

THE EFFECT OF CORTISONE, ISONIAZID, AND ESTROGEN ON  
THE HEALING RATE OF BONE DEFECTS FILLED WITH  
VARIOUS BONE GRAFTING MATERIALS

by

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## INTRODUCTION

Veterinary orthopedic surgery has made tremendous advancements in the last twenty years. Many of these were due to the modification of ideas and procedures from human orthopedic surgery. Although many techniques and appliances have been applied, bone transplantation has been ignored.

Brinker (1953) in a report to the American Animal Hospital Association stated that "Although the principles and techniques of bone grafting were developed using dogs as experimental animals, very little reference to bone grafting is found in veterinary literature. Apparently the veterinary profession is lagging in this phase of orthopedic surgery." He recommended that more work be done on the problem of bone storage and the value of homogenous bank bone as an aid in veterinary orthopedics.

Reported indications for bone grafting include the following:

1. Treatment of early fractures (Frantz et al. 1953).
2. Treatment of delayed and non union fractures (Murray 1944, Meekison 1945, Carpenter et al. 1946, Phemister 1947, Farrow 1948, Cleveland and Winant 1952, Taylor 1959).
3. Filling of bone defects following trauma, cysts, osteomyelitis and the removal of tumors (Frantz et al. (1953, Hazlett 1954).
4. Correction of bone deformities (Moore 1944).
5. Arthrodesis (Sorrel et al. 1947, Bosworth et al. 1953).

6. Reconstruction of defective bone (Moore 1944, Davis 1944, Flannagan and Buren 1947, Gibson 1948).
7. Plastic surgery (Peer 1955).
8. Whole or partial joint or bone replacement (Herndon and Chase 1952, Capurro and Pedemonte 1953, Clark 1959).

Bone grafts may be either cancellous or cortical, depending on the architectural arrangement of their bone lamellae. They are classified as autogenous, homogenous, or heterogenous depending on the species of the donor and recipient.

Cancellous or spongy bone consists of a loosely arranged network of bone trabeculae separated by interconnecting marrow spaces. The flat bones such as the skull, ribs, and pelvic girdle are cancellous bone covered with a thin cortical layer.

The loose arrangement of bone lamellae makes cancellous bone the ideal grafting material when rapid vascularization and replacement are important and strength is not necessary, (Stringa 1957, Abbot et al. 1947, Brinker 1953, Gallie and Robertson 1918).

This type of bone is best used to fill bone defects and cavities. It is used to pack around fracture sites and cortical transplants (Holmstrand 1957).

Clinically varied opinions exist on the use of cancellous grafts. Some surgeons advocate the exclusive use of cancellous bone (Cleveland and Winant 1952, Hazlett 1954, Phemister 1947), while others expressed

the belief that it is of value only where no strength is required (Gallie and Robertson 1918, D'Aubigne 1949). Cancellous or spongy bone has been used in conjunction with metallic plates (Cleveland and Winant 1952, Horowitz and Lambert 1945), pins (Spira 1954) or massive cortical grafts (Abbott et al. 1947, Gallie and Robertson 1918).

Cortical or compact bone has a decidedly different structural arrangement than cancellous bone. It consists of concentric layers of bone substance tightly arranged in tubular columns around the blood conducting unit of the Haversian canal. These tubules are closely united by interstitial lamellae. There are no marrow spaces in cortical bone. The walls of the shafts of long bones are cortical. Because of its density and vascularization, incorporation and replacement are slowed. However, cortical bone has the advantage of greater tensile strength. It is, therefore, better suited to use in fracture treatment, joint fusions and other procedures where stability is essential (Gibson and Loadman, 1948). Although some investigators state that the density of cortical bone inhibits incorporation and leads to sequestration (Cleveland and Winant 1952), Gallie and Robertson (1918) successfully transplanted massive cortical transplants, and Herndon and Chase (1952), Capurro and Fedemonte (1953), Clark (1959) successfully transplanted whole and half joints.

Cortical bone grafting techniques include: The simple inlay and onlay grafts (Moore 1944, Meekison 1945, Farrow 1948, Setzel 1956,

Taylor 1959) intramedullary grafts (Moore 1944, Farrow 1948, Temizer 1956), barrel stave grafts (Moore 1944), circumferential grafts (Heiple et al. 1963, Fitts et al. 1955), diamond grafts (Blair 1951), dual grafts (Moore 1944, Bishop et al. 1947), latch grafts (Rizzo and Lehmann 1947) and sliding grafts (Hughes-Davis 1954) have been described and evaluated.

In autogenous bone grafting the patient is both the donor and recipient. Grafts of this type are favored in orthopedics because it is believed that they heal more rapidly, show greater cell survival and produce less tissue reaction (Gallie & Robertson 1918, Inclan 1942, Peer 1955). Frantz et al. (1953) surveyed 577 leading orthopedic surgeons and reported 377 used only autografts while 200 used both homografts and autografts.

Numerous clinical reports of successful autografts were found in the literature (Farrow 1948, Gibson and Loadman 1948, Spira 1954). The usual sites for securing the transplants are the tibial crest for cortical bone and the rib (Peer 1955) or wing of the ilium (Horowitz and Lambert 1945, Dick 1946) for cancellous bone.

The disadvantages of autografts include the necessity of two operations which prolong the surgery time and thereby increases the incidence of patient discomfort and operative infection (Kreuz 1951). In children and in cases of generalized bone disease, sufficient quantities of suitable bone may be unavailable (Henry 1948). After taking the autograft, the donor bone may be seriously weakened, predisposing

it to fracture.

Homogenous bone grafts are obtained from another individual of the same species as the graft recipient. The use of this type of graft dates back to the early days of bone transplantation. Macewen (1881) successfully used homografts and temporarily stimulated the use of homogenous bone grafting but it was not until the advent of adequate storage methods that homografts became popular.

Many clinical and experimental investigators report comparable results with homografts and autografts (Inclan 1942, Bush 1945, Reynolds and Oliver 1950, Herbert 1951, Kreuz et al. 1951, Weaver 1949, Wilson 1951a, 1951b, Bosworth 1953, Lloyd-Roberts 1952, Tucker 1953, Carr and Hyatt 1955). Other reports recommended homografts be used only when autogenous bone was not available (Reynolds and Oliver 1949, Reynolds et al. 1951, Brav 1953, Rocher 1953, Carnesale and Spankus 1959).

Some workers feel that homografts have failed due to either immunologic rejection (Curtiss et al. 1956, 1959, Chalmers 1958, 1959, Burwell et al. 1963, Burwell 1964), or the inability of the osteoblasts to survive transplantation (Campbell et al. 1953).

Heterogenous bone grafts are obtained from a member of a species other than that of the recipient. These have been used with satisfactory results by veterinary surgeons in Europe. Guillamenet et al. (1953) reported that frozen sheep and calf heterografts were comparable to homografts. Clayton (1955-1956) and Tucker (1956) considered cultured



calf bone equal or superior to human autografts. Successful results from the use of bovine, canine, lapine, avian, porcine, equine, and simian bone were obtained (Peer 1955).

Alloplastic transplants are derived from a substance other than bone. The use of ivory, cow horn, stag antler and animal teeth has been reported, however these are inferior to grafts of bone tissue.

Reynolds and Oliver (1950) stated that fracture and graft healing are identical processes which take place by a uniform and orderly series of events. The cells involved and the mechanism of their activation is at present unsettled. As reported by Chase and Herndon (1955), Heine, Ollies, Bonome, Axhausen, and Phemister formulated the osteoblastic theory of bone repair. This theory proposes that the healing of transplants is due to the osteogenic activity of the cells of the cambium layer of the periosteum, the endosteum, and the fibrous elements of the Haversian canals of both the host and graft.

Lerichi and Policard proposed the mesenchymal or metaplastic theory of bone repair. They postulated that osteogenic cells originated from the surrounding connective tissue. The transplant induced the osteogenic activity of the host mesenchymal cells and acted as a scaffolding and local calcium source (Chase and Herndon 1955).

There are many proponents of the metaplastic theory. Some have tried to prove the existence of induced metaplastic bone formation by transplanting osseous tissue into extra skeletal sites such as subcutaneous

pouches, skeletal muscles and the anterior chamber of the eye (Hutchinson 1952, Urist and McLean 1952, 1955, De Bruyn and Kabisch 1955). Still other workers (Levander 1938, Eskelund and Plum 1950, 1953, Moss 1958) have attempted to isolate an osteogenic inducer substance from emulsions of bone, periosteum and various non-osseous tissues. The results have been inconclusive.

Others have attempted to discredit the metaplastic theory by proving the viability of transplanted osteoblasts (Reynolds and Oliver 1950, Ham 1952, Ham and Gordon 1952, Ray et al. 1952).

Cooley and Goss (1958) demonstrated that bone healed in the absence of actively participating connective tissue and that connective tissue alone cannot produce callus formation. A series of experiments was performed in which a fractured ulna was inserted into a subcutaneous pouch on the lateral surface of the thigh. In one series the grafts were irradiated and in another the recipient site was irradiated. The radiation dose was sufficient in both series to stop cellular proliferation. No healing was found in the irradiated grafts in normal host beds, but in all the non-irradiated grafts in irradiated beds callus was evident.

Auxhausen (1956) after extensive experimentation with autografts and homografts advanced the diphasic theory of bone healing. He claimed osteogenesis occurred in two phases. The first and physiologically most important started a few days after transplantation. It originated from the surviving cells of the transplant. The second phase required several

weeks to develop. It was reportedly the result of differentiation of non-specific connective tissue cells. Burwell (1964) expressed similar ideas.

The present investigation was designed and undertaken for the following reasons:

1. To determine the histologic processes and healing rate of circumscribed cortical bone defects in the dog.
2. To determine the histologic processes and healing rate of circumscribed cortical bone defects in the dog when filled with fresh autogenous cortical bone, frozen stored cortical homogenous bone, plasma stored cortical homogenous bone, autoclaved cortical bovine bone and polyurethane foam.
3. To determine the effect of prednisolone, isoniazid and stilbesterol on these histologic processes and healing rates.

This data would be of aid to the veterinary surgeon in establishing the value of bone grafting in the treatment of canine bone defects and would indicate the most useful materials for this purpose.

The observations of the effect of drugs on graft healing would also be important since these drugs must at times be used in the treatment of superimposed conditions. If stimulation or inhibition of bone or graft healing was noted, the use of these drugs should be re-evaluated.

The author wished not only to add to the scientific knowledge in the field of bone transplantation, but also to stimulate the use of bone

grafting in veterinary surgery. It is only through extensive clinical use that a practical evaluation of bone grafting can be developed.

## REVIEW OF LITERATURE

### Bone Grafts

The history of bone transplantation dates to the early days of surgery nearly two centuries before the knowledge of anesthesia or asepsis. In 1668, van Meekern (Tucker 1956) repaired a cranial defect in a Russian soldier by using a plate of bone removed from the calvarium of a dog. Healing was satisfactory but the graft was removed by order of the church. After this incident the science of bone transplantation was dormant until 1809 when Merrem (Peer 1955) reported successful results with animal autografts. This caused a revival of interest in the experimental aspects of bone transplantation and soon after many similar reports appeared (Peer 1955). The early experimentation, although in animals, was not concerned as much with the surgical implications as with the study of the cells involved in osteogenesis (Chase and Herndon 1955).

Probably the most notable early clinical work was by Macewen (1881) who, by means of a series of homografts reconstructed a humerus which had been destroyed by osteomyelitis.

Chase and Herndon (1955) in a historical review of the literature

concerning bone grafting reported 45 clinical cases of autografts and 10 of homografts in the 1890's. In the first 10 years of the 1900's 162 reports of autografts and 29 of homografts were found. After 1910, an increase in bone grafting popularity was evident and after 1920, the literature shifted from isolated case histories to reports of surgical experiences with hundreds of cases. These figures indicate acceptance of bone grafting as a clinical procedure.

The practical value of human homografts was limited until adequate storage methods were developed.

Bauer in 1910 (Chase and Herndon 1955) showed that canine bone could be used successfully after three weeks storage under ordinary refrigeration. Gallie and Robertson (1918) preserved homografts and heterografts by boiling. This procedure has been advocated by others (Lloyd-Roberts 1952, Temizer 1956). Christopher in 1923 (Peer 1955) stored cadaverous bone in alcohol at 80 C. He reported good results in three cases and concluded this procedure to be ideal for the storage for bone homografts.

Orell (1937) in an attempt to develop a better method of bone preservation experimented with what he called "os purum" and "os novum". "Os purum" was bone of either human or bovine origin. It had undergone a chemical process by which the fat, connective tissue and protein were removed leaving only an inorganic framework. The other substance, "os novum" consisted of a piece of "os purum" which had been imbedded on the

anterior surface of the host tibia for a period of time. Here it was invaded by new host bone. "Os novum" was then used as a transplant. Although Goff (1944) and Orell (1952) have reported excellent results with the use of these substances, they have not been widely accepted.

Inclan (1942) used refrigerated homografts and heterografts that were preserved in citrated blood plasma, saline, or blood. Good results were obtained in 74.4 per cent of 52 cases. The use of this method of preservation was modified by Tucker (1953) who stored human cadaverous bone in plasma to which sulfathiazol and penicillin had been added. The bone was held at 5 C for up to 11 months with satisfactory results. Tucker stated that these grafts contained viable osteocytes. Their healing rate was clinically comparable to that of fresh autografts.

Haas (1957) experimentally compared frozen homografts and fresh autografts with homografts which were refrigerated in citrated blood or serum. The superiority of the autogenous bone and the inferiority of the frozen homograft were shown. Homografts which had been stored in citrated blood healed more slowly than autografts but did show cellular proliferation when placed in extrasketal sites.

The early work of Tucker (1953) with plasma stored homogenous grafts stimulated continued investigation of the plasma storage technique. This led to the development of cultured calf bone as a heterografting material (Tucker 1956). Tucker (1956), found that when calf bone was stored in bovine plasma at 40-45 C for 6 weeks or more the osteocytes



appeared to retain their viability for at least 6 months. At the same time the graft seemed to lose its antigenic characteristics.

Experimentally, cultured calf bone was superior to frozen homografts. Tucker (1956) used cultured calf bone successfully in 22 orthopedic cases. Similar clinical results were obtained by other workers (Clayton 1956).

Bush (1947) and Wilson (1947) found that freezing was a suitable method for storing bone. As a result of these reports many bone banks were formed. An adequate supply of suitable bone for all grafting procedures was then possible.

Weaver (1949) used frozen cadaverous bone in 46 patients. The grafts had been stored an average of 134 days. The surgery consisted of massive inlays, pegs, dual onlays and Phemister type grafts. The results indicated that homogenous frozen bone was satisfactory under clinical conditions.

Herbert (1951) stored homografts and heterografts at temperatures below -30 C. He found little difference between autogenous, homogenous, and heterogenous transplants of bone.

Wilson (1951a, 1951b) was successful in 85 per cent of 284 homograft operations. He concluded:

1. With careful technique homogenous bone grafts may be preserved for surgical use.
2. Such grafts are well tolerated by human tissues and the risk of infection is no greater than with fresh

autogenous grafts.

3. The healing of such grafts takes place by a process of invasion, absorption, and replacement similar to that of autogenous bone grafts.
4. The results obtained are identical to those of autogenous grafts, except that in some instances the healing appears to be a little slower.
5. The operation of a bone bank is safe and practical. It offers great advantages to the patient and the surgeon from the standpoint of availability, abundance, and surgical economy.

The freeze-drying method for preserving bone was developed by Kreuz et al. (1951). He stated that this process minimized the protein denaturization, the dehydration, and the harmful concentration of salts. After freeze-drying, these grafts may be stored at room temperature for several months. Experimental work showed that freeze-dried bone was slower to incorporate than autogenous bone but more rapid than simple frozen bone. The superiority of freeze-dried grafts over frozen bone was also reported by Turner et al. (1955) with canine homografts.

Carr and Hyatt (1955) reported the healing rate of 125 freeze-dried homografts was as rapid as with fresh autografts.

Reynolds and Oliver (1949) discussed the practicability and efficiency of storing homogenous bone in a merthiolate solution. The



advantages were simplicity, low cost of preparation, asepsis of the graft, and excellent toleration by the host.

Reynolds et al. (1951) observed no allergic reaction in 212 cases where merthiolate stored grafts were used. The incidence of infection was lower than in autograft procedures. Still, a 30 per cent higher failure rate was reported with the merthiolate stored bone than with autografts.

Over 100 experimental and clinical reports comparing homografts, autografts, and heterografts were found. The evaluation of these works is difficult since many variable factors complicate comparison. Some of the variable factors noted were: (1) host species, (2) types of bone, (3) recipient sites, (4) surgical techniques, (5) graft storage method, (6) presence or absence of periosteum, and (7) final results. Most reports agreed that fresh autogenous bone was superior to fresh or stored homografts and heterografts (Reynolds and Oliver 1950, Ray et al. 1952, Campbell et al. 1953, Bonfiglio 1958, Chalmers and Sisson 1959, Heiple et al. 1963).

A histologic study of cortical transplants of fresh autografts, merthiolate-stored homografts, frozen homografts and boiled homografts was performed by Reynolds and Oliver (1950). They found that autografts healed more rapidly in the early stages but by 10 weeks homograft and autograft healing was identical.

Chalmers and Sisson (1959) reported fresh autogenous bone grafts were superior to frozen, autoclaved, freeze-dried, deproteinized, decalcified and freeze-dried irradiated homografts.

Ray et al. (1952) studied the cellular survival of fresh autografts, fresh homografts, frozen homografts and embryonic bone placed in the anterior chamber of the eye. Only the cells of fresh autografts and embryonic homografts survived and produced new bone.

Campbell et al. (1953) placed fresh autografts, fresh homografts, boiled homografts, "os purum", air preserved frozen autografts, air preserved frozen homografts in contact with an intact rib. All 141 grafts united strongly into the host bed. Fresh autografts were superior and fused more rapidly than the others. A foreign body reaction was found with all homografts.

Ray and Holloway (1957) observed organic bone matrix healed into bone defects more rapidly than either inorganic bone salts or frozen homogenous bone.

Holmstrand (1957) applied the techniques of microradiography, x-ray micro diffraction autoradiograph and polarized light in a comparison of autograft and homograft healing. These demonstrated that the most rapid absorption and replacement of minerals was in autografts.

Bonfiglio (1958) reported pronounced inflammatory reaction around preserved homografts. The union of the homograft and host bone was slightly retarded.

Brav (1953) had 84 per cent success with 75 autografts and 75 frozen homografts used in fresh fractures, major arthrodesis, nonunion and delayed union. The healing rate was equal.

Kawamura (1958) reported 303 cases where bone grafts had been used. The success rate was 81.8 per cent with fresh autografts and 86.3 per cent with frozen homografts.

Carnesale and Spankus (1959) reviewed their clinical experience with 143 homografts and 137 autografts and 24 mixed operations. The success rate was 88 per cent with autografts, 77 per cent with homografts and 79 per cent with mixed. They stated that homogenous bone should be used only in cases where there was an insufficient supply of autogenous bone.

#### Synthetic Bone Substitutes

The use of synthetics in bone surgery has been limited. Scales (1953) discussed the requirements necessary for any synthetic substance used as a substitute for bone. These requirements were:

1. It must be biologically compatible both immediately and for the life of the patient.
2. It must be readily invaded and replaced by the host bone and not interfere with healing.
3. It must not interfere with the blood supply to the area.
4. It must not stimulate fibroplasia or electrolysis.

Reynolds and Ford (1953) placed tricalcium phosphate soaked gelfoam in bone defect. These had no effect on healing of the defect.

Calcined calcium sulfate was used by Peltier and Orn (1958) to fill the defects between homografts and host bone. This substance did not inhibit healing or produce a foreign body reaction. A higher percent of homograft union was noted in cases where the plaster of paris was used. A plaster of paris-epoxy resin mixture proved incompatible with tissues (Gourley and Arnold 1960).

Cobey (1956), after extensive experimentation, was unsuccessful in developing satisfactory synthetic bone.

Mandarino and Salvatore (1959a) claimed polyurethane polymer fulfilled the requirements for a practical bone filling substance. It was recommended for the treatment of bone cysts and fractures. They reported polyurethane was easy to apply, nontoxic, light weight, strong, adhesive and was readily replaced by bone. In 220 human cases in which this substance was used 93 per cent were successful.

Examination of the experimental results (Mandrina and Salvatore 1959b) in the dog revealed that about 25 per cent of the plastic was still present at the end of one year.

Bloch (1958) used an ethoxlyline resin in conjunction with an amine curing agent to immobilize fractures in sheep and man. Good results were obtained in 16 of 25 sheep and six humans. Endosteal and periosteal bone growth and invasion of the plastic was observed.

### Effect of Medicinal Agents on Bone Repair

Estrogen. Early investigators demonstrated extensive endosteal new bone formation in birds was associated with high levels of estrogen (Kyes and Potter 1934, Pfeiffer and Gardner 1938, Gardner 1940). Similar changes were described in mice (Urist et al. 1950). Increased subperiosteal and medullary density also has been noted (Pfeiffer and Gardner 1938, Edgren and Calhoun 1956).

The increase in endosteal bone noted in rats given estrogens was not due to new bone formation. Instead metaphyseal bone resorption was retarded (McLean and Urist 1955).

Pollock (1940) reported a slight stimulation of fracture healing in rats treated with estrogen.

Brush (1945) working with rats, and Moffatt and Francis (1955) with rabbits, found stilbestrol retarded fracture healing.

Estrogens have been used clinically in cases of senile and post menopausal osteoporosis (Reifenstein and Albright 1947).

Cortisone. The effect of cortisone on bone is not fully understood. Animals subjected to high levels of cortisone showed bone resorption and inhibition of bone growth (Follis 1951, Storey 1957, 1958a, 1958b).

Blunt et al. (1950) demonstrated retarded healing of fractures in cortisone treated rabbits. Poor organization, vascularization and

resorption of the hematoma were found.

Sisson and Hadfield (1951) reported that the antianabolic effect of cortisone was responsible for retarded fracture healing in rabbits. They found that the cartilage cells of the callus degenerated rather than matured.

Fontaine et al. (1954) speculated that corticosteroids interfered with phosphatase metabolism at the fracture site.

Kowalewski and Lyon (1958) demonstrated that the uptake of radioactive sulphur in fractured bones was significantly inhibited by cortisone treatment.

Curtiss and Wilson (1953) compared the healing of homogenous bank bone and fresh autogenous bone in dogs with and without cortisone treatment. Cortisone was given at the rate of 1 mg. per pound body weight once a day. This drug inhibited healing at two and four weeks after grafting. From six to 16 weeks no difference could be observed.

Key et al. (1952) reported no retardation of fracture healing in cortisone treated rats or humans.

Isoniazid. Isoniazid is a drug used in the treatment of tuberculosis. Pulmonary lesions and draining bone sinuses appeared to heal more rapidly after treatment with this drug (Spain 1953, Wilkinson 1954).

Spain (1953) found isoniazid effectively reduced inflammation but did not stimulate skin healing.

Martyn and Campbell (1963) experienced clinical results with isoniazid in cases of wounds and indolent ulcers. They postulated that isoniazid stimulated the growth of normal granulation, increased the tensile strength of scars and was effective against antibiotic resistant organisms.

No reports dealing with isoniazid in bone healing were found.

## MATERIALS AND METHODS

### Experimental Animals

Twenty-four male dogs were used in this investigation. All were between two and four years of age. They were of mixed collie or hound breeding. Clinical examination showed these dogs to be free from disease.

### Preparation of Grafts

The bone homografts were obtained from a healthy one year old male mongrel. The animal was euthanatized. The skin over both femurs was prepared for aseptic surgery by clipping the hair, washing the skin with soap and water, defatting the surface with ether, and disinfecting the area with alcoholic-roccal. The cadaver was then moved to the operating room where the femoral shafts were removed surgically. These were cut into grafts which were approximately one centimeter square. Care was taken not to remove the periosteum.



One-half of the grafts were placed in empty sterile tubes and sealed. They were immediately placed in a freezing unit at  $-16^{\circ}\text{C}$  and held at this temperature for at least 30 days before they were used as frozen stored homografts.

The remaining grafts were placed in sterile tubes which contained a plasma citrate solution. These grafts were stored in an ordinary refrigerator at  $5^{\circ}\text{C}$  for at least 30 days before they were used as plasma stored homografts.

The plasma citrate solution was prepared in the following manner:

1. Five hundred cubic centimeters of blood were drawn from three healthy mongrel dogs. This was mixed with a standard anticoagulant solution.<sup>1</sup>
2. This mixture was stored at room temperature for three days to allow settling of the cellular elements.
3. The plasma was decanted from the blood cells and pooled.
4. The pooled plasma was diluted to a 20 per cent solution with sterile physiologic saline and stored at  $5^{\circ}\text{C}$ .
5. The sterility of the solution was checked by inoculation of thioglycolate broth. This was incubated at  $37^{\circ}\text{C}$  for seven days before it was considered negative for bacterial growth.

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<sup>1</sup>A.C.D.<sup>R</sup>, Abbot Laboratories, Chicago, Illinois.



6. When the solution was found to be sterile 1,5000 mg. of tetracycline and 150,000 units of mycostatin were added to one liter of the 20 per cent plasma solution.
7. The solution was added to sterile tubes just prior to collection of the grafts. The tube contained at least four times as much plasma as bone.
8. The sterility of each tube was determined before the graft was used.

Heterogenous bone was obtained from the metacarpus of a 200 pound calf within one hour after death. This bone was cut into strips six centimeters long and one centimeter wide. The strips were placed in tubes and autoclaved for 20 minutes at 20 pounds pressure. Storage was at room temperature.

Autogenous bone was harvested at the time of grafting. A skin incision was made over the medial side of the proximal one-third of the tibia. A piece of the tibial cortex about eight millimeters square was removed from the flat surface medial to the tibial crest. The periosteum was not removed from the graft.

Polyurethane foam<sup>1</sup> was supplied in two bottles. One contained the catalyst and the other the prepolymer. These were autoclaved for five minutes and allowed to cool to 150 C. These were then mixed in a ratio

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<sup>1</sup>Ostamer<sup>R</sup>, Wm. S. Merrell Co., Cincinnati 15, Ohio.

of one part catalyst to four parts prepolymer. After stirring for about two minutes the mixture became a uniform milky color. The plastic was then spooned into the dry bone defect. The plastic was allowed to expand and harden. In about 10 minutes the excess ostamer was trimmed with scissors.

#### Surgical Procedure

The animals were anesthetized with intravenous sodium pentobarbital. The skin over both femurs and the left tibia was prepared for surgery in the manner described for the homograft collection.

Aseptic technique was employed in all surgical procedures.

The femoral cortex was exposed by the standard lateral approach (Leonard 1960).

Three circumscribed cortical bone defects six millimeters in diameter were drilled in the lateral cortex of each femur. These were about one and one-half centimeters apart. Drilling was done slowly with a hand drill to prevent heat necrosis of the surrounding bone. Bone chips were removed and the defects were flushed with saline.

The defects in the left femur were then filled with inlay bone grafts of plasma stored homogenous bone, frozen stored homogenous bone and autoclaved bovine bone.

The defects in the right femur were filled with polyurethane foam and autogenous bone. One defect was not filled.

The muscle closure was with simple interrupted sutures of ophthalmic catgut. The skin was reapposed with 32 gage monofilament stainless steel wire.

All animals received 300,000 units of penicillin and .25 grams of streptomycin once a day for three days.

#### Medicinal Procedure

After surgery the dogs were placed in one of four groups.

Group I was the control and received no medication.

Group II was given 25 mg. of diethylstilbestrol<sup>1</sup> intramuscularly immediately after surgery. This was repeated every seven days until euthanasia.

Group III received 20 mg. prednisilone<sup>2</sup> intramuscularly immediately after surgery and 5 mg. a day for 14 days.

Group IV received 100 mg. of isoniazid<sup>3</sup> orally once a day for 14 days.

One dog from each group was euthanatized at seven days, 14 days, 28 days, 42 days, 56 days and 84 days. The femoral shaft was immediately removed and examined grossly. This was then placed in 10 per cent buffered

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<sup>1</sup>Repositol Diethylstilbestrol, Pitman-Moore Company, Division of Allied Laboratories, Inc., Indianapolis, Indiana.

<sup>2</sup>Delta-cortef, Upjohn Company, Kalamazoo, Michigan.

<sup>3</sup>Nydrizid<sup>R</sup>, Squibb, New York 22, New York.

formalin. After three days the bone was radiographed using non screen film.

The excess bone was trimmed from the defects.

The bone was decalcified in nitric acid, cut longitudinally, and embedded in paraffin. Sections were cut to 6 microns and were stained with hematoxylin and eosin.

#### EXPERIMENTAL RESULTS

##### Gross and Radiographic Examination

No information about the healing of the canine bone defects was gained from the gross and radiographic examination of the tissues.

##### Healing in the Drug Treated Animals

The histologic picture of bone and graft healing was not varied by treatment of the host with prednisilone, estrogen, or isoniazid.

##### Healing of the Unfilled Defect (Seven Days Postoperatively (Plate I, Figs. 1 and 2))

Defect. The most prominent feature at this stage of healing was the presence of a hematoma. This filled the surgical defect and the adjacent medullary space. The central one-third consisted of red blood cells loosely interwoven with fibrin strands. Peripherally fibroblasts almost entirely replaced the hematoma, leaving only small aggregations

of erythrocytes trapped within the newly formed granulation tissue. The organized mass was penetrated by many blood vessels, varying in size from fine capillaries to moderately large vessels. This picture was therefore one of acute granulation tissue.

Periosteum. Both the fibrous and cambium layers were thickened by cellular proliferation. Flump osteoblasts from the cambium layer lined the surface of the cortex but no new bone formation was evident.

Femoral cortex. Cortical necrosis was evident adjacent to the defect. There were no osteocytes in the lacunae. The blood vessels were empty. Osteoclasts were eroding the dead bone along the defect and in the Haversian canals. Inflammatory cells were absent.

Endosteum. Arising from the endosteal trabeculae and penetrating the edges of the organized clot were fine "finger like" projections of new bone. These trabeculae were about one-third the thickness of mature endosteal trabeculae. Large cuboidal osteoblasts covered their surface. The internal structure consisted of a loose fibrillar arrangement of lightly stained bone matrix which surrounded many large darkly stained osteocytes (Fig. 2).

Fourteen Days Postoperatively (Plate I,  
Figs. 3 and 4, Plate II, Fig. 5)

Defect. The hematoma was completely invaded by fibroblasts. The area below the endosteal surface was replaced by new bone.

Periosteum. The thickness of the periosteum was increased to about five times normal. The fibrous layer was complete over the bony defect and extended over the edges into the superficial one-half. The cambium layer was markedly proliferated. It formed several layers of cuboidal osteoblasts. These cells produced a loosely arranged cuff of immature bone along the periosteal surface of the femoral cortex. This resulted in elevation of the periosteum (Fig. 5).

Subperiosteal new bone formation extended some distance from the rim of the defect. The newly formed trabeculae were arranged at right angles to the long axis of the femur.

Some of the subperiosteal bone grew into the cortical defect and toward the endosteal osseous callus. This was not a major factor in the healing process.

Femoral cortex. Osteoclastic resorption of the necrotic bone along the edges of the defect and in the vessels of the old Haversian systems continued. This process produced large pits in the cut surface of the femoral cortex. New bone from the endosteum was laid down in some of these cavities and canals. This process produced a cement line at the junction of new and old bone.

Endosteum. The endosteal new bone increased to about four times the amount seen at seven days. The trabeculae formed a lattice work of new bone containing large marrow spaces. Callus bridged the gap on the endosteal surface but extended only slightly into the defect.

Endosteal trabeculae were loosely arranged perpendicular to the surface of the femur.

Twenty-eight Days Postoperatively (Plate II, Figs. 6 and 7)

Defect. A small amount of granulation tissue still remained. This was in the center of the defect directly under the periosteum.

Periosteum. The thickness was not increased from 14 days. New bone from the cambium layer became more dense due to the deposition of bone matrix on the surface of the trabeculae.

Subperiosteal bone grew over the edges of the defect and down along the sides to the endosteal callus. At that point, fusion was noted. It was at this stage difficult to distinguish endosteal from subperiosteal callus.

Femoral cortex. Union of the endosteal and subperiosteal new bone to the host cortex was evident along the margins of the defect. Vascularization and erosion of the cortex in these areas was noted.

Endosteum. Endosteal new bone filled about one-half of the defect. The other half was filled by the fibrous layer of the periosteum. Bone matrix was deposited on the surface of endosteal trabeculae making the new bone more dense than at 14 days.



Forty-two Days Postoperatively (Plate II, Fig. 8  
and Plate III, Fig. 9)

Defect. The granulation tissue was replaced by new bone.

Periosteum. The fibrous layer was of normal thickness except over the defect where it was about four times normal. It filled the central portion of the defect at that point.

The cambium layer was proliferated. Bone produced by this structure contributed only about one-tenth of the total mass of new bone. The junction of subperiosteal new bone with endosteal new bone was marked by a cement line.

Femoral cortex. The bone at the margin of the defect was highly vascularized. Attachments of new bone to the defect wall were more numerous and solid than at 28 days. In some areas only the cement line marked the edges of the defect. This cement line was penetrated by many vessels which eroded both new and necrotic bone.

Endosteum. Endosteal bone filled nearly two-thirds of the femoral defect. This bone was dense and lamellated. Osteoblasts deposited new bone within the marrow spaces nearly obliterating them. Only a vascular space remained.

The general orientation of the large trabeculae was transverse to the long axis of the femur.



Fifty-six Days Postoperatively (Plate III, Figs. 10 and 11)

Periosteum. There were no significant changes from 42 days observations.

Femoral cortex. There were no significant changes from 42 days observations.

Endosteum. The trabecular pattern was at approximately a 110 degree angle to the long axis of the femur.

Eighty-four Days Postoperatively (Plate III, Fig. 12  
and Plate IV, Fig. 13)

Periosteum. The fibrous layer of the periosteum was normal thickness except over the cortical defect where it dipped into the outer one-fifth. This left a crater-like defect in the cortex.

The cambium layer was inactive.

Femoral cortex. The vessels in the area of the cement line were more numerous and larger than in previous sections. Much of the cement line and dead femoral cortex was eroded by these vessels.

Endosteum. The excess endosteal callus below the defect had been resorbed leaving only a few large trabeculae within the medullary canal.

Healing of the Autogenous Bone Graft Filled Defect  
(Seven Days Postoperatively)

Defect. Hematoma filled all interspaces between the graft and host cortex as well as the medullary canal beneath. The portions of the clot between the graft and host bone were replaced by fibroblasts as was the periphery of the clot below.

In these connective tissue areas arteries, veins, and capillaries were numerous.

Periosteum. The fibrous layer was complete over the surface of the graft. Proliferation of the osteogenic layer produced an increased number of large cuboidal cells over the host cortex and graft. New bone formation was absent.

Femoral cortex. The lacunae were devoid of cells for a variable distance away from the margin of the defect.

Graft. The appearance of the graft was similar to the appearance of the margins of the femoral cortex. There were no cells in the lacunae. Most of the vessels of the haversian canals were degenerate although a few large ones appeared functional.

Endosteum. The surface of the mature trabeculae of the medullary canal were covered with active osteoblasts. This new bone extended away from the parent trabeculae and penetrated the granulation tissue surrounding the clot. The new bone was morphologically similar to that seen in the unfilled defect at seven days.

Fourteen Days Postoperatively (Plate IV, Fig. 14)

Defect. The hematoma was almost entirely replaced by granulation tissue. This produced a fibrous callus which united the host cortex and graft.

Periosteum. The fibrous layer was slightly thickened near the edge of the defect. The cambium layer proliferated and formed several layers of active osteoblasts. These cells produced loosely arranged trabeculae of new bone which were deposited on the surface of the host and graft. This thin layer of bone bridged the defect over the graft and extended peripherally for some distance.

Femoral cortex. There were some areas of mild osteoclastic activity along the edges of the defect and in the haversian canals. New bone deposition followed the osteoclastic resorption and filled the cavities produced.

Graft. The autograft was undergoing changes similar to those seen in the necrotic host cortex.

Endosteum. New endosteal trabeculae were more numerous than at seven days. They permeated the granulation tissue remnants of the hematoma and extended into the spaces between the graft and defect margins. They grew toward the periosteum and in a few places united with subperiosteal new bone, host cortex and graft.

The trabecular pattern was lacy and contained many marrow spaces.

Twenty-eight Days Postoperatively (Plate IV, Fig. 15)

Defect. The organized hematoma was completely replaced by new bone which filled the defect. The new trabeculae were perpendicular to the long axis of the femur.

Periosteum. The fibrous portion was normal in thickness. The cambium layer consisted of proliferating osteoblasts. The new bone produced by these osteoblasts made a thin plate of dense bone which formed a continuous layer over the graft and the edges of the host defect. Only a small amount grew toward the endosteal surface in the space between the graft and host. A cement line separated the subperiosteal bone from endosteal and cortical bone.

Femoral cortex. Osteoclastic bone resorption produced marked erosion of the haversian lamellae. Some of these were vascularized.

Graft. Changes similar to those in the host cortex were evident in the autograft.

Endosteum. Endosteal new bone was more dense than at 14 days due to the deposition of bone matrix on its surface. It filled the small gaps between the edges of the graft and host cortex. Trabeculae branched to the junctional and endosteal surfaces of the host and graft and solidly adhered to both. A cement line was produced by this union.

The trabeculae were at right angles to the long axis of the femur.

Forty-two Days Postoperatively (Plate IV, Fig. 16)

Defect. Similar to 28 day sections.

Periosteum. No significant difference from the 28 day observations.

Femoral cortex. There was marked vascularization of the cortical margins of the bone defect. Osteoclasts eroded the necrotic bone adjacent to the defect. The pits thus formed were filled with new endosteal bone. Only a small portion of the necrotic bone was replaced.

Graft. About one-fifth of the autograft was eroded by osteoclasts and replaced by new endosteal bone. Vascularization of the autograft was moderate in amount.

Endosteum. One-fifth of the graft and small portions of the host cortex adjacent to the defect were replaced by endosteal new bone. This produced a solid union of endosteal new bone to host cortex and graft. There was no orderly pattern of bone replacement. Instead, it continued in a random manner.

Fifty-six Days Postoperatively (Plate V, Fig. 17)

Defect. No change from 42 days.

Periosteum. No change from 42 days.

Femoral cortex. Vascularization and osteoclastic erosion of necrotic bone was more marked.

Graft. About one-half of the transplant was replaced by new bone. The process was irregular and disorderly.

Endosteum. The trabeculae in the space between the host and graft were reorientated nearly parallel to the long axis of the femur.

Eighty-four Days Postoperatively (Plate V, Fig. 18)

Defect. No change from the 42 day was noted.

Periosteum. No change from the 42 day was noted.

Femoral cortex. The necrotic bone was nearly replaced by new bone.

Graft. The bone transplants were almost completely replaced by new bone. Only small islands of necrotic graft remained within the callus of new bone. Vascularization was marked.

Endosteum. The new bone was almost entirely endosteal in origin. It was more vascular and more loosely arranged than the host cortex but the lamellar pattern was similarly oriented.

Healing of the Plasma Stored Homograft Filled Defect

The healing processes and rate were identical in sections of fresh autografts and plasma stored homografts.

Healing of the Frozen Stored Homograft Filled Defect

The healing processes and rate were identical in the sections of the fresh autograft and frozen homograft.

### Healing of the Autoclaved Calf Bone Filled Defect

The general pattern of the healing process was similar to that of the fresh autograft. Therefore, only differences will be reported here.

#### Seven Days Postoperatively

Defect. No visible change from the seven day autograft section was noted.

Periosteum. No visible change from the seven day autograft sections was noted.

Femoral cortex. No visible change from the seven day autograft sections was noted.

Graft. The graft fragments appeared more basophilic when stained with hematoxylin and eosin than were the host cortex or the fresh autograft. All organic elements were absent having only a "moth eaten" network of mineral containing lamellae.

Endosteum. There was no visible difference from the 7 day autograft sections.

#### Fourteen Days Postoperatively (Plate V, Fig. 19)

Defect. There was no visible difference from the 14 day autograft sections.

Periosteum. There was no visible difference from the 14 day autograft sections.



Femoral cortex. There was no visible difference from the 14 day autograft sections.

Graft. The graft was surrounded by a dense layer of connective tissue which prevented any bone attachments to the graft. Many leucocytes and multinucleated giant cells were seen in this granulation tissue. Osteoclasts were more numerous and active than around the autograft.

Endosteum. There was no visible difference from the 14 day autograft sections.

Twenty-eight Days Postoperatively (Plate V, Fig. 20)

Defect. There was no visible difference from the 28 day autograft sections.

Periosteum. There was no visible difference from the 28 day autograft sections.

Femoral cortex. There was no visible difference from the 28 day autograft sections.

Graft. A thin layer of dense connective tissue "walled off" the graft. The graft was very porous due to marked osteoclastic erosion. Leucocytes aggregated around the edges of the graft.

Endosteum. Invasion of the connective tissue mass by new bone was similar to that seen in the autograft but there were no boney attachments to the graft.



Forty-two Days Postoperatively (Plate VI, Fig. 21)

Defect. There was no visible difference from the 24 day autograft sections.

Periosteum. The only visible difference from the 24 day autograft sections was the increased thickness of the subperiosteal new bone layer which covered the graft and a portion of the host cortex.

Femoral cortex. There were no visible differences from the 24 day autograft sections.

Graft. New bone surrounded the graft and invaded it in a few places on the endosteal surface. Vascularization and erosion were more marked than in the autograft, although replacement by new bone was retarded. There was no solid union of the graft.

Endosteum. Endosteal new bone resembled that of the autograft sections but no replacement of the graft was noted.

Fifty-six Days Postoperatively (Plate VI, Fig. 22)

Defect. There was no visible difference from the 56 day autograft sections.

Periosteum. New bone increased in density from the 42 day sections.

Femoral cortex. There was no visible difference from the 56 day autograft sections.

Graft. The connective tissue seen at 42 days was entirely replaced

by new bone from the endosteum and subperiosteum. A few endosteal trabeculae invaded the graft from the sides and below.

Endosteum. Endosteal new bone covered the junctional and medullary surfaces of the graft and adhered to its surface in a few places.

#### Eight-four Days Postoperatively (Plate VI, Fig. 23)

Defect. There was no visible difference from the 84 day autograft sections.

Periosteum. This layer increased in density from the 56 day sections.

Femoral cortex. There was no visible difference from the 84 day autograft sections.

Graft. Changes from the 56 day heterograft sections were a slight increase in vascularity and bony invasions of the graft.

Endosteum. There was no visible difference from the 84 day autograft sections.

#### Healing of the Polyurethane Foam Filled Defect

The general healing pattern was similar to that of the fresh autograft. Therefore, only differences will be reported here.

#### Seven Days Postoperatively

Defect. Polyurethane foam completely filled the cortical defect

preventing the formation of an organized hematoma. It mushroomed above the periosteal surface as well as filled the medullary canal.

Periosteum. There was no visible difference from the seven day autograph sections.

Femoral cortex. There was no visible difference from the seven day autograft sections.

Polyurethane foam. The plastic appeared as a light pink lattice work of fine homogenous acellular trabeculae. It was not vascularized.

Endosteum. Polyurethane foam filled the medullary canal and interfered with the endosteal proliferation below the defect. Some new bone formation was noted along the femoral cortex on either side.

#### Fourteen Days Postoperatively (Plate VI, Fig. 2h)

Defect. The defect was filled with polyurethane foam. No clot or connective tissue was present.

Periosteum. There was no visible difference from the 14 day autograft sections.

Femoral cortex. There was no visible difference from the 14 day autograft sections.

Polyurethane foam. The polyurethane foam was unchanged from the seven day sections. There were no connective tissue or bone invasion of the plastic.

Endosteum. The amount of endosteal new bone was increased from

seven days but did not invade the polyurethane foam or bridge the endosteal surface of the defect. It did, however, grow into the junctional space between the host cortex and plastic. There it covered the host bone with a thin layer of new endosteal bone.

Twenty-eight Days Postoperatively (Plate VII, Fig. 25)

Defect. There was no change from 14 day polyurethane foam sections.

Periosteum. There was no visible difference from the 28 day autograft sections.

Femoral cortex. There was no visible change from the 28 day autograft sections.

Polyurethane foam. There was no change from 14 day polyurethane foam sections.

Endosteum. Changes seen in the endosteum were similar to those seen in the 28 day autograft except there was only slight invasion of the plastic.

Forty-two Days Postoperatively (Plate VII, Fig. 26)

Defect. There was no change from 28 day polyurethane foam sections.

Periosteum. The subperiosteal new bone was much thicker than in autograft sections.

Femoral cortex. There was no visible difference from the 42 day autograft sections.

Polyurethane foam. No change from 28 day polyurethane foam sections.

Endosteum. There were only slight invasions of the plastic by endosteal new bone.

Fifty-six Days Postoperatively (Plate VII, Fig. 27)

Defect. There was no change from 42 day polyurethane foam sections.

Periosteum. There was no visible difference from the 56 day autograft sections.

Femoral cortex. There was no visible difference from the 56 day autograft sections.

Polyurethane foam. There was no change from 42 day polyurethane sections.

Endosteum. These sections were similar to the 56 day autograft except new bone did not invade the polyurethane foam.

Eighty-four Days Postoperatively (Plate VII, Fig. 28).

Defect. There was no change from 56 day polyurethane sections.

Periosteum. There was no visible difference from the 84 day autograft sections.

Femoral cortex. There was no visible difference from the 84 day autograft sections.

Polyurethane foam. A few boney trabeculae invaded the plastic but very little was replaced.

Endosteum. These trabeculae were similar to those of the autograft sections at 84 days except only a few invaded the graft.

## DISCUSSION

### Unfilled Defects

The present investigation showed the healing of circumscribed cortical defects in the dog was predominantly a function of the osteoblasts which covered the surface of the mature endosteal trabeculae. These cells formed new bone which ultimately filled the defect. The periosteum produced only a small portion of the final callus. These findings were similar to those of Ely (1927), Bourne (1944) and Melcher (1962). As far as is known, the present report is the only one describing the healing of circumscribed cortical bone defects in the dog.

Ely (1927) reported on five cats with cortical defects. The stage of trabecular maturity and defect healing was comparable to similar sections in the dog. Rat (Melcher 1962) and guinea pig (Bourne 1944) defects healed much more rapidly than those of the dog. At 21 days these defects were equivalent to the 84 day canine defects.

Ely (1927) believed that new bone was formed from the cut ends of the cortex adjacent to the defect. This was not observed in the canine defects. The endosteal new bone migrated into the defect and fused with the bone at the edges.

#### EXPLANATION OF PLATE I

- Fig. 1. Unfilled defect 7 days postoperatively, H and E, 10x.
- Fig. 2. Unfilled defect 7 days postoperatively, H and E, 45x.
- Fig. 3. Unfilled defect 14 days postoperatively, H and E, 10x.
- Fig. 4. Unfilled defect 14 days postoperatively, polarized light, 10x.



Fig. 1



PLATE I

Fig. 2

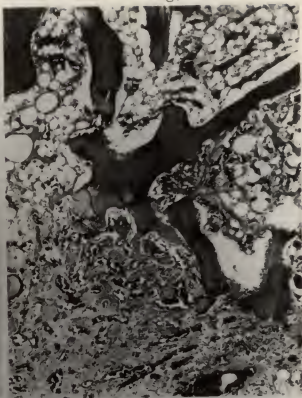


Fig. 3

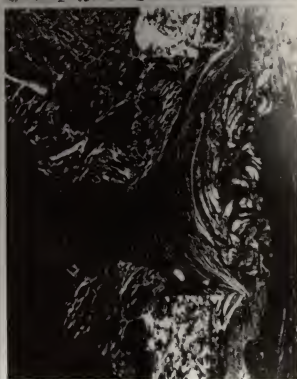


Fig. 4

#### EXPLANATION OF PLATE II

- Fig. 5. Unfilled defect 14 days postoperatively, H and E, 45x.
- Fig. 6. Unfilled defect 28 days postoperatively, H and E, 10x.
- Fig. 7. Unfilled defect 28 days postoperatively polarized light, 10x.
- Fig. 8. Unfilled defect 42 days postoperatively, H and E, 10x.

Fig. 5



Fig. 6

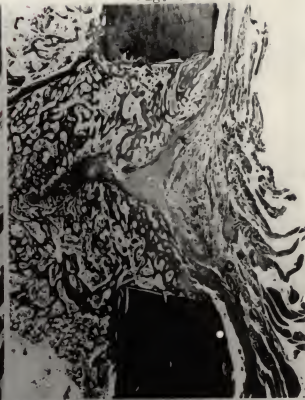


Fig. 7

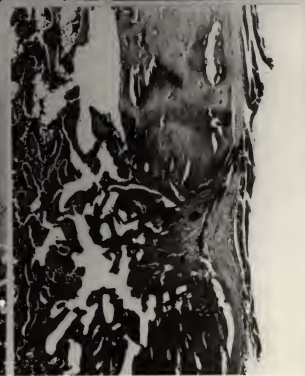


Fig. 8

EXPLANATION OF PLATE III

- Fig. 9. Unfilled defect 42 days postoperatively, polarized light, 10x.
- Fig. 10. Unfilled defect 56 days postoperatively, H and E, 10x.
- Fig. 11. Unfilled defect 84 days postoperatively, polarized light, 10x.
- Fig. 12. Unfilled defect 84 days postoperatively, H and E, 10x.

Fig. 9



Fig. 10

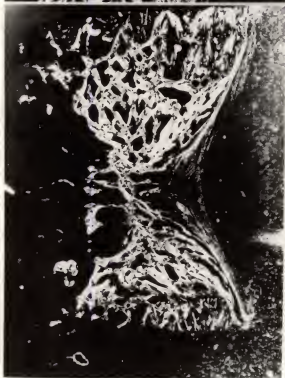


Fig. 11

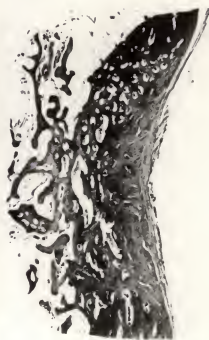


Fig. 12

EXPLANATION OF PLATE IV

- Fig. 13. Unfilled defect 84 days postoperatively, polarized light, 10x.
- Fig. 14. Autograft 14 days postoperatively, H and E, 10x.
- Fig. 15. Autograft 28 days postoperatively, H and E, 10x.
- Fig. 16. Autograft 42 days postoperatively, H and E, 10x.



Fig. 13



Fig. 14



Fig. 15



Fig. 16



EXPLANATION OF PLATE V

- Fig. 17. Autograft 56 days postoperatively, H and E, 10x.
- Fig. 18. Autograft 84 days postoperatively, H and E, 10x.
- Fig. 19. Autoclaved heterograft 14 days postoperatively, H and E, 10x.
- Fig. 20. Autoclaved heterograft 28 days postoperatively, H and E, 10x.

## PLATE V

Fig. 17



Fig. 18



Fig. 19



Fig. 20

EXPLANATION OF PLATE VI

- Fig. 21. Autoclaved heterograft 42 days postoperatively, H and E, 10x.  
Fig. 22. Autoclaved heterograft 56 days postoperatively, H and E, 10x.  
Fig. 23. Autoclaved heterograft 84 days postoperatively, H and E, 10x.  
Fig. 24. Polyurethane foam 14 days postoperatively, H and E, 10x.

Fig. 21



Fig. 22

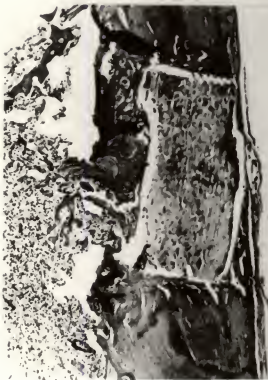


Fig. 23



Fig. 24

EXPLANATION OF PLATE VII

- Fig. 25. Polyurethane foam 28 days postoperatively, H and E, 10x.  
Fig. 26. Polyurethane foam 42 days postoperatively, H and E, 10x.  
Fig. 27. Polyurethane foam 56 days postoperatively, H and E, 10x.  
Fig. 28. Polyurethane foam 84 days postoperatively, H and E, 10x.

## PLATE VII

Fig. 25



Fig. 26



Fig. 27



Fig. 28

Although Melcher (1962), Ely (1927) and Bourne (1944) reported complete filling of the boney defect, it was not observed in the present investigation. At 84 days when the periosteal callus was complete, and the endosteal callus had undergone extensive remodeling, a depression remained in the center of the defect. Such a depression is clinically significant, as it is a weakened area in the bone cortex and predisposes to cyst formation.

Melcher (1962) found cartilage formation as an intermediate stage in defect healing of rats. In the dog, there was no cartilage in the healing defects.

The healing of unfilled cortical defects was in contrast to the classical descriptions of bone healing in which the periosteum was responsible for the bridging, sealing and uniting portions of the callus. In defects the endosteum performs these functions. The reason for the reported difference is unknown. Melcher (1962) hypothesized a drilled defect was a weaker stimuli to the periosteum than an actual fracture. The superior immobilization of a drilled defect would seem to be a major factor in dampening periosteal response and promoting rapid endosteal callus formation.

There was no evidence of metaplasia or independent bone formation from undifferentiated connective tissue. Instead, all bone appeared to arise from either the osteoblasts of the endosteum or the cambium layer of periosteum.



### Filled Defects

The host reaction peripheral to the filled defects was identical to the reaction noted in the unfilled defects. This process produced incorporation and replacement of the transplants and polyurethane foam. These filling substances functioned mechanically. They provided immediate filling of the defect and formed a scaffold through which the new bone grew. Only the time of incorporation into the host and replacement by new bone varied.

Many investigators (Phemister 1914, Ham and Gordon 1952, Campbell et al. 1953, Axhausen 1956, Haas 1957, Bonfiglio 1958) believe that at least some of the osteogenic cells of the fresh living autograft survived transplantation and were active in the production of new bone. This was not observed in the present investigation. Most lacunae were devoid of cells by seven days and all were empty by 14 days. Similarly, the osteoblasts of the transplanted periosteum and endosteum were absent in histologic sections. There were no osteogenic areas in association with the grafts. New bone arose primarily from the host endosteum and to a lesser degree perisosteum. This new bone extended around and into the transplants. These results are in agreement with those of Reynolds and Oliver (1950), Urist and McLean (1952), Hutchinson (1952), Urist et al. (1958), Chalmers and Sisson (1958).

Experimental (Campbell et al. 1953, Stringa 1957, Bonfiglio 1958,

Chalmers and Sisson 1959, Heiple 1963) and human clinical (Brav 1953, Carnesale and Spankus 1959) studies report the superiority of fresh autografts over fresh or stored homografts. Reynolds and Oliver (1950) and Wilson (1951a, 1951b) found that early healing was slightly more rapid and orderly in autografts than homografts but by 10 weeks no difference was noted. In the present study it was impossible to distinguish any gross or histologic difference in the healing rate or processes of fresh autogenous, frozen homogenous, or plasma stored homogenous grafts even in the early postoperative sections.

Foreign body reactions around homografts as reported by Campbell et al. (1953) and Bonfiglio (1958) were absent.

Although it is generally assumed that bone heterografts are inferior to both autografts and homografts, many reports, however, claim excellent results with specially treated or stored heterografts (Gallie and Robertson 1918, Goff 1944, Orell 1952, Guilleminet 1953, Fischer and Clayton 1955, Tucker 1956, Temizer 1956, Clayton 1956, Hurley and Rosomonoff 1958).

Boiled heterografts in this investigation were incorporated and replaced much more slowly than homografts but the new bone formation and maturation around the graft was the same. The grafts seemed to repel the new bone trabeculae. Osteoclasts were most numerous adjacent to the bovine graft. Giant cells indicating a foreign body reaction were noted.

At 12 weeks only slight invasion of the transplant was noted, in the heterografts, whereas at that time the autografts and homografts were almost entirely replaced.

Tucker (1953) reported living osteocytes in bone grafts which were stored in homogenous plasma for up to 28 months. He also expressed the opinion that this storage period made the graft more compatible with the tissues of the host. The present study indicated that plasma and frozen stored homografts were tolerated as well as autografts. No cells were present in the lacunae or on the endosteal or periosteal surface of the graft.

This plasma storing method is adequate for the storage of bone homografts in the dog. The advantages over frozen homografts are:

- (1) they are softer and more easily shaped, (2) no freezer is needed,
- (3) no thawing is necessary, (4) unused bone can be restored.

Polyurethane foam was completely walled off by host connective tissue and new bone. It was only rarely invaded at 12 weeks by a new bone trabeculae. Bone does not, as was suggested (Mandrino and Salvatore 1959a), "grow through the plastic as would be expected with an autograft." This substance retarded defect healing. Mandrino and Salvatore (1959b) previously reported that the plastic was about 75 per cent replaced by new bone at the end of 12 months. They did not study the early healing processes. In the present investigation, it was noted that frozen and plasma stored homografts were comparable to fresh autografts.

All autografts and homografts were superior to the unfilled defect because they were quickly incorporated and replaced without retarding the healing rate. These grafts also provided a framework for new bone formation and prevented the formation of a "dimple like" depression in the host cortex.

Homografts in this study were as useful as fresh autografts. They had the following advantages: (1) being readily available; (2) saving the patient and the operator a second surgical procedure. The two storing methods outlined here are practical for the veterinarian with a large surgical practice.

The unfilled defect healed more rapidly than the boiled heterograft or the polyurethane foam.

There seems to be no clinical value in polyurethane foam or boiled bovine bone; in fact, they would seem to be contraindicated in the filling of cortical bone defects.

#### Effect of Drugs

Medication with prednisilone, estrogen, and isoniazid in the dosages used here neither stimulated or inhibited the healing rate. Administration of the three drugs did not result in variable healing time when defects were filled with fresh cortical autografts, plasma stored and frozen homografts, autoclaved calf bone or polyurethane foam.

Retardation of fracture healing in cortisone treated animals has

been reported by Blunt et al. (1950), Sisson and Hadfield (1951), Fontaine et al. (1954), and Kowalewski and Lyons (1958). Curtiss and Wilson (1953) had similar results in early healing of bone homografts and autografts. Cortisone appeared to inhibit organization of the clot vascularization, fibroplasia, and osteogenesis. None of these changes were noted in this study. This is in agreement with Key et al. (1952) who found no retardation of fracture healing in cortisone treated animals.

Reports of stimulated fibroplasia in wounds on patients who received isoniazid were found in the literature (Spain 1953, Wilkinson 1954, Martyn and Campbell 1963).

It was postulated that this drug might stimulate osteogenesis as well. This was not found to be true in this study.

Estrogenic hormones produce osteomyelosclerosis in a number of species (McLean and Urist 1955). Neither an increased healing rate nor an increased amount of endosteal new bone production was noted in this investigation.

#### SUMMARY AND CONCLUSIONS

Three circumscribed cortical bone defects were experimentally produced in the lateral cortex of each femur of 24 dogs. In each animal five of the six defects were filled with a different substance. The substances investigated were fresh autogenous bone, frozen stored homogenous

bone, plasma stored homogenous bone, autoclaved bovine bone and polyurethane foam. One defect was not filled.

The dogs were divided into four groups. One group was treated with prednisilone, one with estrogen, and one with isoniazid. One group was untreated and served as a control.

One dog from each group was sacrificed at seven, 14, 28, 42, 56, and 84 days. The femurs were then studied grossly, radiographically and histologically.

Based on these studies the following conclusions were made:

1) The healing of both filled and unfilled cortical bone defects was a function of the host bone.

2) A cartilage precursor was not found in any of the healing sections. Most of the callus was endosteal in origin. The periosteum produced only a small amount of the new bone.

3) The grafts functioned mechanically by filling the defect and furnishing a template for new bone growth. Autografts and homografts did not impede or stimulate the healing rate.

4) The site of the healed unfilled defect was marked by a central depression.

5) Cortical grafts of fresh autogenous bone, frozen stored homogenous bone, and plasma stored homogenous bone were united with the host and replaced at the same rate and by identical processes.

6) Autoclaved bovine bone and polyurethane foam prevented the healing of cortical defects for at least 84 days.

7) Prednisilone, estrogen, and isoniazid in the dosages used had no effect on the healing of filled and unfilled bone defects.



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THE EFFECT OF CORTISONE, ISONIAZID, AND ESTROGEN ON  
THE HEALING RATE OF BONE DEFECTS FILLED WITH  
VARIOUS BONE GRAFTING MATERIALS

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AN ABSTRACT OF A MASTER'S THESIS

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MASTER OF SCIENCE

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Although bone grafting is a widely used technique in human surgery, its applicability has not been fully investigated by the veterinary surgeon.

The present investigation was designed to determine the healing rate of unfilled cortical bone defects and similar defects filled with fresh autogenous bone, plasma stored homogenous bone, frozen stored homogenous bone, autoclaved bovine bone, and polyurethane foam. The effect of cortisone, estrogen and isoniazid on that healing rate was also studied.

Twenty-four male dogs of mixed breeding were secured for this study. They were anesthetized and the lateral cortex of both femurs surgically exposed. Three circumscribed defects, six millimeters in diameter, were drilled in each femoral shaft. One defect was not filled. The remaining defects were filled as follows: (1) fresh cortical autograft, (2) frozen stored cortical homograft, (3) plasma stored cortical homografts, (4) autoclaved bovine cortical heterograft, and (5) a synthetic bone filling substance polyurethane foam.

The dogs were then placed in four groups. Group I was given no medication. Group II was given 25 mg. of diethylstilbesterol (Repositol<sup>R</sup>) after surgery and at weekly intervals until euthanasia. Group III received 20 mg. prednisilone after surgery and 5 mg. each day for 14 days. Group IV was given 100 mg. of isoniazid once a day for 14 days.

One dog from each group was euthanatized at seven, 14, 28, 42, 56, and 84 days. The femoral shaft was removed, examined, and fixed in 10 per cent buffered formalin. The bone was radiographed. It was then decalcified in nitric acid, embedded in paraffin, cut to six microns and



stained with hematoxylin and eosin.

The unfilled and filled defects healed by identical processes. A hematoma filled the spaces around the graft and in the unfilled defect. This was invaded by blood vessels and loose connective tissue. No cartilage was formed. Instead, the osteoblasts of the endosteum proliferated and formed new bone trabeculae. These trabeculae united the graft to the host bone. In the case of the unfilled defect, this endosteal new bone restored the continuity of the cortex. The periosteum played only a minor role in the healing of these defects.

The autograft and homograft filled defect healed at the same rate. Their healing was superior to the unfilled defect. Cortical restoration was incomplete in the healed unfilled defect. A dimple-like depression remained centrally.

Autoclaved bovine bone and polyurethane foam prevented defect healing for the length of the investigation.

Prednisilone, estrogen and isoniazid neither stimulated nor inhibited the healing of filled or unfilled circumscribed cortical bone defects.