AN EXPERIMENTAL STUDY OF SURGICAL BONE GRAFTING IN THE CANINE UTILIZING AUTOCLAVED AUTOGENOUS NORMAL BONE

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ABSTRACT

AN EXPERIMENTAL STUDY OF SURGICAL BONE GRAFTING IN THE CANINE UTILIZING AUTOCLAVED AUTOGENOUS NORMAL BONE

by Bryan Roger Coupland

The purpose of this experiment was to study the efficacy of surgical bone grafting using autoclaved autogenous normal canine bone. Segments of normal bone were removed from areas of the extremities where osteogenic tumors are commonly found. These segments were then autoclaved at sterilization temperatures and firmly replaced. Results were observed by monthly radiographs. At a uniform end-point the dogs were sacrificed, and the graft areas examined histologically.

The tibia and ulna were chosen as sample bones. Since the latter bears little of the actual support of the foreleg, both front legs were operated on. One ulnar graft included the periosteum, while the other ulnar graft was removed leaving the periosteum intact in the host bed. With the tibial grafts, an attempt was made to maintain the continuity of the entire perosteum. The ulnar grafts were stabilized by means of stainless steel wires at each end. The tibial grafts were stabilized with a combination of intramedullary pins, stainless steel wires and Thomas splints.

Radiographs were taken at monthly intervals. After a period of 9 months, the dogs were sacrificed and the grafts removed to include one-half inch of host bed bone. Histologic slides were made and observed for replacement by new bone.

With the tibial grafts, union occurred in all cases between 7 and 9 months, including one case in which osteomyelitis developed. Histologically, the union of the tibial grafts was confirmed by the presence of many active capillaries and immature osteocytes throughout the graft. There was no sign of an inflammatory reaction microscopically in the graft that appeared to have osteomyelitis on x ray. Radiographically, the ulnar grafts appeared to have been almost universally reabsorbed to some extent, except for 3 that had been incorporated with the host bone at one end. However, on microscopic examination 5 of the grafts had considerable osteoid formation which seemed to fill the defect between the host bone and the graft. The center of each graft consisted of many bone lamellae, with no sign of live osteocytes.

A viable periosteum surrounding the bone graft did not appear to play a significant role in bone regeneration, since at least 2 of the grafts that showed evidence of new bone growth had no viable periosteum following surgery.

Three of the ulnar grafts contained an area of localized inflammation, somewhere within the graft bed. Microscopically, and under polarized light, a piece of nylon suture material was seen in the middle of one of these areas of inflammation. Two of the three ulnar grafts that were without new bone formation showed evidence of an inflammatory reaction.

It was concluded that autoclaving is a satisfactory means of sterilizing an autogenous graft of normal canine bone. Replacement of the autoclaved bone was seen histologically by sheets of immature osteocytes and neo-capillaries that formed a perimeter around a core of devitalized bone. Results indicated that although 9 months was an adequate period

for the tibial grafts to become stabilized, it was not sufficient time for the ulnar grafts.

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By

Bryan Roger Coupland

A THESIS

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Dedicated to

Delphine

"If any of you lack wisdom, let him ask of God, that giveth to all men liberally, and upbraideth not; and it shall be given him."

James 1:5

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INTRODUCTION

The idea for this project arose from a problem that develops in selected cases of bone cancer in humans, especially of the mandible. In many instances the mandible is unilaterally removed and a prosthesis is withheld for a period of 6 months to a year, pending examination of the soft tissues adjacent to the bone for metastasis. By the time the bone bed is considered to be free of neoplasia there is often an obvious healing deformity. Not only is it difficult at this time to fit a prosthesis which approximates the individual's original appearance, but there are often considerable psychologic manifestations.

Therefore, it was decided to explore the possibility of maintaining the continuity of the bone involved. This might be accomplished by removing the diseased segment of bone, devitalizing it and replacing it while the patient was still anesthetized. In order to accomplish this, devitalization of all cells in the segment of bone must be a rapid process. Autoclave sterilization is one of the few means of meeting this criterion.

Before using diseased bone, a logical sequence is to first learn how the body will respond to normal, healthy bone that is autoclaved and replaced in situ. The question of whether union occurs between the treated bone and the host bone can then be answered.

The idea was conceived of surgically approaching the long bones of clinically normal dogs. A 2-inch segment of bone would be aseptically removed, autoclaved, and firmly fixed back in place. Radiographs would

be used to determine if the body replaced the graft with normal bone. If cases of bone tumors ammenable to total excision were presented to the MSU Small Animal Clinic, this technique would be used, and the results compared with those using normal bone.

If such a technique were successful, it would fulfill several important functions:

1. Avoid amputation if an extremity is involved.
2. Maintain structural function of the limb or area.
3. Treat the pathologic condition on initial surgery, by destroying the causative agent.
4. Prevent the psychologic manifestations, resulting from complete ablation of the area.

The idea is not completely original, since such a procedure has been successfully used several times in the past. One wonders why more bone tumors are not treated in this manner when the outcome of these few is so promising. The explanation lies in 2 areas. First, with the quantity of literature that is avilable today, there is little space allotted for techniques that fail, or for patients that do not live. Secondly, as cases of bone tumors of the extremities are examined for the application of this technique, one can see that very few are ammenable to total excision. Many bone tumors arise from the ossification centers, and the latter are generally in the epiphyseal area. Therefore, the portion involved often spreads to the articular cartilage of the adjacent joint by the time symptoms are noticed. It is wise to consider the following criteria before adopting this surgical technique:

1. Histologic type of tumor
2. Tumor size

3. Soft tissue and lymph node involvement
4. Radiographic or other evidence of metastasis

In order to study the value of autoclaved autogenous normal bone as a grafting material in the canine, several questions must be answered:

1. Will autoclaved bone provide the necessary function, when replaced, that it did previously?
2. Will autoclaving the bone segment so change its size and shape as to make union difficult with the host bone?
3. How will the body react to the bone once it has been autoclaved and all viable cells are killed? Will the body replace this graft with normal osseous tissue, or will it be rejected as an inert foreign body?
4. Is autoclaved bone more susceptible to infection than normal bone, and will its use result in sequestration or impeded union?
5. How long a period is required before it can be assumed that sufficient mineralization has occurred to allow normal function of the operated limb?
6. After compilation and examination of data, what ramifications will this procedure have? Does it appear to warrant further experimentation with whole or partial joints, cartilage or osteomyelitic bone?

LITERATURE REVIEW

A. REVASCULARIZATION AND RECALCIFICATION OF THE BONE GRAFT

Controversy regarding the response by the body to bone grafts has existed since the mid-1700s. The main argument at that time was regarding the role of the periosteum in osteogenesis. Some experimenters thought that the periosteum served only to transport blood vessels to the bone diaphysis. Others maintained that the periosteum was the chief force in new bone formation.

The end of the 19th century saw this problem still unresolved, but experimentation had produced another division in thinking. One group stated that, especially in fresh autogenous grafts, osteogenic properties persisted in the bone graft. The other group thought that all elements of the bone graft were reabsorbed by the body following implantation and that new bone was substituted from the host tissue.

During the early 1900s results of bone grafting were variable and inconclusive. Experimenters continued to produce opposing results. It was almost the middle of the 20th century before the mechanism of bone graft replacement was agreed upon.

Abbott (1947) stated that the only tissues that remained viable were the endosteal layer and the cambium layer of the periosteum. Horwitz (1949) also found that most of the graft died except for the endosteal layer and a few elements of the cambium layer of the periosteum. This was somewhat refuted by Hutchinson (1952) from results of bone transplanted into muscle, bone and the anterior chamber of the eye. He deduced that

although empty lacunae of the autograft inferred that the graft was dead, it recovered from the initial period of devitalization, to survive as active healthy bone. Homografts, he stated, if placed in a host bone bed, were replaced entirely by host bone. Here the distinction appeared to be the graft type.

Maatz et al. (1954) stated that they had positive proof that some of the soft tissue elements of the autograft survived and that this graft had the capacity to regenerate. They summarized by saying that autografts relied on their own regenerative powers and on those of the host, while the homograft and the heterograft were replaced solely by the host. Chase and Herndon (1955), who are today leaders in this field, stated that following massive transplants of bone the periosteum usually survived and retained its osteogenic ability, while most of the bone and marrow died. However, they still favored the beliefs of the opposite group, namely that the majority of recalcification occurs from the host bone.

Burwell (1964) did an ingenious experiment, whereby he washed the marrow from a homogeneous bone graft and replaced it with the recipient's marrow, producing a fresh "composite homograft-autograft". From this work he deduced that new bone was derived mainly from the contained marrow of the graft.

Burwell (1966) advanced the theory of the "induction" system and the "reacting" system. He said one should use treated bone of high inductive potential (i.e., frozen or freeze-dried), and a connective tissue host bed of high osteogenic potential or reaction (i.e., cancellous bone or red marrow).

Many experimenters believed that a graft died in its entirety and that it was replaced by elements from the host bed. One of these was

McWilliams (1914), who felt that the periosteum of the graft does not persist in the host and thus the viability of the graft soft tissue elements is not necessary.

Davis and Hunnicutt (1915) found that removal of soft tissue elements from a graft had little if any effect and that the periosteum seemed only to protect against early reabsorption.

Willich (1926) maintained that osteogenesis was due to the presence of the periosteum and endosteum of the host.

Albee (1944) was even more specific, by claiming that 75% of revascularization of the graft was by the marrow and endosteum, and 25% by the periosteum.

Reynolds and Oliver (1950) found no evidence that any of the bone elements of an autogenous bone graft lived or retained osteogenic powers, but rather that healing was "appositional bone-growth" from the host bone.

Campbell et al. (1953) stated that all elements of the transplanted bone died and were replaced by the host. This group was only able to get osteogenesis with fresh autogenous grafts, and then mainly on the endosteal surface of the graft. With onlay bone grafts, they noticed "creeping substitution" from the host to the onlay-graft. "Replacement" was more obvious in autogenous grafts, whereas "absorption" prevailed in preserved grafts.

As time went along, experimenters began to realize that there was some truth in both arguments. Not only did vascularization appear to occur from the host bed tissue, but also certain elements of the graft were seen to retain some osteogenic activity. Cohen and Lacroix (1955) stated that the success of bone grafting depended on: (1) the mechanical

support the graft provided, (2) the amount of "repair tissue" elaborated by the host, and (3) the osteogenic potency of the graft. In summary, they implied that the bone graft acted as a triggering device for bone formation from the host and that it also provided a framework to sustain the newly formed bone. They further proved that it was not just a question of having an adequate blood supply, since the grafts placed under the capsule of the kidney did not produce as much osteogenesis as in the tibial bone bed.

Although Siffert (1955) stated that grafts were mainly passive scaffolds along which new bone grew, he concluded that there were some bone transplants that survived, provided they were revascularized early. Therefore, he concluded that it was the cells nearest the host bed of bone that survived, since they were revascularized first. The simple vascular pattern of cancellous bone (i.e., the ilium) allowed it to be more readily revascularized than the complex Haversian system of dense cortical bone.

Axhausen (1956) stated that the bone graft, rather than becoming revitalized, became necrotic. It was the presence of necrotic bone, he theorized, that stimulated the pleuri-potential cells of connective tissue in the host bed to form osteogenic cells.

Cohen et al. (1957) reported that by radioactive tagging of calcium they could prove that the calcium found in the callus surrounding a graft came from the graft itself.

Probably the most theoretical work done that combined the best of both previous groups was by Ray and Sabet (1963). In an attempt to determine the source of osteogenesis in new bone grafts, they devised 2 separate experiments in which labeled thymidine was used to label the

graft bone cells and the host cells. They concluded that the living homograft and autograft served as a direct source of bone forming cells, as well as acting as an inducing agent to cause the connective tissue cells of the host bed to differentiate into osteogenic cells. Therefore, the authors proposed that cells from both graft and host contributed to new bone formation, although during the first 2 weeks new bone was formed primarily from the graft.

B. THE PERIOSTEUM AND BONE GRAFTING

One of the secondary aspects of this project has been the influence that periosteum has on bone grafting. This history would be severely lacking without mentioning the previous work done on the osteogenic ability of the periosteum and the present theories on its value.

A significant piece of work was done by Haldeman (1933), who used a variety of osteo-periosteal grafts, on defects produced in the radius of rabbits. He concluded that (1) the periosteum was the most important part of the graft, and (2) comparison of cortical grafts with and without periosteum showed clearly that the presence of periosteum favored early closure of the defect and survival of the graft.

Very different results were obtained by Pollock and Henderson (1940). They placed grafts in opposite legs, and retained the periosteum with the graft on only one leg. In those cases where the periosteum had been removed a fibrous periosteum developed from the surrounding connective tissue. They concluded that there was no advantage gained by retaining the periosteum on the graft and that, in fact, removal of periosteum possibily resulted in formation of a more active periosteum from the surrounding connective tissue. Flanagan and Burem (1947) stated,

> "By separation of the periosteum only at the site of the bone graft to be removed, with the periosteum and muscle attachments left undisturbed on sound portions of the shaft, an adequate blood-supply is maintained for the successful revascularization of grafts."

Flinn (1951), in ununited fractures of the tibia, cut a ribbon-shaped piece of periosteum, leaving it still attached in the center to a chip of bone and at one end to healthy periosteum. Once the fracture was reduced, this graft spanned the fracture and was found to aid appreciably in bone repair. Dingman (1952), in experiments with periosteum, found no regeneration of bone in 12 cases of extraperiosteal resection of the ulna (i.e., removal of the bone plus periosteum). In 12 cases of subperiosteal excision of the same bone, 9 showed definite evidence of bone regeneration. Campbell et al. (1953) maintained that all components of the bone graft died, and were replaced by bone from the host bone. They also stated that minimal osteogenesis occurred from the host periosteum with the majority being formed from the endosteum.

Several years later Debruyn and Kabisch (1955) did a comprehensive study in comparing fresh and frozen autogenous and homogenous transplants of bone and bone-grafting material. Fresh, autogenous transplants of compact bone resulted in 86% regeneration. Fresh autogenous periosteum was successfully grafted in 58% of the cases. The same year, Cohen and Lacroix (1955) reported on periosteal grafts (1) on the denuded surface of the tibia, (2) in the anterior chamber of the eye, and (3) on the subcapsular surface of the kidney. Examination of the results showed that the tibia produced the most osteogenesis, the kidney produced moderate osteogenesis, and the eye almost no reaction at all. They reported that the main influence was chemical rather than vascular or mechanical.

Axhausen (1956), who felt necrotic bone was the stimulus for bone regeneration, claimed that autogenous bone with the periosteum intact was undebatably the best for bridging bone defects. He said,

> "When the periosteum (in which active osteogenesis originates) is separated from its underlying bone, a loss of intensity of the first osteogenic phase occurs."

Khoury et al. (1963) found in dogs and monkeys that by stripping the femoral periosteum to the epiphyseal plates, they were able to get an increase in longitudinal growth in 63% of the animals. They maintained that this was due to interrupting the blood supply to the diaphysis, thereby increasing it to the epiphysis. This procedure resulted in an increased incidence of shaft fractures. This same reaction was reported 2 years later by Yabsley and Harris (1965), who said that the changes were initiated mainly by damage to the nutrient artery.

Richany et al. (1965) corroborated Campbell's findings that endosteal callus formation, in the presence of bone grafts, preceded and exceeded periosteal callus formation. They emphasized the importance of bone marrow and said that when it was absent the periosteum provided the main source of bone.

Gage et al. (1966) reported on in situ freezing of bone. In successful cases where the bone was devitalized, repair began from the periosteum and medullary cavity of the host at the border of live and dead bone. Ross (1966) concluded that the filling of cortical bone defects was a function of the host bone, and that most of the callus was endosteal in origin, although the periosteum produced a small amount of new bone.

C. BOILED AND AUTOCLAVED BONE IN BONE GRAFTING.

Stewart (1934) removed a segment of radius and boiled it for 10 minutes. He was unable to find any production of new bone, within or about the graft.

Orell (1937) wrote a paper which even today is considered to be an authoritative comparison of boiled bone, with (1) os purum (i.e., bone with everything removed except the calcium framework) and (2) os novum or immature living bone with great proliferative power. The latter is produced by placing a piece of os purum subperiosteally on the surface of the tibia for 2 months. The os novum endproduct is soft, very vascular bone. (Boiled bone differs from os purum in that the former still contains fat, connective tissue, and proteins.) This same author found that, whereas an autogenous bone graft stimulated new bone formation within 14 days, boiled bone grafts took a minimum of 3 months. The main use of boiled bone as grafting material, he maintained, was when the bone was pathologic (i.e., chronic osteomyelitis or neoplasia). Orell claimed that the periosteum should not be separated from the soft tissue overlying the bone, but that the best way to expose a bone was to loosen the periosteum from it.

Reynolds (1950), contrary to previous findings, claimed that at the end of 10 weeks he could find no microscopic difference between autogenous and homogenous bone grafts. He stated that boiled bone did proceed to union much slower than homogenous bone preserved by most other means. In his work he was careful to peel the periosteum back before removing the bone.

In 2 separate studies, Kiehn et al. (1950) and Reynolds and Oliver (1950) concluded that boiled bone grafts proceeded to union much more slowly than most types of preserved homografts and autografts.

Lloyd-Roberts (1952) strongly favored the use of boiled cadaveric bone, and stated that failures by others were due to deviation from the cardinal grafting rules:

1. Have the host bone in contact with the graft as vascular as possible.
2. Avoid any bending stresses.
3. Obtain firm apposition of the graft to its bed.

He was able to get successful "takes" by 3 months and found no rejection of the graft by the host.

Campbell et al. (1953) described a foreign-body reaction of dense fibrous tissue around preserved homografts. Maatz et al. (1954) described their findings concerning the reaction of host bone to boiled bone grafts from which all organic substance had been removed: (1) the remaining inorganic substance was replaced by new bone, (2) there was no trace of inflammatory reaction (contrary to the findings of Campbell and others), and (3) the mineral elements of the graft incited the formation of callus, often furnishing the minerals and thereby surpassing the function of mere scaffolding. Contrary to the views of Lloyd-Roberts, Maatz et al. stated that boiling is the least desirable method of preserving bone. Their process of choice was deep freezing, since it preserved the biological value.

Baird et al. (1958) compared autoclaved bone with bone sterilized in beta-propiolactone, but got such a variety of results that they were able to make only one conclusion, that autografts were superior to homografts.

Devries et al. (1958) did considerable work on the preservation of bone grafts. They noticed a high incidence of postoperative infection and generally poor results with frozen or autoclaved bone, especially since the former is an excellent method of preserving many bacteria and viruses. The authors claimed autoclaving denatures the osseous protein, thereby changing the basic physical properties of the bone and retarding revascularization. They summarized by saying,

> "Boiled bone, though capable of carrying out the function of fresh or frozen bone, does so more slowly than autogenous bone."

Urist and McLean (1963) reported at length on the immune response towards transplants generally, and the rejection of homo- and heterografts of bone, specifically. They found that prolonged storage, chemical coagulation, and autoclaving reduced the antigenicity of homogenous and heterogenous bone, allowing it to act as a passive scaffold into which new bone grew from the host bed. Brooks et al. (1963) showed that previously implanted homogenous bone grafts sensitized an animal to reject a subsequent skin graft from the same donor at an accelerated rate. Compared to a fresh homograft, which produced a definite acceleration, autoclaved bone produced an "indeterminate effect". Heterogenous bone has definitely been shown to be antigenic and to produce circulating antibodies by Burwell and Gowland (1961). There is some question whether homogenous bone produces circulating antibodies, although its antigenicity has been adequately demonstrated by Nisbet et al. (1960).

Heiple et al. (1963), in a study comparing the healing process of bone grafts, used a variety of grafts and preservation techniques. They obtained generally poor results by deproteinizing bone in ethylene diamine and then autoclaving the graft.

Williams (1964) lauded the use of boiled cadaveric bone for the repair of fractures. Although autogenous bone undeniably heals the quickest and produces the least reaction, he cited several disadvantages: (1) it involves a second incision, (2) the quantity of bone you can take is limited, and (3) there is a risk of infection, fracture, or deformity, depending on the site of the donor tissue. Since the author felt osteocyte survival is not important to the success of the graft, by boiling a prospective bone graft he eliminated any chance for a foreign-body reaction.

Burwell (1966), who worked extensively on the immune response of bone transplantation, reported that he impregnated various types of treated bone grafts with the marrow from the recipient, giving composite "homograft-autografts". He then embedded them in the vertebral muscles of the canine. Since boiled bone without marrow never produced new bone, and some of the composite grafts containing recipient's marrow did produce new bone, the author concluded that the osteogenic activity must reside in the marrow. In addition, he noticed that boiled bone formed less new bone than other types of treated bone, but produced more new bone than marrow transplanted alone.

D. EXPERIMENTAL TECHNIQUE.

Parrish (1966) stated that where a graft replaces a section of bone it should be fixed with a "step-cut" at both ends, have screws to stabilize it, and finally an intramedullary pin if at all possible.

Rhinelander and Brahms (1961) emphasized the value of wire for stabilizing bone grafts, since it does not cause erosion, and the bone callus can grow over or around wire loops. However, he cautioned that the following points be remembered:

1. A sufficient number of wires should be used.
2. Wires should be properly placed.
3. Wires must be sufficiently strong (i.e., 18 gauge for the femur and tibia, and 20 gauge for the humerus, radius, ulna and fibula).
4. Wire should not be over-strained on tightening.
5. Small ancillary pins should support the wire.
6. Periosteum should not be stripped away from the bone.

There are many ways of destroying all living cells in a segment of bone. It is important to have a technique that may be completed while the dog is maintained under anesthesia. A comparison of steam under pressure with other sterilization procedures shows the former to be the method of choice.

To better exemplify the value of autoclaving in this procedure a few basic rules of sterilization should be outlined. According to Sykes (1958) the minimum recommended temperature for sterilization with steam is 115 C, under pressure for 30 minutes. Another way of stating this is that no living cell can survive 10 minutes' exposure to saturated steam at 121 C.

The choice was made in favor of steam under pressure over dry air heating, since according to Perkins (1956) the former possesses a singular quality of being able to heat materials and permeate porous substances (i.e., bone) by rapid condensation, as opposed to the slower method of heat absorption in the case of hot air. The period of exposure is measured from the time the pressure gauge reaches 250 F (121 C) and should be no less than 12 minutes. It is generally reported that it is not advantageous to raise the temperature above 121 C. If in doubt as to sterilization

efficiency, Perkins concluded one should lengthen the time, since:

1. Above this temperature, many substances undergo deterioration.
2. The "blow-off" valve on the autoclave is usually set for 20 lb./square inch.

McCulloch (1945) also compared steam sterilization to dry heat. He found that heat alone up to 140 C for 3 hours gave a comparable effectiveness of 100 C for 5 minutes with steam sterilization. This, he said, was certainly below the minimum requirements, as was "intermittent heating" on successive days. Finally, sterilization in boiling water and free-flowing steam required many hours to be effective. Even then it could not produce the results of steam under pressure, since the powers of penetration were significantly reduced.

E. AUTOCLAVED BONE GRAFT CASE HISTORIES.

The following are cases found in the literature in which autoclaved bone was successfully used as a grafting material.

Parrish (1966) stated that,

> "Surgical therapy continues to be the most important tool in the management of bone tumors, but this method must be refined. Use of a surgical attack that is too timid has permitted the recurrence rate for all bone neoplasms to be too high."

This author found that a wide resection is the only way to get beyond the confines of the bone tumor into normal bone. Also, he stated that one must remember the medullary spread of certain tumors (i.e., central chondroma). In tumors that extend to the articulation replacement of the joint with a homologous duplicate can be accomplished. From the work of Chase and Herndon (1959), it was made clear that half-joint replacement was more successful than whole-joint, and it was preferable to have a joint graft too small rather than too large. Brodey (1965),

who amputated the limb at the first visible sign of canine osteosarcoma, still had a failure rate of 80%.

In human beings, McKenna et al. (1966) found an interesting paradox, in that with cases of osteogenic sarcomas there was a greater percentage of success with a longer time interval between diagnosis and amputation. In the slower growing tumors (i.e., chondrosarcomas, fibrosarcomas) it was just the reverse.

There are 2 recorded cases of traumatic bone loss that were amazingly identical in history, treatment, and outcome. The first was reported by Kirkup (1965), who had a patient involved in a motorcycle accident. A 9-inch section of the left femur had been driven distally out through the patella and snapped off. Police dogs located the extruded bone 24 hours later, and the author found neither ligamentous attachments nor periosteum on the bone, for the latter had remained in the limb as an intact envelope. The bone segment was boiled and cultured for bacteria. When it was found to be sterile, it was autoclaved and placed into its original position within the periosteal tube. The author drilled holes through the devitalized bone to facilitate revascularization. The treated bone was fixed in place with an intramedullary pin and wire loops where the autoclaved bone contacted the normal femoral condyles. Radiographs indicated callus formation within 23 days, and the individual returned to work after one year.

A similar accident between a motorcycle and a truck was reported by Abell (1966). It too involved the left femur. In this case a 7-1/2-inch segment was extruded through the patellar area, leaving the periosteum virtually intact. The bone, found wedged in the truck bumper, was scraped, scrubbed with soap and water, autoclaved, and placed in a

sterile container of zephiran chloride. Nine days after the accident the bone was replaced and fixed in place with an intramedullary Kuntscher rod. The fractured patella was subsequently removed. Ten months after surgery, full weight bearing was possible on this leg. Abell claimed the success was due to the following factors:

1. It was basically a sterile wound.
2. The fragments were extruded with such force that the periosteum was cleanly stripped off and remained intact.
3. Good fixation was obtained of the extruded fragments.

Orell (1937) reported that he had used this method of sterilizing bone on 4 cases of chronic osteomyelitis and had obtained complete healing in from 6 months to a year. The author also resected, subperiosteally, a malignant osteogenic sarcoma by removing the proximal two-thirds of the humerus, including the shoulder articulation. Before being replaced, the bone was boiled for 15 to 20 minutes, scraped, and perforated to facilitate revascularization. The limb was cast. The patient died 5-1/2 months after surgery from metastasis to the lungs, but no recurrence was found in the humerus at post-mortem examination.

A case was reported by Thompson and Stegall (1956) involving a chondrosarcoma of the femoral head, neck, and intertrochanteric region. There was no joint involvement or periosteal reaction, despite one small area of cortical perforation. The proximal 16 cm. of the left femur was resected in one piece, along with the soft tissue attachments of tendons and muscles. The entire coxofemoral joint capsule and ligamentum teres were removed and the acetabulum was denuded to bleeding bone. The bone specimen was then autoclaved for 30 minutes. The femoral neck broke within a short period, so the treated bone was replaced with the greater

trochanter in the acetabulum, and multiple, fresh, autogenous bone chips were placed about the coxofemoral joint. The only follow-up surgery was 12 months later, when a tibial autograft was placed from the ilium across the hip joint in vascular bone. Full solid bony union occurred 2 years after the initial surgery. The authors stated that the autoclaved specimen was used because it was "structurally perfect for the situation".

Several cases in which neoplastic bone tissue was resected, autoclaved, and replaced were reported by Williams (1960) to the Australian Orthopedic Association. His first success involved an osteosarcoma of the femur of a woman. He removed the distal 1/6 of the femur, autoclaved it, and replaced it, fixing it by means of a long Kuntscher nail across the knee joint. The patient was able to walk one year after surgery, with only 3/4-inch shortening of the leg. The second case was a large osteoclastoma involving the proximal end of the tibia. He resected the neoplastic bone, replaced it with a similar autoclaved piece from a cadaver, and fixed it with a Kuntscher nail.

MATERIALS AND METHODS

A. INTRODUCTION

Eight male dogs of undetermined age were obtained for use in this project. They were housed in individual kennels at the Michigan State University Veterinary Clinic and exercised in an indoor run twice daily. The following table identifies each dog in this experiment.

Table 1. Animal identification key

Dog No.	Clinic Case No.	Weight at beginning of experiment (lb.)	Breed
1	106975	22	Mixed breed
2	107863	58	Mixed breed
3	107927	48	Springer Spaniel
4	107932	45	Mixed breed
5	108088	50	Boxer
6	108228	42	Mixed breed
7	108227	60	Mixed breed
8	108260	34	Cocker Spaniel

B. SURGICAL APPROACH TO THE ULNA

In dogs 1 through 4 (see Table 1), the following approach was undertaken on the ulna of the left leg.

Each dog was anesthetized with pentobarbital sodium* (1 gr./ml.) at the dosage of 1 ml./5 lb. body weight. The left foreleg was clipped, scrubbed with a liquid antiseptic solution,** and a tourniquet was applied above the elbow using flexible rubber tubing. Strict asepsis was observed throughout.

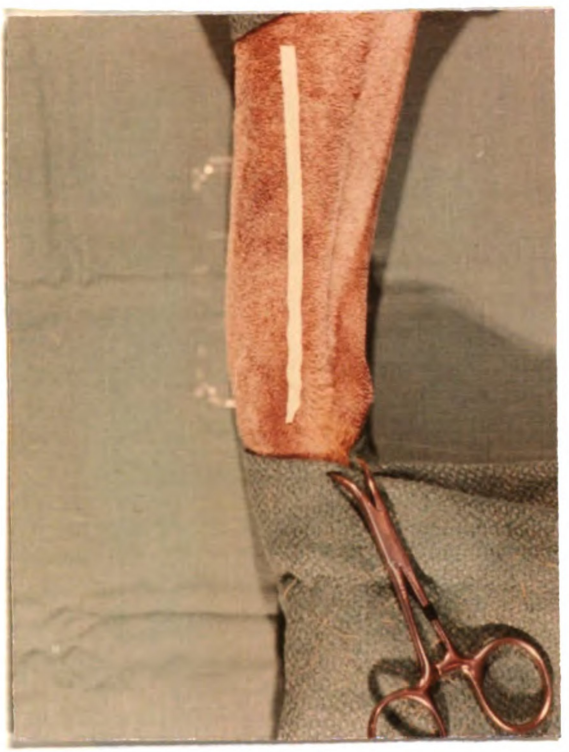

Fig. 1. Lateral approach to the ulna. (Tape indicates incision line.)

A 4-inch incision was made on the lateral surface of the mid third of the ulna parallel to its long axis (Fig. 1). The subcutis was separated by blunt dissection, exposing the extensor muscles (Fig. 2). The fascia between the extensor carpi ulnaris and the lateral digital extensor muscles was cut with scissors exposing the ulna in its entirety (Fig. 3).

In order to preserve the periosteum, the abductor pollicus longus muscle on the anterior surface of the ulna, and the deep digital flexor

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* Diabutal, Diamond Laboratories, Inc., Des Moines, Iowa.

** Germicidal Detergent, Parke, Davis and Co., Detroit, Mich.

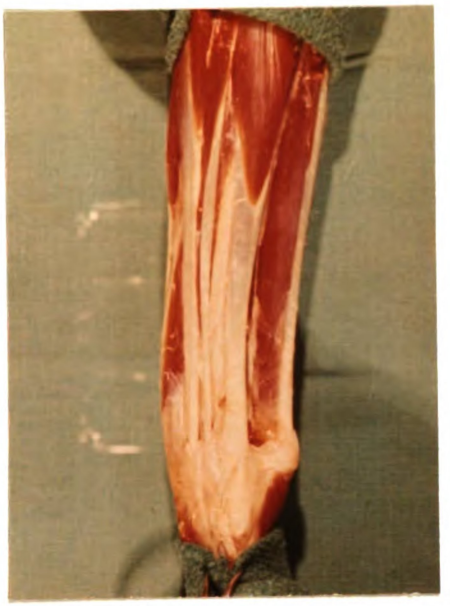

Fig. 2. The lateral surface of the left foreleg with skin and fascia removed.

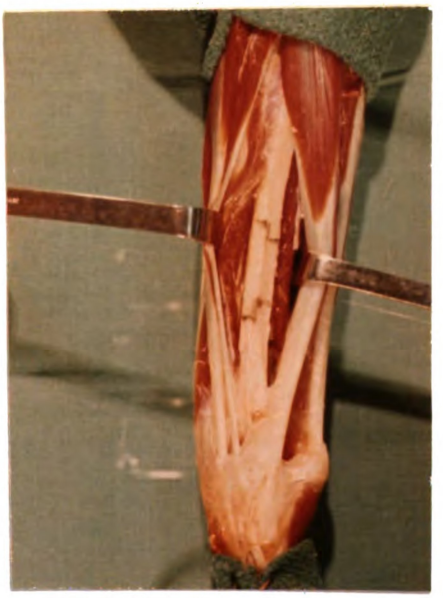

Fig. 3. Separation between extensor carpi ulnaris and lateral digital extensor muscles, exposing the ulna.

(ulnar head) on the posterior surface of the ulna, were separated from the bone by means of an osteotome to expose a midsection of the ulna about 4 inches in length.

A Hall air drill* was used at a pressure of 110 lb. of compressed air to remove the segment of bone plus periosteum. The bone was cut in step fashion at each end, to prevent rotation when fixed in place. This bone was then autoclaved for 15 minutes in a high pressure autoclave** reaching a peak temperature of 250 to 270 F (121 to 132 C) and maintaining a pressure of 29 to 31 lb. The bone was maintained in a sterile condition, replaced in its original position, and fixed at each end with a 4-0 stainless steel wire by means of a loop secured by a square knot around the step. The

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* Zimmer Co., Warsaw, Indiana.

** Ortho-Vac, Wilmont Castle Co., Rochester, N.Y.

muscles were then apposed and the fascia and skin sutured with simple interrupted sutures of type A nylon. There was no secondary immobilization utilized, other than a loose bandage over the incision. Sutures and bandages were removed permanently after 10 days.

The approach to the right ulna was exactly the same as that for the left, down to the separation between the extensor muscles. Thus, for the sake of brevity, the description will continue from this point.

With the mid-ulna exposed, a 4-inch longitudinal incision was made on the bone, just through the periosteum. Then, with an osteotome, the periosteum, with the overlying muscles attached, was peeled off the graft portion of the ulna leaving the former as an intact envelope.

The Hall drill was used to remove a 2-inch segment of ulna in step fashion similar in size to that of the left ulna. The bone segment was autoclaved for the same period of time and replaced into the periosteal sheath. Stainless steel wire (4-0 gauge) was again used to hold the bone segment in place. Due to a certain amount of drying during the operation, the cut edges of the periosteum retracted, making suturing of the edges an impossibility. Because the periosteum was in fact still adhered to the overlying muscles, it was felt that by relocating the latter in their original position, the periosteum would be in approximately the normal location. The fascia and skin were sutured by means of simple interrupted sutures of type A nylon. A temporary bandage was used to cover the incision until such time as the sutures were to be removed.

The time varied between operations on the 2 forelegs from a maximum of 3 months (Dog 1) to a minimum of 2 days (Dog 4), with the mode being one week (Dogs 2 and 3).

C. SURGICAL APPROACH TO THE TIBIA

To give the tibial grafts the greatest opportunity for successful revascularization, the periosteum was not autoclaved with the graft, but rather retained in the host bed. Since the tibia bears the majority of the support between the femoro-tibial and the tibial-tarsal joints it was suggested that the original technique of wiring be supplemented by a Thomas splint and an intramedullary pin.

Dogs 5 through 8 were used for this part of the experiment. They were anesthetized and the right hind legs prepared for surgery in a manner similar to the previous group. The only exception to the latter was the tourniquet, which was applied 2 inches proximal to the patella. A 5-inch incision (Fig. 4) was made on the medial surface of the distal half of the tibia, exposing the underlying musculature (Fig. 5).

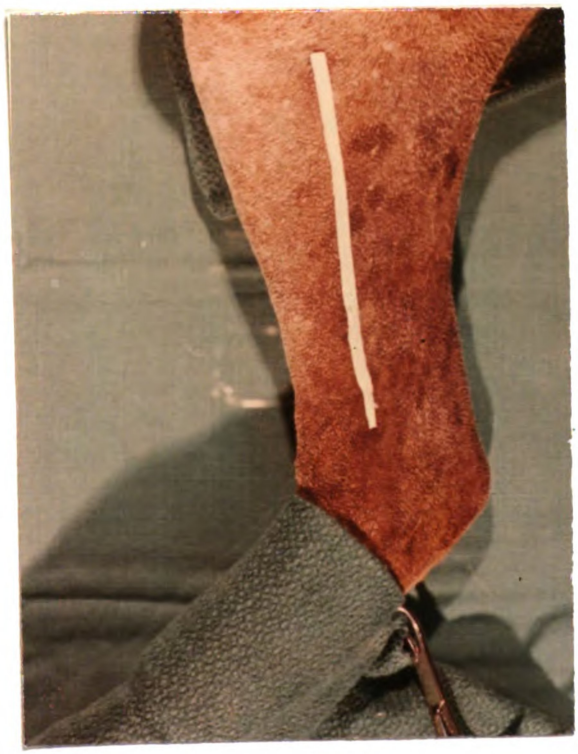

Fig. 4. Medial approach to the shaft of the tibia (right hind leg).

The tibia was found to be much more free of musculature (Fig. 6) and therefore dissection was continued between the tibialis anterior muscle (anteriorly) and the deep digital flexor, on the posterior surface of the tibia.

In a manner similar to that used for the ulna, a 4-inch longitudinal incision was made through the overlying periosteum. The latter was peeled away from the bone with an osteotome, without removing the 2 attached muscles.

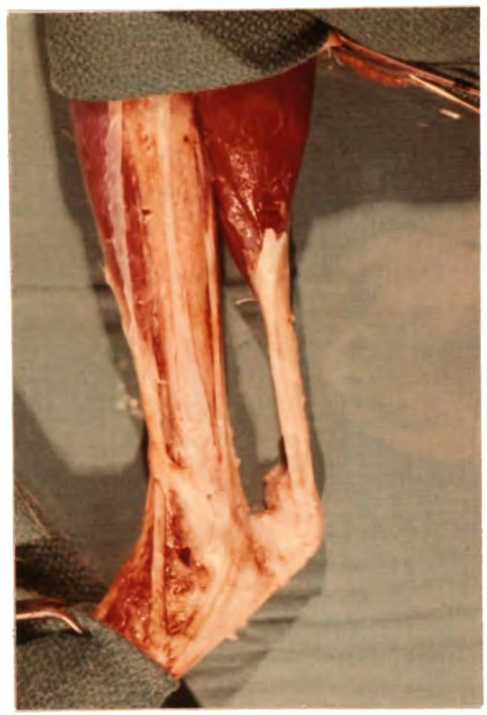

Fig. 5. Skin and superficial fascia removed to show the underlying musculature.

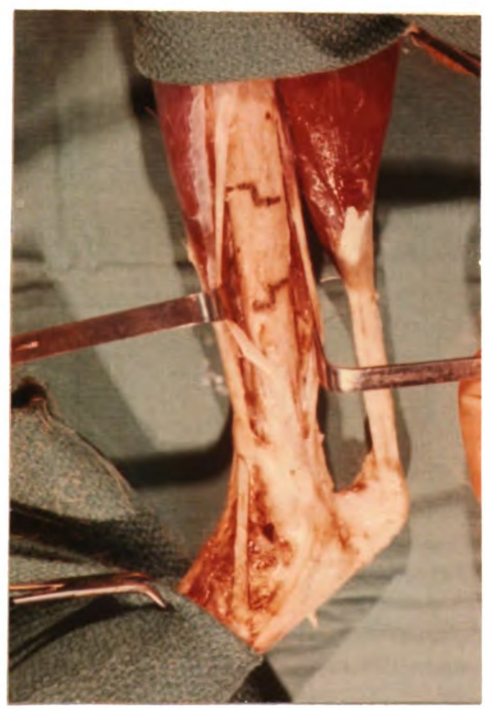

Fig. 6. Medial view of the right rear leg indicating the limited muscle covering over the tibia.

Using a Hall air drill, a 2-inch segment of tibia was removed in step fashion and autoclaved for 15 minutes at the same temperature and pressure as was used for the ulna.

Prior to replacing the devitalized bone segment into the bone bed a 4/32 stainless steel, trochar pointed Steinman pin was placed retrograde up the medullary cavity of the proximal tibial segment. The pin was placed so as to exit from the tibia just proximal to the crest of the tibial tuberosity. The distal end of the pin was positioned even with the cut edge of the proximal portion of the tibia. The bone was replaced and the pin was run distal through the segment and into the distal portion of the tibia until adequate resistance was felt. The proximal end of the pin was then cut off as close to the tibia as possible.

To minimize motion of the bone segment, 4-0 stainless steel wire was looped around both junctions between live and treated bone and secured with one square knot. Following this the muscles were allowed to fall back into place and the fascia and skin were sutured with simple interrupted sutures of type A nylon.

To further stabilize the tibia, a Thomas splint was affixed to the operated leg while the animal was still anesthetized. The splint was left in place for 6 weeks and then removed. The Steinman pins were not removed until the animals were sacrificed.

D. RADIOGRAPHIC TECHNIQUE

The first group of dogs, in which the ulna was the surgical site, were radiographed immediately upon finishing surgery. A second radiograph was taken after one week to be sure the bone graft had not been displaced. Monthly radiographs were taken of the left and right ulna in both lateral and antero-posterior planes for 9 months following surgery.

Prior to removal of a segment of the tibia, a radiograph was taken of the entire tibia to determine the size of the medullary cavity of the bone, the angulation if any, and the approximate area of bone that might be best to remove. The next radiograph was taken directly after surgery and from here on, both a lateral and an antero-posterior radiograph were taken monthly of the right tibia until 9 months had elapsed from the time of surgery.

At the experiment's conclusion the results of all 12 bone grafts were compared, at a similar end-point of 9 months.

E. HISTOPATHOLOGIC TECHNIQUE

Approximately 9 months following the bone grafting procedure, each dog was euthanatized with pentobarbital sodium* (2.75 gr./ml.). The graft area was removed to include 1/2-inch of host bone at each end of the graft. Each specimen was then put immediately into a formalin-acid alcohol fixative.

The ulnas were removed full thickness, but owing to the diameter of the tibial grafts, the latter were cut in half lengthwise for better infiltration of preservative and better decalcification. The decalcification, dehydration, and embedding of the tissue with celloidin were carried out, as recommended in the U.S. Armed Forces Institute of Pathology Manual (1960).

The stain used for the tissue sections was Galigher's Alum - Hematoxylin Stain. This is used most frequently by the Michigan State University Anatomy Department for bone tissue embedded in celloidin. This stain is described by Galigher and Kozloff (1964).

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* Toxital, Jensen-Salsbery Laboratories, Kansas City, Missouri.

RESULTS

A. Dog 1 (106975)

I. RIGHT ULNA, GRAFT AND PERIOSTEUM

a. Radiography. There was evidence of new bone, beginning from the proximal portion of the untreated ulna. This new bone extended at least 2/3 of the way across the defect resulting from reabsorption of the graft. This immature spicule was considerably less dense than the remainder of the untreated ulna. The only remnant of the graft was a very small triangular piece of bone. The distal ulna had become thinner in the area of the graft (Fig. 9).

b. Histology. A small piece of original bone, made up of empty lacunae, was visible in the center of the graft area. There was a localized area of inflammatory cells present, although there was no evidence of either bacteria or foreign material. At one end of the graft area, there was a sheet of osteoid tissue, and a few osteoclasts were visible.

II. LEFT ULNA, GRAFT DEVOID OF PERIOSTEUM

a. Radiography. The remnant of the graft, although no less dense than before, was about 1/2 its original length. This bone appeared to be barely attached to the proximal ulna by a thin strand of osseous tissue. The ends of the untreated bone, adjacent to the graft, also underwent reabsorption, as the ends of the bone were quite thin. There was no evidence of callus formation (Fig. 12).

b. Histology. The remainder of the graft appeared to be attached to host bone by a few threads of immature connective tissue, rather than by dense bone. There were no new areas of bone formation, nor were there any new capillaries or osteoclasts visible in the graft section. The only bone visible was a segment of dead bone, composed solely of circular lamellae with empty lacunae.

B. Dog 2 (107863)

I. RIGHT ULNA, GRAFT AND PERIOSTEUM

a. Radiography. A longitudinal split was visible down the center of the graft (this occurred during surgery, when it was attempted to remove the graft with an osteotome). The proximal end of the graft became well united with the host bone by 9 months, and it appeared that the longitudinal split was filling in with bone (Fig. 15). The graft was considerably more dense than it was on radiographs taken at 3 months (Fig. 13).

b. Histology. One end of the graft was well united to the host bone by osteoid made up primarily of young osteocytes. Polarization showed very few collagenous fibers. New capillaries, filled with blood cells, were visible throughout the entire graft. The other end of the graft did not appear to be as well incorporated with the host bone.

II. LEFT ULNA, GRAFT DEVOID OF PERIOSTEUM

a. Radiography. A gradual decrease in size and density of the graft appeared over the 9-month period. There was little callus formation from the proximal ulna, but this did not apparently proceed to surround the graft. The ends of the untreated ulna became quite thin and rounded, showing radiographic evidence of reabsorption (Fig. 18).

b. Histology. There was microscopic evidence of osteoid formation between the graft and the host bone at both ends of the graft. The graft itself appeared to be well vascularized throughout its substance. There were many osteoclasts around the entire perimeter of the graft indicating recanalization. In the center of the graft there was a small remnant of dead bone made up of lamellae with no osteocytes and no capillaries.

C. Dog 3 (107927)

I. RIGHT ULNA, GRAFT AND PERIOSTEUM

a. Radiography. By 2 months' time, there was good evidence of callus formation from the distal ulnar segment (Fig. 19). At 5 months, the graft appeared to have decreased in density, but there was evidence of union between the graft and the host bone at the distal end (Fig. 20). Although there was some rounding of the ends of the ulnar graft bed, there was no obvious reabsorption by 9 months (Fig. 21).

b. Histology. Histologically, the defects at both ends between the graft and the host bone were filled with osteoid. However, under polarizing lenses there appeared to be a greater preponderance of collagenous fibers, rather than osteocytes. Many osteoclasts were present around the original treated bone, giving it a ragged edge.

II. LEFT ULNA, GRAFT DEVOID OF PERIOSTEUM

a. Radiography. Very good callus formation was visible by 2 months, especially at the proximal end (Fig. 22). Even at this time the graft was almost completely reabsorbed. The graft area was relatively unchanged by 9 months, when the dog was sacrificed, since it was just a large defect with several remnants of bone. From the distal ulnar

segment there was a spicule of new bone (Fig. 24).

b. Histology. The most obvious histologic reaction in the graft area was one of inflammation, since there was a large area of inflammatory cells present. There was no sign of new osteocytes, although there were some thin bands of collagen present. These bands were quite different from the wide sheets of collagen and osteoid seen in many of the previous sections. Spread throughout the graft defect were several small islands of dead bone.

D. Dog 4 (107932)

I. RIGHT ULNA, GRAFT AND PERIOSTEUM

a. Radiography. According to the radiograph taken at 9 months, the bone graft had been almost completely reabsorbed, except for 2 spicules of bone. There was no apparent stimulation of the periosteum of the host bone to produce a callus. Also, there had been reabsorption and thinning of the host bone ends (Fig. 27).

b. Histology. There was no sign of new bone formation in the graft defect. The only trace of bone was several pieces made up of lamellae with empty lacunae. There was only a small amount of collagen present. Under polarized light, a piece of nylon suture material was seen in the middle of a localized area of inflammation.

II. LEFT ULNA, GRAFT DEVOID OF PERIOSTEUM

a. Radiography. By 6 months the bone graft was completely united, at its distal end, to the host bone (Fig. 29). When the final radiograph was taken, just prior to sacrificing the dog, the graft was relatively unchanged. There was a limited amount of union at the proximal end.

Also, there was some degree of reabsorption and rounding at the cut end of the proximal ulna (Fig. 30).

b. Histology. The periosteum had grown from the host bone over the graft, since the graft periosteum was autoclaved along with the bone. The graft itself appeared to be viable, as it was composed primarily of young osteocytes, and there were many capillaries containing blood cells. One end of the graft was well attached to the host bone by osteoid, while the other end of the graft was primarily collagenous tissue.

E. Dog 5 (108088)

I. RIGHT TIBIA, GRAFT AND PERIOSTEUM

a. Radiography. The graft was relatively unchanged after 3 months, but there appeared to be a severe periosteal reaction, especially of the distal tibial segment. At this time, there was some callus formation at both ends of the graft (Fig. 31). Three months later, the callus from the proximal tibia had joined with that of the distal tibia, in the area of the fibula. The periosteal reaction appeared to be centered in the area where the point of the intramedullary pin was pushing through the tibial wall (Fig. 32). At 9 months the graft was well incorporated into the tibia, except for a small area at the distal end of the graft (Fig. 33).

b. Histology. The graft appeared to be well infiltrated with osteoid and firmly united with the host bone. New capillaries were seen through most of the graft substance. There was no sign of inflammatory reaction.

F. Dog 6 (108228)

I. RIGHT TIBIA, GRAFT AND PERIOSTEUM

a. Radiography. Callus formation at both ends of the graft was quite obvious by 3 months (Fig. 34). By 6 months the callus completely surrounded the graft and filled the gap between the graft and the host tibia (Fig. 35). The last radiograph showed the callus to be in the process of remodeling, as it began to decrease in diameter. Delineation of the graft from the main body of the tibia was impossible (Fig. 36).

b. Histology. The dead bone of the tibial graft was well infiltrated with new, healthy osteoid and revascularization of the graft had occurred. Complete union of the graft and the host bone had taken place, although some of the graft bone was not yet mature.

G. Dog 7 (108227)

I. RIGHT TIBIA, GRAFT AND PERIOSTEUM

a. Radiography. In this case the callus was well established by 3 months (Fig. 37). After 6 months the callus formed at each end of the graft had almost completely spanned the autoclaved bone, giving it the appearance of a "callus fracture". At this time the graft appeared to have been united with the untreated bone, at the distal junction (Fig. 38). By 9 months union of the graft appeared to be complete, with remodeling of the callus well under way (Fig. 39).

b. Histology. The graft appeared to be almost entirely new bone, not yet mature enough to form lamellae. However, there were still a few remnants of devitalized bone lacking viable cells or blood vessels. In the medullary cavity of the graft there was considerable fatty marrow tissue.

H. Dog 8 (108260)

I. RIGHT TIBIA, GRAFT AND PERIOSTEUM

a. Radiography. The incorporation of the graft by the host, in this case, appeared to be the best of the whole experiment. The callus did not appear to cover the entire graft but rather just the 2 junctions between the autoclaved bone and the untreated bone. Therefore, at 9 months the graft appeared to be completely united with the host tibia. There was no sign of callus at this time (Fig. 42).

b. Histology. This graft also appeared to have been gradually infiltrated with osteoid, made up of immature osteocytes and collagenous fibers. Capillaries were visible throughout the graft. The medullary canal contained marrow, but along with the fat cells it also contained hematopoietic cells.

Dog 1 (106975) - right ulna

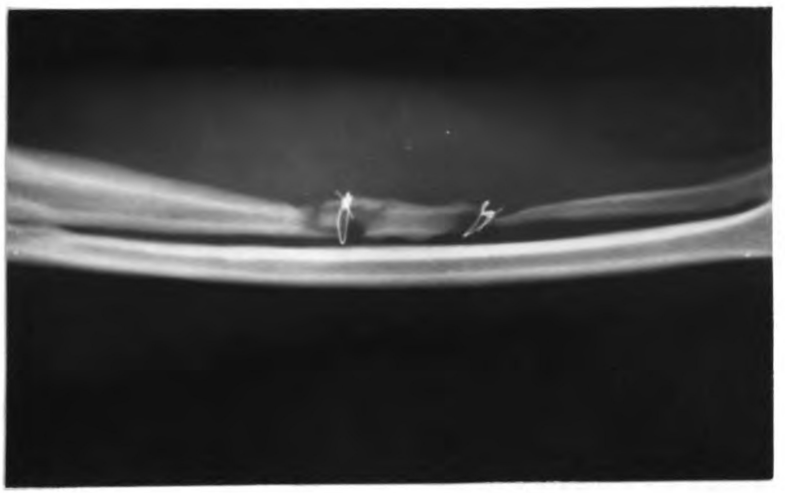


Fig. 7. (3 months) Early reabsorption of both graft and host bone bed, especially at the distal end of the graft.

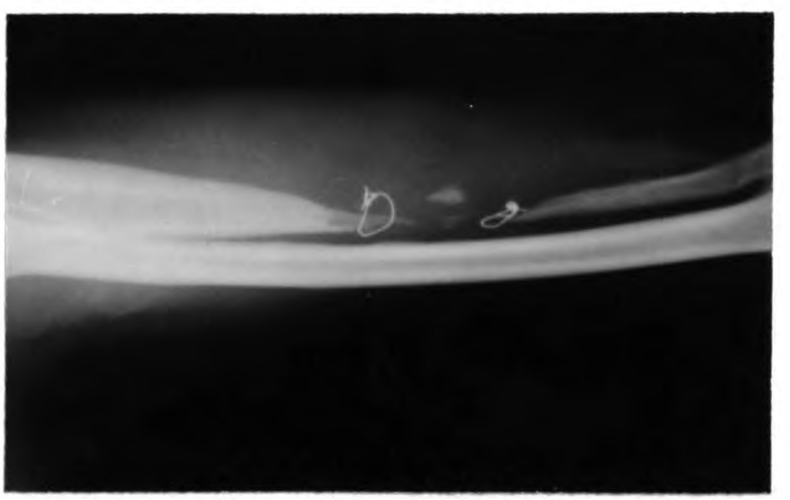


Fig. 8. (6 months) Almost complete reabsorption of the graft, with evidence of new bone formation at the proximal ulnar end.

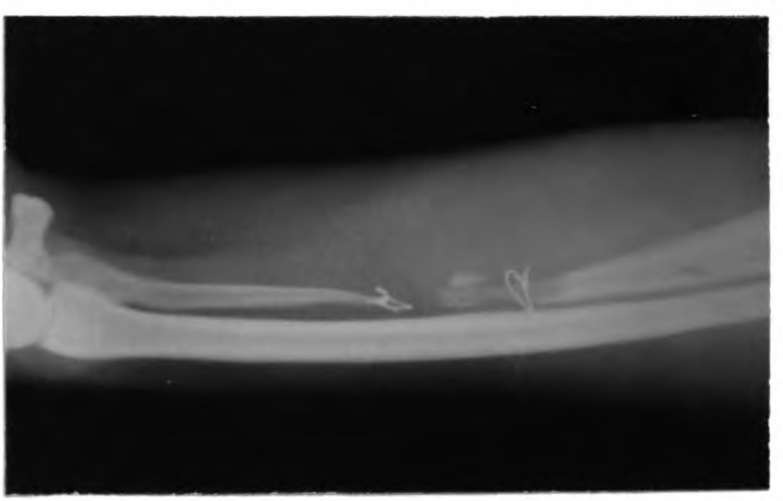


Fig. 9. (9 months) Proliferation of new bone at the proximal end of the ulna, filling up to 2/3 of the defect resulting from reabsorption of the autoclaved bone.

Dog 1 (106975) - left ulna

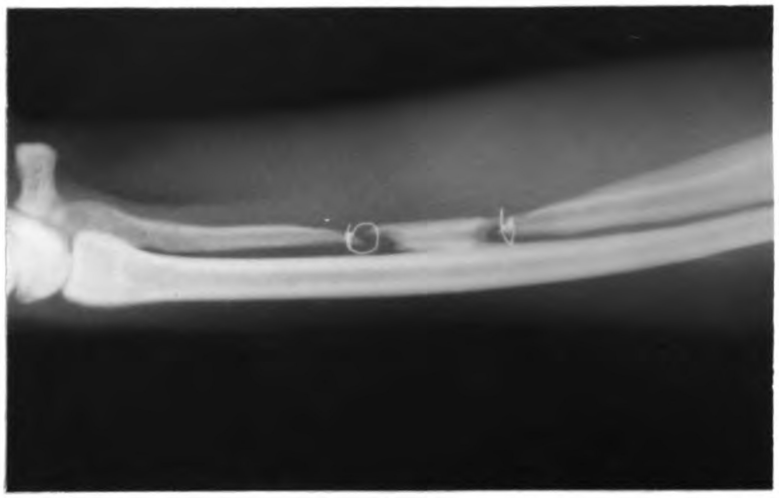
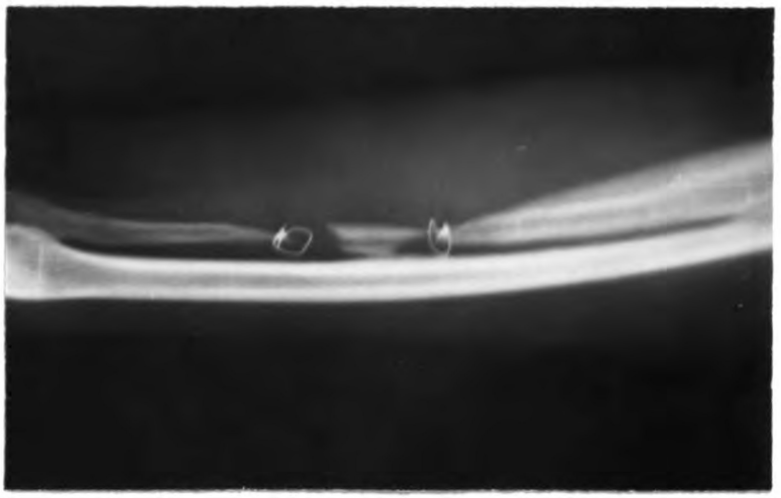


Fig. 10 (3 months) Early reabsorption of the ends of the graft, as well as rounding of the normal ulnar bone ends.

Fig. 11. (6 months) Progressive reabsorption of the graft, with no sign of callus formation or recalcification of the graft.



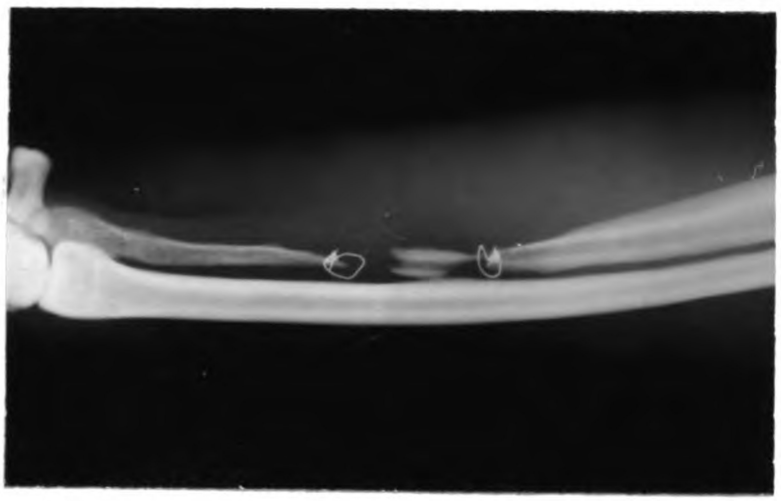


Fig. 12. (9 months) The graft is about one-half the original length, with decalcification of bone ends adjacent to the graft. A thin strand unites graft and host bone at the proximal end.

Dog 2 (107863) - right ulna

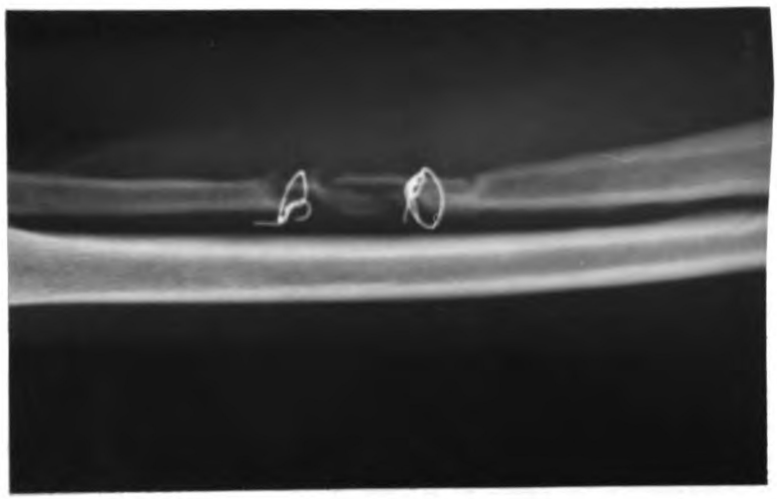
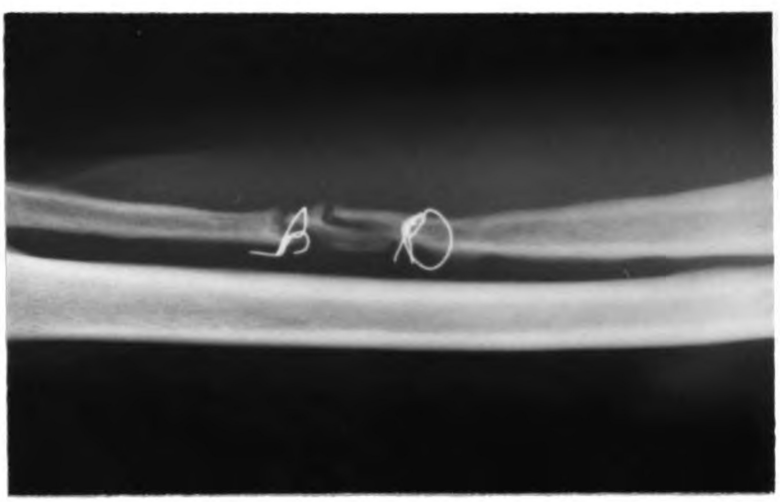


Fig. 13. (3 months) Decalcification of midportion of the graft and longitudinal splitting of same. (Graft split during removal prior to autoclaving.)

Fig. 14. (7 months) Evidence of union between the graft and the proximal portion of the ulna.



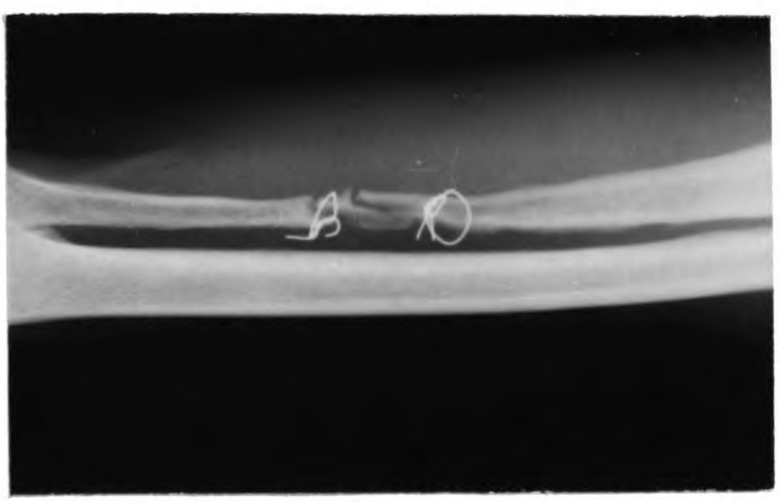


Fig. 15. (9 months) The graft is considerably more dense. The longitudinal split is beginning to fill in.

Dog 2 (107863) - left ulna

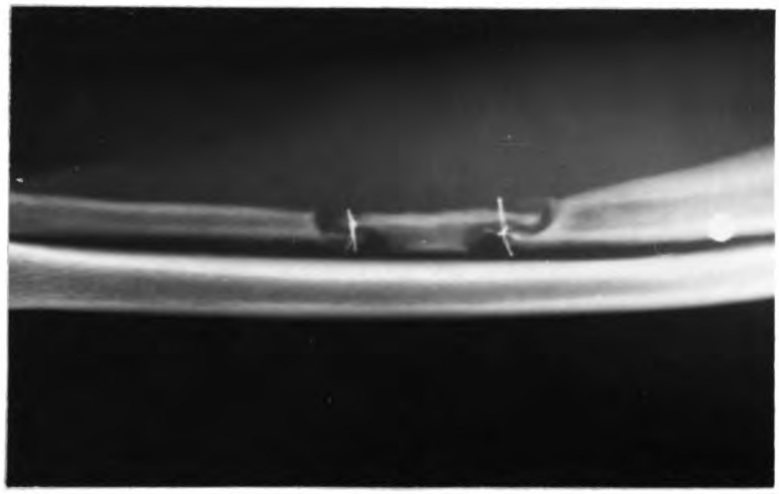
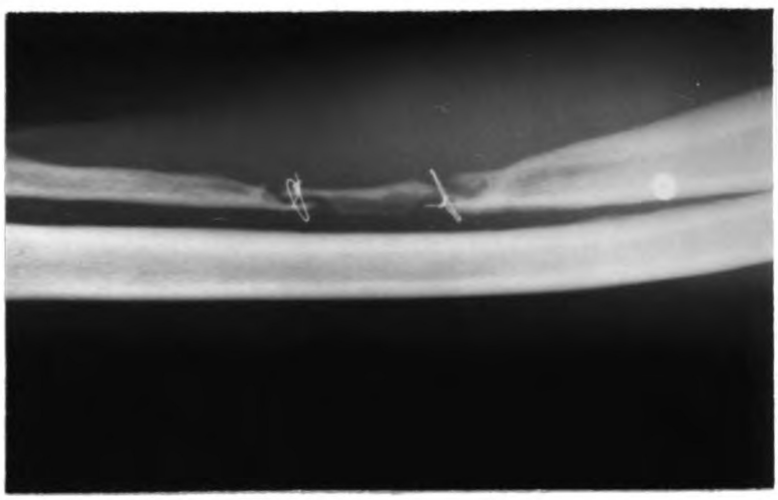


Fig. 16. (3 months) Early reabsorption of both the graft ends and the limits of the host bed. There is some evidence of callus beginning from the proximal normal ulna.

Fig. 17. (7 months) Further reabsorption, with loss of graft density.



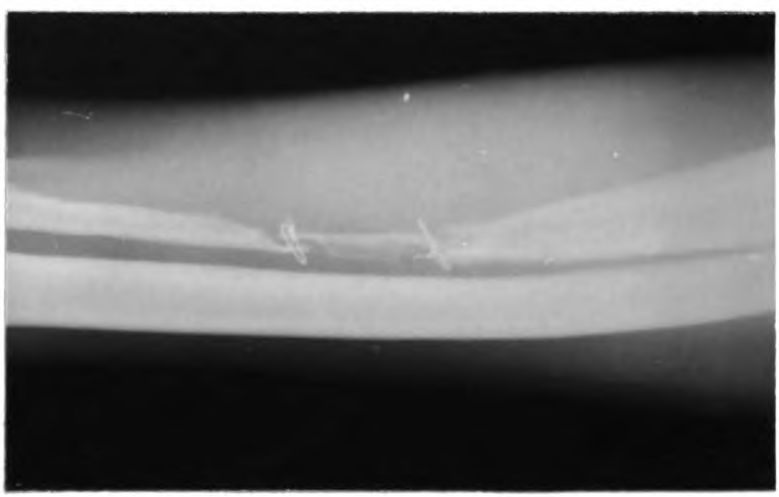


Fig. 18. (9 months) Rounding of cut ends of both the graft and the host bone, with almost complete decalcificiation of the graft. There is little progress of the callus.

Dog 3 (107927) - right ulna

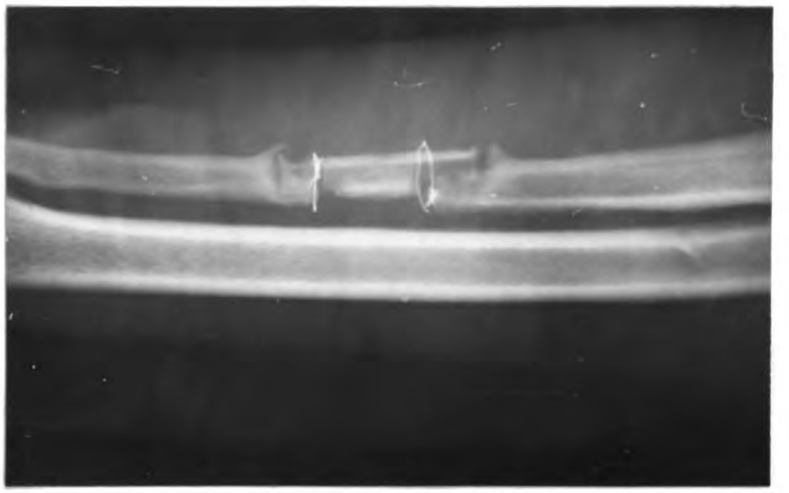
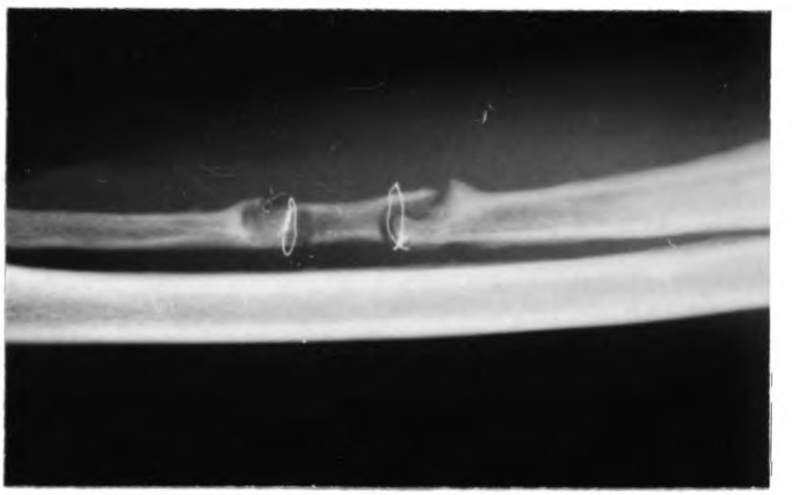


Fig. 19. (2 months) Beginning of callus formation from the distal ulnar segment. There is no evidence of reabsorption.

Fig. 20. (5 months) Increased callus formation at both ends of the graft. There is some acceptance of the graft at its distal end.



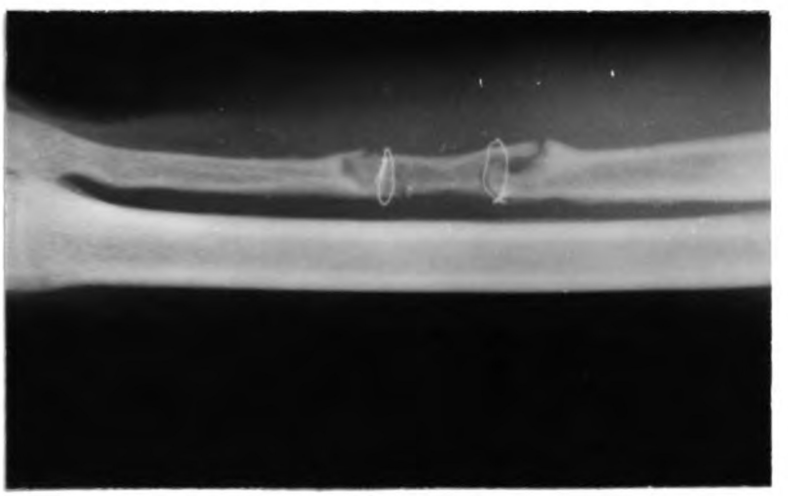


Fig. 21. (9 months) Increased calcification of the graft, with gradual union at the distal junction. There is little or no reabsorption of the ulnar host bone.

Dog 3 (107927) - left ulna

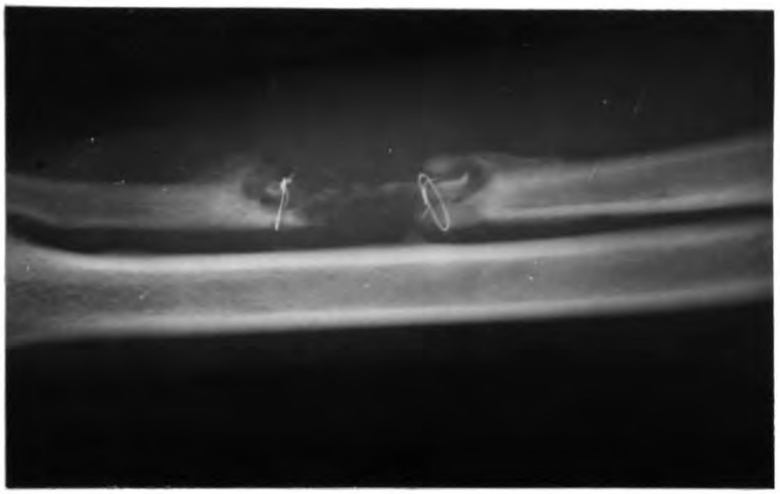
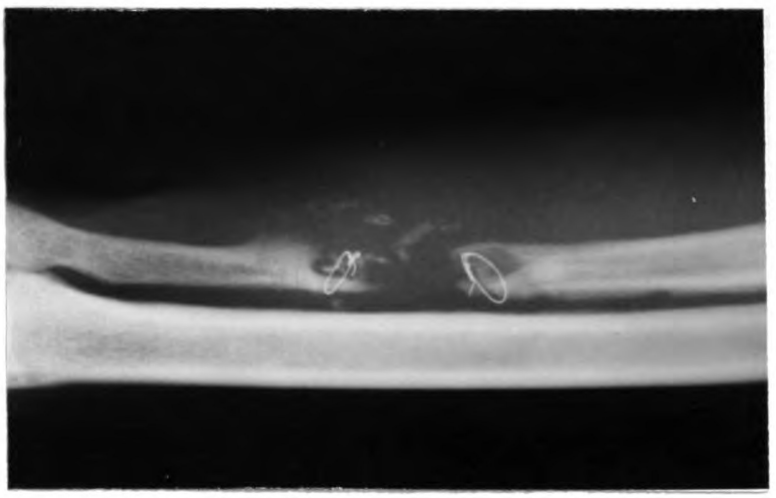


Fig. 22. (2 months) Complete decalcification of the grafted bone, despite a good callus beginning at both ends of the graft.

Fig. 23. (5 months) No evidence of the graft remaining except for a few isolated islands of poorly calcified bone.



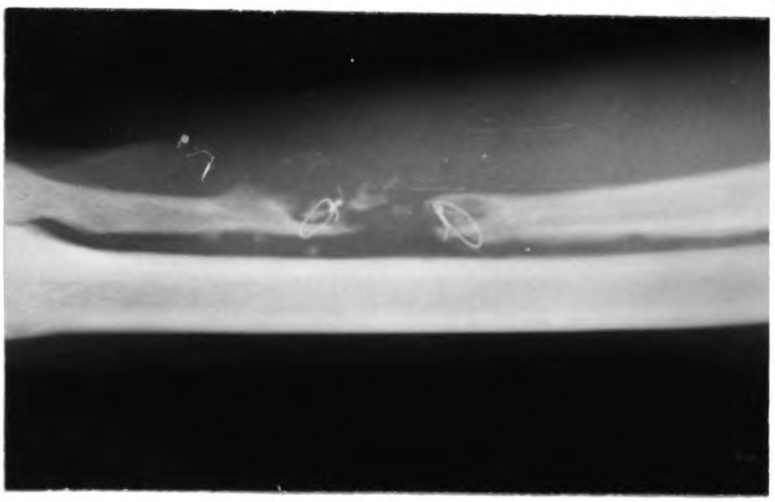


Fig. 24. (9 months) A spicule of new bone beginning from the distal ulnar segment. There is only a minimum of reabsorption of the host bone ends.

Dog 4 (107932) - right ulna

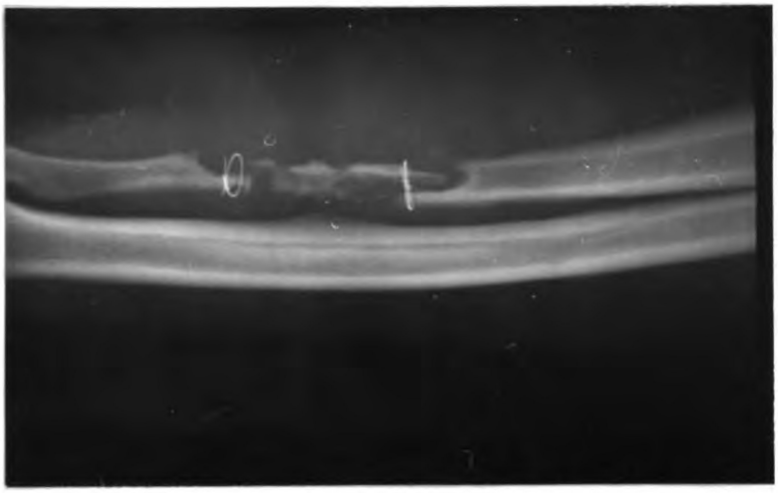


Fig. 25. (2 months) Even within this short period of time, the graft has undergone considerable demineralization.

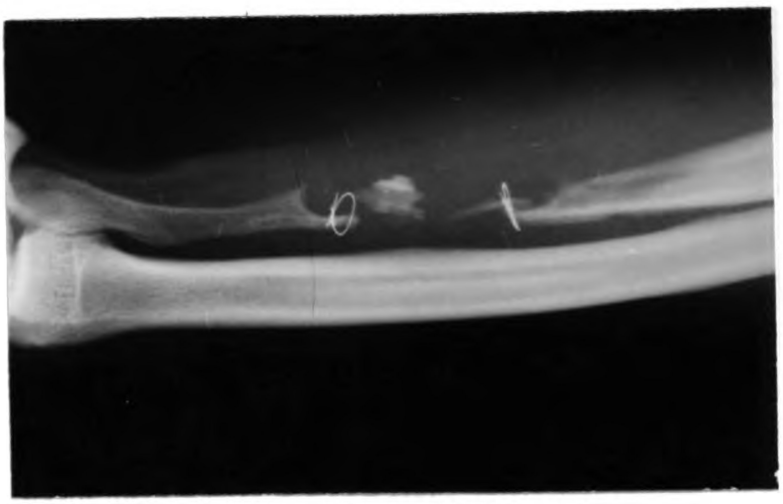


Fig. 26. (6 months) The inert remains of reabsorption persisting along with rounding of the host bed bone ends adjacent to the graft.

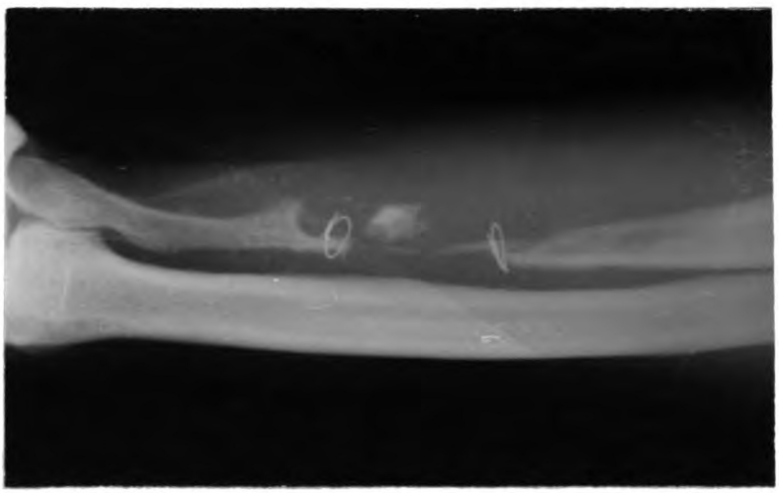


Fig. 27. (9 months) Relatively unchanged from the radiograph taken at 6 months. Calcification of the defect appears highly unlikely at this point.

Dog 4 (107932) - left ulna

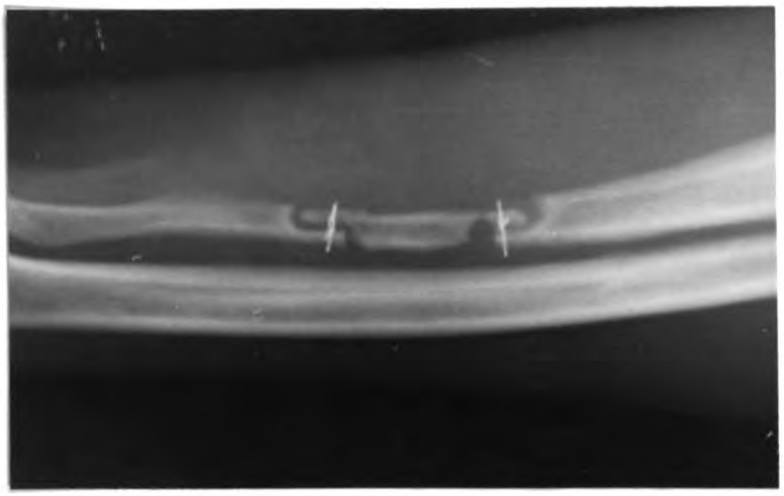


Fig. 28. (2 months) Only slight reabsorption at the graft-host junctions. A few spicules of early callus can be seen from the normal bone.

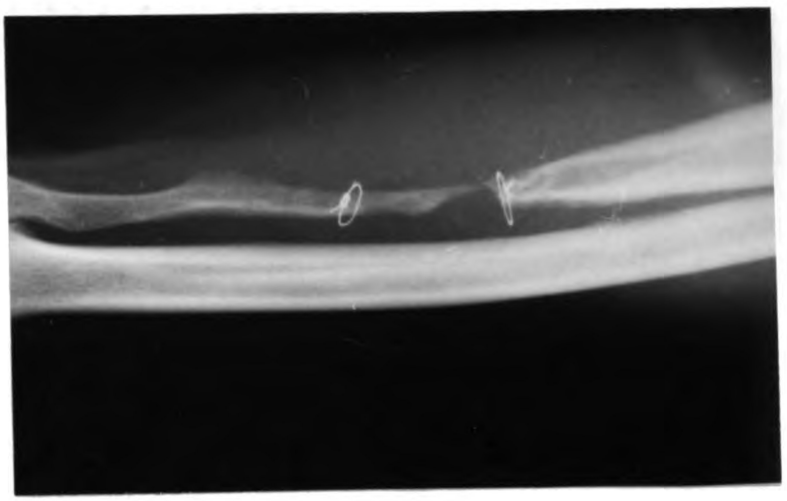


Fig. 29. (6 months) Complete bony union at the distal end of the graft. The proximal end of the graft is attached by a thin strand to the normal bone.

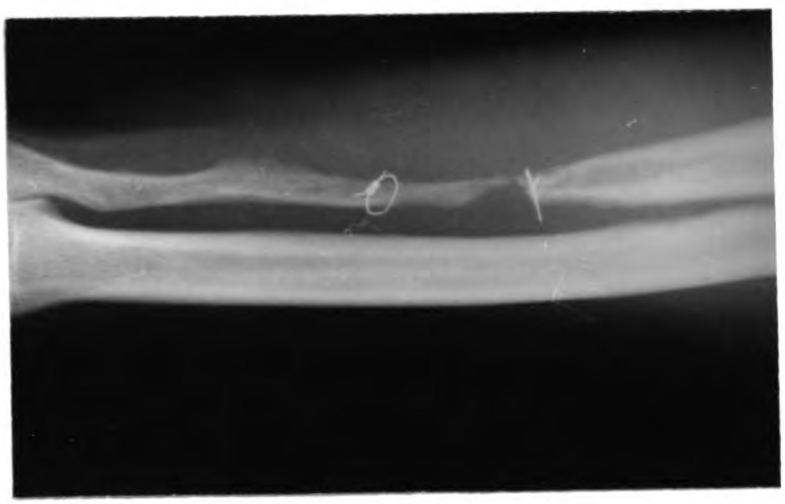
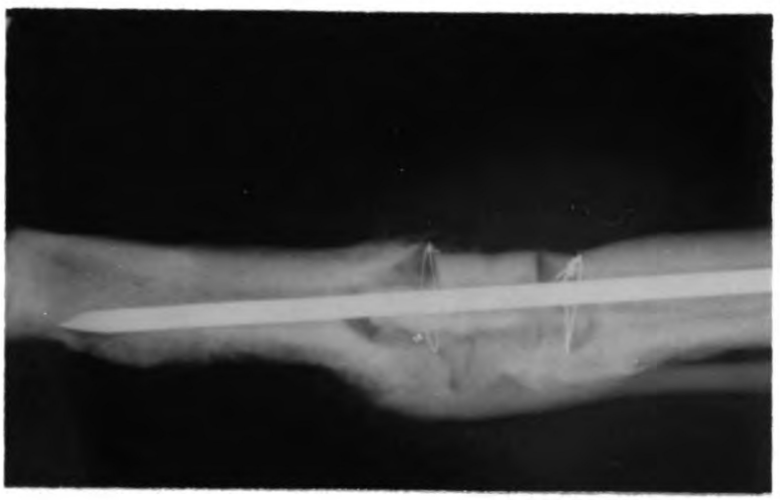


Fig. 30. (9 months) The graft is unchanged from the previous radiograph. There is some loss of substance at the proximal graft junction.

Dog 5 (108088) - right tibia

Fig. 31. (3 months) Signs of periosteal reaction exist. The graft is relatively unchanged. Early callus is beginning at the distal host-graft junction.

Fig. 32. (6 months) Graft not yet incorporated by the host bone, although a solid callus formed between the tibia and the fibula.



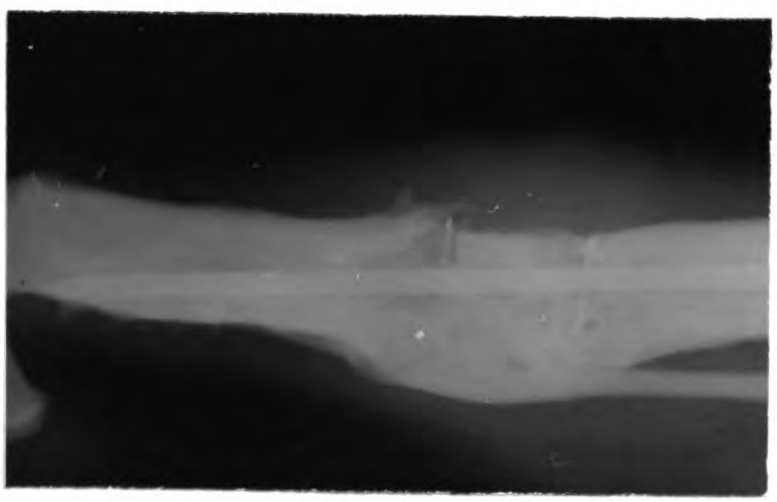


Fig. 33. (9 months) Partial union at the proximal graft limit. There is decreased periosteal reaction and complete calcification of the callus, uniting the entire tibia around the graft.

Dog 6 (108228) - right tibia

Fig. 34. (3 months) No sign of decalcification of the graft, nor reabsorption of the host bone. Callus is beginning at both ends of the graft, from the healthy tibia.

Fig. 35. (6 months) Good callus formation spanning the entire graft. One can see an increased density in the material filling both step junctions.

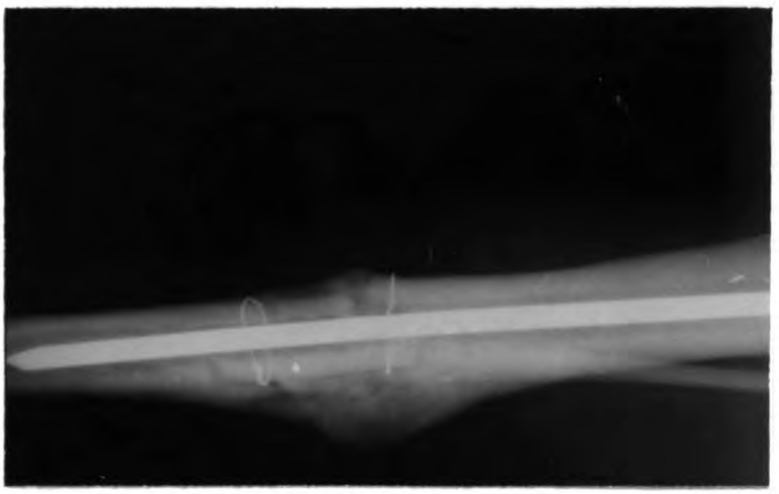


Fig. 36. (9 months) Complete union between the graft and the host bone. Also, there is some indication of remodeling of the callus.

Dog 7 (108227) - right tibia

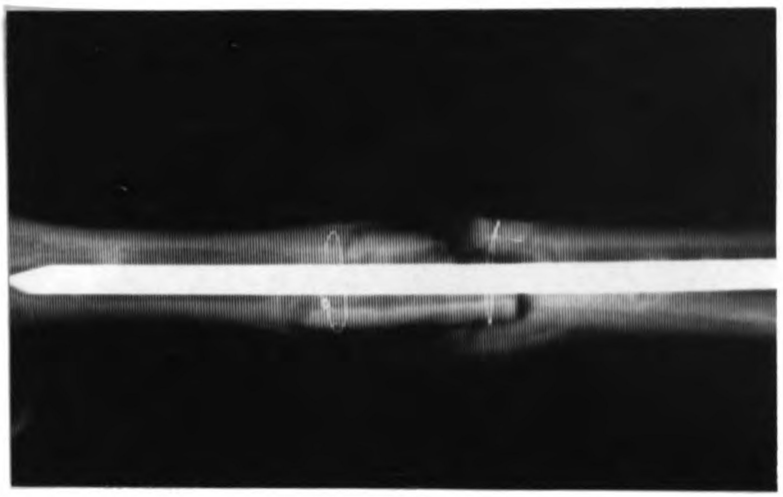


Fig. 37. (3 months) Callus formation well established at both ends of the graft, with no sign of reabsorption of either the graft ends or the host bone ends.

Fig. 38. (6 months) The graft is completely incorporated at the distal end. Callus from each end almost entirely spans the graft, giving the appearance of a "callus fracture".

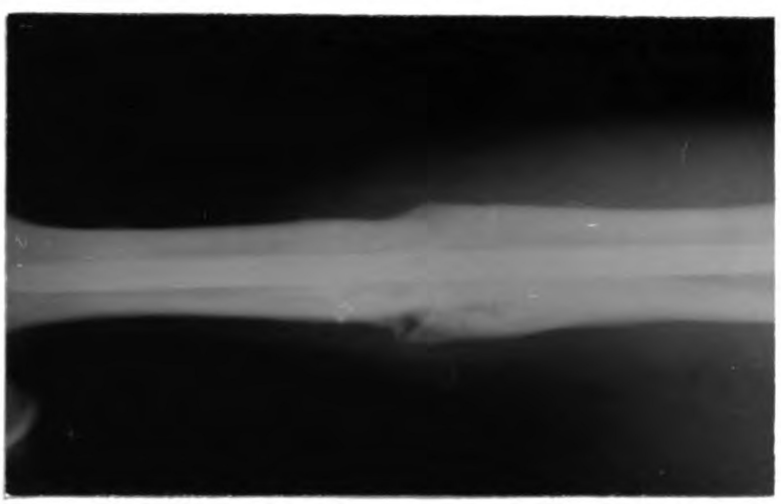


Fig. 39. (9 months) The graft is united with the host bone at both ends. The callus is complete and remodeling is under way. Only a small area is not dense bone.

Dog 8 (108260) - right tibia

Fig. 40. (3 months) Callus has already begun to form at the graft limits, from the healthy bone. No demineralization of the graft has occurred, nor rounding of the host bone ends.

Fig. 41. (6 months) The graft is well accepted at the proximal end. Callus is covering the distal end.

Fig. 42. (9 months) The graft limits are imperceptible. The tibia is apparently back to normal condition, with remodeling of the callus complete.

DISCUSSION

A. ULNAR GRAFTS

In most cases examination of radiographs of the ulnar grafts did not reveal the anticipated amount of callus formation. In the case of the left ulnas, where the graft periosteum was autoclaved, only 1 of 4 grafts was well united with the host bone, and then mainly at the distal end of the graft (Fig. 30). Union between graft and host bone had begun at the proximal end of the graft in one case, although there had been some decalcification of the implanted segment according to the radiograph (Fig. 18). The remaining 2 cases of left ulnar grafts showed various stages of reabsorption. One case had lost about 1/2 its original length and it is questionable whether this segment would have remained inert or would have completely disappeared (Fig. 12). In the fourth case of this group the graft was almost completely reabsorbed. At the proximal end of the distal ulnar segment a spicule of new bone had begun to form from the host bone and might have conceivably filled the defect in time (Fig. 24).

Radiographically, the grafts that were autoclaved without periosteum (right ulnas) showed results almost identical to the grafts that were autoclaved with periosteum, although not in the same animals. These results lead one to wonder if healthy periosteum at the graft site is really a necessary factor in bone regeneration.

As in the left ulnas, only one graft in the right ulna achieved solid union between graft and host bone, and then mainly at the proximal

end of the graft (Fig. 15). One graft had changed very little in size but had active osteoid which was not visible radiographically (Fig. 21). In another case new bone formation from the proximal ulna had united with the remains of the graft despite the presence of a localized area of inflammation (Fig. 9). The last of the right ulnas showed almost complete reabsorption of the grafted ulnar segment on radiograph (Fig. 27), and histologic examination of the tissue failed to show any sign of new bone formation.

In reviewing the results of the ulnar grafts, the formation of new bone does not seem to depend on the presence of a viable periosteum surrounding the autoclaved bone graft. In many cases, even though areas of young osteocytes seemed to project from the host tissue into the graft, reabsorption and thinning of the host bone ends appeared to occur. The absence of callus in response to the autoclaved ulnar grafts has previously been mentioned. These results are similar to those of Campbell (1953), who said that the ulna is one of the most difficult environments for a graft.

B. TIBIAL GRAFTS

In order to give the tibial grafts the best postsurgical vascular supply, the graft portion was removed devoid of periosteum, and after autoclaving was replaced into a healthy periosteal sheath.

Radiographs taken at 9 months indicated almost 100% success with these grafts, except for the case that appeared to have developed osteomyelitis, on radiographic examination. Even it showed evidence of good callus formation. Taking a closer look at this case, it was noted that at 3 months there was extensive periosteal reaction along most of the

tibial shaft and a considerable reaction around the graft (Fig. 31). By 6 months the host bone had retracted away from the graft, but there was solid callus uniting the tibia and incorporating the adjacent fibula (Fig. 32). When the final radiographs were taken at 9 months, there appeared to be only a minimum of reaction. A solid callus united the entire tibia (Fig. 33).

Comparing this case to another without complications, radiographs indicated callus beginning from the untreated bone at 3 months (Fig. 34). By 6 months the spaces between the graft and the host bone were filled with a radiopaque material, and a large callus encompassed the entire graft (Fig. 35). By 9 months there was no visible delineation of the graft, and the callus was beginning to become narrower in diameter (Fig. 36).

One case that had developed a very good early callus by 3 months (Fig. 37) had completely incorporated the distal end of the graft by 6 months, and the periosteal callus was almost complete (Fig. 38). At the time of the final radiograph union was complete, and the graft was indistinguishable from the normal bone (Fig. 39).

The final case produced the best results of the whole experiment. The radiographs taken 3 months after surgery showed about the same amount of callus at the graft-host bone junction as the previous case (Fig. 40). The difference in this case, radiographically, appears to be that there was not a large external callus over the entire graft as there was in the other 3. At 6 months the graft had become incorporated with the proximal tibial portion (Fig. 41). At the distal end of the graft there was some callus formed around the entire step junction. The final radiograph, taken prior to sacrificing the dog, showed almost

perfect conformation of the tibia, with no evidence of graft limits or of a callus (Fig. 42).

C. GENERAL CONSIDERATIONS

In critically analyzing the results of the bone grafts, several observations were made.

First, the tibial grafts were uniformly successful, whereas after the same length of time all the ulnar grafts showed some degree of reabsorption. Infiltration of the ulnar grafts with new bone was somewhat slower than for the tibias, and where there was osteoid present it generally only involved one end of the graft.

One wonders at the obvious difference here. Did the results improve with the technique, since the ulnas were done first? Is the degree of graft union dependent in any way on the vascularity of the host bone? Is a certain amount of motion necessary at the graft site to ensure callus buildup?

Except for the first set of ulnas operated on, all the surgery for the experiment was done over a 2-week period, and the technique was virtually identical. As far as the vascular supply to the graft site is concerned, Lloyd-Roberts (1952) stated that for a successful graft union the host bone must be as vascular as possible. It has been previously mentioned that the bone marrow and endosteum of the host bone assume major roles in revascularization of the bone graft. The ulna has a very small medullary cavity, proportionately, to that of the tibia. However, there was evidence to indicate that given more time many of the defects left in the ulnas by reabsorption of the graft would have filled in with new bone, formed at the cut surface of the healthy ulna. Pertaining to

motion at the site of a healing bone lesion, it was felt by Abell (1966), as well as Cohen and Lacroix (1955) that in bone grafts, as well as fractures firm apposition results in earlier healing with less callus buildup.

Regarding the value of an intact periosteum, the ulnar radiographs show that the results were comparable when the periosteum was maintained with the host bone and when it was removed with the graft segment and autoclaved. The fact that even one case without a viable periosteum became united with the host ulnar shaft indicates that union can occur without an intact periosteum surrounding the graft. The significance of viable periosteum surrounding the tibial grafts could only be determined by a similar comparative study. If radiographic results of the tibias could be projected to a situation where the periosteum was removed with the bone graft, the graft could quite conceivably be surrounded by callus, similar to that which extended rapidly from the healthy tibia in this experiment.

There does not appear to be a particular tendency for the graft to be accepted at one end more readily than the other, although this is a clinical observation rather than a statistical one. In both the ulnar and tibial grafts, some appeared to be united with the host bone at the proximal end first while others were united first at the distal end.

It is interesting to notice, in the histologic sections, that in 3 of the 4 ulnar grafts that failed to show new bone formation there was an area of inflammation within the original graft area.

SUMMARY AND CONCLUSIONS

A section of bone removed from the diaphysis of the ulna or tibia, autoclaved, and replaced, can be converted by the adjacent, untreated bone into viable osseous tissue. This appears to occur by gradual reabsorption of the treated bone and replacement with sheets of young osteocytes and collagenous fibers. Throughout this osteoid tissue are many capillaries filled with blood cells.

In the center of several grafts, a remnant of the autoclaved bone was visible. On histologic section, this remnant was made up of the typical circular lamellae of compact bone. In this case both live osteocytes and a Haversian vascular system were absent.

Satisfactory surgical approaches are described for easy removal and replacement of bone sections in the diaphyseal area of the tibia or ulna of the canine.

A section of bone from the canine ulna or tibia is unchanged in size and shape by autoclave sterilization. Therefore, if the segment is carefully removed in step fashion, fixation of the graft is relatively easy.

There is no indication of a foreign-body reaction to an autoclaved autograft of bone, as indicated by the absence of a fibrous tissue layer around the graft. However, in 3 of the 12 grafts there was a small localized area of inflammatory cells, one area of which contained a piece of nylon suture material.

Despite the fact that one of the 12 surgical sites appeared to have developed osteomyelitis radiographically, it does not appear that autoclaved bone is more susceptible to infection than normal bone. On the contrary, autoclaving has a decided advantage over other means of preparing bone grafts (i.e., freezing and freeze drying), since it renders the graft completely free of any pathogenic organisms. In one case, osteomyelitis was not severe enough to hinder healing of the graft.

According to the radiographs of the tibias, the callus was not complete at 6 months. Union between graft and host bone was complete by 9 months in 3 of the 4 cases, with remodeling of the tibial shaft well advanced. Depending on the individual dog, the latter length of time could be considered an adequate period before removing internal fixation of the graft. In the case of ulnar grafts, it appears that a great deal more time is required to determine if complete union of the graft and the host bone will occur.

Considering the success of the tibial grafts, many possible clinical uses become evident: (1) half-joint resection of unhealed osteochondritic lesions of the femoral and humeral heads, (2) replacement of chronic, localized, osteomyelitic bone with autoclaved homologous bone, and (3) use of autoclaved, homologous or heterogeneous bone to replace badly deformed bone, due to neoplasia or congenital malformations.

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