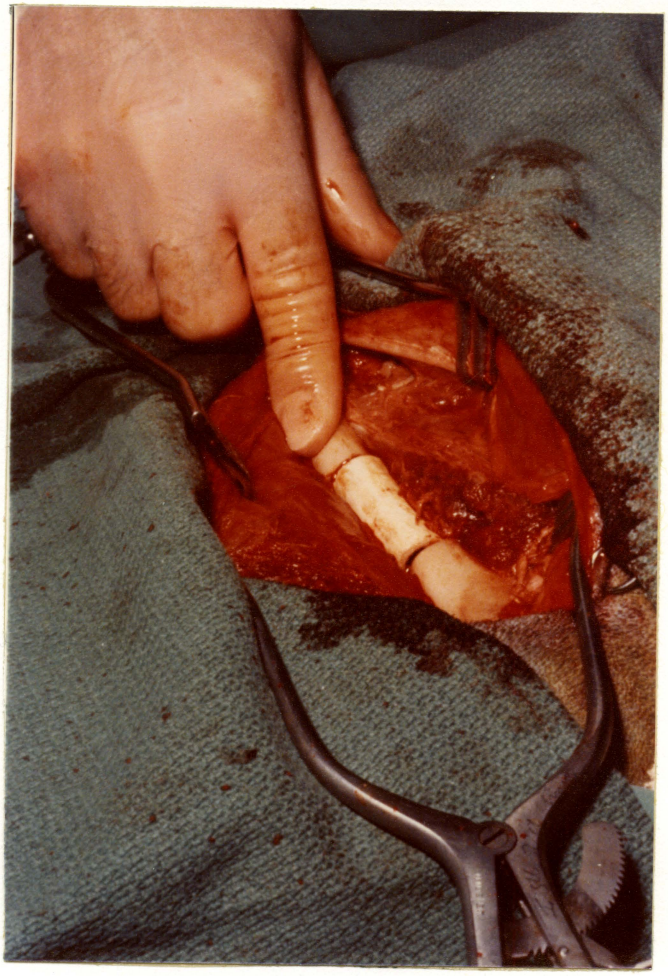
Figure 13. The bone graft following its insertion and in preparation for adjustment at each interface.



minor adjustments were made in both the graft and host bone to insure maximum contact between them (Figure 14).

The Dynamic Compression Plate was placed over the lateral surface of the femur, and immobilized with the aid of bone holding forceps (Figure 15). Two 3.2 mm. holes were drilled into the graft segment. These were tapped with a 4.5 mm. bone tap, and the plate was secured to the graft with two 4.5 mm. cortical bone screws.

Even after rehydration, the freeze-dried bone grafts proved to be extremely dense and brittle, and were subject to cracking during the drilling process unless well bathed in saline. Therefore, a change in technique was devised for the drilling of these grafts. Following the final adjustment phase, the overlying bone plate was securely fixed to the freeze-dried graft with a bone holding forceps. The graft segment and attached bone plate were removed from the operative site and immersed in sterile saline (Figure 16). The graft was subsequently drilled and tapped "under water", and the plate was secured to the graft with two 4.5 mm. cortical bone screws. The plate and graft were returned to the operative site, and the procedure continued. From this point, each procedure was carried out in an identical manner.

Starting with the proximal host bone segment compression was achieved at the graft-host interface in the manner previously described. When possible at least two holes per

Figure 14. Minor adjustments were made to insure maximum contact of the graft to the host bone at each interface.

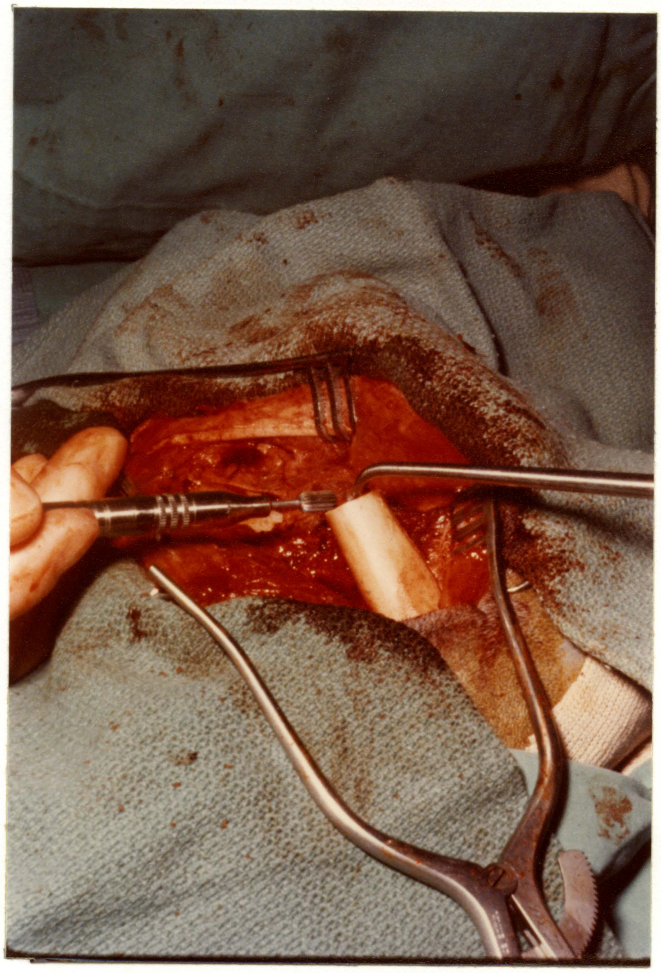


Figure 15. Immobilization of the Dynamic Compression Plate (DCP) over the lateral graft-host bone surface.

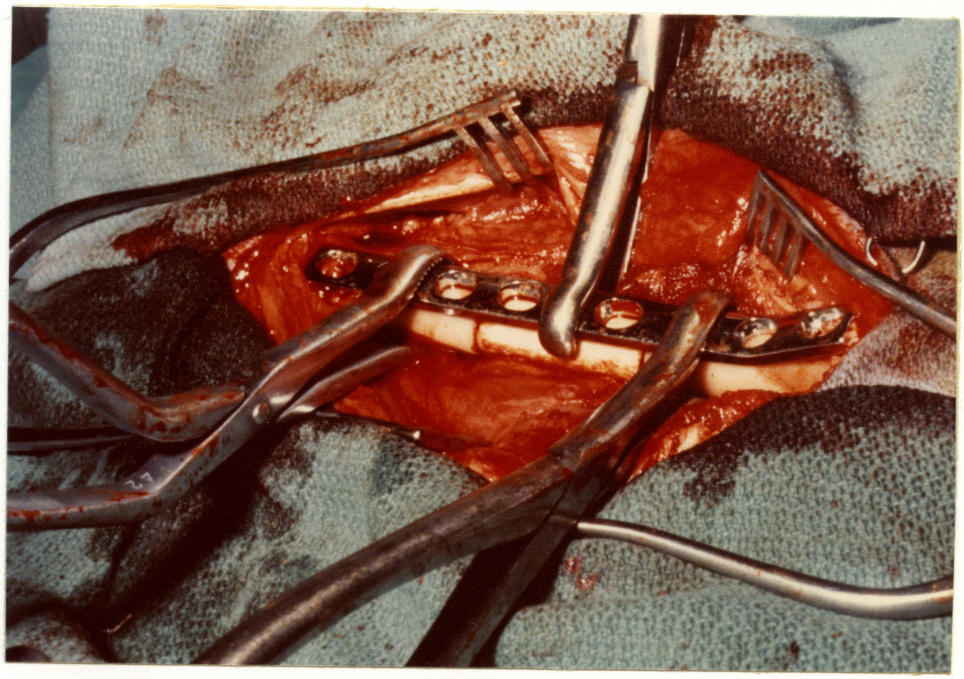


Figure 16. Drilling of the freeze-dried cortical allograft during total immersion in saline.

segment were drilled eccentrically while the most proximal and most distal holes were drilled neutrally. This could only be accomplished with an eight-hole bone plate, however. In the instances where a six-hole plate was utilized, self compression was achieved through only one hole in each host segment.

Following plate application, each bone screw was checked for tightness with thumb and forefinger pressure, and the surgical field was irrigated with a saline-neomycin[1] solution (Figure 17). Tissue closure was initiated with the reapposition of the biceps femoris to the vastus lateralis with 2-0 chromic catgut in a simple interrupted suture pattern. This was followed by closure of the subcutaneous tissue layer with the same size of suture material using a simple interrupted, inverting suture pattern. A running subcuticular closure with 3-0 chromic catgut was followed by skin sutures with 4-0 monofilament[1] wire in a simple interrupted pattern.

Postsurgical Procedures

Immediately after surgery, each animal was given 20,000 units of procaine penicillin G[2] per pound of body weight intramuscularly, and intravenous fluid administration continued until they were in sternal recumbency. Ampicillin[3] was

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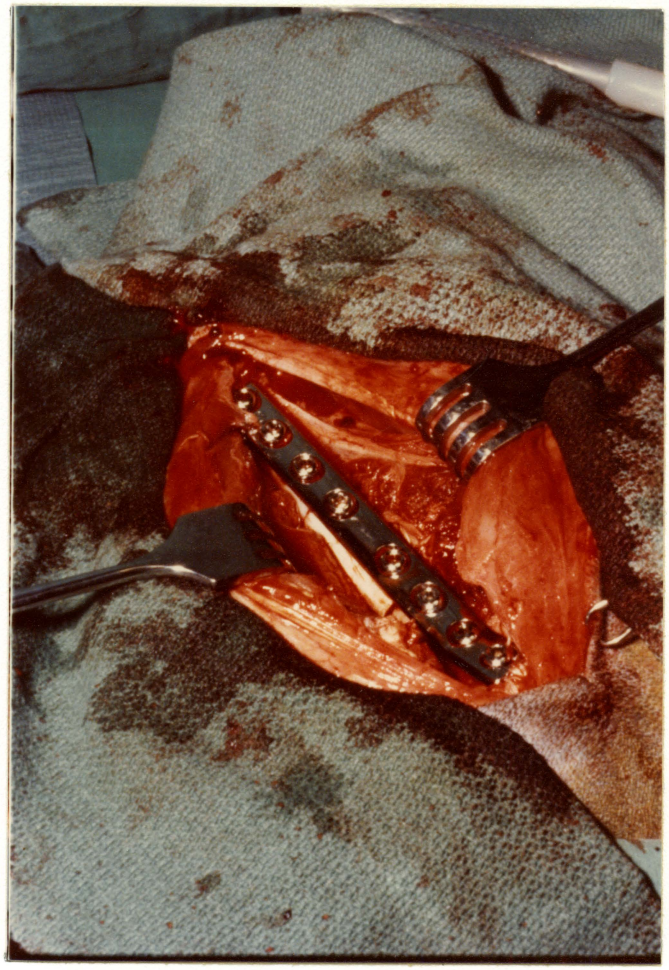
[1]Ethicon, Inc., Somerville, N.J.

[2]Pfizer, Inc., New York, N.Y.

[3]Wollins Pharmaceutical Corp., Melville, N.Y.

Figure 17. The appearance of the graft following its insertion into the host bone segmental defect and application of the Dynamic Compression Plate (DCP).



given per os at a dosage rate of 10 mg. per pound every eight hours for the next ten days.

Radiographs were taken immediately after surgery, and once every two weeks for the ensuing three months. Following this, they were taken once a month until the experiment reached its completion at one year.

A blood hemogram including blood-urea-nitrogen (BUN) and serum alkaline phosphatase level was taken once a week for six weeks following surgery.

Each animal was examined daily for any complications, and the skin sutures were removed fourteen days post-surgically.

At the end of the respective observation periods, each animal was euthanatized, and the test limb was again prepared in a manner as that described in the presurgical procedure. The limb was draped appropriately, and the entire right femur was aseptically harvested and aerobic and anaerobic bacterial cultures were taken. The popliteal lymph nodes of both rear limbs and the superficial and deep inguinal lymph nodes on both sides were also harvested.

Radiographs were taken of the harvested femur before and after plate removal. The bone was then cut longitudinally on a band saw and re-radiographed. These half-sections were again cut longitudinally and once more submitted for radiographic evaluation.

Prior to being fixed in 10% neutral buffered formal-saline, the quarter-sections were cut transversely in a plane perpendicular to the diaphysis in the following areas: through the middle of the graft and in an area of the proximal and distal host bone segments that was at least one screw hole removed from the respective graft-host interface. Although this yielded two sections that had to be analyzed separately, processing proved to be much easier when compared to handling one large segment of bone.

The sections were immersed in the fixative solution for one week and then were decalcified in ethylene diamine tetracetic acid (EDTA) saturated aqueous solution for a period of three months. This solution was changed weekly.

The sections were then processed on a 24 hour cycle in an autotechnicon. Following this, they were embedded in paraplast[1], mounted on wooden spindles, and cut on an A O Rotary Microtome[2] to a thickness of 6-7 microns. Routine hematoxyline and eosin staining was utilized.

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[1]Fisher Scientific Co., Itasca, Ill.

[2]American Optical Corp., Scientific Instruments Div., Buffalo, N.Y.

RESULTS

The following is a compilation of data on the radiographic and corresponding histologic appearance of the test grafts at planned time intervals. Radiographic union was considered to be complete when cortical continuity existed across the graft-host interface and the periosteal and endosteal callus were undergoing active remodeling (Tables 2, 3, and 4). A summary of the radiographic appearance of the individual grafts at the time of harvest has been formulated (Tables 5, 6, and 7). The term radiopaque, as used in the context of this description, refers to the relative density of cortical bone, and is meant to infer that the processes of resorption and revascularization were either non-existent or had just begun. Similarly, the term radiolucent was utilized when the graft cortex appeared less dense and more like normal host bone. It was felt that this coincided histologically with active resorption and replacement of the graft.

In the histological description of the different sections taken at the time of harvest, the term necrotic has been utilized to describe those regions of the graft or host bone cortex that were characterized by an absence of osteocytes within lacunae. Resorption cavity formation in these areas was usually minimal or absent.

A summary of the histologic appearance of the individual grafts according to their type has been formulated (Tables 8, 9, and 10). In addition, a summary of untoward occurrences that influenced the results is given in Table 11.

Group 1: 120 Day Observation Period

This group consisted of one fresh cortical allograft (#4), one freeze-dried cortical allograft (#657), and one fresh cortical autograft (#914) which served as the control. Wound healing was uneventful in each, and no swelling of either the superficial inguinal or popliteal lymph nodes was seen. A weekly blood profile taken for five consecutive weeks following surgery revealed only a mild but transient elevation of the serum alkaline phosphatase. All three dogs were bearing their full weight on the test limb within six to seven weeks following surgery. Aerobic and anaerobic cultures taken at the time of graft harvest were negative for all three dogs (Table 1).

Radiographic evaluation

Fresh cortical autograft (#914) At four weeks, rounding of the edges of both graft and host bone occurred as did the start of periosteal reactivity over the host bone. Early bridging with periosteal new bone at the proximal interface was initiated at six weeks and was complete at ten weeks. Cortical union occurred two weeks later.

A nonunion was encountered at the distal interface due to the progressive loosening of the cortical bone screws in the distal host bone.

Radiographs taken at the time of bone harvest revealed lysis around the bone plate with no evidence of healing at the distal interface except for minor fibrous bridging.

Fresh cortical allograft (#4) The edges of both the host bone segments and the graft showed evidence of resorption or rounding two weeks after surgery. A periosteal reaction over the host bone segments occurred two weeks later. Endosteal thickening was apparent during the sixth week as was the start of a periosteal reaction over the graft. Periosteal bridging across each interface was complete at twelve weeks, and cortical union followed two weeks later.

Radiographs taken of the harvested bone revealed excellent union at each interface. Both the cranial and caudal cortices of the graft appeared quite radiopaque, and trabecular ingrowth from each host segment was equal but minimal in quantity. The center of the graft contained an amorphous looking material (Figure 18).

Freeze-dried cortical allograft (#657) Proliferation of periosteal new bone over the host bone segments together with the rounding of the graft and host bone edges occurred two weeks postoperatively. Endosteal thickening was first noted at each interface during the fourth week. A periosteal bridge of callus that was first observed at the proximal interface during the sixth week was completed two weeks later. Cortical union occurred at the proximal interface twelve weeks after surgery.

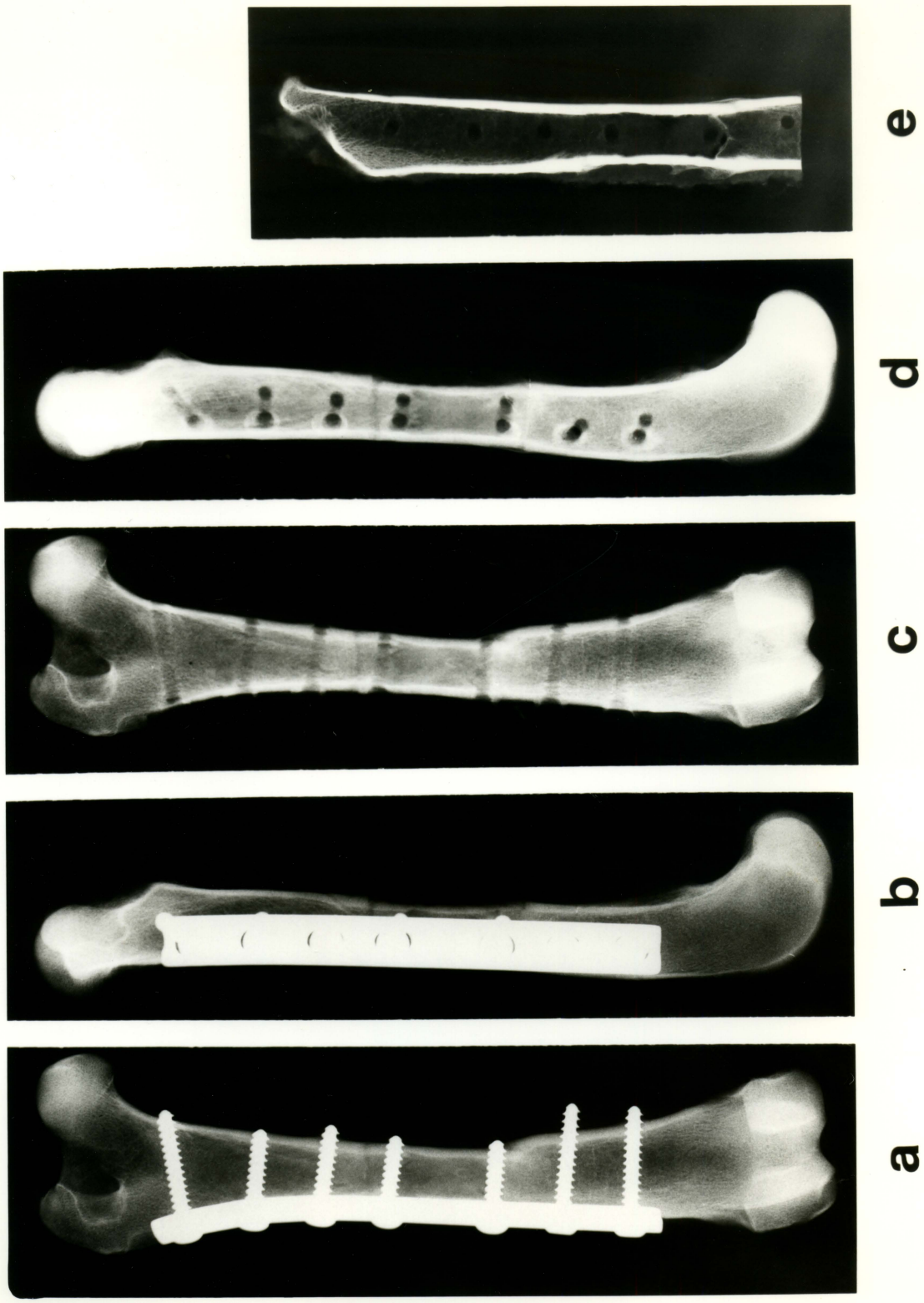
Figure 18. Serial radiographs of the harvested femur of dog #4 (fresh cortical allograft) taken at 120 days. Cortical union occurred at each interface.

a. Anterior-posterior view of the femur with attached bone plate; the proximal interface of the graft is located between the third and fourth cortical bone screws; the distal interface is located between the fifth and sixth cortical bone screws.

b. Medial-lateral view of the femur and attached bone plate.

c. Anterior-posterior view of the femur without the bone plate.

d. Medial-lateral view of the femur following longitudinal sectioning to 1/2 its thickness.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Excellent union at the proximal interface was confirmed radiographically at the time of bone harvest (Figure 19). Endosteal trabeculae ingrowth from the proximal host bone segment had advanced into the upper one-fourth of the graft. This was in contrast to a very minor ingrowth of trabeculae into the distal portion of the graft. Cortical union had not yet occurred while an excellent bridge of periosteal bone was seen across the posterior aspect of the graft. The anterior cortex had become remodeled in its entirety, while a large portion of the posterior cortex remained radiopaque. A zone of lucency was seen between the posterior cortex and the periosteal new bone.

Histologic evaluation

Fresh cortical autograft (#914) The proximal host cortex appeared to be filled with osteocytes and few resorption cavities were seen. The medullary cavity was filled with hematopoietic and fatty marrow with scattered bone spicules. Fibrous granulation tissue and a partial ring of osteoid surrounded the host screw hole.

At the proximal interface there existed complete bony bridging due to bone deposition between resorption cavities in the distal end of the host bone and the proximal end of the graft (Figure 20). The graft cortex was characterized by a generalized loss of osteocytes. Resorption cavities were few in number, and marrow could be seen in many of them. This

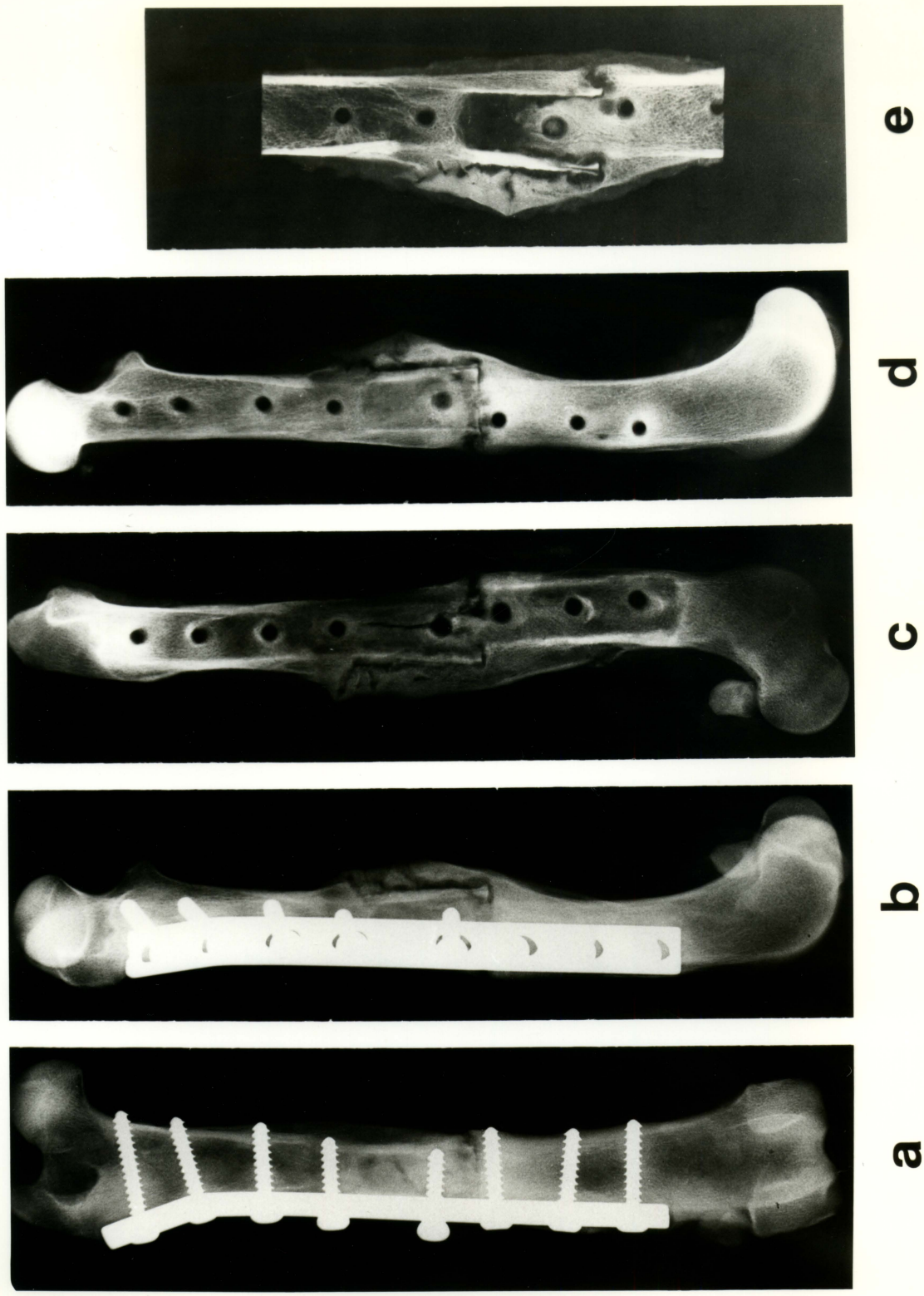
Figure 19. Serial radiographs of the harvested femur of dog #657 (freeze-dried cortical allograft) at 120 days. Cortical union was achieved at the proximal interface, however, a delayed union existed at the distal interface.

a. Anterior-posterior view of the femur and the attached bone plate; the proximal interface is located between the third and fourth cortical bone screws; the distal interface is located between the fifth and sixth cortical bone screws. Note the loosened screw within the graft.

b. Medial-lateral view of the femur and attached bone plate.

c. & d. Following plate removal and longitudinal sectioning of the femur to 1/2 thickness.

e. Following longitudinal section of the femur to 1/4 thickness.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was also true of the resorption cavities of the graft that were situated close to the interface. The only sites of viable osteocytes within the graft were located close to or immediately adjacent to resorption cavities containing blood vessels and marrow.

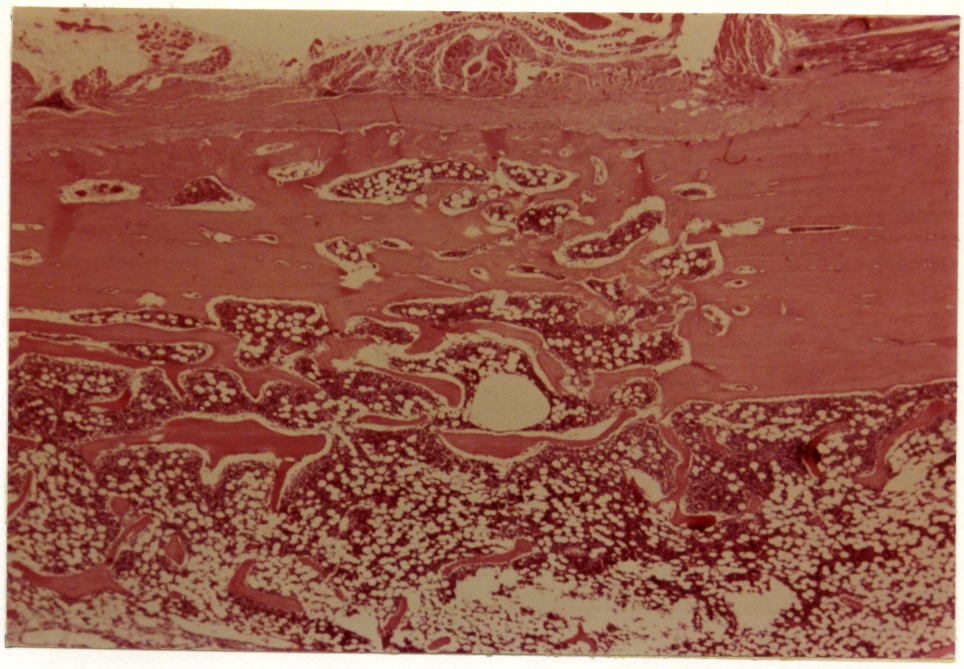
The graft screw hole was surrounded by an interrupted ring of osteoid and granulation tissue. Hematopoietic and fatty marrow extended across the interface and gave way to a loose fibrous and fibrovascular marrow at the middle portion of the graft.

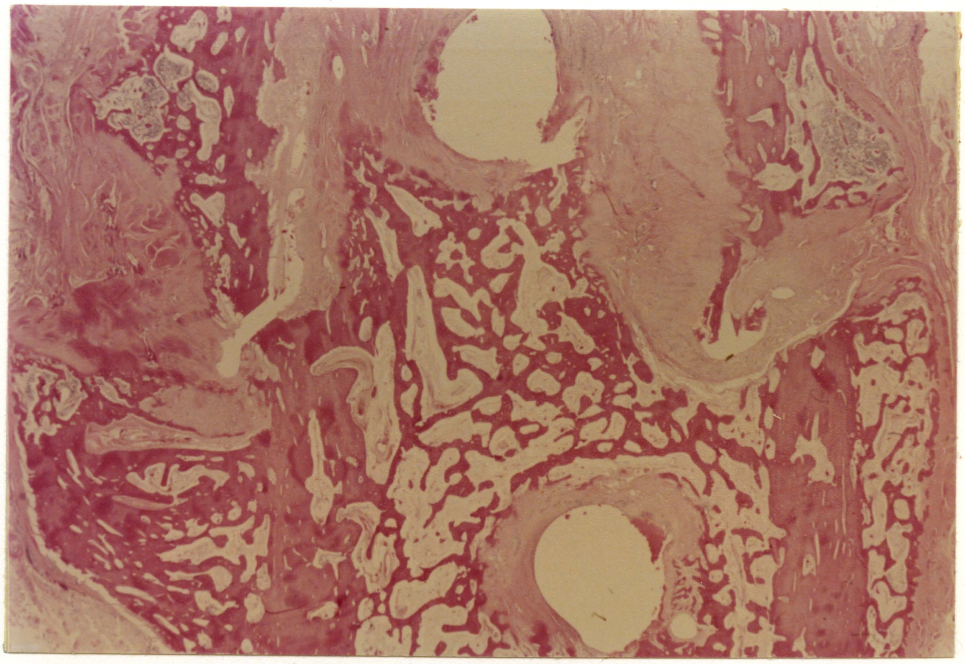
A generalized loss of osteocytes was also noted in the distal half of the graft cortex, and there were only occasional areas in which remodeling had occurred near the interface. The marrow cavity within the graft was filled predominantly with a vascular and loose fibrous connective tissue with scattered areas of desmoplasia. The graft screw hole was partially surrounded with vascular and necrotic fibrous connective tissue.

The distal interface was characterized by a fibrous nonunion which consisted of fibrous connective tissue as well as regions of fibrocartilage (Figure 21). In the region of the pseudoarthrosis, there was an infiltration of mononuclear cells that consisted of lymphocytes, plasma cells, and some macrophages. Neutrophils were also present, but there was no indication of active infection.

Figure 20. The appearance of the proximal interface of dog #914 (control) at 120 days; cortical union has occurred at the interface and the graft has undergone active resorption. Hematoxylin and eosin stain. x4.

Figure 21. Fibrous nonunion at the distal interface of dog #914. Hematoxylin and eosin stain. x4.





The lacunae within the host bone were filled with osteocytes. The host screw hole was encapsulated by a large amount of osteoid tissue. The medullary cavity was filled with loose fibrous connective tissue as well as numerous wide spicules of bone. A considerable quantity of new bone formation was noted at the plane of the interface.

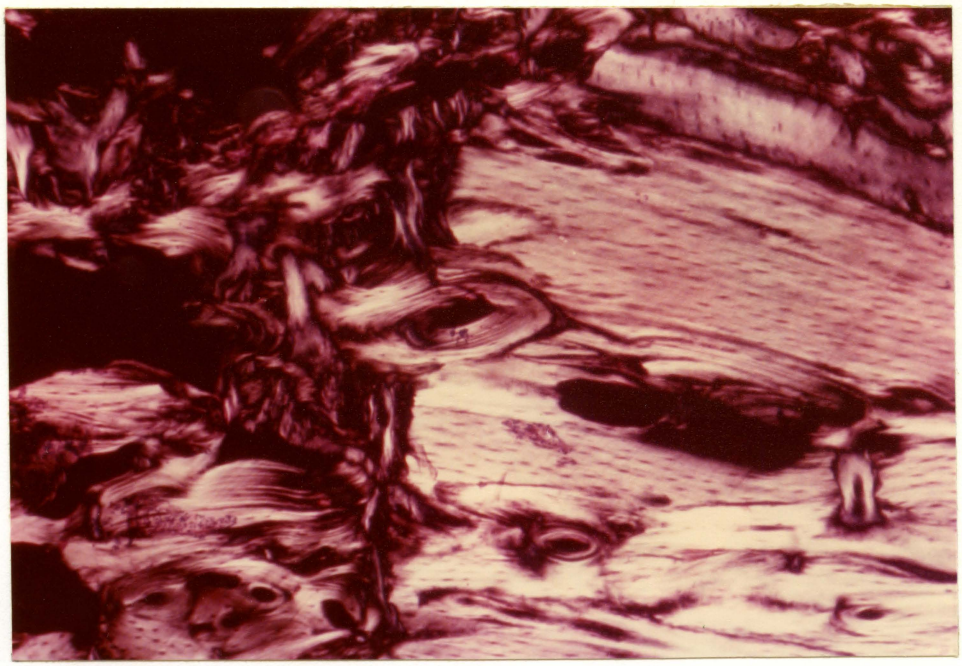
Fresh cortical allograft (#4) The proximal host cortex was filled with osteocytes except near the graft-host interface, and the medullary canal contained fatty and hematopoietic marrow. The host screw hole was almost completely surrounded by a ring of osteoid and fibrous granulation tissue. An interconnecting trabecular pattern of bone was continuous from the region of the screw hole to the line of interface where it became more dense.

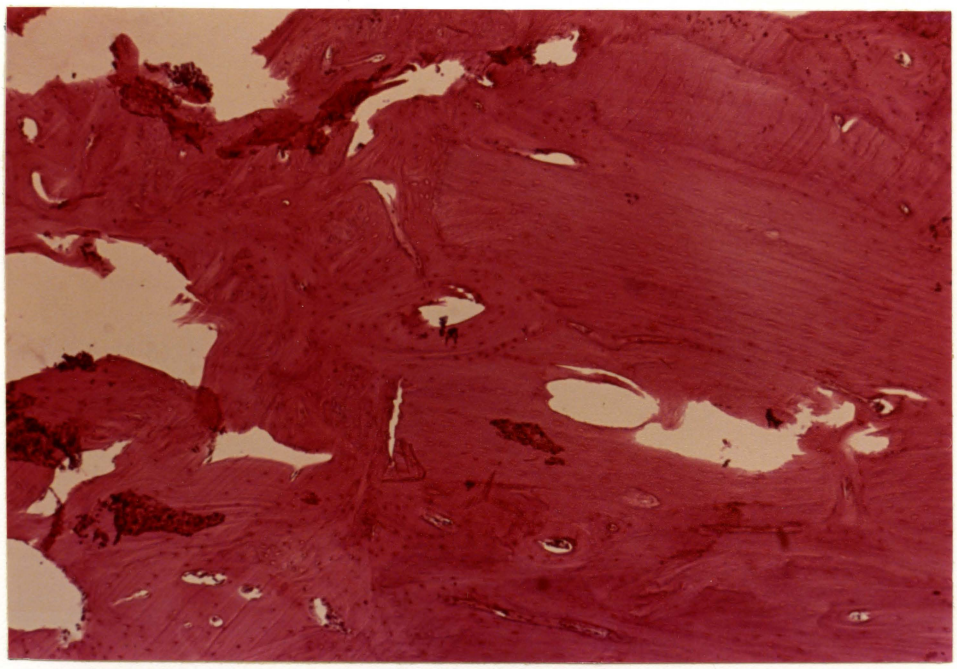
Osseous bridging had occurred at the proximal interface, and the new bone had been deposited perpendicularly to the plane of the diaphysis (Figures 22 and 23). Endosteal and periosteal new bone deposition had also occurred at that site.

The graft appeared to be undergoing active revascularization, resorption, and replacement along the proximal interface. Distal to this, however, much of the graft remained necrotic, as was evidenced by the numerous empty lacunae. Only isolated resorption cavities were seen within the cortex. Periosteal new bone containing marrow elements covered the outer surface of the graft.

Figure 22. Polarized view of the osseous bridging and ongoing graft resorption at the proximal interface in dog #4 (fresh cortical allograft) at 120 days. Hematoxylin and eosin stain. x16.

Figure 23. The same view without polarization. New bone has been deposited within resorption cavities. Hematoxylin and eosin stain. x16.





Practically all of the lacunae within the graft were empty. Revascularization of many of the larger Haversian and Volkman's canals had occurred, but there was minimal osteogenesis at their periphery.

Proximal to and surrounding the distal screw hole within the graft there existed a loose and moderately well vascularized connective tissue. The distal screw hole within the graft was also surrounded by a dense fibrous capsule.

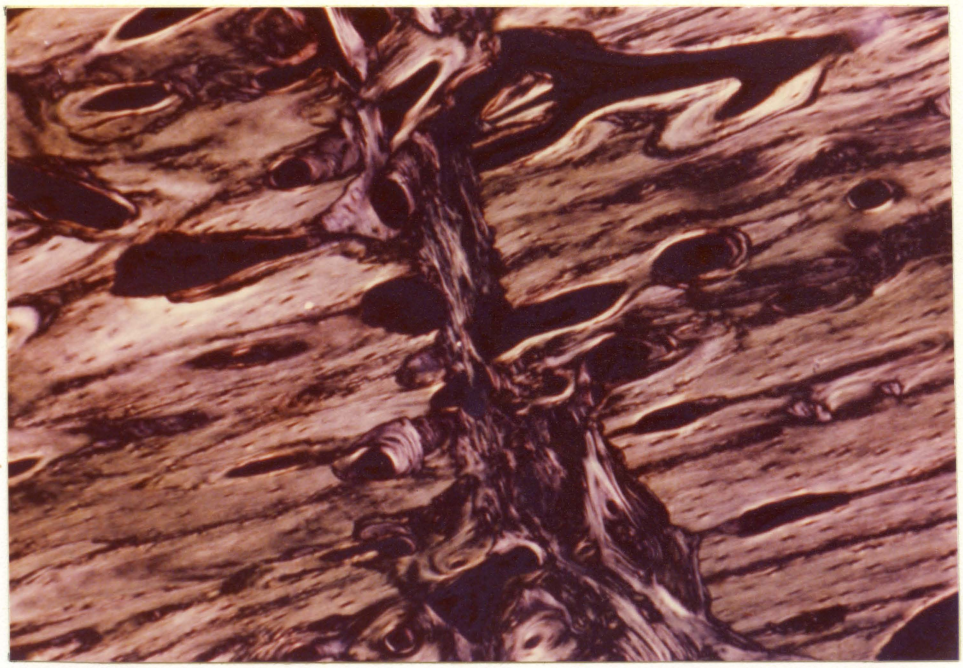
The distal interface was characterized by very small resorption cavities and some radially arranged cavities and osteons (Figures 24 and 25). The host cortex contained a number of empty lacunae, but most were filled with osteocytes. The marrow cavity was filled with dense and interconnecting spicules of bone and fatty and hematopoietic marrow. The host screw hole was completely surrounded by bone.

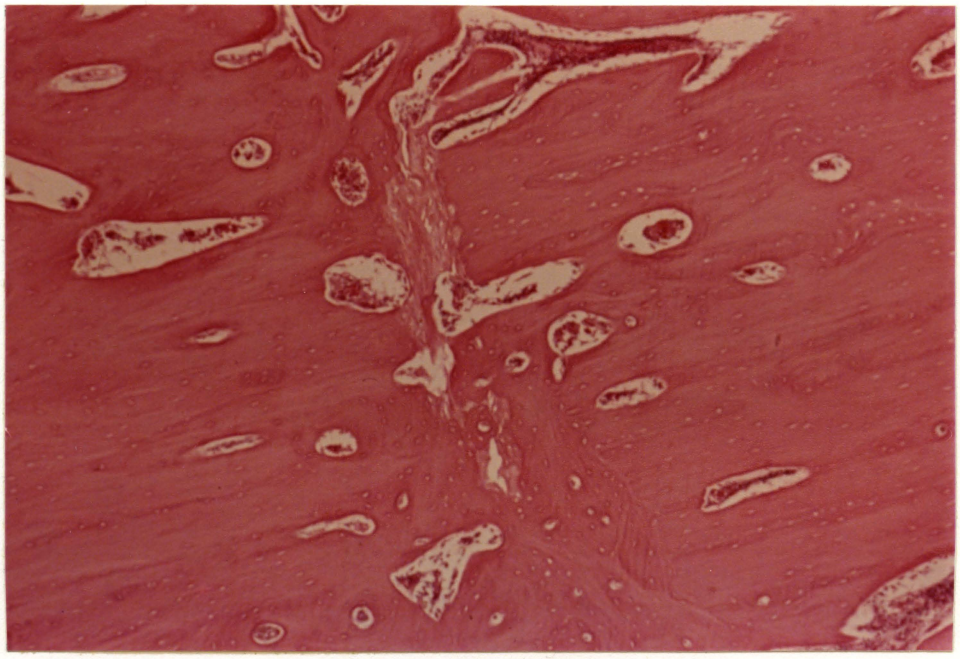
Freeze-dried cortical allograft (#657) In the cranial cortex of the proximal host bone segment, there was a general lack of osteocytes along with a vasculitis in and around vessels located within the resorption cavities. While some of the resorption cavities contained fatty and hematopoietic marrow, others contained marginated neutrophils and vessels with thickened and hyalinized walls. The opposite cortex was replete with osteocytes and was covered by a thick callus of periosteal new bone.

The proximal interface contained somewhat older and

Figure 24. Polarized view of the osseous bridging and relatively few resorption cavities within the graft (to the left of the interface) at the distal interface in dog #4. Hematoxylin and eosin stain. x16.

Figure 25. The same view without polarization. Hematoxylin and eosin stain. x16.





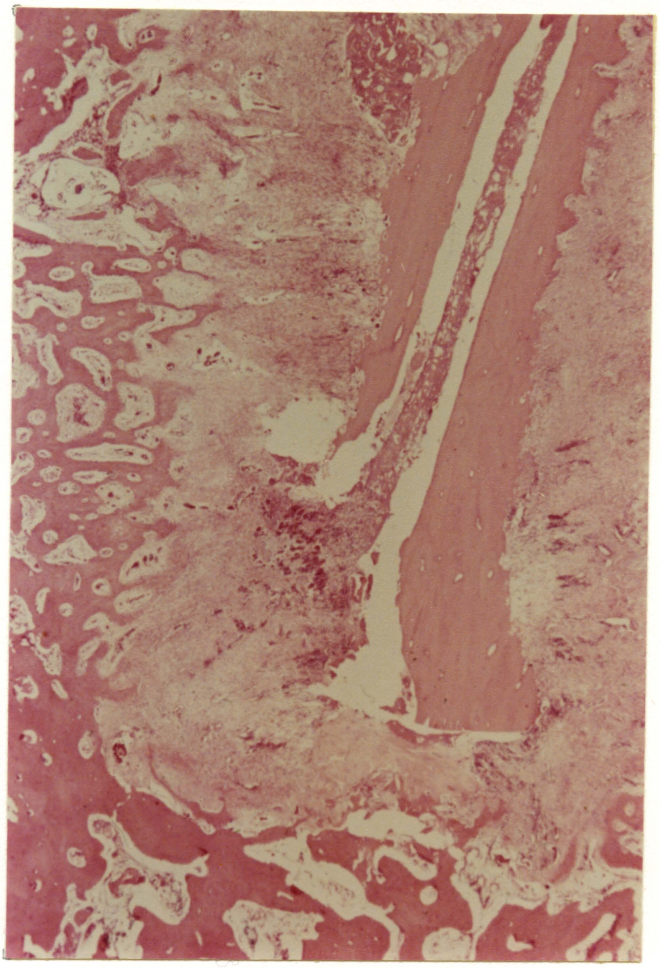
more radially arranged lamellae of bone. The resorption cavities tended to be longitudinal and continued into a heavily remodeled graft cortex which became poorly defined because of its porosity. The endosteal trabeculae were very thick and appeared to be continuous with the inner surface of the graft. The posterior cortex of the graft was almost completely remodeled, with only occasional plates and regions of the original graft cortex remaining.

Distally the posterior surface of the graft contained a split in the cortex which was filled with fibrin and cellular debris. This region of the graft remained unvascularized and contained many empty lacunae and had been walled off endosteally and periosteally by vascularized granulation tissue. The opposite cortex of the graft was heavily remodeled and contained few areas that were devoid of osteocytes. The graft screw hole was lined with a thick, fibrous capsule that was surrounded by a nearly continuous trabecular pattern of bone.

A well vascularized fibrous connective tissue that appeared heavily desmoplastic in some areas had grown across the distal interface. This indicated the presence of a delayed union at that site (Figure 26).

The distal host cortex was filled with osteocytes, and dense, intercommunicating trabeculae of bone were seen within the marrow cavity. The screw hole was lined with fibrous connective tissue that was almost totally enclosed by bone.

Figure 26. The distal interface of dog #657 (freeze-dried cortical allograft) at 120 days; the graft is surrounded by vascularized fibrous connective tissue indicating delayed cortical union. Hematoxylin and eosin stain. x4.



Group 2: 240 Day Observation Period

This group consisted of one fresh cortical autograft (#23), three fresh cortical allografts (#698, #568, and #3), and three freeze-dried cortical allografts (#671, #670, and #731). Wound healing was uneventful in each, and only a transient swelling was seen in the popliteal lymph nodes postoperatively. A weekly blood profile revealed only a mild but transient elevation in serum alkaline phosphatase in #23, #3, and #671. Every dog except #731 was bearing their full weight on the test limb within four to eight weeks following surgery. Dog #731 went from partial weight-bearing two weeks after surgery to complete disuse of the limb thereafter. Aerobic and anaerobic cultures taken at the time of graft harvest were negative for every dog except #731. This dog was cultured positive for Staphylococcus aureus, and, interestingly, never developed an elevation of temperature or of white blood cell count.

Radiographic evaluation

Fresh cortical autograft (#23) Periosteal and endosteal new bone formation together with rounding of the host bone segments was first observed two weeks following surgery. The graft edges showed evidence of rounding two weeks later, and periosteal new bone was deposited on its surface. At six weeks, the start of bridging across each interface with periosteal callus was noted, and appeared to be

complete at the distal and proximal interface at ten and twelve weeks respectively. Cortical union was complete at the distal interface at twelve weeks and at the proximal interface at fourteen weeks. The distal screw in the proximal host bone segment appeared to have loosened during the fourth postoperative week. Beyond this point, no further loosening was noted.

Radiographs taken at the time of bone harvest revealed excellent union at each interface. A small gap between the medial surface of the graft and the overlying periosteum was noted. The graft cortex appeared to be of the same density as the host cortical bone.

Fresh cortical allograft (#698) Rounding of the edges of the proximal host bone segment together with the deposition of small amounts of periosteal new bone at each interface was seen two weeks postoperatively. Further periosteal proliferation with endosteal thickening occurred during the ensuing two weeks. Bridging of the proximal interface with callus was first observed during the sixth week. It also appeared that the periosteum was forming a "sleeve" around the graft with a gap existing between the two. The graft cortex was noticeably thinner. The periosteal bridge across the proximal interface was complete at ten weeks, and cortical union occurred two weeks later.

Bridging across the distal interface was first observed

during the fourteenth postoperative week and appeared to be complete four weeks later. Cortical union at this site was seen during the twenty-second week (Figure 27).

Radiographs of the harvested bone revealed equal and advancing amounts of endosteal trabecular formation. An amorphous material was noted lying in the center of the graft medullary canal. For the most part, the caudal surface of the graft had undergone apparent revascularization and replacement. The cranial cortex, however, appear radiopaque except at each interface.

Fresh cortical allograft (#568) Minimal rounding of the host bone at the distal interface together with proliferation of periosteal new bone over the proximal host bone segment was seen two weeks postoperatively. Further rounding of the graft and host bone segments was noted two weeks later. Also, the graft cortex appeared to be generally less dense than previously. Periosteal bridging across the proximal and distal interface was complete eight and ten weeks respectively. Cortical union was noted at the proximal interface twelve weeks postoperatively while union at the distal interface occurred two weeks later.

At the time of harvest, none of the graft cortex appeared radiopaque. Instead, both cortices appeared to have been partially resorbed and were undergoing active replacement with host bone. There was excellent growth of

Figure 27. Serial radiographs of the femur of dog #698 containing a fresh cortical allograft (medial-lateral view).

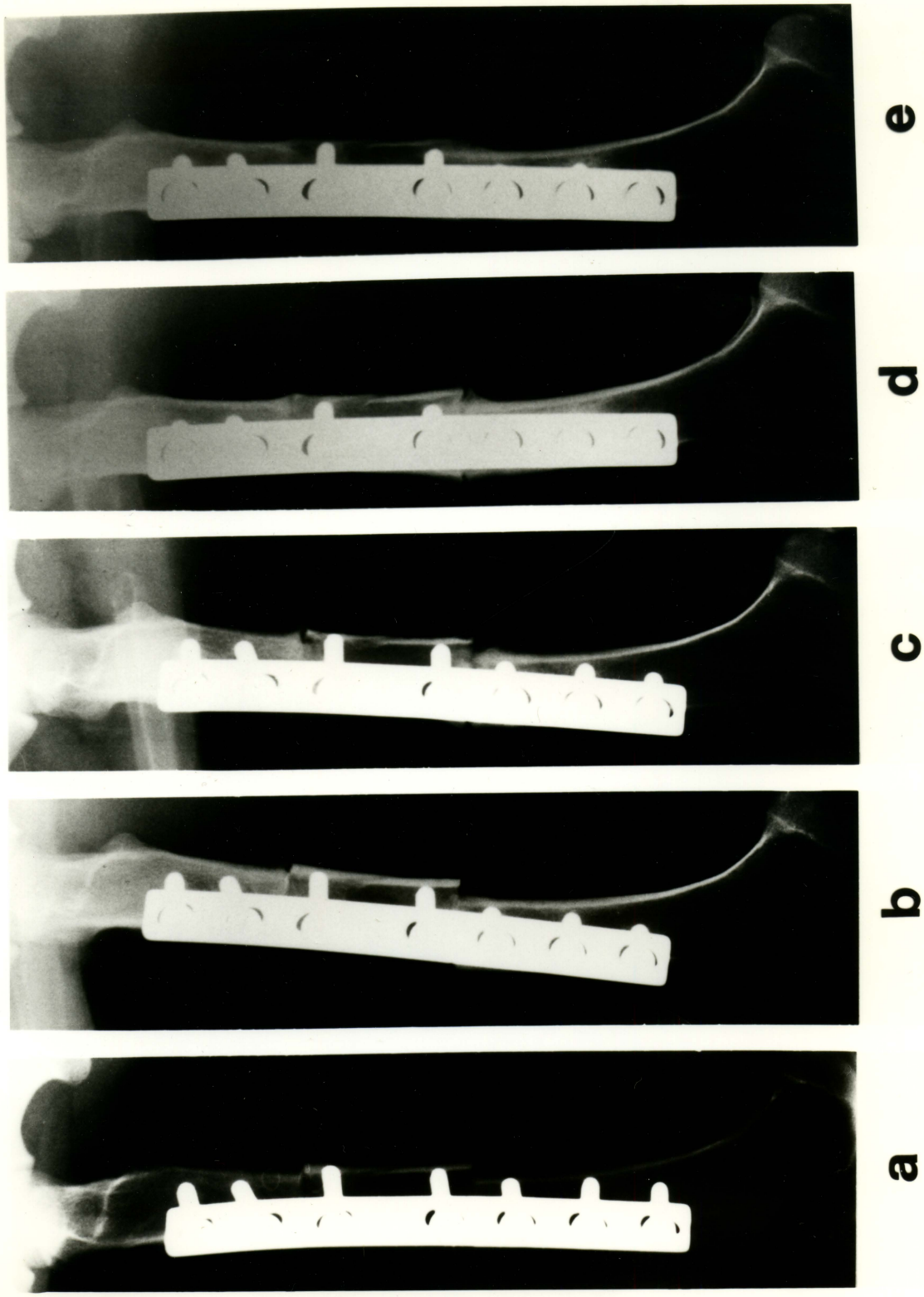
a. Postoperatively.

b. 4 weeks.

c. 8 weeks.

d. 14 weeks.

e. 22 weeks.


a
b
c
d
e

endosteal trabeculae across each interface, and an amorphous substance could be seen within the center of the graft.

Fresh cortical allograft (#3) A longitudinal fracture of the graft with caudal and medial displacement at the distal interface was seen two weeks postoperatively. The distal screw within the graft appeared loosened, and there was rounding of the host bone at the distal interface. Further rounding of the host and graft cortical edges was apparent two weeks later. A periosteal reaction was noted over the graft at six weeks, and early bridging occurred over the distal interface two weeks later. Evidence of a periosteal bridge over the proximal interface was first seen during the fourteenth week, and cortical union was noted at this site twenty-two weeks after surgery. The periosteal bridge over the distal interface was complete at this time also. Cortical union at the distal interface was evident four weeks later.

Radiographs taken at the time of bone harvest revealed excellent cortical union at the proximal interface. Due to the displacement of the graft following its fracture, its distal surface never abutted solidly with the distal host bone. As a result, union occurred due to periosteal and endosteal new bone deposition. Regions within the cranial and caudal cortical segments remained radiopaque. Good endosteal trabecular ingrowth had occurred across each interface, and an amorphous substance was noted in the center of the graft.

Freeze-dried cortical allograft (#671) Rounding of the host bone edges at the distal interface was apparent two weeks postoperatively along with a very slight production of periosteal new bone over the proximal and distal host bone segments. Rounding of the graft segments and the proximal host bone at the interface never occurred. During the sixth week, it was noted that a periosteal bridge of callus began to bridge the proximal interface and was complete at fourteen weeks. The distal screw in the graft appeared to loosen during the eighth week and became progressively more displaced during the ensuing weeks with an ever increasing zone of lysis around it. Cortical union occurred at the proximal interface sixteen weeks postoperatively, and a periosteal bridge of callus across the distal interface was complete at this time. The distal cortex became progressively more radiolucent, and a fracture occurred across the distal interface twenty-four weeks postoperatively.

The harvest films revealed the existence of an excellent cortical union at the proximal interface. The growth of endosteal trabeculae had proceeded across the interface from the host bone and appeared to be attached to the endosteal surface of the graft. The only portion of the graft exhibiting radiopacity was the posterior segment near the distal interface. The appearance of the distal host bone segment at the distal interface closely resembled the "elephant foot" type of callus seen in many nonunion fractures.

Freeze-dried cortical allograft (#670) Rounding of the host bone segments at each interface as well as the distal surface of the graft was noted during the second week following surgery. The same process occurred at the proximal surface of the graft two weeks later. Periosteal new bone was first seen attempting to bridge each interface during the sixth postoperative week, and was completed proximally during the tenth week and distally during the twenty-fourth week. There occurred a continuous proliferation of periosteal new bone that appeared to adhere to the surface of the graft during the eighth week followed by a thinning or resorption of the distal portion of the graft four weeks later. While cortical union was seen at the proximal interface fourteen weeks postoperatively, union at the distal interface remained delayed up to the time of harvest.

Radiographs taken at the time of bone harvest revealed excellent healing at the proximal graft-host interface. The entire cranial cortical segment of the graft appeared to be undergoing active replacement while a small, radiopaque area remained in the caudal cortex near the distal interface. While cortical union was nonexistent at the distal interface, endosteal growth from each host segment into the graft was very good. A large periosteal callus had bridged the distal interface over the anterior and posterior surfaces. A cortical fissure fracture extending from the middle of the

graft into its distal screw hole was noted.

Freeze-dried cortical allograft (#731) Periosteal new bone formation was seen over the caudal aspect of the distal host bone segment four weeks postoperatively. This callus became increasingly roughened in appearance during the next two weeks, and a zone of radiolucency was noted forming around the tip of the distal screw within the graft. The graft began a progressive rate of resorption from the eighth postoperative week until the time of harvest.

Radiographs taken of the bone following harvest revealed that a zone of lucency existed around every bone screw except the most proximal and most distal ones. The area under the bone plate was also lucent. The graft and much of the host bone had undergone a significant amount of resorption, and the screws within them were very loose. A small trabecular union was thought to exist across the proximal interface (Figure 28).

Histologic evaluation

Fresh cortical autograft (#23) The marrow of the proximal host bone segment and the upper half of the graft contained hematopoietic and fatty marrow. The screw holes within these respective regions were encapsulated with a thin sheet of bone and a small amount of accompanying fibrous connective tissue. There was a higher proportion of bone spicules within the host marrow as opposed to the graft

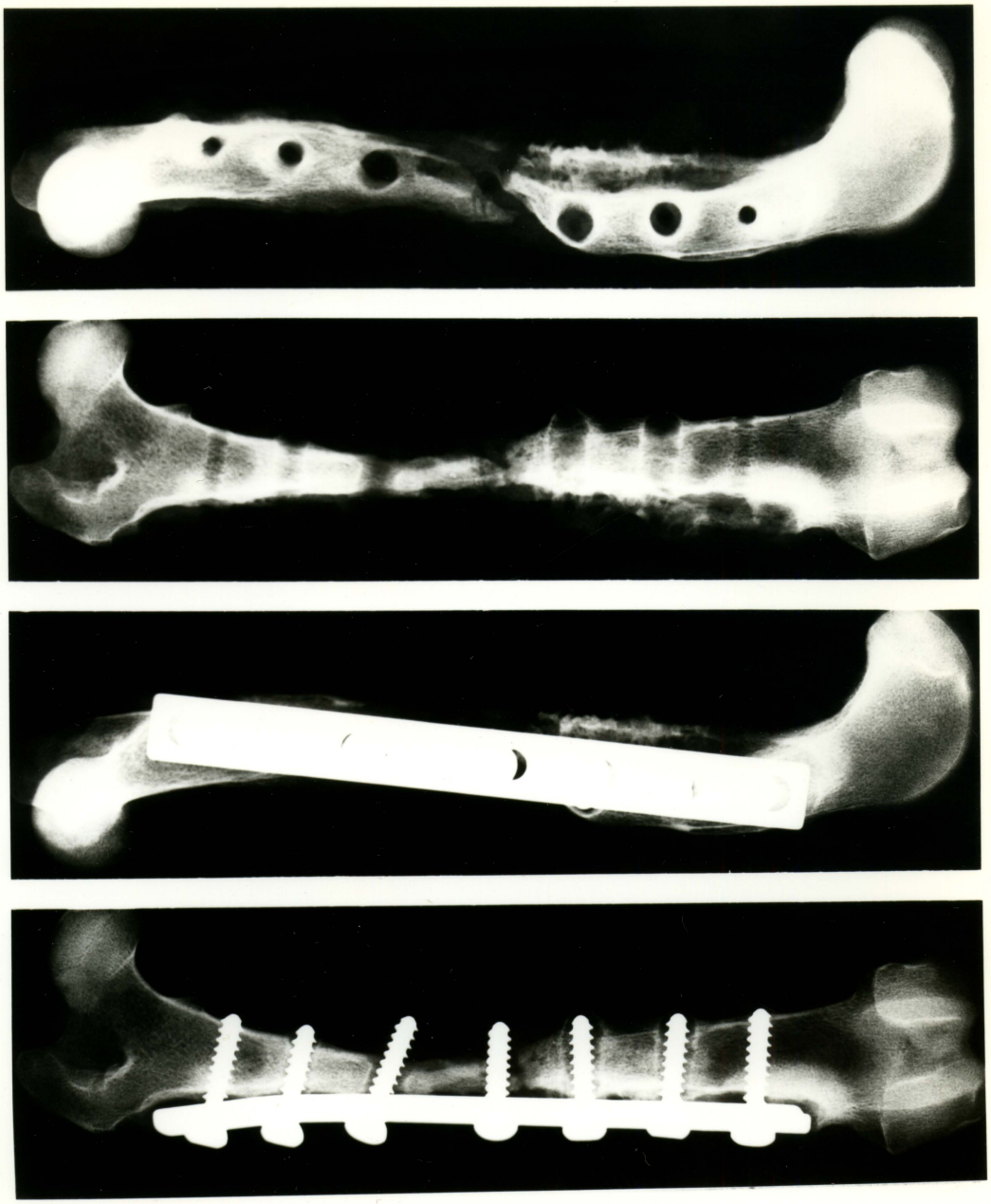
Figure 28. Serial radiographs of the femur of dog #731 containing a freeze-dried cortical allograft and harvested at 240 days.

a. Anterior-posterior view of the femur with bone plate attached; note the lucency around the cortical bone screws and the bone plate.

b. Medial-lateral view with bone plate.

c. Anterior-posterior view without the bone plate.

d. Medial-lateral view without the bone plate; note the extensive resorption of the graft and host bone.

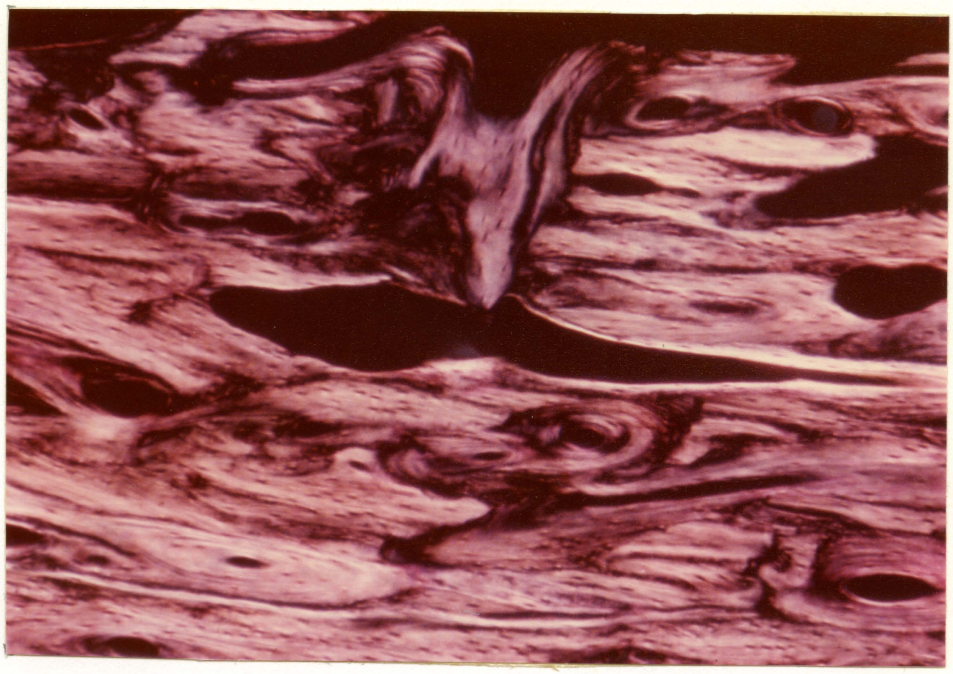

a
b
c
d

marrow although small spicules do appear in the more peripheral portions of the graft marrow. The resorption cavities appeared longitudinally oriented and were greatest in number in the presumed region of the interface (Figures 29 and 30). Most regions of the graft cortex contained viable osteocytes although some areas containing empty lacunae were still present. In comparison, however, the host cortex contained a proportionately greater number of empty lacunae.

The marrow canal within the distal half of the graft contained some fatty marrow but was characterized mainly by fibrous connective tissue with a large encapsulated area of hemorrhage. The adjacent screw hole was incorporated in the fibrous connective and contained no bony encapsulation. In the distal host bone segment, the screw hole was also encapsulated with fibrous connective tissue which contained a thin capsule of lamellar bone. The marrow in the host end was predominantly fatty with peripheral hematopoiesis and large bone spicules. There was considerable remodeling and bridging of the interface with dense lamellar bone. Most of the resorption cavities were oriented longitudinally, and merely suggested the original plane of interface (Figure 31). Hematopoietic marrow was seen in some of the larger resorption cavities close to the interface. Although most of the lacunae in both the graft and host cortices contained osteocytes, there was a somewhat higher proportion of empty lacunae in the graft.

Figure 29. Polarized view of the osseous bridging that has occurred at the proximal interface at 240 days in dog #23 (control). Hematoxylin and eosin stain. x16.

Figure 30. Same view without polarization. The graft is located to the left of the interface. Note the number of osteocytes that are present within the lacunae of the graft. Hematoxylin and eosin stain. x16.



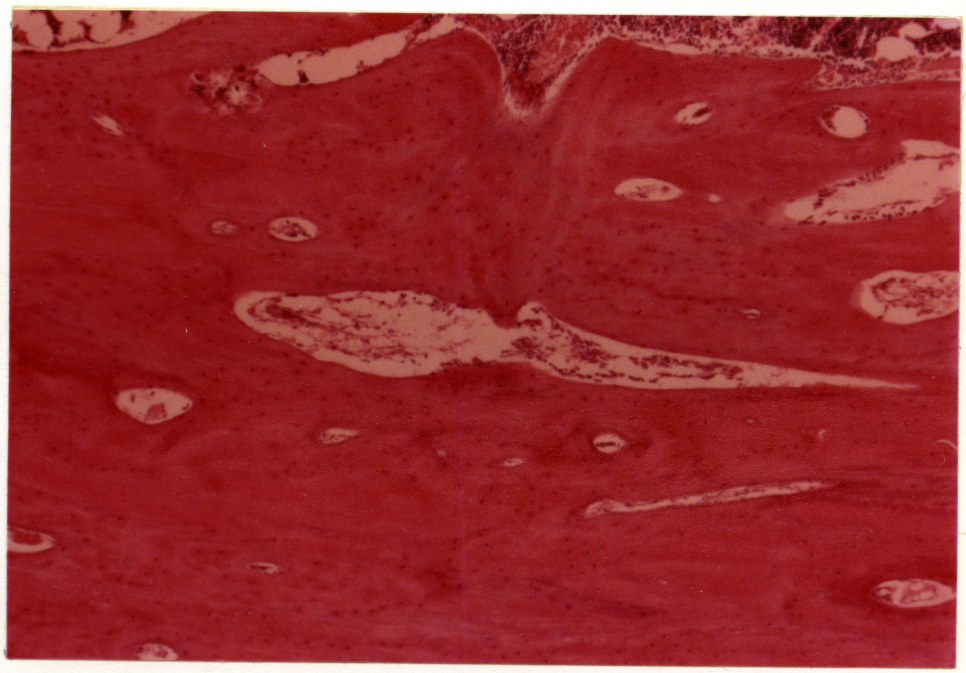
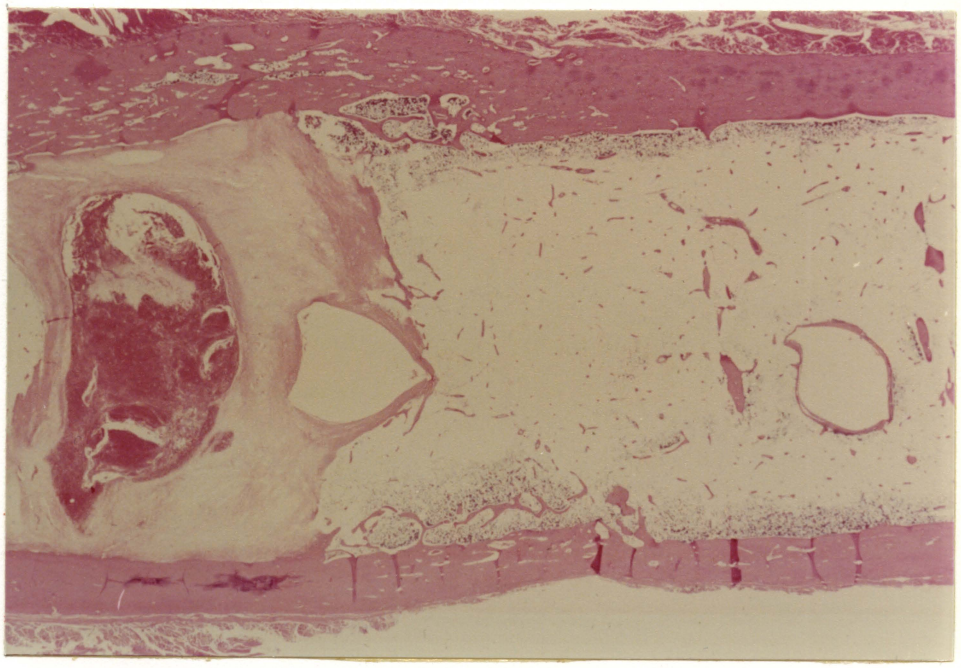


Figure 31. The appearance of the distal graft-host interface in dog #23. The interface is located approximately between the screw holes, and the caudal cortex is located at the top of the photograph. Note the greater number of resorption cavities within the caudal cortex of the graft and the quantity of fibrovascular connective tissue within the medullary cavity of the graft. Hematoxylin and eosin stain. x4.



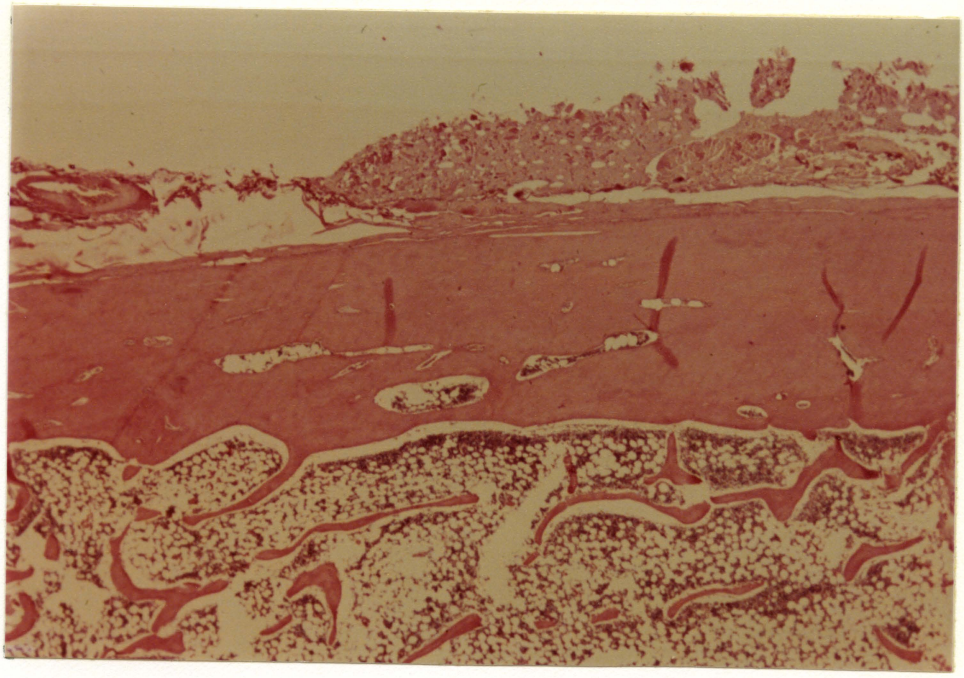
Fresh cortical allograft (#698) The cortex of the proximal host bone segment appeared dense and was filled with osteocytes in lacunae. The graft-host interface was poorly defined and was abutted by a dense layer of periosteal bone. In general, the resorption cavities at that site were oriented longitudinally, and many contained hematopoietic marrow. Although the proximal half of the graft cortex remained recognizable by the numerous empty lacunae, there also existed many reformed osteons that contained vessels and several areas with lamellae of new bone filled with osteocytes. It was noted that the cranial cortical segment containing the graft-host interface contained far fewer resorption cavities, and the interface was more densely bridged (Figure 32).

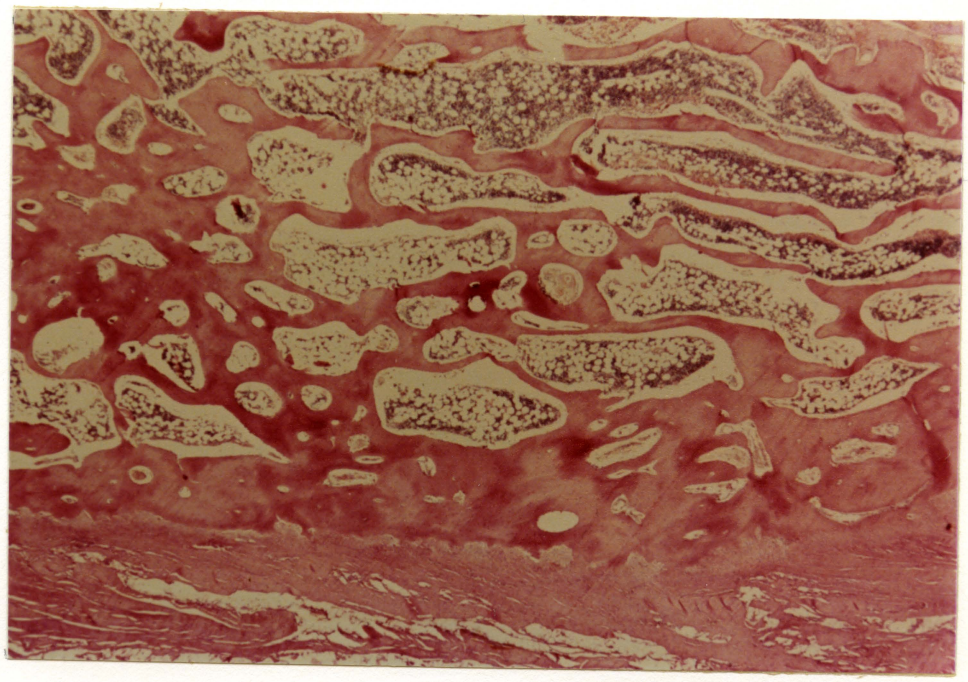
The marrow of the graft appeared to be made up of loose to dense fibrous connective tissue which abutted with hematopoietic marrow that progressed distally from the host bone. The adjacent screw hole was lined by dense fibrous connective tissue which was in turn enclosed by thick trabeculae of bone. The host screw hole was close to abutting on the endosteum and appeared almost totally surrounded by dense lamellar bone.

The distal half of the graft cortex also contained many empty lacunae. It was noted that the resorption cavities were markedly larger in the posterior graft cortex (Figure 33). The marrow cavity contained loose to dense fibrous connective tissue and an area of fibrillar to amorphous fibrin. The

Figure 32. A portion of the cranial surface of the fresh cortical allograft of dog #698 at 240 days. Note the relative lack of resorption that had occurred up to that time. Hematoxylin and eosin stain. x4.

Figure 33. In contrast to Figure 32, the number of resorption cavities within the caudal cortex of dog #698 are quite marked. Hematoxylin and eosin stain. x4.





distal interface was relatively smooth and contained numerous long, slender and sometimes intercommunicating spicules of bone. A combination of fibrous tissue and spicules of bone surrounded the distal screw hole of the graft.

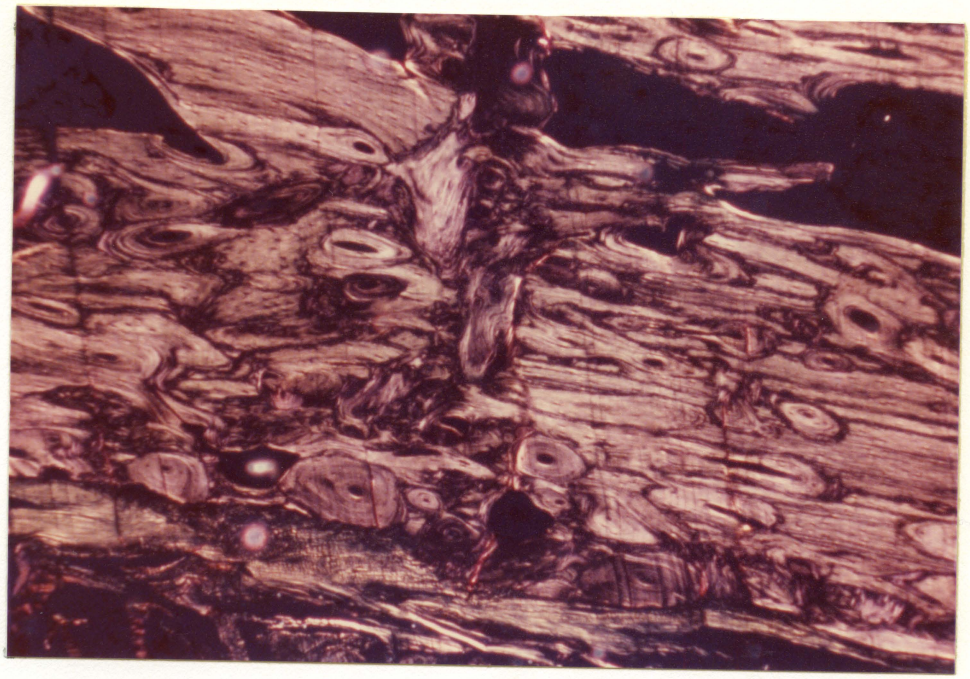
The distal interface was suggested in region only by the numerous resorption cavities. Many of these contained marrow and a few dense endosteal spicules. The cortex of the distal host bone segment closely resembled the proximal one. The screw hole on the host side was surrounded on one side by thin plates of bone and on the opposite side by a thin fibrous capsule.

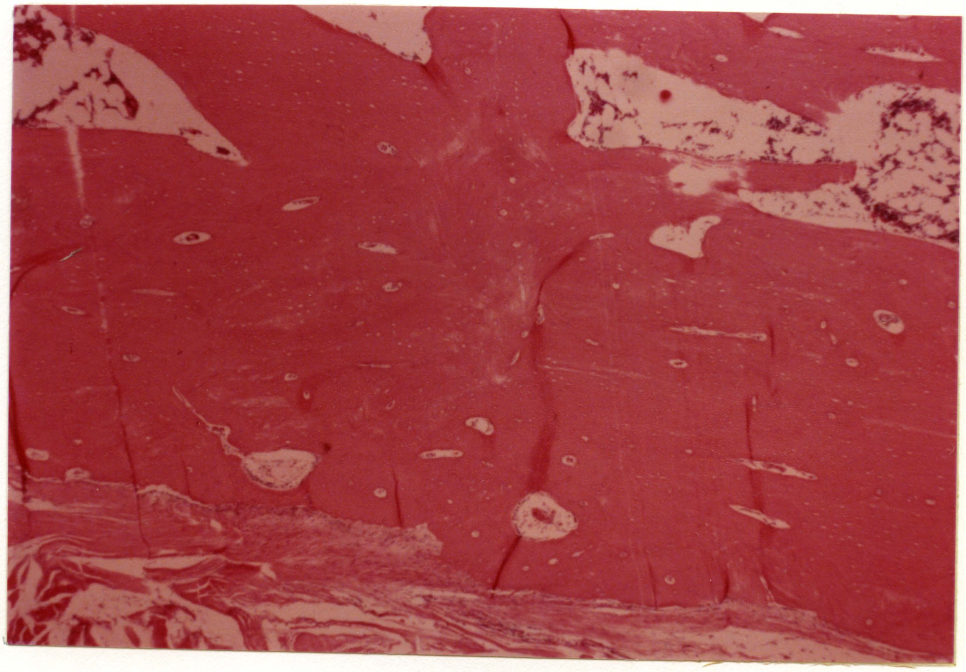
Fresh cortical allograft (#568) This specimen was nearly identical to that described by #698. Of particular interest was the relatively greater number of osteocytes that were seen in the posterior cortex of the host and graft segments. Also, the graft host interfaces were difficult to identify because of the deposition of new bone at these sites and the reorientation of osteons (Figures 34 and 35).

Fresh cortical allograft (#3) The proximal host bone segment and the proximal half of the graft also resemble the previous fresh cortical allografts in this group. The host bone was replete with osteocytes while the graft contained numerous empty lacunae. The posterior cortex of the graft appeared to possess a greater number of osteocytes and new lamellar bone than did the anterior cortex. In addition,

Figure 34. Polarized view of a graft-host interface in dog #568 (fresh cortical allograft) at 240 days. The interface has become more difficult to identify because of the reorientation of osteons across it. Hematoxylin and eosin stain. x10.

Figure 35. The same view without polarization. Hematoxylin and eosin stain. x10.





there was some periosteal new bone formation which increased in thickness and diameter as the approximate region of the interface was approached. The graft component was marked by an obvious paucity of osteocytes in lacunae. The only osteocytes seen were those located around some revascularized resorption cavities which are beginning to fill in with some lamellar osteoid. The number of resorption cavities seen were only slightly greater in number in the posterior cortex as opposed to the anterior cortex.

Although the distal interface was poorly defined, the cortex was by no means established at this point. This was probably a consequence of the longitudinal fracture that was seen two weeks postoperatively. Instead, the interface had become bridged by periosteal new bone formation in the form of spicules that met with a new, thin, reduplicated cortex peripherally. This was evident on both sides of the bone. On the posterior surface, there was a region of fibrous connective tissue interposed between the host cortex and the graft which indicated that a site of localized fibrous nonunion had formed. The host cortex was replete with osteocytes in lacunae. There were a few noticeable resorption cavities close to the interface, but it was covered on the outside with a zone of periosteal new bone formation similar to that seen at the interface with a thin, reduplicated cortex at the surface.

The remaining portion of this section closely resembled those previously described in this group.

Freeze-dried cortical allograft (#671) In the proximal host bone segment, both cortices were replete with osteocytes in lacunae. The marrow cavity and screw hole within this segment closely resembled those previously described. Except for just below the line of interface, only a few osteocytes were seen around the many longitudinal resorption cavities (Figure 36). Most of these cavities, however, contained hematopoietic marrow. The posterior surface of the host and graft were covered with periosteal new bone as was the anterior surface of the graft near the distal interface.

The proximal screw hole in the graft was completely surrounded by a ring of osteoid. There were thick and sometimes interconnecting spicules of bone in the medullary canal with only a minimum of hematopoietic marrow. Further distally a thick network of fibrovascular tissue was noted.

At the distal interface, the graft had become rounded in appearance over its anterior and posterior surfaces. Many resorption cavities were located in this area, and, although most of the graft remained necrotic, there were numerous osteocytes within lacunae around these cavities. A thick layer of fibrous granulation tissue covered a thin layer of periosteal new bone which, in some areas, appeared to be forming by endochondral ossification. The distal screw

hole in the graft was immediately surrounded by a well vascularized fibrous granulation tissue. In the area closest to the interface, this tissue had been replaced by hyaline cartilage. The marrow cavity around the screw hole was filled with thickened fibrovascular tissue and fibrous granulation tissue (Figure 37).

The distal host bone segment was, for the most part, replete with osteocytes. Both cortices were covered with periosteal new bone and endosteal trabeculae were thick and interconnecting. The screw hole was completely encircled by a thickened layer of osteoid which was surrounded by the trabeculae. The marrow elements appeared scant and mainly fibrovascular.

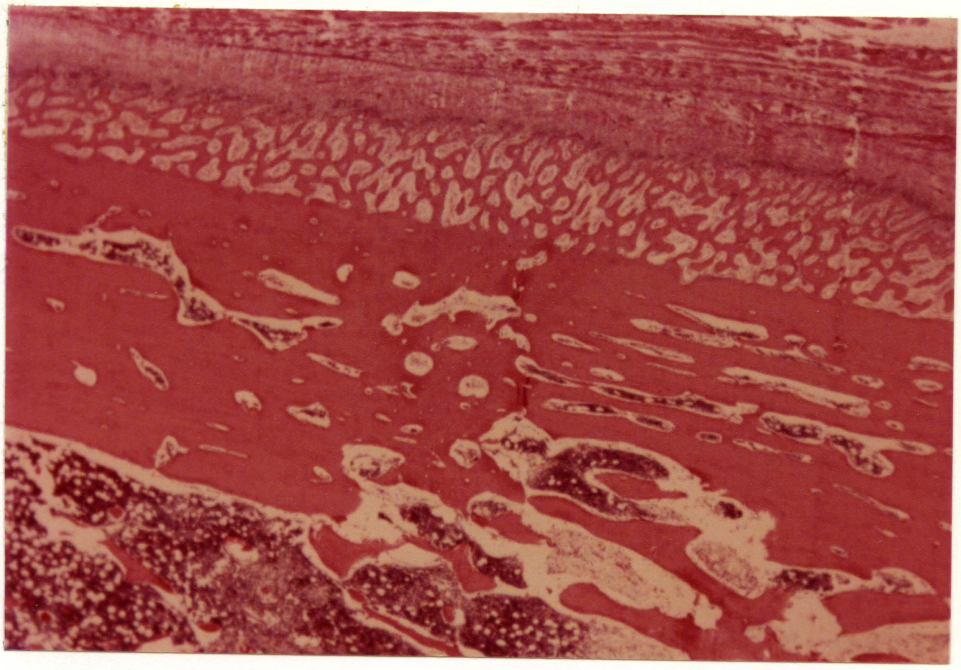
The interface portion of the distal host bone segment had a concave appearance. A layer of fibrocartilage covers both cortical surfaces and extends toward the center of the interface for a short distance. A portion of this cartilage over the anterior surface had previously undergone ossification. Overlying the fibrocartilage was a thick layer of fibrous connective tissue. A large blood vessel was seen within this layer over the posterior host cortex.

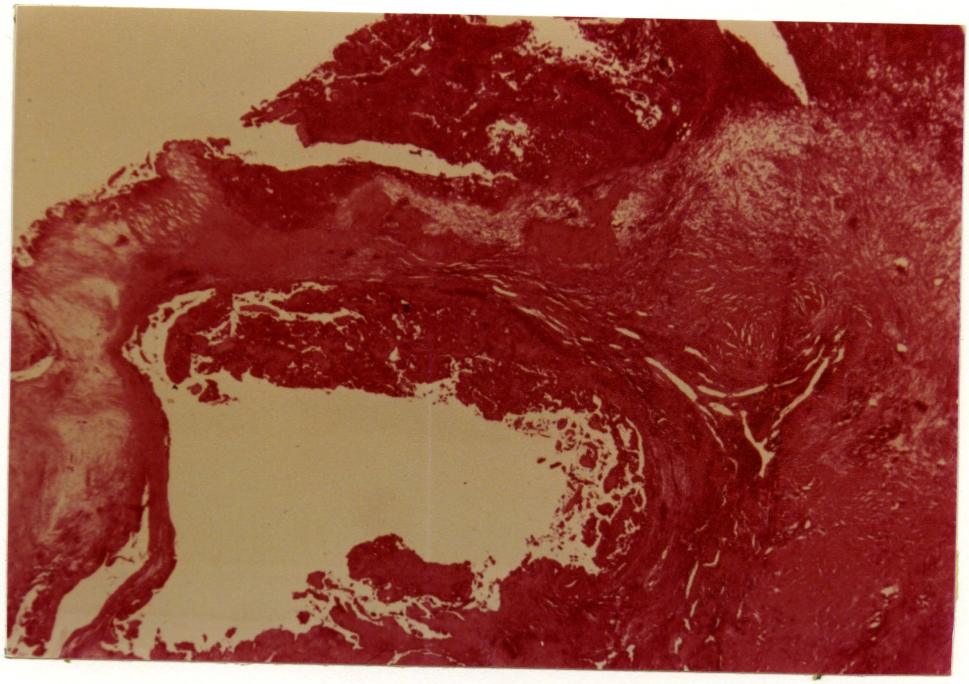
Pseudoarthrosis existed at the distal interface.

Freeze-dried cortical allograft (#670) In the proximal host bone segment, the anterior cortex was generally devoid of osteocytes while the posterior cortex appeared

Figure 36. The proximal interface of dog #671 (freeze-dried cortical allograft) at 240 days. The interface has become difficult to identify because of the reorientation osteons across it. The graft is located on the right side of the photograph and contains many longitudinally oriented resorption cavities. Note the deposition of periosteal new bone that has bridged the interface. Hematoxylin and eosin stain. x4.

Figure 37. Fibrous granulation tissue that had formed around the distal screw hole in dog #671. Hematoxylin and eosin stain. x10.





replete. The anterior cortex, however, was covered by a thick wall of periosteal new bone which extended over the proximal interface.

The graft-host interface was dense and could be identified only by irregularities in the cement lines. The host bone remained recognizable at the interface but was beginning to become remodeled. Remodeling in the graft cortex was pronounced and contained numerous longitudinally oriented resorption cavities many of which were filled with marrow (Figure 38). While many empty lacunae existed within the graft, many osteocytes within lacunae were noted around the resorption cavities throughout each cortex.

The general appearance of the host marrow and screw hole as well as the graft marrow and screw hole were similar to those previously described for the fresh allografts.

The distal half of the graft had undergone much resorption and contained many resorption cavities with marrow elements. Both cortices appeared to contain fewer osteocytes than was evidenced in the proximal half of the graft. While the marrow at this level was similar to that found in the proximal portion, the screw hole appeared differently. Although it was encircled with fibrous connective tissue, the distal aspect was covered by bone and some hyaline to fibrous cartilage which was continuous with

a cartilaginous interface between the graft and the host.

Periosteal new bone formation existed on both sides of the graft and host cortices. The interface between the periosteal new bone and the cortices of the graft and host was comprised in some areas by fibrous connective tissue which graded into fibro-cartilage and hyaline cartilage (Figure 39). This cartilage callus was relatively thin and formed a recognizable band that essentially crossed the entire specimen but followed an irregular course. There were numerous channels of vascular invasion into the cartilage, and osteoid had been laid down in most, if not all, of these invasion channels.

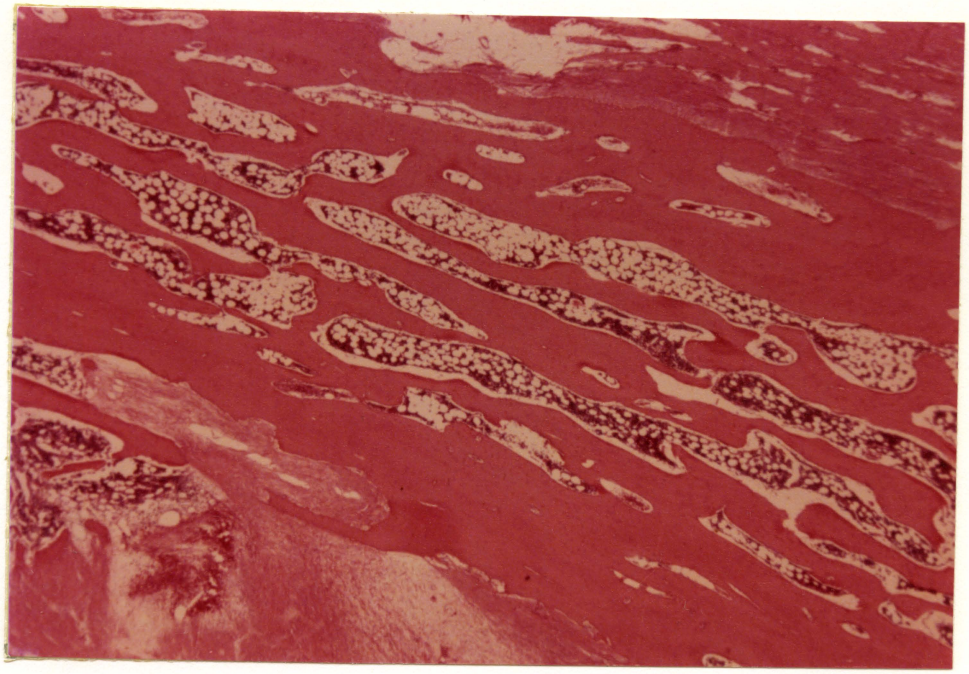
The host cortex again contained fewer osteocytes in the anterior cortex. The marrow and screw hole were similar in appearance to those previously described in the proximal host segment.

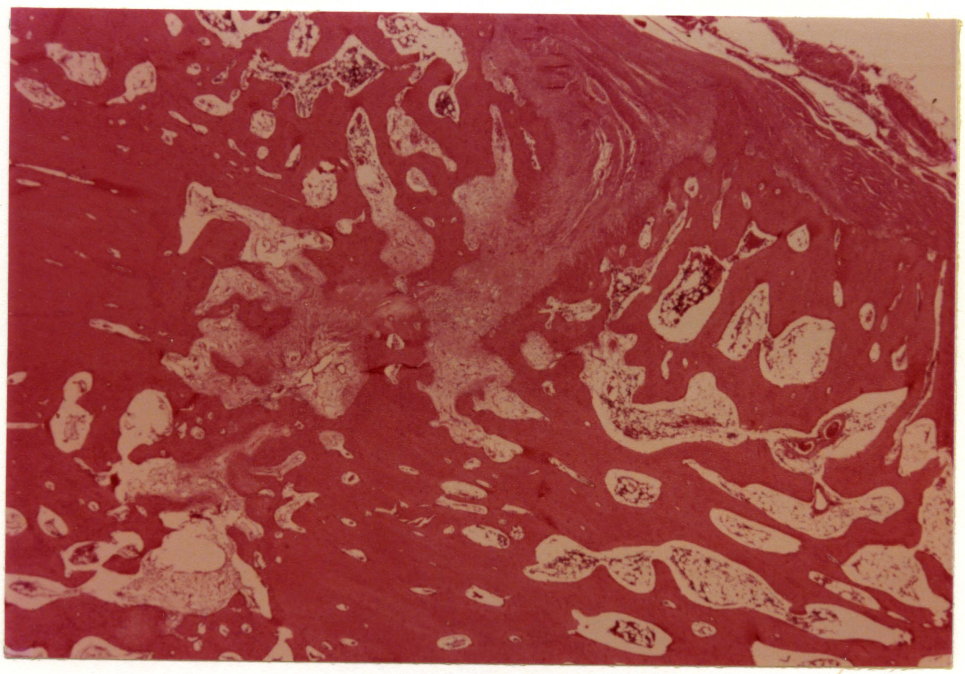
The histological appearance of the distal graft-host interface suggested a delayed cortical union.

Freeze-dried cortical allograft (#731) The outstanding feature of this section was graft resorption due to infection. Periosteal new bone formation was seen on one side of the proximal host bone segment and the graft. The proximal interface was only suggested in one cortical area by an interface between dense cortical bone with empty lacunae adjacent to a considerably remodeled area of cortical bone

Figure 38. The caudal cortex in dog #670 (freeze-dried cortical allograft) at 240 days. Remodeling is pronounced and the resorption cavities are filled with bone marrow. Hematoxylin and eosin stain. x4.

Figure 39. The distal interface in dog #670 contained areas of fibro-cartilage and hyaline cartilage and was classified as a delayed union at the time of harvest. Hematoxylin and eosin stain. x4.





which contained osteocytes in lacunae. The marrow cavity in the host bone appeared normal and was sparse in hematopoietic elements with approach to the screw hole in the host component. This screw hole was lined with fibrous connective tissue and inflammatory cells consisted primarily of lymphocytes, plasma cells, and a few neutrophils. The connective tissue capsule of the screw hole varied from dense to loose connective tissue and contained numerous vascular spaces. Peripheral to this there existed a complete bony capsule composed of dense, interconnecting spicules of bone.

The proximal screw hole in the graft appeared similar to the one just described. Inflammatory cells made up of lymphocytes, plasma cells, eosinophils, neutrophils and macrophages were seen in the regions adjacent to the screw hole.

The distal half of the graft and host bone segment had been sectioned at a place that is 90^{o} perpendicular to the proximal section. The graft bone had undergone a great amount of remodeling and was increased in density. Distinct separation between cortical bone and endosteal or marrow spicules was unclear. The graft surface, which had been considerably remodeled, was undergoing osteoclasia and was surrounded at several sites by granulation tissue. The granulation tissue had produced finger-like projections or villi into these spaces. These villi were incompletely

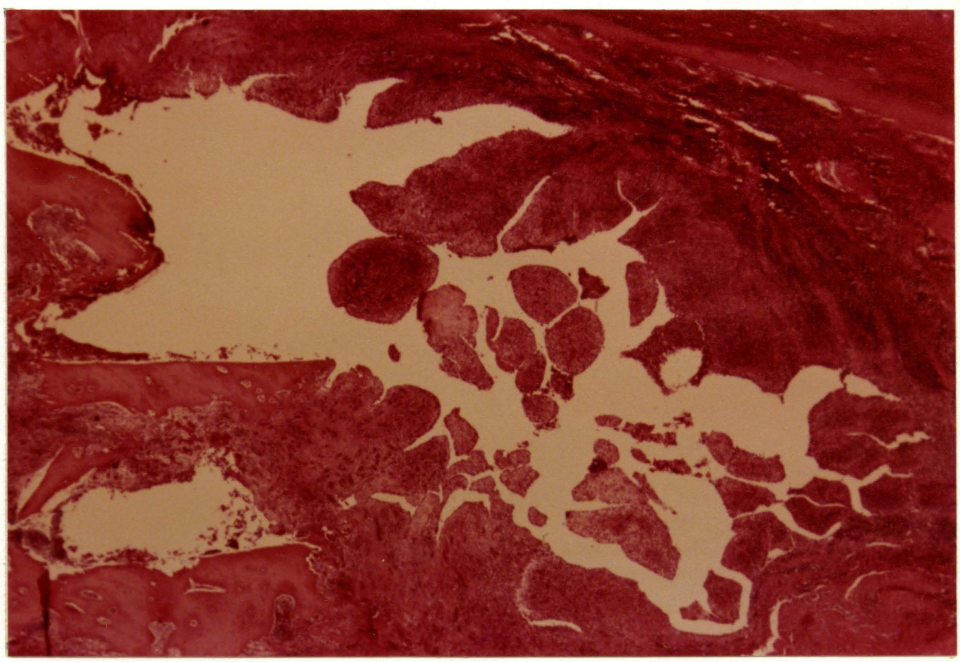
covered with mesothelial cells. Similar spaces could be found between the end of the graft bone and the fibrous connective tissue which separated it from the host bone. Villus proliferation covered with mesothelium projected into these spaces as well. This was synovial tissue formation in a newly formed joint space or pseudoarthrosis (Figure 40).

The space between the graft and the host was filled with connective tissue that varied in its degree of density. In the region characterized by loose connective tissue., there had occurred an inflammatory cell infiltration comprised of neutrophils, eosinophils, and a heavy admixture of macrophages. In other regions, there was an increased number of lymphocytes and plasma cells.

The screw hole within the host bone was filled predominantly with dense to loose fibrous connective tissue. The previously described inflammatory cells were found infiltrating regions of this tissue around the screw. Peripheral to this there appeared dense, irregularly connected spicules of bone.

The general impression of this slide was one of previous severe osteomyelitis at the surgical site which resulted in a nonunion and pseudoarthrosis formation.

Figure 40. Villus proliferation that projected into areas of osteoclasia observed in dog #731 (freeze-dried cortical allograft) at 240 days. Hematoxylin and eosin stain. x10.



Group 3: 365 Day Observation Period

This group consisted of one fresh cortical autograft (#692), two fresh cortical allografts (#1 and #725), and two freeze-dried cortical allografts (#2 and #734). This group resembled the previous two groups in that wound healing was uneventful, and all five dogs were bearing their full weight on the test limb within six weeks following surgery. Also, there was a transient elevation of the serum alkaline phosphatase in #1, #2, and #734. Aerobic and anaerobic cultures taken at the time of graft harvest were negative for all five dogs.

Radiographic evaluation

Fresh cortical autograft (#692) Evidence of graft and host bone resorption in the form of rounding of their respective edges was first observed two weeks post-operatively. A slight periosteal proliferation over the medial surface of the proximal host bone segment was observed at this time also. The periosteum continued to proliferate and appeared attached to the surface of the graft two weeks later. Endosteal thickening occurred simultaneously at each interface, and early bridging of periosteal new bone appeared at these sites six weeks after surgery. The periosteal callus underwent progressive remodeling, and the periosteal bridge was completed at eight weeks. Radiographic union occurred ten

weeks following surgery. Beyond this point, no significant changes occurred except for periosteal and endosteal remodeling.

The entire femur was harvested one year after surgery. Radiographs revealed the presence of a small gap between the periosteum and the surface of the graft. Endosteal trabecular formation had advanced equally from each host segment to almost completely fill the medullary canal of the graft. A small region of amorphous material was seen within the center of the graft between the proximal and distal trabecular walls. The caudal cortical surface of the graft appeared slightly radiolucent in comparison to normal host bone as if it had undergone almost complete revascularization and was being replaced with new bone. A small area of radiopacity existed in the cranial cortex which was presumed to be necrotic (Figure 41).

Fresh cortical allograft (#1) The immediate post-operative impression of the femur was one of poor alignment with posterior angulation at the proximal interface. Two weeks later it was noted that a further change in angulation had occurred. Also, periosteal new bone had been deposited on the proximal anterior surface of the host and at each interface. Rounding of the host bone edges at each interface had occurred as well. The periosteal new bone began to increase in thickness over the next two weeks and was

Figure 41. Serial radiographs of the harvested femur of dog #692 (control) taken at 365 days. The proximal interface is located between the second and third cortical bone screws and the distal interface is located between the fourth and fifth cortical bone screws.

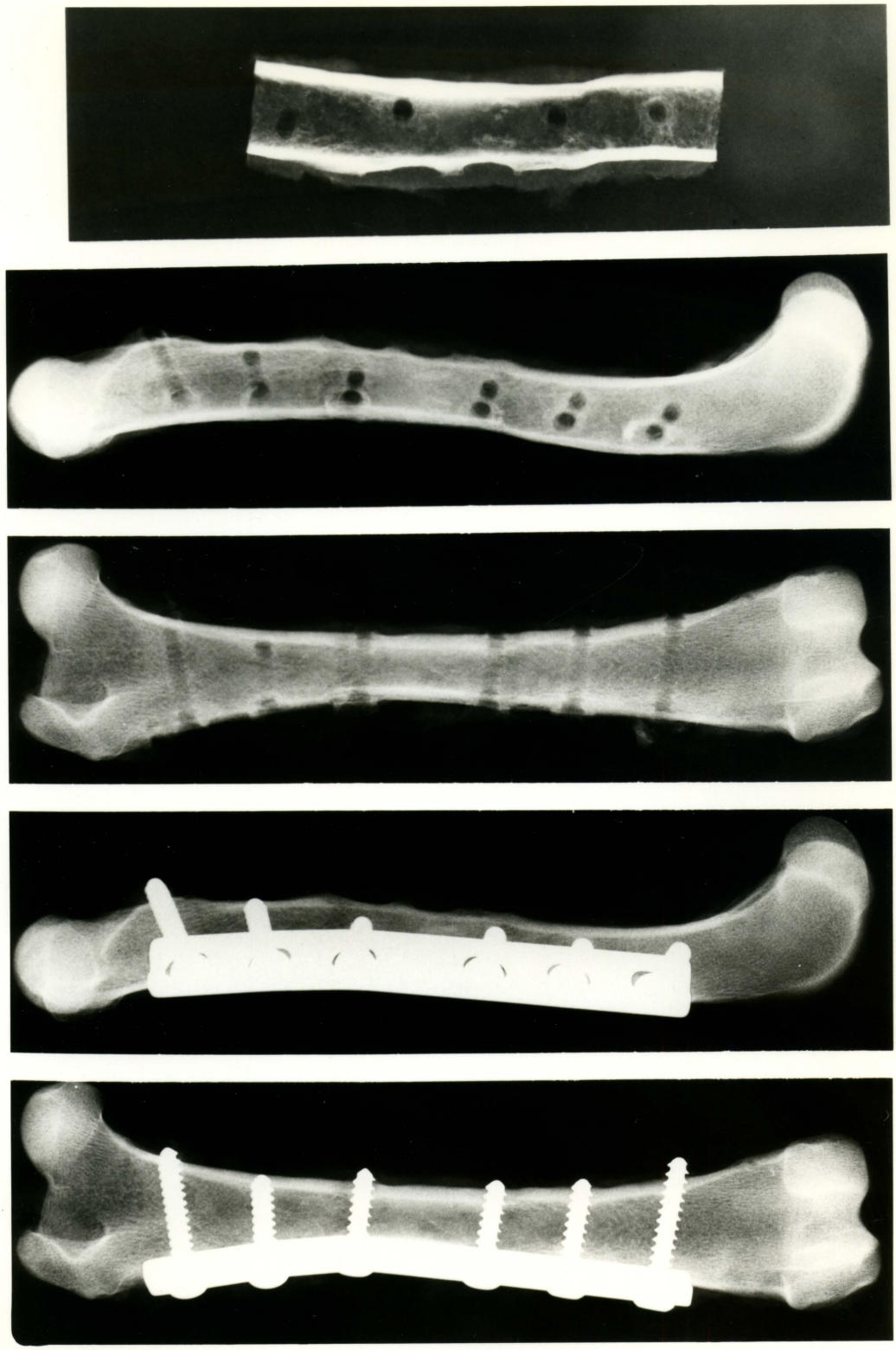
a. Anterior-posterior view of the femur with bone plate attached.

b. Medial-lateral view with the bone plate.

c. Anterior-posterior view without the bone plate.

d. Medial-lateral view without the bone plate.

e. Longitudinal section of the femur (1/2 thickness) showing the proximal and distal graft-host interfaces; the caudal surface is on the left side.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characterized as being extremely rough in appearance.

The first bone screw within the proximal host bone segment appeared to have loosened during the sixth post-operative week which resulted in elevation of the bone plate. During the next four weeks, progressive thickening of the callus continued, and a distinct area of lucency was apparent between the surface of the graft and the overlying periosteal new bone. The second cortical screw within the proximal host bone segment fractured during this time interval also.

A bridge of periosteal new bone formation over the distal interface was complete by fourteen weeks, and cortical union occurred four weeks later. The callus over the proximal interface continued to remodel, and bridging was noted at twenty-six weeks. Cortical union occurred during the ensuing sixteen weeks and was noted on the radiographs taken forty-two weeks after surgery.

The radiographs taken of the harvested specimen revealed an excellent ingrowth of endosteal trabeculae across each interface. The cranial surface of the graft remained very dense, and trabecular union rather than cortical union had occurred on that side at the proximal interface.

Fresh cortical allograft (#725) A disparity in size between the graft and host bone, especially at the distal interface, was noted in the postoperative radiographs. Although the graft was smaller than the host bone, its

positioning appeared to be excellent.

Early periosteal new bone formation occurred over the distal host bone segment which became roughened in appearance four weeks after surgery. Rounding of the edges of each host bone segment was also seen at that time. Periosteal new bone continued to proliferate in an attempt to fill the gap on the medial surface of the distal interface where the graft and the host bone had the largest discrepancy in size. Rounding of the edges of the graft was noted at six weeks, and periosteal new bone was being deposited over the distal end of the bone plate.

The proximal interface was bridged by periosteal new bone at twelve weeks, and cortical union was seen two weeks later. A complete bridge of callus across the distal interface never reached completion, and a delayed union was noted at the end of one year.

The harvest films revealed good cortical union at the proximal interface with only a minimum of trabecular growth into the medullary cavity of the graft. The graft has remained identifiable, especially at the distal interface. Although cortical union failed to occur at this site, a minimum of endosteal new bone was noted crossing the interface.

Freeze-dried cortical allograft (#2) An interfragmentary screw had to be utilized because the graft fractured

while being drilled. Two weeks postoperatively, the graft became displaced posteriorly and medially at the distal interface. A periosteal reaction was noted over the proximal and distal host bone segments and over the graft itself.

At four weeks, further periosteal new bone proliferation had occurred at the distal interface, and the graft and host bone edges appeared to have undergone some resorption. Although the periosteal callus over the graft became increasingly thickened, it did not attach to the graft for another two weeks. Endosteal trabecular growth across each interface and into the graft was excellent at ten weeks.

The proximal interface was bridged with periosteal new bone at twelve weeks, and cortical union occurred at each interface eight weeks later.

During the following months, the large callus overlying the graft began to remodel and take on a smoother appearance. The posterior cortex near the distal interface of the host bone segment began to slowly disappear (Figure 42).

The radiographs taken at the time of bone harvest revealed an excellent endosteal trabecular growth into the graft bone. The cortices of the graft remained only partially identifiable, and a small radiopaque segment of the cranial cortex was clearly visible. Cortical union was present at the proximal interface and at the anterior portion of the distal interface. Due to the fact that the posterior graft and host

Figure 42. Serial radiographs of the femur of dog #2 containing a freeze-dried cortical allograft (medial-lateral view).

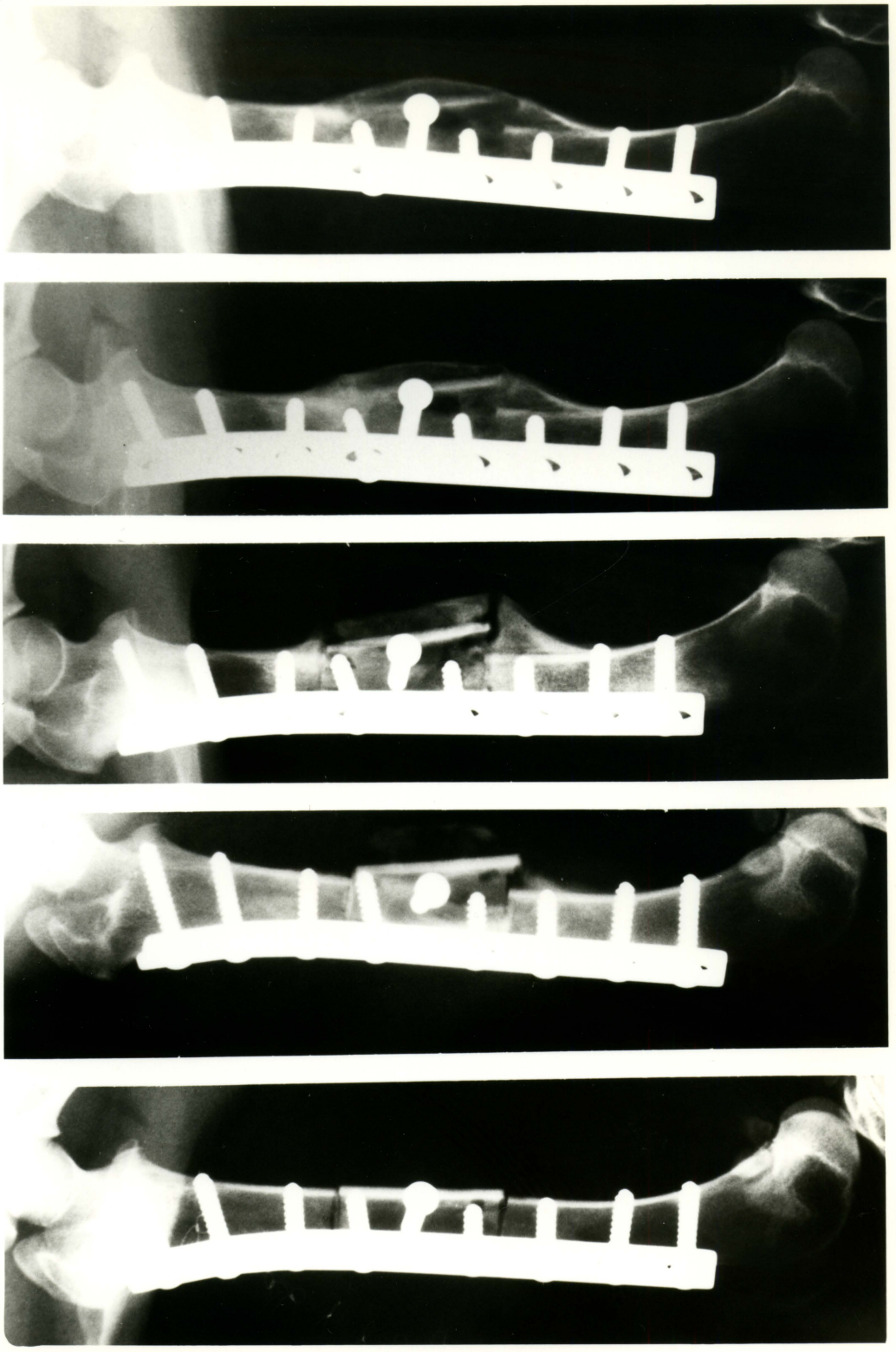
a. Postoperatively; note the interfragmentary bone screw within the graft.

b. 2 weeks; the graft had fractured and a portion of it has undergone posterior displacement.

c. 10 weeks; a bridge of periosteal new bone has attempted to bridge the fragment and each interface.

d. 20 weeks; a periosteal bridge has formed and is undergoing progressive remodeling; the graft cortex has become more radiolucent.

e. 40 weeks; cortical union has been achieved by the deposition of periosteal new bone.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cortices at this level were not in contact, union was facilitated through periosteal and endosteal new bone formation. The posterior cortex of the graft was no longer discernable in its proximal half and appeared to be undergoing excellent resorption in its distal half.

Freeze-dried cortical allograft (#734) Only a very slight periosteal reaction was noted during the entire period of study on this animal. Two weeks postoperatively a small amount of periosteal new bone was seen over the proximal host bone segment along with rounding of the host bone at the interface. The same sequence of events occurred at the distal interface two weeks later. Resorption of the edges of the graft also occurred at this time.

Periosteal new bone was first noticed to be attempting to bridge each interface six weeks postoperatively. Completion of this bridge of callus proximally and distally was noted at ten and twelve weeks respectively. Cortical union of the proximal interface was seen at fourteen weeks, and union of the distal interface occurred two weeks later.

The radiographs taken at the time of bone harvest revealed the presence of an excellent cortical union at each interface. The cranial and caudal graft cortices had remodeled throughout their entire length, and there was almost full incorporation of the medullary cavity of the graft with endosteal trabeculae (Figure 43).

Figure 43. Serial radiographs of the harvested femur of dog #734 (freeze-dried cortical allograft) taken at 365 days. The proximal interface is located between the third and fourth cortical bone screws, and the distal interface is located between the fifth and sixth cortical bone screws.

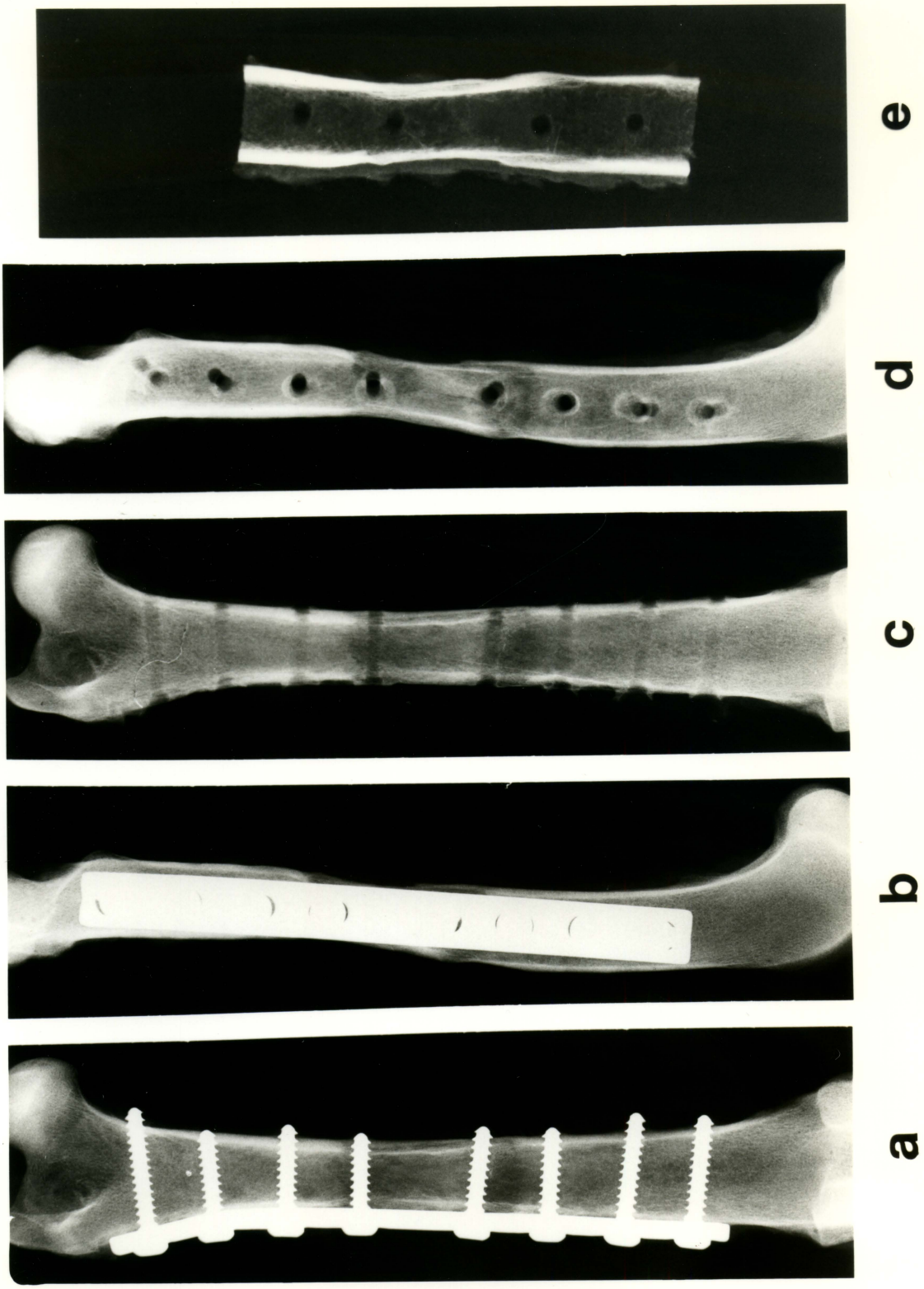
a. Anterial-posterior view of the femur with bone plate attached.

b. Medial-lateral view with the bone plate.

c. Anterior-posterior view without the bone plate.

d. Medial-lateral view without the bone plate.

e. Longitudinal section of the femur (1/2 thickness) showing the proximal and distal graft-host interfaces; the caudal surface is on the left side.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Histologic evaluation

Fresh cortical autograft (#692) The proximal host cortex contained longitudinally arranged osteons, and practically all of the lacunae were filled with osteocytes. The medullary canal contained a large amount of fatty marrow, however, there existed subendosteal hematopoietic marrow as well. Numerous well-formed and occasionally anastomosing spicules of bone were seen. The screw hole was filled by a fibrous jacket and had a few bone spicules adjacent to it. Dense osseous bridging was present at the proximal interface (Figure 44), and there were many longitudinally oriented resorption cavities with some containing hematopoietic marrow. The proximal portion of the graft cortex contained a number of empty lacunae. Only those areas in close association with revascularized channels contained osteocytes. Within the medullary canal, the proximal screw hole of the graft was surrounded by and connected with sparsely vascularized fibrous connective tissue. Occasional spicules of bone were present within the fatty marrow in the proximal portion of the graft. Only a small amount of hematopoietic marrow was seen.

The distal half of the graft cortex also contained a large number of empty lacunae. The lamellar bone containing osteocytes was only present around resorption cavities. The graft medullary cavity contained sparsely vascularized dense

to loose fibrous connective tissue. Some hematopoietic marrow with occasional irregular and non-anastomosing bone spicules was also present. The screw hole was totally enclosed with dense fibrous connective tissue.

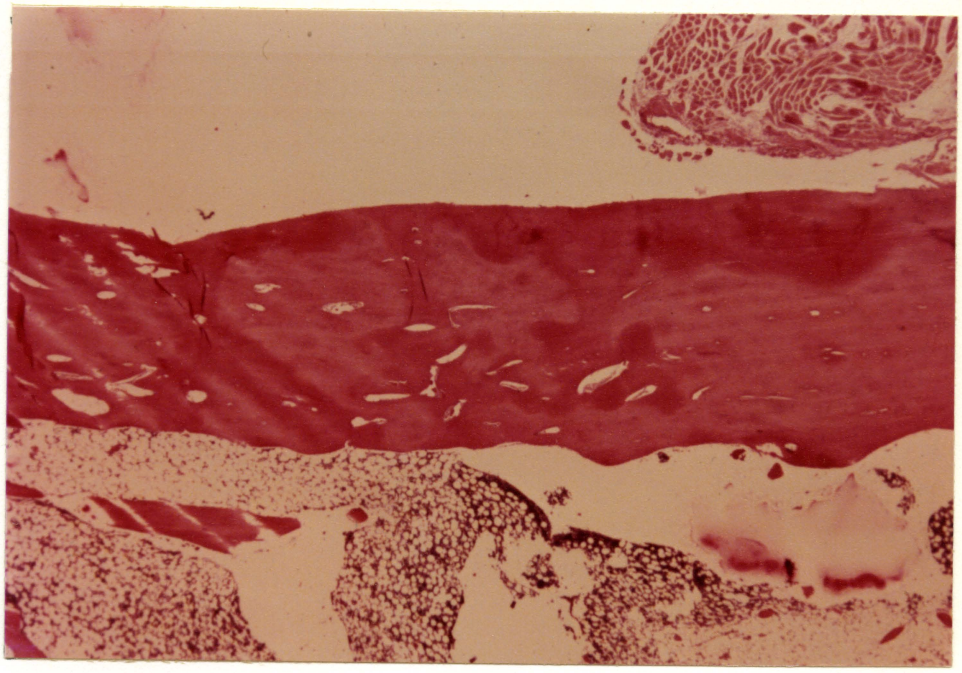
The distal graft-host interface was characterized by large resorption cavities with marrow that were continuous with that of the myeloid cavity (Figure 45). The host marrow cavity primarily contained fatty marrow with occasional large to small bone spicules, and a thin fibrous wall of dense, compact, lamellar bone, relatively few resorption cavities, and few empty lacunae.

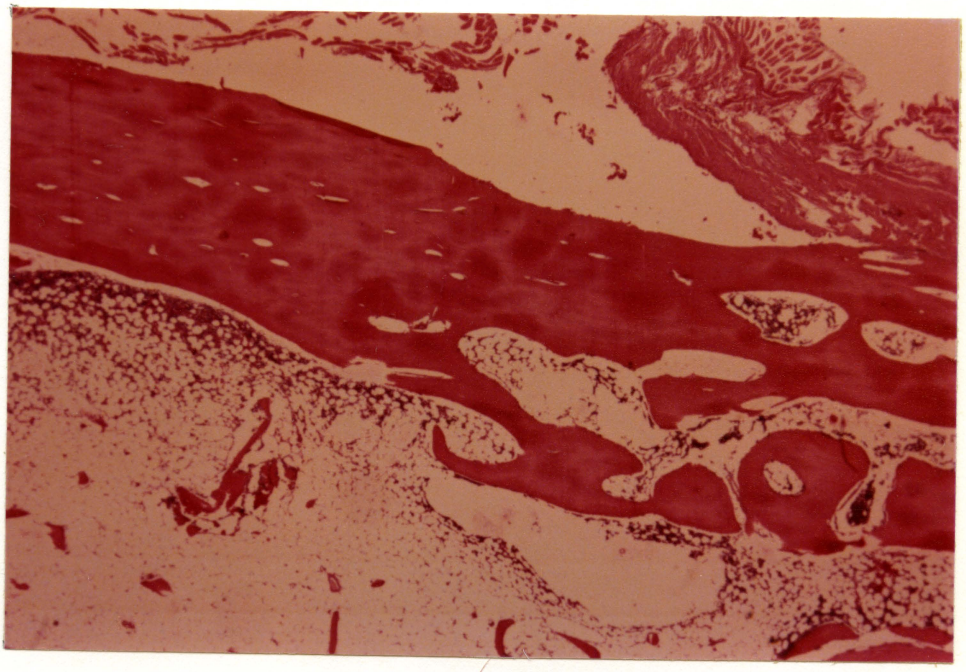
Fresh cortical allograft (#1) A large periosteal callus was noted lying over the anterior surface of the proximal host bone segment. This callus appeared to be undergoing cortical reduplication at its periphery. The original cortex deep to this had undergone considerable remodeling as was evident by the large longitudinal resorption cavities The original marrow cavity remained essentially normal, and the included screw hole was surrounded by bone and fibrous connective tissue. Both the anterior and posterior cortices were replete with osteocytes, however, there was no periosteal new bone over the posterior cortex.

The proximal interface contained many resorption cavities within both graft and host bone cortices. The cranial surface of the graft was also covered by an extension

Figure 44. The proximal interface of dog #692 (control) at 365 days. The interface is not identifiable and the cortex has a more normal appearance. Hematoxylin and eosin stain. x4.

Figure 45. The distal interface of dog #692; large resorption cavities still remain within the graft. Hematoxylin and eosin stain. x4.





of the periosteal callus covering the host bone. Within this area, there was found a region of cartilage or osteocartilage that appeared to be undergoing osseous replacement. The cranial cortex of the graft had undergone practically no remodeling, and the original Haversian canals and nearby lacunae were empty. There was evidence of fibrocartilage formation within the approximate area of the interface indicating that some movement had occurred during the healing process. Considerably more remodeling had occurred within the caudal cortex of the graft, and many lacunae were filled with osteocytes. The screw hole within the graft was lined by fibrous connective tissue and was enclosed by rather dense lamellar bone. A loose to dense fibrous connective tissue was found filling the marrow space.

The distal half of the graft contained cortices that appeared to have undergone minimal remodeling. The Haversian canals were revascularized in some areas, but for the most part, they remained empty. The posterior cortex contained a few more revascularized canals and viable osteocytes.

The distal interface was only suggested by the considerable amount of resorption cavity formation in both the host and graft bone. Longitudinal layers of bone appeared to communicate freely with dense spicules of what essentially constituted the endosteal callus.

Both cortices within the distal host bone contained

longitudinal osteons that were replete with osteocytes. The medullary cavity contained primarily fatty marrow, and the screw hole had a fibrous capsule.

Fresh cortical allograft (#725) The most characteristic features of this section were the lack of graft remodeling and the existence of a delayed union at the distal interface.

The proximal host bone segment was relatively normal in appearance and was filled with osteocytes. The corresponding marrow cavity extended distally into the medullary cavity of the graft, and formed an interface with loose to dense fibrous connective tissue that was poorly vascularized.

The proximal screw hole in the graft was lined entirely with a fibrous connective tissue capsule. The graft cortex contained some resorption cavities with only a few viable osteocytes around them. There were, however, a number of original Haversian canals that awaited revascularization, and an appreciable amount of the cortex contained empty lacunae.

The proximal interface was easily identified by the remains of the original cut ends of the proximal end of the graft. There were small clefts which appeared to run perpendicular to the long axis of the bone and to line up in a single plane. Numerous sites existed where these clefts were traversed by new lamellar bone that contained viable osteocytes. Those portions of lamellar bone of the graft

which abutted directly onto the clefts remained devoid of osteocytes, indicating they were original graft bone. Dense bony bridging was noted on both sides giving apparent reconstitution of cortical strength and structure.

The cortices of the distal host bone segment were of apparent decreased density and contained large resorption cavities. Some of these cavities were filled with marrow and were continuous with a considerable amount of periosteal callus with marrow at the interspicular spaces. This callus extended proximally and partly covered the nonunited junction site at the distal interface.

The screw hole in the distal host bone segment was lined with a mature fibrous capsule which was surrounded by very dense trabecular bone.

An irregular interface was noted between the host and the graft that was comprised primarily of hyaline to fibrocartilage. In numerous regions along both the host and graft surfaces, enchondral ossification was occurring. The ends of the graft cortices extended into distally oriented depressions that had been formed by the host periosteal new bone and the dense endosteal compact bone (Figure 46).

The distal half of the graft cortex appeared to be poorly revascularized. Periosteal new bone was noted at the distal end of the graft cortex which contained focal regions of hyaline to fibrocartilage.

Freeze-dried cortical allograft (#2) The marrow cavity and screw hole within the proximal host bone segment appeared similar to those previously described. This was also true of the marrow cavity and screw hole within the proximal half of the graft.

This section was characterized by the existence of a thickened layer of periosteal new bone that extended over the posterior cortex from just above the proximal interface to well beyond the distal interface. This callus contained numerous cavities that were filled with hematopoietic marrow and possibly had formed due to the marked resorption of both the host and graft cortex following the longitudinal fracture and displacement of the graft.

The cranial cortical surface was not covered by periosteal new bone. Although the proximal interface between the graft and host bone remained clear, it is best described as a complete bony union. As the line of interface was approached, the host cortex exhibited a lack of osteocytes in lacunae in spite of the numerous small resorption cavities that were present and remodeling. There was somewhat more resorption in the graft cortex near the interface, but the number of resorption cavities decreased further distally.

In contrast to the cranial cortex, the caudal cortex of the proximal host bone segment was filled with osteocytes.

The same held true, but to a lesser extent, within the graft.

The anterior cortex of the distal host bone segment had a sizeable number of osteocytes within lacunae. The posterior cortex had been considerably resorbed, and only a small remnant of it remained. The remnant had lost many osteocytes and had undergone resorption and remodeling as well. Distal to that remnant of cortex was a rather wide zone of periosteal new bone formation with reduplication of cortical bone at its periphery. This periosteal callus bridged the plane of union.

The distal interface on the anterior surface was only suggested by a region of resorption. On the posterior surface, cortical remnants were absent, and callus had formed the total structural component of that area. There were some remnants of the graft cortex in this area which had undergone much resorption and early remodeling.

The host marrow cavity, with fatty and hematopoietic marrow, had extended into the graft marrow space and interfaced irregularly with a loose to dense fibrous connective tissue. There existed, however, some areas within the graft medullary cavity that contained fatty and hematopoietic marrow.

Freeze-dried cortical allograft (#734) The proximal host bone segment was well populated with osteocytes, and the marrow cavity contained fatty and hematopoietic marrow.

The screw hole was surrounded by a thin, dense, fibrous capsule, and the proximal interface was suggested merely by the onset of the presence of longitudinally oriented resorption cavities which also contained fatty and hematopoietic marrow.

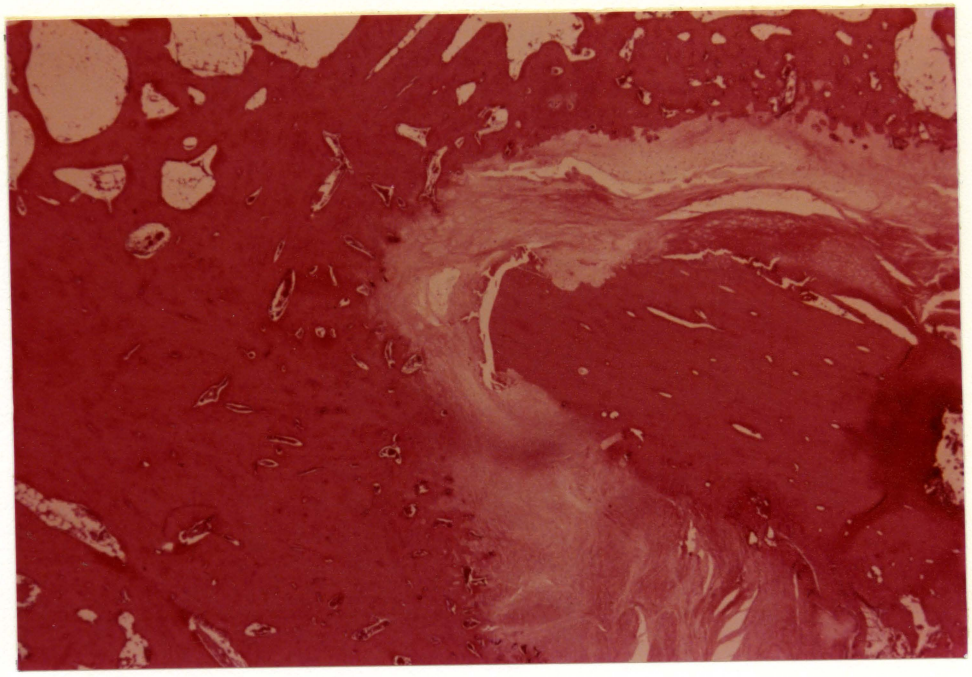
Although there were regions within the reorganizing graft cortex where osteocytes were absent, new bone formation was occurring in areas adjacent to resorption cavities (Figure 47). It was felt that there may have been a slightly greater preponderance of osteocyte formation in the caudal cortex of the graft. Host marrow elements had extended well into the medullary cavity of the graft, and the screw hole was lined with a fibrous capsule.

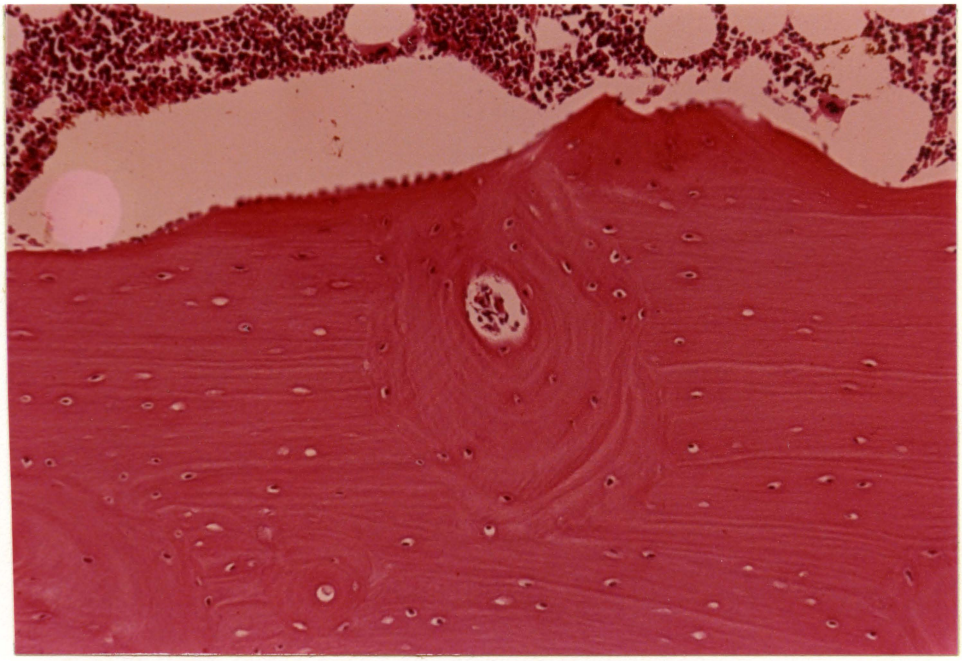
This distal half of the graft cortex was still characterized by longitudinally oriented resorption cavities containing marrow. Within the posterior cortex, these resorption cavities were fairly evenly distributed. On the opposite side, however, they were seen mainly in the outer one-half to two-thirds of the cortex. In that region the inner one-third of the cortex contains many empty lacunae.

The marrow cavity of the graft contained fibrous connective tissue which extended only part way through the graft and abutted with the host marrow. At that level of abuttment, near the level of the distal graft screw hole, there was some mild infiltration of the fibrous marrow tissue with

Figure 46. The distal interface of dog #725 (fresh cortical allograft) at 365 days. The end of the graft cortex is surrounded by fibrocartilage and remains necrotic. This was classified as a delayed union. Hematoxylin and eosin stain. x16.

Figure 47. A resorption cavity within the freeze-dried cortical allograft of dog #734 at 365 days. New bone formation has occurred within the cavity. Note the empty lacunae around the cavity indicating the presence of necrotic bone. Hematoxylin and eosin stain. x40.

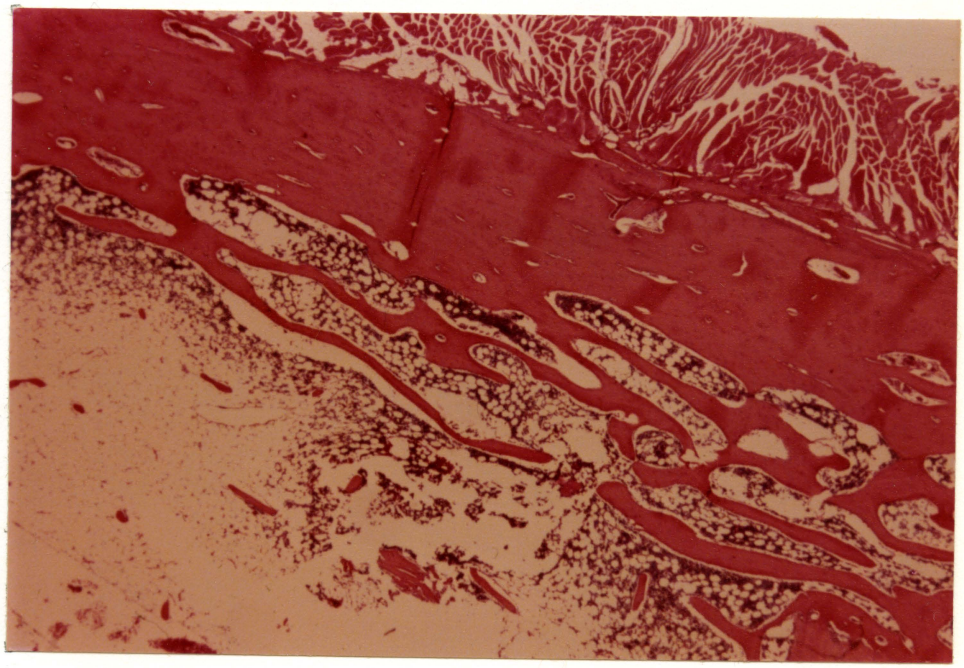




lymphocytes. Several vascular channels in this area contained lymphocytes, neutrophils, and eosinophils. The screw hole within the graft was surrounded by fibrous connective tissue.

The distal graft-host interface was suggested predominantly by the termination of the major resorption cavities within the graft and the onset of dense cortical bone replete with osteocytes (Figure 48). The host screw hole was surrounded by a dense fibrous connective tissue capsule, and the marrow was predominantly fatty.

Figure 48. The distal interface of dog #734; resorption cavities were still evident but the cortex has assumed a more normal conformation. Hematoxylin and eosin stain. x4.



DISCUSSION

The radiographic interpretations of the various stages of graft incorporation are summarized in Tables 2, 3, and 4. Although difficulties have been noted by others regarding the correlation of the radiographic appearance of a graft to that which actually exists histologically, Gresham formulated a useful guide for this purpose (15,71,198). He divided the incorporation and replacement of nonviable freeze-dried cortical bone allografts into three phases. The first phase lasts from three to six weeks and involves a passive relationship between the host and the graft. The graft appears radiolucent and does not change in morphology. During the second phase, early resorption of the graft occurs. The sharp edges of the graft become rounded in appearance, and, if immobilization is inadequate, resorption will be seen around the bone screws. The third phase lasts for many years and involves a continuous replacement of the graft with a concurrent decrease in its size (71).

The progressive radiographic changes observed in this study are analogous to those described by Gresham and have been applied to all three types of grafts. In general, bone resorption occurred one to two weeks earlier in the host bone segments than in any of the grafts studied. This was particularly true of the proximal host bone segment near the interface. Presumably this was due primarily to the active

circulatory supply present in the host bone cortex. Certainly heat necrosis from the bone saw and tissue inflammation may have contributed to this phenomenon. Although graft resorption was noted as occurring almost simultaneously at each interface, this process was seen first in the autograft series three weeks postoperatively and then in the freeze-dried and fresh allograft series at four and five weeks respectively.

The formation of a progressive wall of periosteal new bone as an attempt to bridge each interface was also seen in every animal studied. Although this process was usually first observed near the distal interface, it reached completion at the proximal interface well in advance of the corresponding completion distally in most cases. This may have been due to the problems of instability observed at the distal interface (see Table 11). Disregarding the untoward events that occurred at either interface, the proximal interface was, in general, completely bridged by periosteal new bone at 10 weeks in the fresh cortical autograft series, 12 weeks in the fresh cortical allograft series, and 11 weeks in the freeze-dried allograft series. The values at the distal interface for the respective grafts were 9,15, and 15 weeks.

Cortical union occurred shortly after periosteal bridging and usually was first observed at the proximal

interface. Again, disregarding untoward events, the approximate time of union at the proximal interface for the fresh autograft series was 12 weeks as compared to 15 weeks in both the freeze-dried and fresh allograft series. At the distal interface, these values were 11 ant 18 weeks. It is emphasized that the averages given for the freeze-dried cortical allografts at this site are inaccurate because of the healing problems that occurred.

Most of the problems encountered in this experiment occurred at the distal interface (see Table 11). A delayed union was noted at this site in all three graft types (Figure 19). A fibrous nonunion was seen in #914 (control, Group 1), and there was evidence of pseudoarthrosis formation in #671 (freeze-dried cortical allograft, Group 2) at least one month before the bone plate fractured at that site. It is of interest to note that delayed union at the distal interface has also been reported by others involved in the study of segmental bone transplants (130,199). Although these problems are directly related to mechanical instability, it is difficult to ascertain whether they were a result of inadequate immobilization at the time of surgery or maladjustment of the graft and host bone surfaces. Since graft resorption occurs ahead of replacement, the graft eventually weakens mechanically and the bone plate may undergo a stress fracture. Even the smallest movement at that site would

impede the revascularization of the graft and result in delayed or fibrous nonunion. Heat necrosis of the host bone segment may also have affected the revascularization process. The only problem encountered at the proximal interface was in #1 (fresh allograft, Group 3) where a fibrous nonunion occurred. This was attributed to poor alignment at the proximal graft-host interface with a subsequent fracture of a cortical bone screw on the host side of the interface.

Loosening of the bone screws was also a problem that was shared by all three graft types and was probably another major reason for the instability seen at the distal interface. Usually bone screws loosen due to a lack of solid purchase within the cortex or if the screw threads have become stripped. In this case, it is felt that the phenomenon of screw loosening was the result of bone resorption around the screw proper. Again, heat necrosis from injudicious use of the power drill cannot be overlooked as a cause. The following dogs were victimized by this problem: #1, #2, #3, #23, #657, #671, and #725. Infection with resorption of the graft and host bone together with loosening of the bone plate and screws occurred in #731 (see Table 11).

120 days

The graft-host interface could still be identified even though radiographic union had occurred in each (except at the distal interface in #914 and #657). Although the host and

graft segments had been compressed at each interface, a small gap was usually apparent upon microscopic examination. The healing attempts by the host were typified by Figure 25. It was observed that blood vessels arising from the host cortex had bridged the interface and had penetrated the graft cortex, presumably along previously resorbed Haversian canals. In addition, periosteal and endosteal blood vessels had entered into the area of the interface region presumably in an attempt to cement the host and graft segments together. This deposition of osteoid appeared to be perpendicular to the longitudinal osteons of the diaphyseal cortex. New lamellar bone containing osteocytes within lacunae had been deposited centripetally within a few of the resorption cavities close to the interface (Figure 23).

The preceding was noticeably further advanced in the fresh cortical autograft than that observed in the other two. The resorption cavities within the freeze-dried cortical allograft resembled the control and were much larger than those seen in the fresh cortical allograft. Perhaps this was an indication of the relative antigenicity of the freeze-dried cortical allografts as opposed to the fresh cortical allografts.

240 days

This stage was characterized by marked resorption of the graft cortex and the presence of large resorption cavities

that, for the most part, were filled with hematopoietic bone marrow. Although much of the graft remained necrotic, as indicated by the presence of numerous empty lacunae, new lamellar bone had been deposited around many of these cavities. There had been a heightened production of periosteal and endosteal new bone as well. This was probably due to the structural demand for support because the cortex, for the most part, closely resembled cancellous bone (Figure 33). The interface could be seen in some sections while in others it was only suggested by the intense activity of resorption and revascularization occurring at that site. The endosteal trabecular pattern often resembled a wave of bone that had spread from the host bone segment into the medullary cavity of the graft. At this point, the graft was being revascularized not only by blood vessels from the host cortex but also from the periosteum and endosteum. Lamellar bone deposition had also occurred around the vessels.

The fresh cortical autograft series had, by far, more new bone deposition at this time. The amount and extent of resorption and revascularization of the freeze-dried cortical allografts remained comparable to the control and ahead of the fresh cortical allograft series.

365 days

The graft-host interface was extremely difficult to locate except in those sections containing a delayed or

nonunion. This was because they now closely resembled normal cortical bone even though longitudinal resorption cavities were still present. The healing process was judged to be far from complete.

The fresh cortical autograft still contained many empty lacunae but had been extensively replaced by new lamellar bone. The freeze-dried cortical allografts had undergone almost complete resorption and revascularization and appeared to be ahead of the fresh cortical allografts. Both contained more necrotic bone and less new bone deposition than the control.

A general finding in all the sections was that the portion of the graft that was covered with soft tissue, i.e., the posterior aspect, was usually further advanced in terms of replacement with new lamellar bone and osteocytes. Much of the anterior graft cortex remained necrotic even in the 365 day series. Small islands of resorption and revascularization existed with osteocyte production around them. Usually there was more periosteal new bone seen over the anterior surface than over the posterior surface. In those cases where there existed a delayed or nonunion (Figures 21 and 46), both cortices were usually almost entirely necrotic. It is proposed that movement at the interface was substantial enough to prevent revascularization directly from the host

cortex and was the major reason for the presence of necrotic bone in those sections.

Endosteally a sequence of growth and repair occurred as follows. At 120 days, the medullary cavity of the graft was filled with fibrovascular connective tissue and only a minute amount of bone trabeculae and marrow elements. By 240 days, this connective tissue appeared to be undergoing a slow and progressive replacement with new bony trabeculae that had originated from each host segment, crossed the graft host interface, and had moved toward the center of the graft. By 365 days, the amount of fibrovascular tissue remaining within the graft was markedly reduced but still present.

The screw holes varied in their appearance between graft and host bone. The screw holes within the grafts were usually surrounded by thick fibrous connective tissue and only a small deposition of osteoid. On the other hand, the host screw holes had markedly less fibrous connective tissue around them and more osteoid.

Inflammatory cells comprised primarily of lymphocytes, plasma cells, neutrophils, eosinophils, and macrophages were noted within a few resorption cavities in #657, around the screw holes in #731, within the vascular channels in #734, and at the site of fibrous nonunion in #914. In addition, some of the blood vessels in #657 were noted as having thickened and hyalinized walls. The reason for the

appearance of these cells is not clear except in the case of #731 from which was cultured Staphylococcus aureus. There may have been enough instability at the distal interface in #657 and #914 to incite a localized inflammatory response that was sufficient enough to attract these cells. The small number of inflammatory cells seen in #734 were not thought to be the result of a host immune response to the graft. Resorption and replacement with new bone were quite substantial in this section, and no clinical signs of an immune reaction were ever seen during the test period. Since no specific tests were made to assess the state of the immune system in any of the experimental animals, perhaps the appearance of these cells should be considered as a form of immune response until proven otherwise. No conclusive data dealing specifically with the type of cellular reactions that could be expected within a cortical allograft undergoing graft rejection was located. Whether this would take the form of a local or generalized response within a bone graft is unknown.

According to Hyatt, an unsuccessful allograft may at first appear to be biologically acceptable, but can become involved in a tissue inflammatory response later on. This response is believed to be mediated through vascular buds, and the predominant inflammatory cells seen are of the lymphocytic variety. There is subsequent graft destruction and

resorption by granulation tissue. During this process, the lymphocyte is thought to have an increased phagocytic capability (90).

Since #734 did not appear to be undergoing any type of tissue destruction, nor was it cultured positive for bacteria, the significance of the presence of these cells is unknown.

Longitudinal fractures occurred in grafts #2, #3, and #657 (see Table 11). In the former two, the fracture extended from one end of the graft to the other. As previously noted, the instability at the distal interface of #657 led to a delayed union at the end of 120 days. Excellent resorption, revascularization and replacement with eventual cortical union occurred in the other two grafts. In view of the marked instability created by these fractures, the final results were completely unexpected. Their success, however, has verified Volkov's theory on segmental bone transplantation. He has advocated the use of thin plates of allograft bone instead of thick cortical segments to achieve a large area of contact between the graft and the muscular bed, blood, and host tissue fluid. In doing so, the rate of reconstruction of the graft is greatly accelerated (187).

The findings of this study are in accord with the axioms that have been previously established regarding bone transplantation, i.e., autogenous cortical grafts are resorbed,

revascularized and replaced more rapidly than either freeze-dried or fresh cortical allografts. Also, the processes involved in the incorporation of graft bone are similar to those seen in fracture repair but are delayed when allografts are utilized (99).

Regardless of the rate at which the different grafts were replaced, each served as a trellis or scaffold for the ingrowth of host tissue and were ultimately either partially or completely reduplicated by new host bone. This process has been referred to as osteoconduction, and it occurs as an ordered and simultaneous piecemeal resorption and replacement of the transplanted cortical segment (60,156,181).

The final disposition of a cortical graft has been discussed at length. It is generally accepted that the circulatory supply of the graft bed and the quality of immobilization of the graft and host bone segments are the two most important factors affecting the fate of the graft (116). Since the revascularization of a cortical graft is more dependent upon the bone surfaces against which it contacts and less upon the surrounding soft tissues, the importance of absolute immobilization at the graft-host interface is imperative (63,194). The dense cortical structure of the graft is slowly transformed into numerous resorption cavities and histologically assumes an appearance that is more akin to cancellous bone. At this stage, the

graft becomes extremely weak and is subject to fracture unless proper stress protection is given for a prolonged period of time (175).

According to Stringa, there is a direct correlation between the rate of vascular penetration of a bone graft and its ultimate incorporation in the host bed (163). Pappas has shown that freeze-dried allografts of bone are more rapidly resorbed than fresh allografts, which is in direct agreement with the findings of this study (133).

Another factor having a direct effect on the final disposition of a cortical graft is stress. Roux's law of functional adaptation as emphasized by Phemister, implies that unless a graft is placed in a useful location, e.g., a segmental defect in a long bone, it will undergo retrogressive changes and will be gradually resorbed (139). Hyatt also emphasized the importance of stress in this regard by stating, "...the ultimate anatomical configuration of the graft after reduplication seems to be determined by the functional demands of the operative site." (90). The results of this study attest to the fact that even the slightest amount of movement at either interface will result in delayed or nonunion or ultimately in the fracture of the internal fixation device.

The term "creeping substitution" has become a popular descriptive term of the processes which take place in the

replacement of dead bone. Many have attempted to redefine the process as "creeping invasion" or "creeping replacement", and Urist has recommended that this terminology be expanded to include the concept of induction (164,177,178,182).

Although this term remains popular today, some have interpreted it as a process which is only end-on in nature, i.e., occurs only across the graft-host interface. In spite of the fact that the blood supply that is derived from adjacent cortical bone is more important in revascularizing the graft, Brookes has shown that the ischemic cortex derives a diffuse arterial blood supply from the periosteal and medullary circulations as well (27). This method of graft revascularization was found to be the norm in this study also (Figure 20). Because of the confusion inherent with the use of this term, it is recommended that resorption, revascularization, and replacement be utilized instead to describe the method of bone graft incorporation by the host.

Although the processes of resorption and replacement are thought to occur simultaneously in the ideal transplant, this was not seen in any of the grafts utilized in this study (175). Instead, resorption and revascularization were seen almost simultaneously and were followed by a latent period before new lamellar bone was deposited. Similar results have been reported by Goldberg (67).

Finally, a long-term follow-up study of any patient receiving an allograft is highly recommended. The results of this experiment indicate that all three graft types still contained sizeable quantities of necrotic bone at the end of one year, and is another major reason that the internal fixation device should be left in place indefinitely. Because of the slow turnover of necrotic bone, Bonfiglio has recommended that follow-up studies be performed for at least ten years in man (19). A three to five year follow-up study in dogs and cats receiving cortical allografts would be desirable. During the first year following surgery, radiographs of the involved area should be taken at least every three months. After one year, these patients should be examined every six months if possible. Through frequent re-examinations the status of the graft, internal fixation device and interface healing can be closely monitored.

In this regard, it is the surgeon's responsibility to utilize fresh or preserved allografts of bone only if it is absolutely necessary. Hyatt has emphasized the importance of this point by stating, "The constant and repeated need for thorough and additional long-term follow-up information quickly overrides the transient advantages, timewise, of a bottle of tissue that is used because it is available." (90).

SUMMARY

1. The purpose of this study was to compare, both radiographically and histologically, freeze-dried and fresh cortical allografts to fresh cortical autografts in the canine femur. A total of 15 dogs were utilized in this investigation, and were grouped into 120-, 240- and 365-day observation periods.

2. The grafts were inserted into 5 cm. segmental defects that had been created in the right femur of each dog. The periosteum was stripped from each graft, and the marrow was thoroughly flushed from the intramedullary canal before insertion. The graft and host bone segments were immobilized and compressed at each interface with the Dynamic Compression Plate (DCP) by Synthes.

3. A residual moisture of 1-2% was obtained in the freeze-dried grafts. Although these grafts had been rehydrated for a period of two hours prior to being drilled, the cortices proved to be extremely dense and brittle. Because of their propensity to fracture during drilling, they were drilled while completely immersed in saline.

4. An account of the surgical procedure, immediate post-operative care, and method of radiographic analysis were given.

5. Aside from a transient rise in the serum alkaline phosphatase level in many of the test animals, no untoward effects were observed. Except for #731, each animal was bearing its full weight on the test limb by the eighth postoperative week.

6. Radiographically, cortical union usually closely followed bridging across either interface with periosteal new bone. These processes generally reached completion at the proximal interface first, and except in the fresh autograft series, occurred at the distal interface four to five weeks later. Union occurred at either interface in the fresh cortical autograft series well in advance of that seen in the freeze-dried and fresh cortical allograft series. In most cases cortical union was observed at the proximal interface at approximately the same time in both the freeze-dried and fresh cortical allografts. Difficulty was encountered in making an accurate comparison between the different grafts at the distal interface because of the problems of instability and delayed or nonunion encountered at that site.

7. Problems encountered during the study period included graft fracture, loosening of the bone screws, especially frequent in the graft, and fracture of the bone plate at the distal interface subsequent to pseudoarthrosis formation. Freeze-dried graft #731 was almost

completely resorbed during the observation period and was subsequently cultured positive for Staphylococcus aureus.

8. A detailed histologic comparison was made between the three types of grafts. The process of resorption, revascularization, and replacement was observed to occur much faster in the control grafts. Resorption and revascularization were very pronounced in the freeze-dried grafts and closely resembled that seen in the control. In general, the replacement process appeared to be further advanced in the posterior cortex of the grafts and was believed to be due to the greater amount of soft tissue covering and corresponding vascular supply in that region. There was still a slight to moderate amount of necrotic bone remaining in the control grafts at the end of one year, and a correspondingly larger amount was seen in the freeze-dried and fresh allografts.

9. An extensive historical review was presented regarding early theories of osteogenesis, bone grafting, and the use of freeze-dried bone grafts in orthopedic surgery.

10. The principle of bone induction was discussed along with theories on the effects of the host immune response on different types of processed bone grafts and bone grafts in general.

11. The term "creeping substitution" was defined in light of its original meaning and how it was subsequently misinterpreted. In view of the persistent confusion that has surrounded the use of this term, it was recommended that it no longer be used to describe the processes of resorption, revascularization, and replacement.

12. A three to five year follow-up study of patients receiving cortical allografts was recommended. This was emphasized because of the increased healing time required of cortical allografts and the constant need to monitor the status of the internal fixation device.

CONCLUSION

Fresh cortical autografts have been demonstrated to be superior to either freeze-dried or fresh cortical allografts. In terms of consistent cortical union and mechanical stability, the fresh cortical allografts were superior to the freeze-dried cortical allografts. Although union at the proximal interface was observed at approximately the same time in both allograft types, consistent difficulty was experienced at the distal interface when the freeze-dried allografts were used. In addition, the process of freeze-drying proved to be time consuming and rendered an extremely brittle product that easily fractured while bring drilled unless totally immersed in saline.

It is recommended that further research be performed in both clinical and experimental situations before the wholesale use of freeze-dried bank bone be performed in veterinary orthopedic surgery. Until that time, fresh cortical allografts may prove to be beneficial for bone replacement in situations demanding greater amounts of bone than is available from the patient.

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APPENDIX

Table 1. Results of bacterial culture assays taken at the time of harvest

Group Number	Dog Number	Graft Type	Aerobic Bacteria	Anaerobic Bacteria
1	914	Fresh Autograft	Neg	Neg
1	4	Fresh Allograft	Neg	Neg
1	657	Freeze-Dried Allograft	Neg	Neg
2	23	Fresh Autograft	Neg	Neg
2	698	Fresh Allograft	Neg	Neg
2	568	Fresh Allograft	Neg	Neg
2	3	Fresh Allograft	Neg	*Propionibacterium acne* Serotype II
2	671	Freeze-Dried Allograft	Neg	Neg
2	670	Freeze-Dried Allograft	Neg	Neg
2	731	Freeze-Dried Allograft	*Staphylococcus aureus*	Neg
3	692	Fresh Autograft	Neg	Neg
3	1	Fresh Allograft	Neg	Neg
3	725	Fresh Allograft	Neg	Neg
3	2	Freeze-Dried Allograft	Neg	Neg
3	734	Freeze-Dried Allograft	Neg	Neg

Table 2. Radiographic appearance and time intervals -- Group 1

	Periosteal reaction over host segment; (start of endosteal callus	Rounding of host bone segment	Rounding of graft edges	Callus proliferation over graft	Periosteal bridge of callus starting between graft and host bone	Periosteal bridge complete	Cortical union
914	2 wks (P)[a]	4 wks (P)	4 wks (P)	6 wks	6 wks (P)	10 wks (P)	12 wks (P)
(control)	2 wks (D)[b]	4 wks (D)	4 wks (D)				Non-union at distal interface
4	4 wks (P)	2 wks (P)	2 wks (P)	6 wks	8 wks (P)	12 wks (P)	16 wks (P)
(fresh)	4 wks (D)	2 wks (D)	2 wks (D)		8 wks (D)	12 wks (D)	16 wks (D)
657 (freeze-dried)	2 wks (P)	2 wks (P)	Not seen	4 wks	6 wks (P)	8 wks (P)	12 wks (P)
	2 wks (D)	2 wks (D)				14 wks (D)	16 wks Delayed union

[a] P = Proximal interface.
[b] D = Distal interface.

Table 3. Radiographic appearance and time intervals - Group 2

	Periosteal reaction over host segment; (start of endosteal callus)	Rounding of host bone segment	Rounding of graft edges	Callus proliferation over graft	Periosteal bridge of callus starting between graft and host bone	Periosteal bridge complete	Cortical union
23 (control)	2 wks (P)[a] 2 wks (D)[b]	2 wks (P) 2 wks (D)	4 wks (P) 4 wks (D)	4 wks	6 wks (P) 6 wks (D)	12 wks (P) 10 wks (D)	14 wks (P) 12 wks (D)
698 (fresh)	2 wks (P) 2 wks (D)	2 wks (P) 4 wks (D)	6 wks (P) 6 wks (D)	8 wks	6 wks (P) 14 wks (D)	10 wks (P) 18 wks (D)	12 wks (P) 22 wks (D)
568 (fresh)	2 wks (P) 4 wks (D)	4 wks (P) 2 wks (D)	4 wks (P) 4 wks (D)	6 wks	6 wks (P) 8 wks (D)	8 wks (P) 10 wks (D)	12 wks (P) 14 wks (D)
3 (fresh)	4 wks (P) 4 wks (D)	4 wks (P) 2 wks (D)	4 wks (P) 4 wks (D)	6 wks	14 wks (P) 8 wks (D)	18 wks (P) 22 wks (D)	22 wks (P) 26 wks (D)
671 (f/d)[c]	2 wks (P) 4 wks (D)	Not seen 2 wks (D)	Not seen	10 wks	6 wks (P) 12 wks (D)	14 wks (P) 16 wks (D)	16 wks (P) 24 wks (D) fx.
670 (f/d)	4 wks (P) 2 wks (D)	2 wks (P) 2 wks (D)	4 wks (P) 2 wks (D)	8 wks	6 wks (P) 6 wks (D)	10 wks (P) 24 wks (D)	14 wks (P) 8 mos DU[d] (D)
731 (f/d)	Infection -- Graft Resorption						

[a] P = Proximal interface.

[b] D = Distal interface.

[c] f/d = freeze-dried.

[d] DU = Delayed union.

Table 4. Radiographic appearance and time intervals -- Group 3

	Periosteal reaction over host segment; (start of endosteal callus	Rounding of host bone segment	Rounding of graft edges	Callus proliferation over graft	Periosteal bridge of callus starting between graft and host bone	Periosteal bridge complete	Cortical union
23 (control)	2 wks (P)[a] 4 wks (D)[b]	2 wks (P) 2 wks (D)	2 wks (P) 2 wks (D)	4 wks	6 wks (P) 6 wks (D)	8 wks (P) 8 wks (D)	10 wks (P) 10 wks (D)
1 (fresh)	2 wks (P) 4 wks (D)	2 wks (P) 2 wks (D)	6 wks (P) 6 wks (D)	8 wks	22 wks (P) 6 wks (D)	26 wks (P) 14 wks (D)	42 wks (P) 18 wks (D)
725 (fresh)	4 wks (P) 2 wks (D)	4 wks (P) 4 wks (D)	6 wks (P) 6 wks (D)	8 wks	10 wks (P) 8 wks (D)	12 wks (P) Delayed	14 wks (P) 1 year - Delayed union
2 (freeze-dried)	2 wks (P) 2 wks (D)	4 wks (P) 4 wks (D)	4 wks (P) 4 wks (D)	6 wks	4 wks (P) 2 wks (D)	12 wks (P) 20 wks (D)	20 wks (P) 20 wks (D)
734 (freeze-dried)	2 wks (P) 4 wks (D)	2 wks (P) 4 wks (D)	4 wks (P) 4 wks (D)	10 wks	6 wks (P) 6 wks (D)	10 wks (P) 12 wks (D)	14 wks (P) 16 wks (D)

[a] P = Proximal interface.
[b] D = Distal interface.

Table 5. Radiographic appearance of fresh cortical autografts (control dogs) at time of harvest.

Dog #	Period of observation (days)	Location	Presence of cortical union	Amount and extent of endosteal trabecular ingrowth into graft	Cranial cortex	Caudal cortex
914	120	P[a] D[c]	CU[b] NU[d]	++ mid-graft +	Radiopaque except at proximal interface	Radiopaque except at proximal interface
23	240	P D	CU CU	++ mid-graft ++ distal 1/4	Radiopaque at mid-graft region	Radiolucent throughout
692	365	P D	CU CU	+++ mid-graft ++ mid-graft	Slight radiopacity at mid-graft region	Radiolucent throughout

[a] P=Proximal interface.

[b] CU=Cortical union.

[c] D=Distal interface.

[d] NU=Nonunion.

+++ = Pronounced.

++ = Moderate.

++ = Slight.

+ = Negligible.

Table 6. Radiographic appearance of fresh cortical allografts at time of harvest.

Dog #	Observation period (days)	Location	Presence of cortical union	Amount and extent of endosteal trabecular ingrowth into graft	Cranial cortex	Caudal cortex
4	120	P[a]	CU[b]	++ proximal 1/4	Radiopaque	Radiopaque
		D[c]	CU	++ distal 1/4		Radiopaque
698	240	P	CU	+++ mid-graft	Radiopaque except near each interface	Radiolucent throughout
		D	CU	+++ distal 1/4		
568	240	P	CU	+++ mid-graft	Radiolucent throughout	Radiolucent throughout
		D	CU	+++ mid-graft		
3	240	P	CU	++++ proximal 1/4	Radiopaque except near each interface	Radiopaque at mid-graft region
		D	CU	++++ distal 1/4		
1	365	P	CU	++++ proximal 1/4	Radiopaque except near distal interface	Radiolucent throughout
		D	CU	++++ distal 1/4		
725	365	P	CU	+++ proximal 1/4	Almost entirely radiopaque	Almost entirely radiopaque
		D	DU[d]	++++ distal 1/8		

[a] P=Proximal interface.

[b] CU=Cortical union.

[c] D=Distal interface.

[d] DU=Dalayed union.

++++=Pronounced.

+++=Moderate.

++=Slight.

+=Negligible.

Table 7. Radiographic appearance of freeze-dried cortical allografts at the time of harvest.

Dog #	Observation period (days)	Location	Presence of cortical union	Amount and extent of endosteal trabecular ingrowth into graft	Cranial cortex	Caudal cortex
657	120	P[a]	CU[b]	+++ proximal 1/4	Radiolucent throughout	Radiopaque except at proximal interface
		D[c]	DU[d]	++ distal 1/4		
671	240	P	CU	+++ mid-graft region	Radiolucent throughout	Radiolucent except near distal interface
		D	F[e]			
670	240	P	CU	+++ mid-graft	Radiolucent throughout	Radiolucent except near distal interface
		D	DU	+++ mid-graft		
731	240	P	Infection -- Graft Resorption			
		D				
2	365	P	CU	+++ mid-graft	Radiopaque at mid-graft region	Radiolucent throughout
		D	CU	+++ mid-graft		
734	365	P	CU	+++ mid-graft	Radiolucent throughout	Radiolucent throughout
		D	CU	+++ mid-graft		

[a] P=Proximal interface.
[b] CU=Cortical union.
[c] D=Distal interface.
[d] DU=Delayed union.
[e] F=Fracture at distal site.

++++=Pronounced.
+++=Moderate.
++=Slight.
+=Negligible.

Table 8. Histologic appearance of fresh cortical autografts (control dogs) at time of harvest.

Dog #	Observation period (days)	Location	Type of union at interface	Amount and extent of endosteal trabecular ingrowth into graft
914	120	P[a]	CU[b]	++ mid-graft
		D[c]	NU[d]	+
23	240	P	CU	++ mid-graft
		D	CU	+ distal 1/4
692	365	P	CU	++ mid-graft
		D	CU	++ distal 1/4

[a] P = Proximal interface.
[b] CU = Cortical union.
[c] D = Distal interface.
[d] NU = Nonunion.

++++ = Pronounced.
+++ = Moderate.
++ = Slight.
+ = Negligible.

Resorption cavity formation within graft:		Estimated amount of new bone formation within resorption cavities		Estimated amount of necrotic bone within graft	
Cranial cortex	Caudal cortex	Cranial cortex	Caudal cortex	Cranial cortex	Caudal cortex
+++	++	++	++	++++	++++
+	++	++	+	++++	++++
++	++	+++	++++	++	+
++	+++	++	++++	+++	+
++	++	++	+++	++	++
++	++	++	++++	+++	++

Table 9. Histologic appearance of fresh cortical allografts at time of harvest.

Dog #	Observation period (days)	Location	Type of union at interface	Amount and extent of endosteal trabecular ingrowth into graft
4	120	P[a]	CU[b]	++ proximal 1/4
		D[c]	CU	++++ distal 1/4
698	240	P	CU	+++ proximal 1/4
		D	CU	++ distal 1/4
568	240	P	CU	++ mid-graft
		D	CU	++ mid-graft
3	240	P	CU	+++ proximal 1/4
		D	CU	++ distal 1/4
1	365	P	CU	++ proximal 1/4
		D	CU	++ distal 1/4
725	365	P	CU	++ proximal 1/4
		D	NU[d]	++ distal 1/4

[a] P = Proximal interface.
[b] CU = Cortical union
[c] D = Distal interface.
[d] NU = Nonunion.

++++ = Pronounced.
+++ = Moderate.
++ = Slight.
+ = Miniscule.

Resorption cavity formation within graft:		Estimated amount of new bone formation within resorption cavities		Estimated amount of necrotic bone within graft	
Cranial cortex	Caudal cortex	Cranial cortex	Caudal cortex	Cranial cortex	Caudal cortex
+	++	+	+	++++	++++
++	+	+	+	++++	++++
++	+++	+	+++	++++	++
++	++++	++	+++	++++	++
+++	+++	+	+++	++++	+
+++	++++	++	++++	+++	+
+	++	+	++	++++	+++
++	++	++	++	++++	++++
+	++	+	++	++++	+++
++	++	++	++	++++	++++
++	++	++	+	++++	++++
+	+	+	+	++++	++++

Table 10. Histologic appearance of freeze-dried cortical allografts at time of harvest.

Dog #	Observation period (days)	Location	Type of union at interface	Amount and extent of endosteal trabecular ingrowth into graft
657	120	P[a]	CU[b]	+++ mid-graft
		D[c]	DU[d]	++ distal 1/4
671	240	P	CU	++ mid-graft
		D	F[e]	+
670	240	P	CU	+++ proximal 1/4
		D	DU	+++ distal 1/4
731	240	Infection -- Graft Resorption		
2	365	P	CU	++ proximal 1/4
		D	CU	++ distal 1/4
734	365	P	CU	+ mid-graft
		D	CU	+ distal 1/4

[a] P = Proximal interface.
[b] CU = Cortical union.
[c] D = Distal interface.
[d] DU = Delayed union.
[e] F = Fracture through interface.

++++ = Pronounced.
+++ = Moderate.
++ = Slight.
+ = Miniscule.

Resorption cavity formation within graft:		Estimated amount of new bone formation within resorption cavities		Estimated amount of necrotic bone within graft	
cranial Cortex	Caudal cortex	Cranial cortex	Caudal cortex	Cranial cortex	Caudal cortex
+++	++++	+++	+++	++	++
+	++	+	++	++++	+++
+++	+++	+	+	++++	++++
+++	+++	+	+	++++	++++
++++	++++	++	+	++++	+++
++++	+++	+	+	++++	++++
++	++++	++	+++	++++	+
+++	+++	++	++	+++	+++
+++	+++	++	++++	+++	+
+++	+++	++	+++	+++	++

Table 11. Untoward occurrences that influenced the results.

Dog #	Graft type	Problems encountered	End result
914	Fresh autogenous	Loosening of cortical screws in distal host bone segment	Nonunion distal interface
23	Fresh autogenous	Loosening of distal screw in proximal host bone segment	Cortical union at each interface
3	Fresh cortical allograft	Longitudinal fracture of graft; loosening of distal screw within graft	Cortical union at each interface
1	Fresh cortical allograft	Loosening of first screw and fracture of second screw in proximal host bone segment	Cortical union at each interface
725	Fresh cortical allograft	Disparity in size of graft and host bone at distal interface	Delayed union at distal interface
657	Freeze-dried cortical allograft	Longitudinal fracture of graft	Delayed union distal interface
671	Freeze-dried cortical allograft	Loosening of distal screw in graft	Fracture at distal interface
731	Freeze-dried cortical allograft	Bacterial contamination	Graft resorption
2	Freeze-dried cortical allograft	Longitudinal fracture of graft	Cortical union at each interface