# WHOLE BONE TRANSPLANTATION OF THE PROXIMAL PORTION OF THE FEMUR USING A SELF-COMPRESSING PLATE

BY

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B.S., UNIVERSITY OF CONNECTICUT, 1966
D.V.M., MICHIGAN STATE UNIVERSITY, 1969

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A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

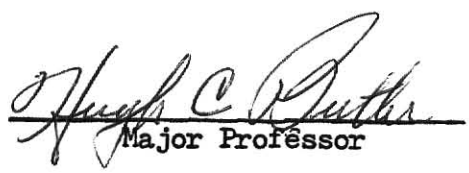
MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

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# INTRODUCTION

Very little information is available regarding whole bone transplantation of the proximal portion of the femur in the dog or man. Although many types of grafting techniques have been studied, little scientific attention has been devoted to the proximal femur.

Limited experience has indicated the results following the transplantation of an entire or proximal portion of a femur. Capurro and Pedemonte (1953)(30) reported the replacement of a cadaveric femur for a femur which had an extensive hydated cyst involvement. Only a ten month follow-up study was given. Poverman (1956)(138) reported on the transplantation of an entire fresh homograft femur for treatment of a persistent osteomyelitis. The results of a two year follow-up evaluation were encouraging. Krupko and Kachenko (1964)(106) reported on the replacement of the upper third of the femur in fifteen patients using semiarticular frozen homografts. No long term follow up studies were given. Smolik and Kubica (1965)(155) studied transplantation of homografts of the femoral heads in forty-three dogs. Within seven to twelve months following surgery evidence of healing and union were seen radiographically. Two and one-half years post operatively good functional results with no signs of degeneration were reported. Ottolenghi (1966)(127) transplanted a whole frozen femur to a thirty-eight year old woman whose entire femoral medullary cavity was affected by hydatid disease. A seven year follow-up radiographs revealed a non-union fracture of the femoral neck with necrosis of the femoral head. The patient was ambulatory.

It was the intent of this investigation to study the effect of transplantation of a whole bone graft of the proximal portion of the femur over an eighteen week period. Three different types of grafts were used (autograft, fresh allograft and frozen allograft). These grafts were studied radiographically and histopathologically and evaluated on the basis of their viability, and rate and nature of incorporation.

# REVIEW OF THE LITERATURE

## Introduction:

A vast amount of literature has been written on the transplantation of bone and cartilage. The review cited has been limited to certain specific areas which are relevant to the investigation to follow. The areas to be discussed include studies which are concerned with: 1. Joint transplants; 2. Cartilage transplants and grafts; 3. Osteocyte survival, induction and osteogenesis and 4. Freezing and immunogenicity.

## Joint Transplants:

Tietz (1902)(157) was the first person to perform an autogenous half-joint transplantation in man. His first procedure dealt with the replacement of a resected distal portion of the radius with the phalanx of the great toe. This same year, he performed three successful autogenous hemitransplants. Four years later, Judet (1908)(96) began his studies on half and whole joint transplantations. He was the first, using rabbits, to transplant whole and half joints with and without capsules to normal and heterotopic sites. The rabbits were euthanized at specific intervals and studied histologically. He reported the success of two cases of homologous knee joints into physiological sites. From his studies, Judet concluded that transplantation would be more successful if the joint with its capsule were used and placed into a normal physiological site.

In 1907, Lexer (1908)(111) performed the first transplantation involving an allograft. He took massive grafts of bone and joint

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from living patients and transplanted them directly after removal. He was (Lexer)(1908)(110) also the first to perform a whole joint transplant in man. In 1908, he reported the transplantation of five homologous whole joint (four knees and one elbow taken from fresh amputations) and four half-joint transfers. In one case, the knee joint was transplanted with its capsule. Shortly after surgery, the capsule became necrotic and the transplant failed. Good healing occurred in the other three knees with excellent mobilbity in two and limited mobilbity in one. He was able to follow two patients for one and one-half to two years, post operatively. Degenerative changes were observed within their knee joints even though good function persisted. By 1925, Lexer (1925)(109) had performed a total of twenty-three whole joint and eleven half-joint transplantations. He was able to follow twelve of these cases six to ten years (nine knee joints and three finger joints) post surgically. Several cases which received half knees had excellent function even though there was radiographic evidence of bone and joint degeneration. From his work, he concluded that transfer of a half-joint without its capsule is preferable to whole joint transfers. In 1929, Burkle-De La Camp (17) reported the histological findings on two of Lexer's whole knee joint transplantation fourteen and sixteen years post operative. Articular cartilage was lost and had been replaced by fibrocartilage. Intra-articular ligaments and menisci were no longer present. A capsule had formed around the joint with bony deposits and extensive marginal ridges lining this capsule. The articular space persisted.

There were others in the early 1900's who contributed to the knowledge of joint transplants. Buchmann (1908)(14) using a closed joint technique substituted an autogenous first metatarsophalangeal joint for an elbow joint. In 1911, Tuffier (1911)(159) evaluated Buchmann's cases and found the results obtained unsatisfactory. Impallomeni (1911)(94) performed eight whole knee joint and four tibiometatarsal transplants in fowl. Four of the former, and one of the latter was successful. Impallomeni also reported the transplantation of whole autogenous and homogenous joints in rabbits. In 1911, Dalla VeDova (41) concluded, based on his studies, that the transfer of a whole homogenous joint without its capsule was superior to a transplant having an intact capsule. Two years later, Pucci (1913)(140) performed twenty-one homogenous whole joint and half-joint transfers in rabbits. Some were done directly and others followed freezing. Favorable results were obtained in eighteen cases. Goebell (1913) (69) was the first to report the replacement of a finger joint by a joint taken from the foot.

One year later in 1914, Obata (1914)(124) working with rabbits performed thirteen autogenous and thirty-two homogenous metatarsophalangeal joint transfers. He found autogenous grafts to be superior. Minoura, (1914)(120) also working with rabbits, reviewed the results of autogenous metatarsophalangeal-joint grafts transplanted to non-physiological sites.

In 1945, Cuthbert (1945)(40) reported a case in which he had substituted a foot joint for a metacarpophangeal thumb joint destroyed by a gunshot wound. It is interesting to note that nine years later, Graham (1954)(72) performed a procedure identical to that of Cuthbert's

in a three year old boy and recorded the undisturbed growth of epiphyseal cartilage.

Herndon and Chase (1952)(82), (1954)(81) reported the fate of autograft and allograft whole knee joint transfers. Each graft consisted of the distal femur, proximal tibia, ligaments and menisci. Synovium and capsule had been removed except in the area of the collateral ligaments. A total of one hundred twenty-nine transplants were performed. Animals were followed clinically and radiographically for a two year period. Animals were sacrificed from one day to two years and histologic sections studied. In the autogenous transplants, good functional results were obtained. Initially the articular surfaces and ligaments appeared to be well preserved. Five to six months post operative degeneration of the articular cartilage was noted in areas of increased pressure. Later these areas were replaced by fibrocartilage. Within the first nine month post operative the entire graft underwent a reorganization. New bone replaced dead bone by the process of resorption and apposition. Both fresh and frozen homogenous transplants displayed an intiial functional result equal to the autogenous transplants. However, in these cases, within the first four to six months a progressive degeneration of the joint appeared with a concommitant reduction in function. Marginal erosions, cystic changes, and fibrosis were seen at necropsy. Degeneration and destruction had taken place faster than replacement and reorganization. Revascularization was almost as rapid as that seen in the autogenous grafts. However, resorption and new bone formation had been delayed considerable. Necrosis was more intense and absorbed articular cartilage was not replaced. Pannus and scar tissue filled some of these vacant areas. Microfractures of the underlying bone occurred. In 1955, Chase and Herndon, (1955)(32)

after reviewing the literature, concluded that half-joint transplantations had been found to be more successful than whole joint transfers.

Harnach (1959)(78) performed a hip joint transplant in one monkey. The head of the femur, and acetabulum was transplanted intact. Six months post operative the joint space was found to be one-third smaller than normal. The femoral head and synovial fluid had a normal appearance.

In 1960, Entin (1960)(53) reported on seven finger joint transplantations which had been performed four to thirty months previously. He found movement following transplantation was limited to from ten to thirty degrees. Two years later, Entin (Entin et al 1962)(54) showed that nonvascularized metacarpophalangeal joint autografts were usually destroyed within nine months following transplantation. Erdelyi, (1963)(56) on the other hand performed eleven transplantations of small joints from the foot to the finger. Results were good. Ankylosis did not occur providing healing by first intension was achieved.

Parrish (1966)(129) described his results with massive autogenous or homologous grafts. He agreed with Chase and Herndon (1955)(32)believing better results were obtained with hemijoint transfers. He felt this resulted from the fact that with this type of transplant the synovial lining was intact and a good blood supply was retained. By treating nineteen patients by means of an en bloc excision, major complications were confined to seven patients who received massive frozen homografts which replaced most, or all of one side of a major joint. Five of these involved

the distal end of the femus, ligamentous instability and bone resorption presented a problem. In all of the homotransplantations of the articular surfaces, degenerative changes were seen radiographically four to six months post operative. In 1973, Parrish reported on an eleven year follow-up study of twnety-one cases. The extremities of sixteen of the twenty-one patients had been saved, although the ultimate functional status of the grafts varied extensively.

In 1966, Volkov (1966)(164) reported favorable results in forty-seven out of eighty operations in which massive frozen homologous bone grafts were used in the construction of large bony and osteochondral defects. Later, Volkov (1960)(165) reported the results of four different types of bone transplantations. The first involved the replacement of part of a joint surface. Here, all fifteen cases reviewed were found to be successful. The second type of transplant involved one hundred five cases in which the whole of one articular surface and supporting bone were transplanted. Because of the inadequate revascularization and failure of incorporation, twenty of these cases resulted in avascular necrosis with collapse of the articular surface of the graft or deeper layer. Another twenty per cent experienced degenerative changes within the joint. Inadequate graft revascularization seemed to be the forerunner of microfractures in the subarticular bone and collapse of the cases involving the articular surface. Transplantation of two joint surfaces showed no evidence of necrosis. The fourth type of transplant recorded was that of a whole joint

complex (knee, hip or elbow). No excellent results were achieved. Volkov's results appeared to indicate that the smaller the portion of the joint which was transplanted, the better the result.

In 1967, Harto-Garofalidis et al (1967)(79) reported on the replacement of the phalanx of the thumb using a tibial bone graft. One year later, Entin and Kahn (1968)(55) wrote of disappointing results following the transplantation of autogenous half-joints to the hand. The poor results of Entin and Kahn may have resulted from inadequate fixation, loss of normal functional stresses, or delay in osseous repair.

Hamilton et al (1969)(77) discussed the changes seen following allotransplantation of femoral and acetabular shell grafts in the dog. Two and one-half years after insertion, a majority of the grafts failed, primarily as a result of loss of fixation. Articular damage was apparent in all grafts. Gross follicular hyperplasia was observed in the lymph nodes of the long term survivors and a round cell infiltration surrounding the graft was observed. Porter and Lance (1974)(136) said that in 1973 Hamilton concluded that the deterioration of the transplant resulted from a low grade immunological rejection.

Kettlekamp (1972)(99) believed the failure of long term whole joint transfers was primarily related to vigorous and faulty osseous repair. He felt the age of the animal, blood supply, and mechanical stress influenced osseous repair. The rapid clearance of dead bone and its replacement by fibrous tissue rather than new bone resulted, when under stress, in collapse of an articular sur-

face. Because both sides of the joint are undergoing the same type of process, collapse of one articular surface lead to collapse of the other. Incongruities and loss of the cartilage in areas of contact resulted. Campbell (1972)(27) transplanted allogenic half-joints and large articular fragments which had been preserved at five degrees Centigrade for periods of one to four weeks. Degeneration of the cartilage similiar to that which had been seen by Herndon and Chase was observed.

Seligman et al (1972)(150) performed whole knee joint transplantions in one hundred fifty-five dogs. Grafts used were similiar to that used by Herndon and Chase (1954)(81). The dogs were divided into four groups: 1. autograft group; 2. frozen allograft group; 3. frozen irradiated allograft group; 4. frozen allograft azathioprine group. Graft failure resulted from collapse of subchondral bone followed by resorption of grafted tissue and mechanical disintegration of cartilage. Cartilage which did not survive freezing and transplantation was replaced by fibrocartilage. No circulating cartilage antibodies or cellbound cartilage antigen-antibody complexes were identified.

Ottolenghi (1972)(126) and (1966)(127) reported sixty-two cases of resection followed by the transplantation of massive frozen homologous and autologous grafts. Included in his study were sixteen osteoarticular and forty-six metadiaphyseal resections. Favorable results were obtained in a total of thirty-seven cases. Failure of the graft resulted most frequently from resorption and infection. Like Herndon, (1955)(83) Ottolenghi found that signs of bone resorption and necrosis of cartilage appeared after several months

following apparently normal clinical and radiographic findings. Changes were more apparent in the homologous than in the autologous grafts. Ottolenghi felt that normal cartilage was very sensitive and required the stimulus of normal function for its integrity. Changes seen in the transplanted joints were similiar to those seen in the atrophic type of neuropathic artropathies (resorption, collapse, etc.) Ottolenghi believed these changes might result from lack of graft innervation.

Bunke (1967)(15) performed autogenous whole joint transplants using microvascular anastomosis on monkey toe and finger joints, and rat knees. He found that joints transplanted by microvascular anastomosis survived completely if the vascular anastomosis was successful. None of the degenerative changes seen in free autogenous or homogenous transplants were seen even after two years. Buncke did not believe that degenerative changes, seen by some investigators, in free joint transplants developed because of lack of nerve supply. In his study, nerves to the digits were not replaced. He believed that because of low antigenicity, cartilage, joint capsule and bone can survive as homografts if they are immediately revascularized.

Slome and Reeves (1966)(153) performed homograft transplantations of vascularized whole knee joints to orthotopic positions in six dogs. The knee joint included 7.5 centimeters of femur and 5.5 centimeters of tibia. Azathioprine and goat antidog lymphocyte serum was used for immunosuppression. The one autograft was successful. Vascular thrombosis and joint destruction resulted in three of the homografts in which either a donor geniculate artery

to host popliteal artery anastomosis or short or long popliteal to host popliteal graft had been performed. Two homografts having long femoropopliteal pedicle anastomosis were successful. Four years later, Reeves (1973)(143) reported on a five year follow-up study of his knee joint transplants. The autograft transplant appeared to be completely normal. Allografts were slower to obtain a bony union and showed changes consistant with an acute or chronic rejection.

Daniel et al (1971)(42) compared dogs which had received free whole joint transplants to those in which the blood supply was preserved. Thirteen out of twenty of the autogenous whole joint transplants in which the vascular supply had been maintained retained their full viability. Daniel suggested that it was the initial post transplantation ischemia present in the subchondral circulation that was principally responsible for articular degeneration in the free grafts. Two years later, Judet and Padovani (1973(97) discussed their study involving the transplantation of whole knee joints in the dog in which popliteal arteries and veins were anastomosed. Of the twenty-one homotransplants performed, only two joints remained viable by the third post operative week. Of the twenty-eight autotransplants performed, only four had a satisfactory appearance to their joint surface at one year. In 1973, Goldberg and Lance (1973) (70) disclosed their results on vascular transplants of the knee joints in Greyhounds. Six autografts had been technically successful. Articular cartilage, ligaments and menisci were normal on necropsy examination. Unsuccessful results were obtained in the eleven allotransplants performed.

Cartilage Transplants and Grafts:

Peer (1938)(131) reported that in 1864, Paul Bert was the first to transplant hyalin cartilage. Fischer's work (1882)(58) showed that cartilage grafts would survive if transplanted with perichondrium. In 1896, Konig (1896)(103) was the first to use cartilagenous grafts in humans. He used grafts for the repair of laryngeal and tracheal cartilage defects. Three years later, Von Mangoldt (1893)(167) successfully transplanted costal cartilage to the nose.

Tuffier (1901)(160) was the first person to use the "shell" principle. He performed a procedure which he called "chondroplastic reconstruction" in which, following a fracture-dislocation of the shoulder, he removed the articular cartilage of the head along with a thin layer of subchondral bone and transplanted it on the shaft. In 1911 Tuffier(1911)(159) performed similiar procedures on two elbows and in (1913)(158) he replaced an ankylosed tuberculous elbow using articular cartilage and subchondral bone from a tibiotarsal joint. Loeb (1926) (112) transplanted autografts and homografts of xiphoid cartilage with perichondrium in guinea pigs. He found both the autografts and homografts survived. This same year, Mannheim and Zypkin (1926)(115) reported their experiments with autocartilagenous grafts. They concluded that following transplantation, cartilage retained its structure and would be useful in plastic repair. The research of Kirkham (1940)(101), Rinehart (1951)(145) and Young (1945)(171) also demonstrated that cartilage retained its normal structure after autotransplantation. In 1948, Moore (1948)(121) reported on the cartilagenous-cup arthroplasty for use in the ununited fractures of the neck of the femur. This procedure was very similiar to Tuffier's. Moore removed

the head of the femur, reamed the cancellous bone down to subchondral bone, and then replaced the cartilagenous cap over the newly-shaped trochanteric area. In nine cases, excellent results were obtained.

Urist and McLean (1952)(162) reported the observation that full-thickness transplants of homogenous cartilage from young rats induced the formation of bone tissue in the anterior chamber of the eye. Gibson and Davis (1953)(66) using surgical bovine implants in man found that cartilage acts as an antigen and that the intensity of the antigen antibody reaction increased in direct proportion to the duration of the hosts exposure to this foreign protein. In (1955)(34) and later in 1958 (36), Craigmyle found that cartilage homografts and heterografts evoked an active immune response in the host. He attributed the antigenicity of the cartilage to the chondrocytes, although he found the chondrocytes to be only weakly antigenic. Moskalewski et al (1966)(123) demonstrated the immunogenicity of the chondrocytes. Heyner (1969)(86) showed them to have histocompatibility antigens in common with other cell types. Craigmyle (1960)(35) pointed out that there was indirect evidence which suggested that the ground substance also may be antigenic. However, Gibson (1965)(65) and Wyburn (1955)(170) have shown the interstitial substance of the allogenic cartilage to be nonantigenic. Seligman et al (1972)(150) found that cartilage homografts did not induce an antigen antibody reaction. Seligman did not believe that degenerative joint changes seen in cartilage resulted primarily from an immune mechanism. In 1974, Elves and Zervas (1974)(51) reported their results in rats grafted subcutaneously with heterografts of sheep osteoarticular tissues. The results obtained indicated that articular cartilage alone is poorly immunogenic and if it does lead to an immune response, the response

is delayed. Elves believed it was the cartilage matrix which formed a protective barrier to effector mechanism of the immune response. Prudden (1881)(139) and Dupertuis (1941)(133) demonstrated that transplants of cartilage into suitable sites in unrelated hosts of the same species survived. Bacsich and Wyburn (1947)(6) and 1955)(7) reported the survival of cartilage homografts in guinea pigs up to three weeks. In 1955, Craigmyle (1955)(34) implanted cartilage autografts and homografts intramuscularly and into the anterior chamber of the eye in rabbits. The cartilage retained its structure and viability for the full one year study. Gibson et al (1958)(67) confirmed the long term survival of cartilage implants for up to two years in man. The work of Curran and Gibson (1956)(38) with mice, and Dziewiatkowsi (1954)(49) with rats have shown, using $S^{35}$, the viability of transplanted cartilage. Schatten et al (1958)(148) have shown that $S^{35}$, was metabolized only by viable chondrocytes in man, rats and mice, and that the uptake process of this isotope was dependent on living enzyme systems.

Peer (1945)(131) attributed the early failure of cartilage autografts to inadequate nourishment of the graft. He felt this resulted from poor revascularization of the graft site which later lead to death of the chondrocytes and absorption of the matrix. Schofield (1953)(149) believed it was the hematoma adjacent to the cartilage graft which predisposed it to absorption. Schofield thought hematomas or dead spaces near the graft interfered with the diffusion of nutritive fluids and thus predisposed the graft to cellular death. Gibson et al (1958)(67) showed that chondrocytes with their low metabolism could, after transplantation, survive at body temperature for a few days. After this time, Erdelyi (1963)(56), Graham (1954)(72) and Honner and Thompson (1971)(88) have indicated that chondrocytes must get their nourishment

from the synovial fluid or underlying bone. Buncke (1967)(15) felt it was unlikely that bone would revascularize rapidly enough to sustain the chondrocytes. If a capsule was transplanted and survived as a free graft, synovial fluid might serve to nourish the transplant.

McKibbin and Holdsworth (1966)(118) and Honner and Thompson (1971)(88) presented evidence which suggested that in growing animals part of the nutrition of the articular cartilage takes place from the subchondral network, and with increasing age and decreasing growth the importance of subchondral circulation in nutrition of the articular cartilage declines. McKibbin has also indicated that the growth of articular cartilage is inhibited by the interruption of the subchondral circulation. The work of Lemperg (1972)(108) indicated that in younger animals the chondrocytes in the deepest layers may be completely dependent upon the subchondral vessels for their nutrition.

Chesterman et al (1968)(33) postulated that it was the avascularity and the protective characteristics of the matrix that was responsible for the prolonged survival of cartilage homografts. Craigmyle (1958)(36) and (1955)(34) also believed the ability of cartilage homografts to survive resulted from the peculiar physico-chemical character of the matrix. He felt the matrix acted as a semipermeable diffusion chamber. Resultantly, foreign cartilage cells survived in an immune host. The matrix was thought not to prevent the release of antigen from the graft, but prevented antibodies from reaching the cartilage cell. The low antigenicity of cartilage was considered such, due to the cell population.

Bocsich and Wyburn (1955)(7) and (1947)(6) believed the survival of homografts untreated cartilage was due to the protective effect of

the-mucopolysaccharides in the matrix. They prevented host antibodies which formed in response to a transplant from reaching cells deep within the transplant. In 1960, Peacock et al (1960)(130) demonstrated that it was the matrix of a whole cartilage graft which prevented the chondrocytes from contacting the immumocompent cells of the host. The net result was that the host failed to become immunized, or if immunized, was unable to reach the graft cells. Experimentally, Heyner (1973)(87) has shown that afferent and efferent blocks exist. Quinn and Cerroni (1957)(141) showed that chondroctin sulfate extracted in vitro from costal cartilage is non-antigenic. The work of DiFerrante (1964)(46) and Loewi (1965)(113) have indicated that cartilage matrix was antigenic. Seligman et al (1972)(150) said that Sandson has shown definitely that it was the protein core of the protein-polysaccaride molecule of cartilage that was antigenic. Sandson fractionated the core into two parts (PPL and PPH), one being species specific similiar to an isoantibody and the other common to several species.

In 1961, Fiala et al (1961)(59) reported their findings with osteochondral shell grafts of the femoral head in dogs. Two millimeters were found to be of optimum thickness for the shell. This same year, Pap and Krompecher(1961)(128) described their results in dogs knees following the transplantation of shell grafts of articular cartilage. Degenerative changes were seen in autogenous and allogenic transplants of bone and articular cartilage over five millimeters in thickness. Two years later, DePalma et al (1963)(45) reported on femoral head transplantations in dogs using a three to five millimeter shell. Autografts, fresh allografts, and plasma stored homogenous grafts were used.

replaced in all cases but was most rapid in the autografts. In most of the autografts the articular cartilage was viable. The cartilage of the fresh allograft showed degenerative changes in the superficial layers of the cartilage after one hundred and ten days. Generally, allografts stored for less than thirty days did comparatively as well as autografts. Allografts, frozen and stored in plasma for longer periods were markedly less successful. Campbell et al (1963)(29) transplanted osteochondral shells of less than five millimeters in thickness to the medial femoral condyle and distal end of the radius in dogs. All long term allografts showed cartilage degeneration. Campbell et al (1963)(104) observed that in the allotransplantation of osteochondral grafts, if the graft bone was thicker than five millimeters, collapse of the subchondral bone occurred later leading to degeneration of the overlying cartilage. If the underlying allograft bone was of proper thickness, the cartilage, whether viable or not, survived structurally for at least one year. De Palma et al (1963)(45) and Pap (1961)(128) have found that the death of cartilage cells per se could not be equated with cartilage degeneration. In contrast to this view, Chesterman and Smith (1968)(33) and Hagerty et al (1960)(76) have insisted that in order for articular cartilage to preserve its function it must retain its viability. These latter workers used small osteochondral fragments or small pieces of articular cartilage for their studies, not whole joints.

In 1964, Krupko et al (1964)(106) reported fifteen operations in which the upper third of the femur was replaced with a frozen semi-articular homograft. A two year follow-up study showed adequate mobility of the coxofemoral joint in all cases. Four years later

Chesterman (1968)(33) found after transplantation of the femoral head shell allografts, no clinical abnormalities were found up to three years post operative in twelve of the twenty-four dogs studied. In 1974, Porter and Lance (1974)(136) reported that early results following transplantation of double shell osteochondral grafts in the canine were encouraging. He felt that, at present, shell grafts had the best chance of survival.

Osteocyte Survival, Induction and Osteogenesis:

In 1739, Duhamel (1974)(47) did the first scientific study on the problem of osteogenesis. He placed silver wires subperiostially and found weeks later that they were covered by bone. Duhamel felt this new bone production resulted from an osteogenic property of the periostium. Von Haller (1763)(166) disagreed, for he believed the periostium served only as support for blood vessels. He thought that blood vessels had an osteogenic role in fracture healing, and osteogenesis resulted from an exudate from arteries. In 1841, Flourens (1847)(61) resolved this difference of opinion. Flourens showed that periostium had osteogenic properties and was a primary agent in bone healing.

Ollier (1867)(125) carried out extensive studies on periostium. He concluded on the basis of his work, that transplanted periostium and bone remain alive and could, under certain circumstances, be responsible for osteogenesis. He believed the best graft was one which was viable and had an attached periostium. He felt that the contents of the Haversian canals and endostium were important in the regeneration of bone.

Barth (1893)(9) and (1898)(10) reported that all elements of transplanted bone, marrow, and periostium die and are replaced by

newly-formed bone arising from the host tissue by appositional new bone growth. In 1908, Barth (1908)(8) recognized the fact that fresh autogenous bone grafts were capable of active new bone formation, but felt from a practical standpoint for filling bone defects in the skull and long bones, bone dust or necrotic bone chips were equally effective. Based on his experiments, Axhausen (1909)(5) believed that autograft periostium had a high survival rate and great osteogenic potential. However, he felt this survival property and osteogenic activity was less in homografts and virtually nonexistant in heterografts. In 1918, Gallie and Robertson (1918)(64) discussed the value of the bone grafts in clinical surgery. They believed it was important to transplant living graft elements, and therefore, autogenous bone was considered to provide the best graft. Living osteoblasts of the fresh autogenous grafts were thought to aid healing. Gallie felt that the endosteal surface of the graft which bore the greatest numbers of free osteoblasts was the most valuable part of the graft. Phemister (1914)(134), Mayer and Wehner (1914)(117) and Haas (1922)(74) showed that in fresh autogenous bone grafts, osteogenic properties persisted and these properties played a significant part in the incorporation of the graft into the host.

Keith (1934)(98) found from his experiments that when small fragments of bone from young dogs were used as autogenous grafts large quantities of new bone resulted. Less marked new bone formation resulted when the autografts involved adult dogs. Keith felt the tendency for proliferation and new bone formation depended on the presence of living osteogenic cells in the grafts.

Abbott (1947)(1) concluded from his studies that the mature elements of either a cortical or cancellous bone graft seldom survived transplantation. The elements which did survive and produce new bone were cells of the endosteal and cambium layer of the periostium. Abbott felt the cortical graft had strength but little osteogenic capability. Cancellous bone, on the other hand, had high osteogenic potential. He believed in most cases cortical bone should be used as a graft when strength is of primary importance and cancellous bone should be used when osteogenesis would be of most value as in the treatment of ununited fractures of the ends of the long bones, repair of defects of bone resulting from tumors and for fusion of joints.

Reynolds and Oliver (1950)(144) studied bone grafts implanted in the tibias of dogs. They found no evidence that any element of the fresh autografts survived. Thus they concluded that the process of fixation and replacement was initiated entirely by the host tissue. Hutchinson (1952)(89) found that in the early period following transplantation mature osteocytes of autografts degenerated and were later replaced by osteoblasts. These osteoblasts were thought to be formed by metaplasia of the cells of the soft tissue elements of the donor bone. Hutchinson felt that both autografts and homografts were capable of inducing new bone formation from the tissues of the host.

Phemister (1951)(135) demonstrated that autografts were incorporated earlier and more completely into the host skeleton and were therefore more successful than homografts. Phemister attributed the superiority of the autograft to the better survival of cells within the graft. One year later, Ray et al (1952)(142) reported their

studies involving the placement of implants into the anterior chamber of the eye in guinea pigs and rats. Ray confirmed the fact that homogenous and autogenous grafts were capable of surviving and proliferating in a suitable ectopic site. Additionally, it was found that following freezing or lypholizing adult cancellous and cortical bone was less able to survive at an ectopic site. One year later, Campbell et al (1953)(28) compared grafts of fresh autogenous, fresh homogenous and preserved bone. Osteogenic cells and osteocytes survived only in the fresh autogenous bone grafts and these contributed prominently in the incorporation of the graft into the host tissue. Maatz (1954)(114) compared autografts, homografts and heterografts by means of the spongiosa test. Grafts were implanted into holes five millimeters in diameter punched into bone spongiosa. Autografts were found to regenerate from their own potential. Homografts appeared to depend on the osteogenic potential of the host. Heterografts appeared to act to a considerable degree as a foreign body in relation to the host. The time required for incorporation and replacement of the fresh homograft by the host was twice that needed by the autographs. Fresh heterografts required three times as much time for incorporation and replacement as did the autografts. Devitilization of the autogenous graft was found to inhibit its incorporation, whereas devitilazation of the homogenous graft aided the incorporation process.

Chase (1955)(32) reported, based on his review of the literature, that following large transfers of viable bone, a large amoung of periostium with its original capacity survived.

DeBruyn and Kabisch's work (1955)(43) dealt with the question of whether bone formed following transplantation of bone marrow or peri-

ostium was the result of production by the osteogenic cells of the transplant or was formed from the host as the result of induction by the transplant. Fresh autogenous transplants whether bone marrow, or periostium were found to be equally osteogenic. Results indicated that osteogenesis produced by the connective tissue surrounding the transplant was primarily due to induction by the transplant causing the transformation of mesenchymal cells into osteogenetic cells into osteogenetic elements. However, some cells of bone marrow transplant origin were also thought to take a small part in the osteogenic process.

Siffert's (1955)(151) studies supported the concept that transplants deserved primarily as a passive scaffold along which new bone marrow grew. He found no evidence of direct osteogenic stimulation by any elements of the transplanted fragments. His results did show that autografts were incorporated more rapidly than homografts. Additionally he found that both autogenous, and in most instances homogenous grafts, augmented new bone formation. However, no reason could be given to account for this latter observation.

Bonfiglio (1958)(12) studied the repair of a three centimeter defect created in the ulna of rabbits. He compared the repair capacity of fresh autogenous, fresh homogenous, frozen, freeze dried, and merthiolate-preserved homogenous bone transplants. The fresh autogenous bone transplants were found superior to all others. Some of the surface cells of the fresh autogenous bone transplants appeared to survive and form new bone. Frozen homogenous bone induced little osteogenic stimulus in the host tissues. Haas (1957)(75) also reported the survival of some of the osteogenic cells of the fresh autograft and indicated he felt they took an active part in new bone production.

Chalmers and Sisson (1959)(31) made a detailed comparison between autograft and homograft (fresh and freeze dried) bone transplants in rats. With the exception of a graft from an identical twin, homografts, whether living or dead were not found to make a significant contribution of living bone tissue. Freeze dried bone induced a less intense inflammatory response because of its altered antigenicity. Freeze dried bone also experienced a prolonged survival time compared to the fresh homograft. Because the homograft did not supply living cells and was slowly absorbed, it was found to be inferior to the autograft.

Heslop et al (1960)(84) found that a certain percentage of osteocytes in both autografts and fresh homografts survived up to two hundred fifty days after transplantation. Osteogensis was found to be copious in the autografts but was negligible or absent around the homografts throughout the course of study. It was felt that this failure of new bone formation around homografts resulted from the presence of an abnormal precursor tissue around the graft unsuitable for ossification. Heslop believed that the survival of osteocytes largely depended upon plasmatic circulation and that the overall vast superiority of the autografts resulted to some degree from its readier establishment of a blood supply. He found no quantitative relationship between osteocyte survival and osteogenesis. However, a qualitative relationship did exist. The presence of living osteocytes were necessary for osteogensis to occur. In 1963, Heiple et al (1963)(80) made a controlled study of the osteogenic properties of the various types of bone grafts in dogs. Fresh autogenous bone grafts were found to be superior to all others, freeze dried received the next

highest rating. The remainder of the grafts were rated as follows: frozen homogenous; decalcified homogenous; frozen irradiated homogenous; freeze-dried irradiated homogenous; fresh homogenous; fresh homogenous; deproteinized heterogenous and deproteinized homogenous.

Sabet et al (1964)(147) working with mice, found that cellular isografts of embryonic bone continued to survive and grow after transplantation. The new osteocytes which developed were both a product of the host via induction and graft as a result of cell proliferation. Further, it was determined that fresh homografts of embryonic bone survived transplantation for only short periods, later undergoing avascular necrosis. In the case of the homograft the cells of the host did not, as a rule, contribute to the scanty new bone formed.

Ross (1966)(146) reported the results of filling three circumscribed cortical bone defects in the lateral cortex of dog femurs with different substances (fresh autogenous bone, frozen stored homogenous bone, plasma stored homogenous bone, autoclaved bovine bone and polyurethane foam). The healing of both filled and unfilled cortical bone defects were found to be a function of the host bone. The grafts appeared only to have the mechanical function of filling the defects and furnish a template for new bone growth. New bone arose primarily from the host endostium. Ross did not find that homografts or autografts impeded or stimulated the healing rate.

Working with rats, Burwell (1964)(19), (1963)(21 and (1965)(18) combined homologous fresh marrow-free iliac bone with the animal's own red marrow in order to form a fresh composite homograft-autograft of cancellous bone and inserted these grafts into a muscular site. This graft formed considerably more new bone than did either component of

the graft transplanted separately. Burwell suggested that the stimulus to osteogensis and cellular source of osteoblasts in fresh iliac autografts resulted from an inductive system and a "triggering" of mitosis in pre-existing littoral osteoblasts. This lead to osteoblastic differentiation and bone formation. He felt the resultant new bone formed by the fresh iliac autografts was derived from two sources, the littoral osteoblasts" of the grafted bone and the "littoral-endothelial cells" of the contained marrow. The greater formation of bone by the composite graft was attributed to either a more extensive necrosis of the marrow, or an added induction effect of bone upon marrow. Burwell (1966)(20) later showed that the capacity of cancellous bone to induce osteogenesis in bone marrow resided in the organic components of the bone. By physical and chemical means, this capacity could be lowered. It was further determined that from the standpoint of induction, cancellous bone treated by freezing or freeze drying was the most suitable devitalized bone for grafting.

Simmons et al (1973)(152) also showed that a composite bone allograft-marrow autograft was a better stimulus to new bone formation than was an autogenous graft. Simmons thought that the new bone formed in the autograft and composite graft was produced, in part, by the osteoblasts which survived transplantation and in part, by the differentation of connective tissue cells which had bone forming capabilities in the host bed. This was also true of the composite graft. Simmons believed additionally that the greater bone formation with the composite graft resulted from a strong interaction between the allogenic bone of the graft and the labile osteogenic source cells present both in the autologous marrow and in the investing connective tissues of the

recipient. The idea that the investing connective tissue had contributed to bone formation was not in agreement with Burwell's findings. Burwell believed osteogenic cells were formed only from the induction of autologous undifferentiated reticulo-endothelial cells in marrow.

Goldberg and Lance (1972)(71) performed full-thickness iliac allograft and autograft transplantation is rabbits. They found autografts to have a superior osteogenic capability. Autografts achieved a significantly higher and more rapid level of revascularization than was seen in allografts. Allografts transplanted into rabbits who had received azathioprine displayed osteogenic and revascularization capabilities comparable with the autograft transplants.

Post et al (1966)(137) demonstrated that a homograft in a millipore filter diffusion chamber implanted subcutaneously in mice was capable of proliferating by means of a diffusable inductor substance. Buring and Urist (1967)(16) and Morihisa (1970)(13) also performed studies involving bone tissues sealed in millipore diffusion chambers placed into a heterotopic site. They too demonstrated the existance of an inductor substance which was released from the bone matrix.

In 1957, Enneking (1957)(52) reported a study indicating that following autologous bone grafting the osteocytes undergo necrosis because of an insufficient blood supply. In 1974, Adelaar (1974)(2) successfully anastomised the periosteal vessels of autologous bone grafts to vessels at the recipient site. Comparing grafts with and without intact periosteal vessels no physiologic difference in healing in osteocyte survival, bone viability or clinical union could be found.

Freezing and Immunogenicity

Freidlaender (1975)(62) reported that in 1867 Ollier described the beneficial effects of cold on the preservation of bone. Grohe (1899)(73) showed that periostium survived refrigeration at 0 - 40 degrees for up to one hundred hours and displayed osteogenic properties following transplantation. Bauer (1910)(11) was the first to demonstrate that homogenous grafts could be preserved by refrigeration for up to three weeks and then be successfully transplanted. In 1942, Inclan (1942)(95) reported the successful preservation of autogenous grafts by refrigeration in Ringer's solution and citrated blood. Five years later, Bush (1947)(25) and Wilson (1947)(169) independently developed a similar procedure for storing bone at -20 degrees Centigrade. Later, Bush and Garber (1948)(26) and Wilson (1951)(168) showed proof in clinical studies that refrigerated homografts are well tolerated by the host. Bush reported only four complications out of one hundred twenty-six operations, and Wilson had an eighty percent success rate in two hundred fourteen cases cited. In 1951, Manziano (1965) reported the first autogenous bone bank in the United States was established.

Kreuz et al (1951)(105) studied the sequential events leading to the incorporation of fresh autografts, freeze-dried allografts and frozen allografts placed in the ulna of dogs. Kreuz found that the incorporation of freeze-dried allografts proceded at a slower rate, but in a comparable fashion to fresh autografts. The early inflamatory response seen with the fresh allografts was markedly reduced in the freeze-dried allografts. Allografts of frozen bone lagged slightly behind those which had been freeze-dried.

In 1955, Bonfiglio et al (1955)(13) found that tissue response to

frozen bone allografts was reduced compared to fresh allografts. He attempted to identify the presence of bone humeral antibodies by using precipitin, compliment fixation and hemagglutination tests. Following the transplantation of frozen allografts, no antibodies were identified. However, in animals which had received prior immunization with bone extract, and later received a frozen bone allograft, low titers of compliment fixing antibody were found.

Burwell and Gowland (1962)(24) studied the response evoked in regional lymph nodes draining the sites of fresh cancellous and homologous bone grafts in rabbits. Based on this study, Burwell determined that the principle antigenic component of the fresh homograft of iliac cancellous bone was the nucleated cell of the red marrow. The treatment of marrow-containing cancellous bone by freezing and freeze-drying impaired the transplantation homograft antigenicity. He concluded that treatments which devitalized bone homografts also reduced their antigenicity. The mechanism by which this occured was unknown. But, he postulated, it could be the result of direct antigenic inactivation, a prevention of antigenic release from dead cells, or result from the antigens failure to reach the site of immune response.

In 1963, Heiple et al (1963)(80) reported that both freezing and freeze-drying appeared to destroy the antigenicity of bone homografts. Immalieu (1960)(92), (1962)(91), (1963)(93) showed that freezing and storage of grafts suppressed transplant antigenicity. Osteochondral allograft transplantation (Imamalieu (1969)(90) was improved by freezing and freeze-drying. He felt reduction in graft immunogenicity resulted in a slower absorption rate allowing faster revascularization and new bone formation.

Langer et al (1975)(107) reported that both fresh and frozen bone elicited a cellular response. This response included the developement of enhancing factors which blocked detectable immunity and protected the graft from rejection. Langer found no evidence which showed that freezing altered bone immunogenicity. Unlike Burwell (1969)(22) and Imamalieu (1969)(90) who indicated that freezing markedly impaired the antigenicity of the corticocancellous graft, Langer found no difference in the degree of induced cellular immunity.

## RESEARCH DESIGN AND METHODOLOGY

### Design

A total of eighteen clinically normal mixed breed dogs were used as recipients in this experiment. The dogs were between one and three years of age, of both sexes and ranged in weight from 15.6 to 23.6 kg. They were radiographed prior to selection to evaluate their freedom from gross bony abnormalities of the acetabulum, as well as head, neck and proximal shaft of the femur.

From the radiographs, four measurements of the proximal portion of the femur were taken (Figure 1): 1) The diameter of the femoral head (AB); 2) The distance from the center of the articular surface of the femoral head to the third trochanter (CD); 3) The distance from the center of the femoral head to a predetermined point on the acetabulum (EF); 4) The distance from the center of the femoral head to a point bisecting the articular surface of the femoral head (FC). Candidates for this experiment were selected so that they might reasonably be suited to serve as donor or recipient candidate.

The eighteen dogs were divided into three groups according to the type of graft each was to receive; 1) The animals in Group 1 were autograft recipients. Here, grafts were taken from the proximal portion of the femur and then replaced to their original location; 2) Those in Group 2 received fresh allografts. In this second group each dog received a whole bone graft directly from a living donor. The graft was given no special treatment prior to transplantation; 3) The dogs in Group 3 received frozen allografts. The animals in this group received whole bone transplants which had been frozen in a specially prepared medium at a temperature of -32 degrees Centigrade for a period of between twenty-four and twenty-eight days.

Methodology

I. Preparation of Frozen Grafts.

Following radiographic evaluation six dogs were selected to serve as whole bone frozen graft donors. The proximal portion of the femur selected from each dog to be used as a graft was taken in aseptic fashion. Each graft was immediately immersed in a specially prepared solution and stored in a sealed container. The aseptically prepared solution consisted of 35% serum, 10% glycerin[1], 5% dimethylsulfoxide[2] and 50% tissue culture medium[3] to which the following antibotics had been added: tylosin[4] (200 micrograms/ml.), chlortetracycline Hcl[5] (500 Micrograms/ml.) chloramphenical sodium succinate[6] (500 micrograms/ml.), kanamycin sulfate [7] (1000 micrograms/ml.), amphoteracin B[8] (4 micograms/ml.), streptomycin sulfate[9] (200 micrograms/ml.), and

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[1]Glycerin USP, Mallinckrodt, Inc., St. Louis, Missouri

[2]Domoso$^{R}$, Diamond Laboratories, Inc., Des Moines, Iowa

[3]Minimum Essential Medium (Eagle), with Earle's BSS, L-Glutamin, $NaHCO_3$, Microbiological Associates, Bethesda, Maryland

[4]Tylocine $^{R}$ Injection, Elanco Products Company, Indianapolis, Ind.

[5]Aureomycin Intravenous, Lederlie Laboratories, Pearl River, New York

[6]Mychel-s$^{R}$, Rachelle Laboratories Inc., Long Beach, California

[7]Kantrim$^{R}$, Bristol Laboratories, Syracuse, New York

[8]Fungizone$^{R}$, Intravenous, E. R. Squibb and Sons, Inc., Princeton, New Jersey

[9]Streptomycin Sulfate Injection, U.S.P.

and penicillin [1] (200 units/ml.). The container was placed in a freezer which was adjusted so as to reduce the temperature of the bone one degree centigrade per hour over a twenty-four hour period. The bone was then placed in a Revco ultrafreeze and held at a temperature between -30° centigrade to -32°centigrade for a minimum holding period of twenty-four days. Prior to use, the graft, still sealed in its container, was placed in a 37°C water bath and brought up to a room temperature over a two hour period. After reaching the required temperature, the graft was immediately implanted.

Beginning the day of surgery and throughout the experiment all the animals were placed on erythromycin[2] (250 mg. B.i.d.) and ampicillin[3] (250 mg. b.i.d.). Furosemide[4] (50 mg. b.i.d.) was administered to each animal on an alternating four day schedule. Tetracycline hydrochloride[5] (800 mg. daily) was given for a four-day period at two week post-operative intervals. During the entire experimental period the involved limb was kept in a flexion bandage.

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[1] Penicillin G. Potassium for Injection U.S.P.,E.R. Squibb and Sons, Inc., Princeton, New Jersey

[2] E-mycin$^{R}$, Upjohn Company, Kalamazoo, Michigan

[3] Polycillin$^{R}$, Bristol Laboratories, Syracuse, New York

[4] Lasix $^{R}$, National Laboratories Corp., Kansas City, Missouri

[5] Tetrachel$^{R}$, Rachelle Laboratories, Inc., Long Beach California

All dogs were radiographed immediately following surgery and at fourteen day intervals. Additionally, animals within each group scheduled for euthanasia at three-week post-operative intervals were radiographed prior to their experimental termination.

Each dog was euthanized using $T_{61}$ euthanasia solution[1]. At necropsy, the femurs were removed and split longitudinally. One half of the bone was fixed in neutral buffered 10% formalin for seventy-two hours. Following fixation, the bone was decalcified in a standard formic acid-sodium citrate solution, embedded in paraffin, sectioned at six microns, stained with H. and E., and examined. The other half of the femur was preserved in 70% ethyl alcohol. Later, photographs of the tetracycline labeled bone was taken using UV light source, and Kodak Daylight Film[2].

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[1] T-61[R] Euthanasia Solution, National Laboratories Corp., Somerville, New Jersey

[2] Kodak High Speed Ektachrome Film, Eastman Kodak Company, Rochester, New York

II. Surgery.

The dogs were premedicated with atropine sulfate[1], induced with thiamylal sodium[2] and maintained on methoxyflurane[3] anesthesia (Figure 2). Depending on the femur selected, the right or left pelvic femoral regions were then clipped with a #40 clipper blade (Figure 3). The animal was placed in lateral recumbency and secured with leg ropes. The skin was given a ten-minute prep using a poloxamer-iodine solution[4] followed by an alcohol scrub. The surgical site was draped with sterile drapes. A skin incision was made from the wing of the ilium coursing posterior to the greater trochanter along the craniolateral aspect of the femur to its distal one-third (Figure 4). The skin was then toweled in (Figure 5). The subcutaneous tissue and superficial fascia was incised in the same line as the skin incision. The tensor fascia lata was incised along the cranial border of the biceps femoris muscle (Figure 6). The biceps femoria was retracted caudally and the vastus lateralis cranially. The superficial gluteal muscle was then isolated and transected through its tendon of insertion on the third trochanter (Figure 7). The piriformis muscle was next cut through its tendon of insertion near the third trochanter. The middle (Figure 8) and deep gluteals (Figure 9) were similarly transected through their tendons of insertion on the greater trochanter. The insertion of

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[1]Atropine Sulfate Injection, U.S.P., Elkins-Sinn, Inc., Cherry Hill New Jersey

[2]Surital, Parke-Davis, Detroit, Michigan

[3]Metofane, Pitman-Moore, Inc., Washington Crossing, New Jersey

[4]Septo Dyne™ Solution, Winthrop Laboratories, New York, New York

the fascia lata muscle was severed. The origins of the vastus lateralis (Figure 11), vastus intermedius and vastus mediallis muscles were severed close to the periostium. A portion of the insertion of the abductor magnus et brevus muscle on the lateral lip of the rough surface of the femur was severed distally to about the midshaft of the femur (Figure 13). Also, the entire insertion of the adductor longus muscle was incised. The insertion of the adductor longus on the proximal end of the lateral lip of the rough surface of the femur was cut at this time. The joint capsule was then circumferentially incised (Figure 14). The insertions of the internal obturator, external obturator and gamelli muscles were then severed through their tendon of insertion on the trochanteric fossa of the femur. The insertions of the quadratus femoris, adductor longus and ileopsoas muscles were severed. Next, the round ligament was cut, freeing the proximal one-third of the femur (Figure 15). An osteotomy of the femur was performed at a point approximately eight centimeters distal to the most proximal point of the greater trochanter (Figure 16). A specially made guide was employed to insure that the angle of the cut made in the recipient femur would be similiar to the cut made in the graft (Figure 17). An oscillating saw[1] was used to perform the osteotomy (Figure 18).

The graft was then placed on its new site. The graft was next fixed by means of a five-hole ASIF narrow DCP plate[2]. The plate was bent to

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[1]Stryker, Stryker Corporation, Kalamazoo, Michigan

[2]Narrow Dyanamic Compression Plate, Synthes Ltd., Wayne Indiana

conform to the normal curvature of the proximal lateral aspect of the femur. Using a neutral guide a 3.2 millimeter drill hole was placed through the plate into the most proximal point in the graft (Figure 21). A 3.6 millimeter tap was employed (Figure 22) and a 4.5 millimeter screw inserted (Figure 23). The procedure was repeated and a screw was placed in the most distal hole, five (Figure 24). Screws were then placed through holes four and two (Figures 25 and 26). Using a load guide, a screw was placed in the most proximal portion of the recipient (third hole) in order to complete the fixation (Figures 27 and 28).

Five #1 chromic gut sutures[1] were then preplaced in the remaining attached portion of the joint capsule (Figure 29). One end of each suture was placed lateral to the proximal femur and medial to the plate. The femoral head was then placed into its normal anatomical position and the preplaced sutures tied (Figure 30). Two mattress sutures of 0 chromic gut[2] were then placed through the tendons of insertions of the internal and external obturator and gamellimuscles, passed lateral, to the femur and medial to the plate and tied. Mattress sutures placed in a similar fashion were then used to hold the deep, middle and superficial gluteals in their normal position (Figure 31). The fascia of the anterior border of the adductor magnus et brevis was then sutured to the fascia of the posterior border of the vastus lateralis (Figure 32). Portions of the superficial gluteals were then sutured to the

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[1] #1 Chromic Ethicon. Ethicon Inc., Somerville, New Jersey

[2] #0 Chromic Ethicon Inc., Somerville, New Jersey

fascia of the posterior border of the vastus lateralis (Figure 32). Portions of the superficial gluteals were then sutured to the surrounding fascia with 00 chromic gut[1]. The tensor fascia lata was sutured to the biceps femoris using 00 chromic gut in a continous pattern. The skin was apposed with 0000 black monofilament nylon[2] using a simple interrupted pattern (Figure 34).

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[1]00 Chromic gut, Ethicon Inc., Somerville, New Jersey

[2]0000 Ethilon, Ethicon, Inc., Somerville, New Jersey

Figure 1: Measurements were taken of the femoral heads and necks of all candidates to determine their transplant suitability. (See Text).

Figure 2: During the procedure the dogs were maintained on metofane anesthesia.

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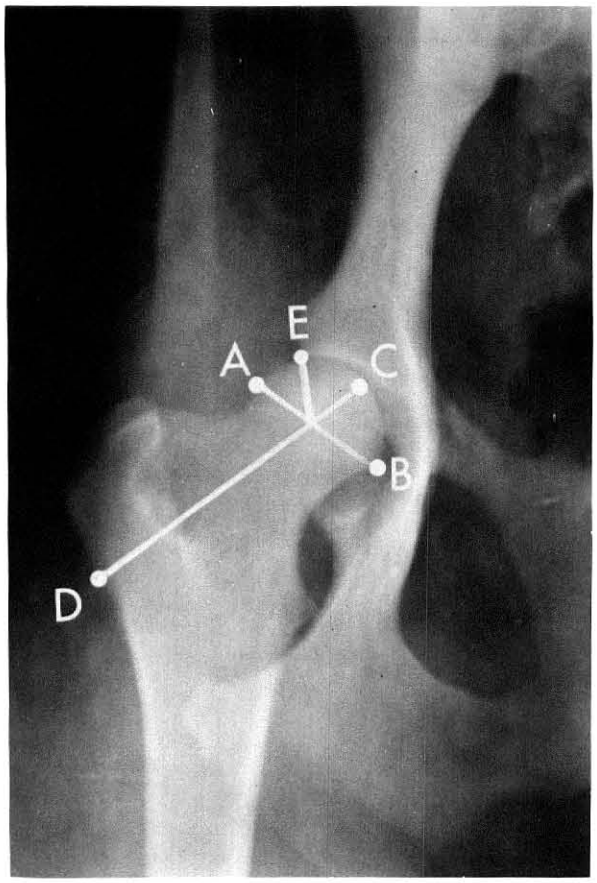



Fig. 1

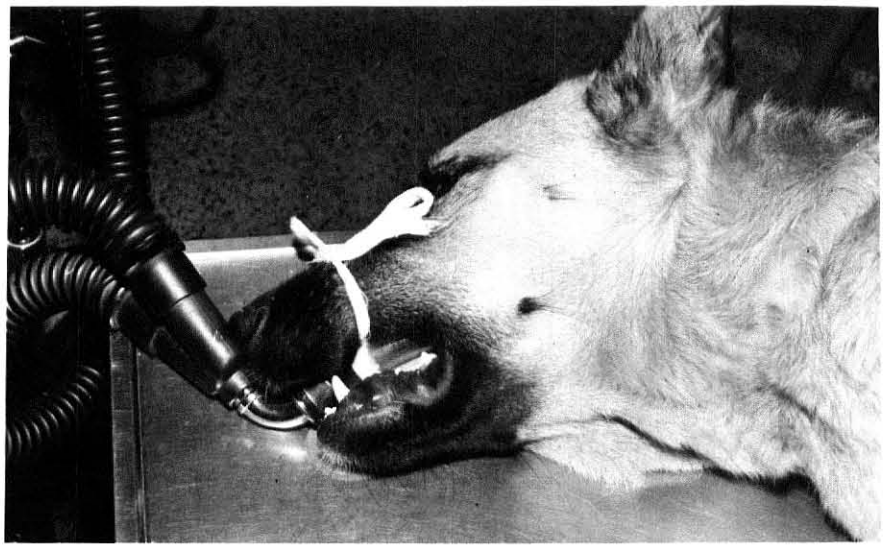


Fig. 2

Figure 3: The left pelvic and femoral region were clipped and prepared for surgery. The wing of the ilium, tuber ischii, greater trochanter and distal lateral condyle of the femur were used as points of reference.

Figure 4: The skin incision was made from the wing of the ilium coursing posterior to the greater trochanter along the craniolateral aspect of the femur to its distal one-third.



Fig. 3

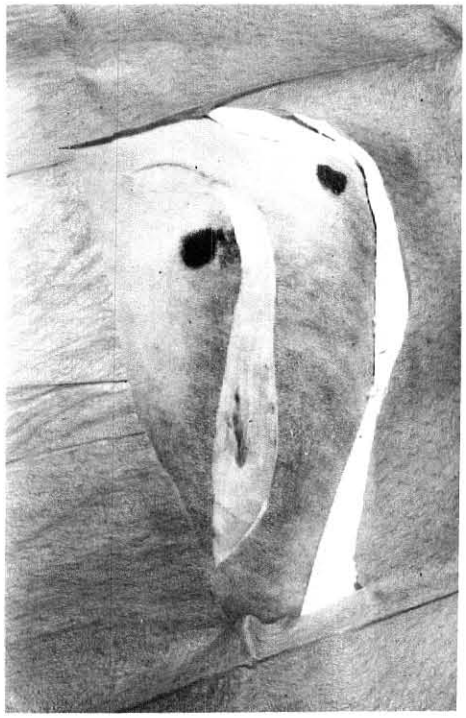


Fig. 4

Figure 5: - The skin was toweled in.

Figure 6: The tensor fascia lata was incised along the cranial border of the biceps femoris muscle.

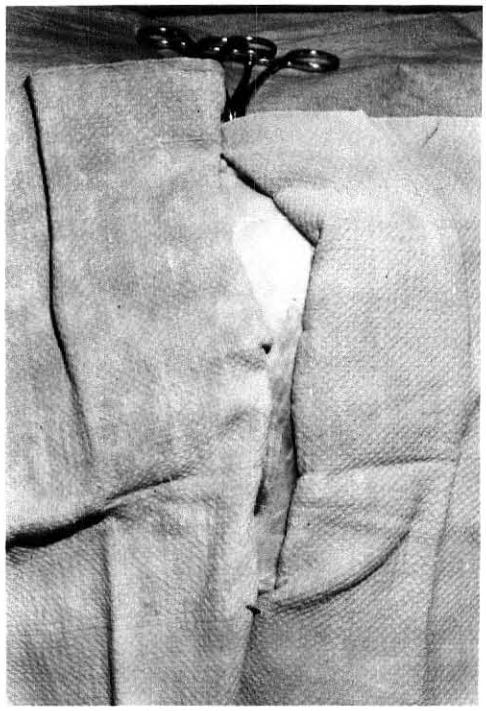


Fig. 5

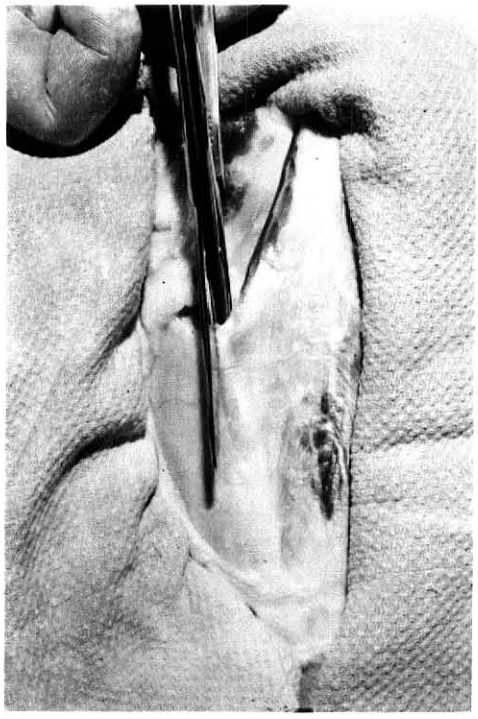


Fig. 6

Figure 7: The superficial gluteal muscle was transected through its tendon of insertion.

Figure 8: The middle gluteal and piriformis muscles were severed through their tendons of insertion.

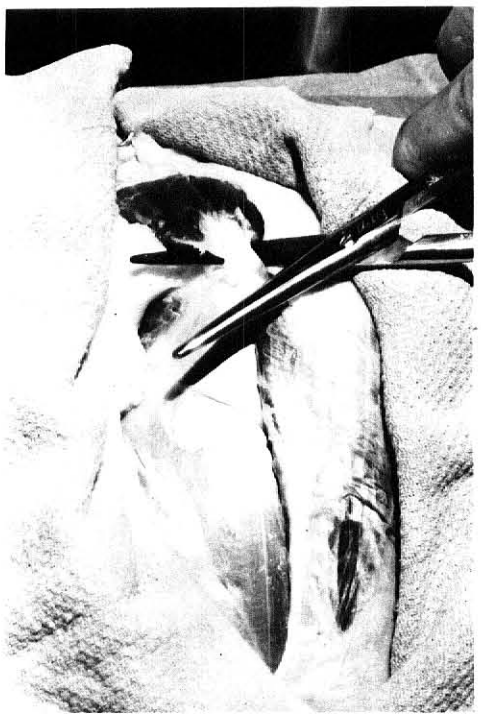


Fig. 7

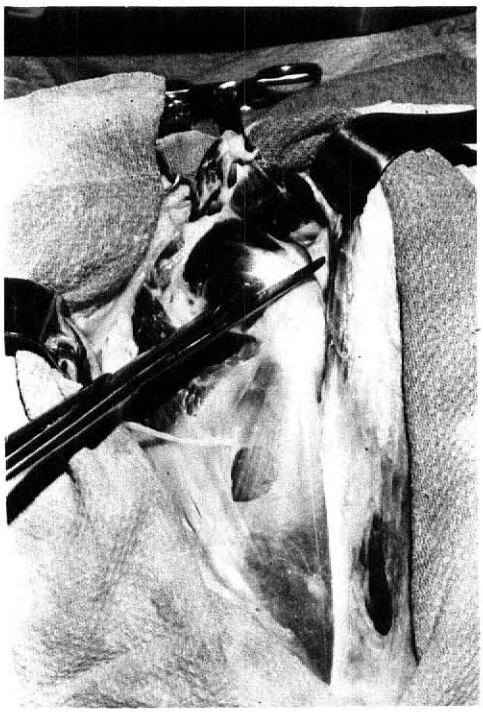


Fig. 8

Figure 9: The deep gluteal muscle was severed close to its tendon on the greater trochanter.

Figure 10: The gluteals were reflected dorsally, tensor fascia lata and vastus lateralis muscles anteriorly, and biceps femoris muscle posteriorly, to expose the proximal one-half of the shaft of the femur and dorsal joint capsule.



Fig. 9

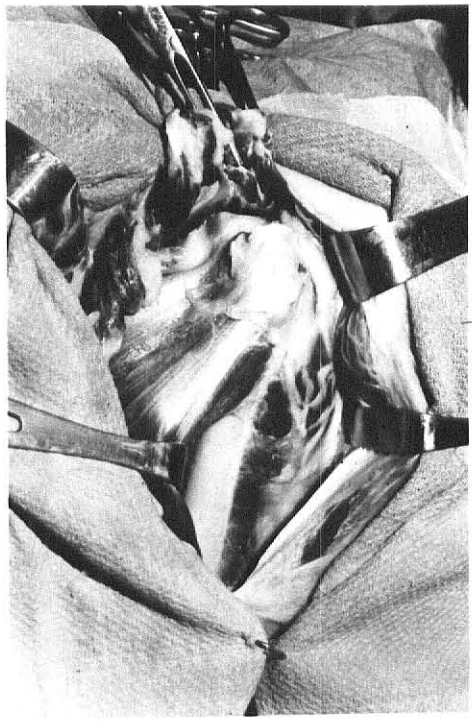


Fig. 10

Figure 11: The vastus lateralis muscle was severed close to its origin on the proximal portion of the lateral lip of the rough surface of the femur.

Figure 12: The vastus lateralis muscle was retracted anteriorly.



Fig. 11



Fig. 12

Figure 13: The proximal insertion of the adductor magnus et brevis was severed and retracted posteriorly.

Figure 14: The joint capsule was incised circumferentially.



Fig. 13

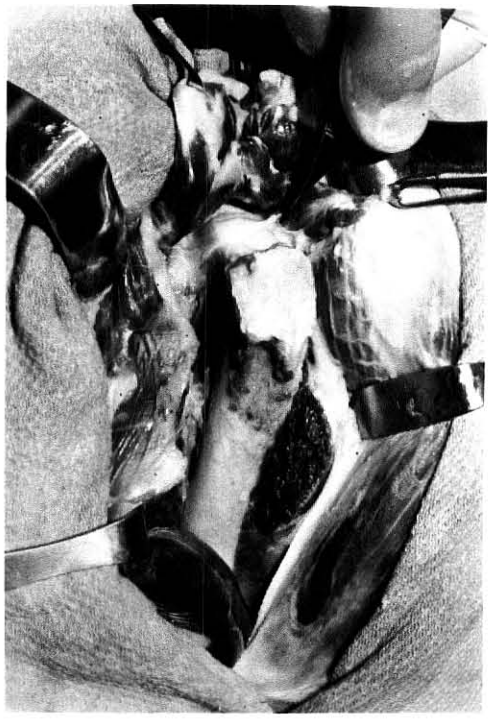


Fig. 14

Figure 15: Following severence of the round ligament and muscles outlined in the text, the proximal one-third of the femur was freed.

Figure 16: An osteotomy was performed at a point eight centimeters distal to the most proximal portion of the greater trochanter.

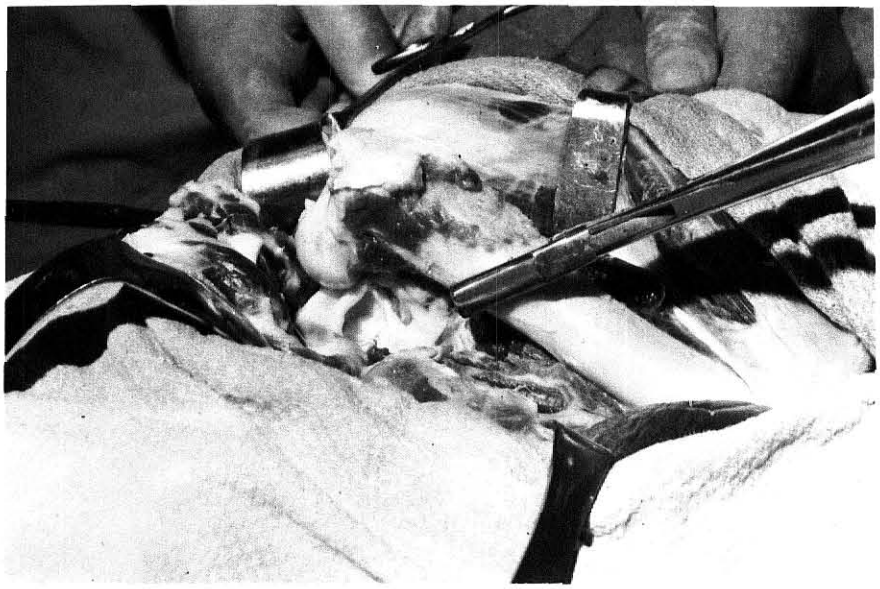


Fig. 15



Fig. 16

Figure 17: A special guide was used to perform the ostectomy.

Figure 18: The guide was placed on the lateral aspect of the femur, and a stryker saw used for the ostectomy.

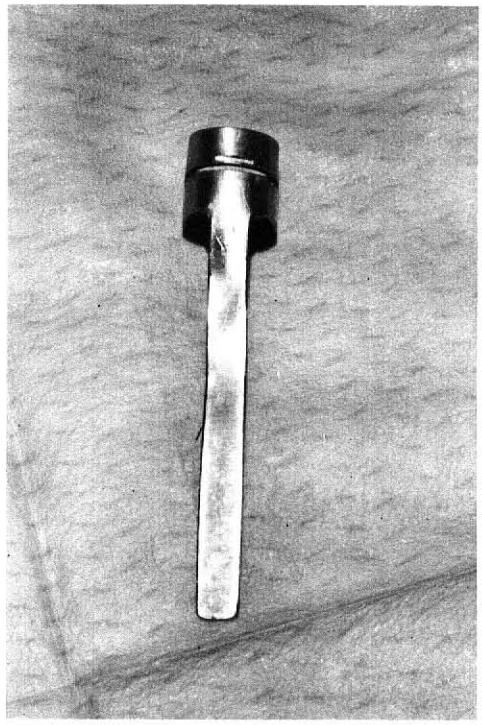


Fig. 17

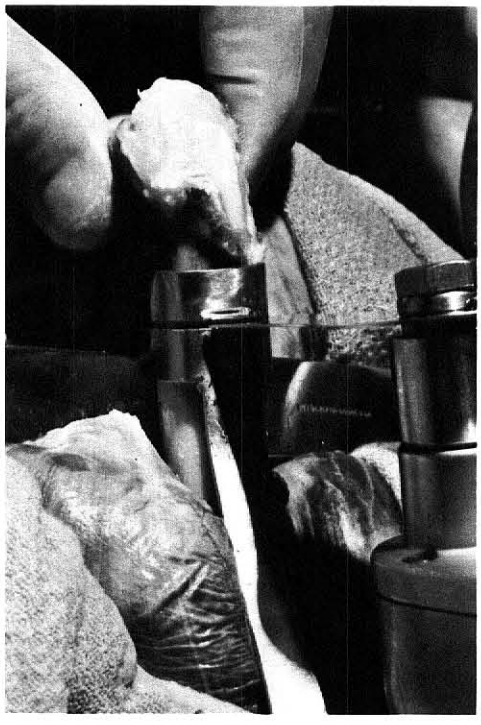


Fig. 18

Figure 19: The guide was removed and the remaining one quarter of the cut was made through the femoral shaft.

Figure 20: Excised portion of bone.

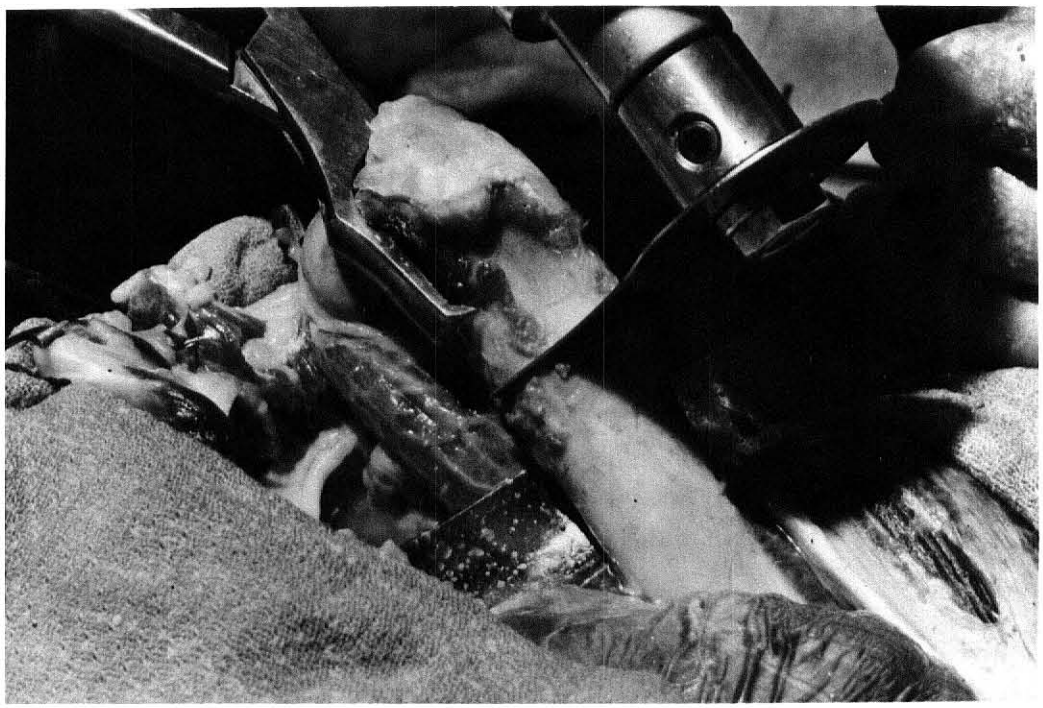


Fig. 19

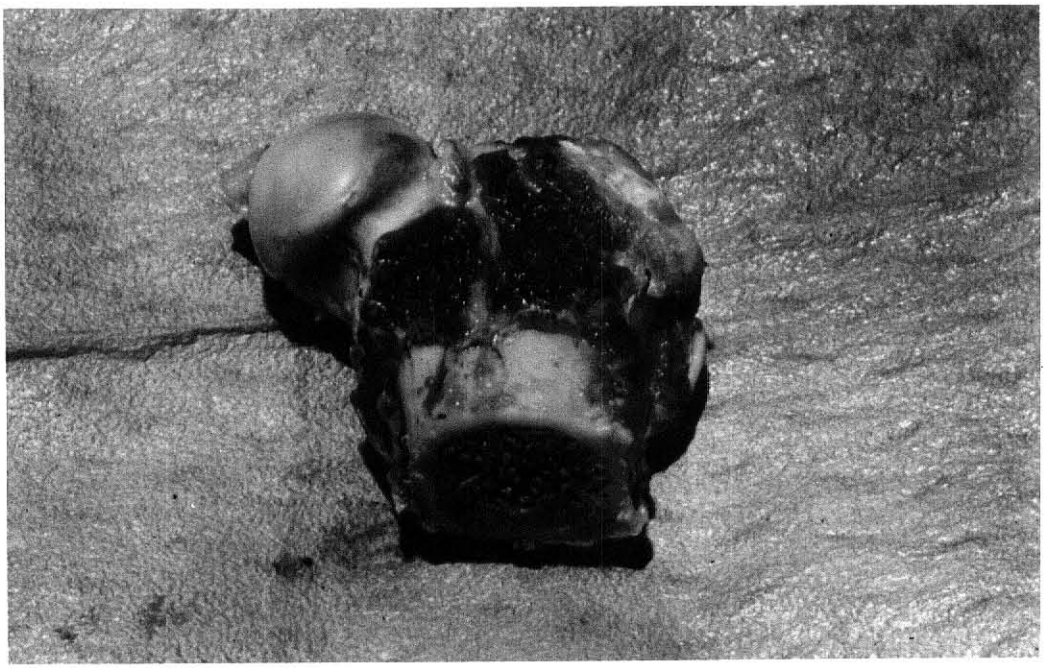


Fig. 20

Figure 21: Using a neutral guide a 3.2 millimeter drill hole was placed through a five-hole DCP plate into the most proximal point of the graft.

Figure-22: The hole was then tapped with a 3.6 millimeter tap.

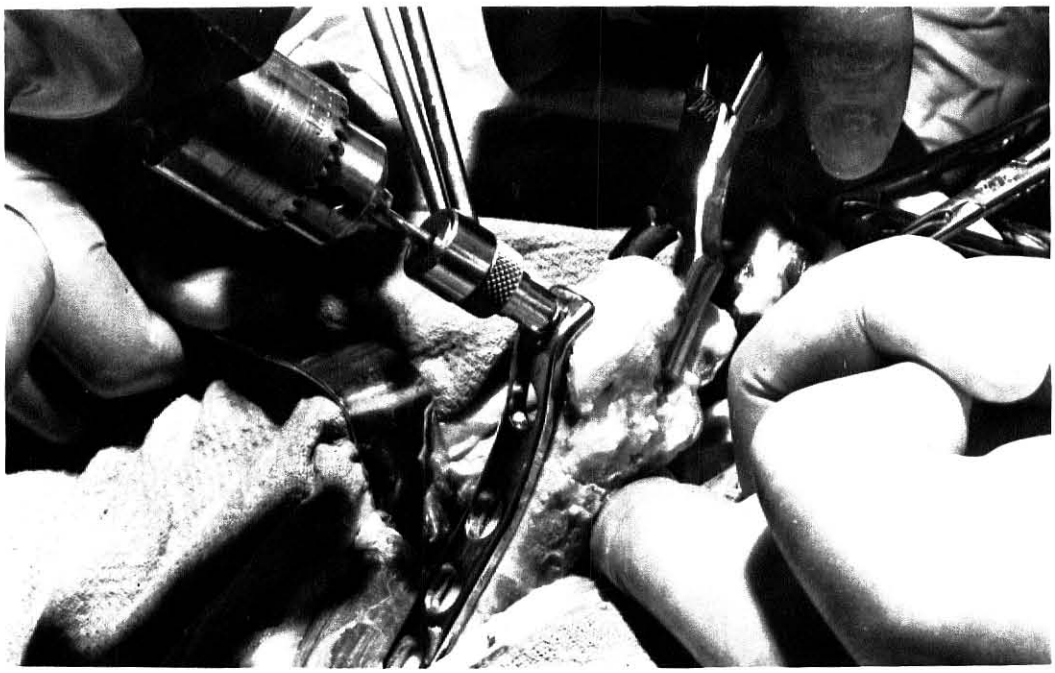


Fig. 21



Fig. 22

Figure 23: A 4.5 millimeter screw was inserted.

Figure 24: The procedure was repeated and a screw was placed in the most distal screw hole (five).



Fig. 23

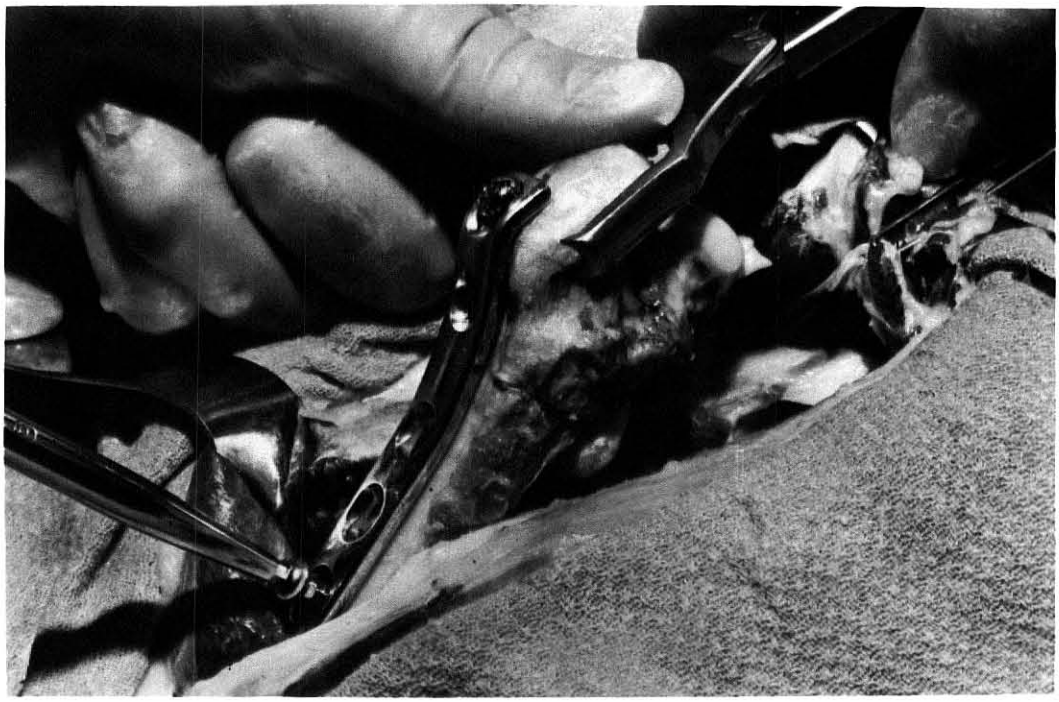


Fig. 24

Figure 25: Screw four was inserted in a neutral position.

Figure 26: Screw two was inserted in a neutral position.



Fig. 25

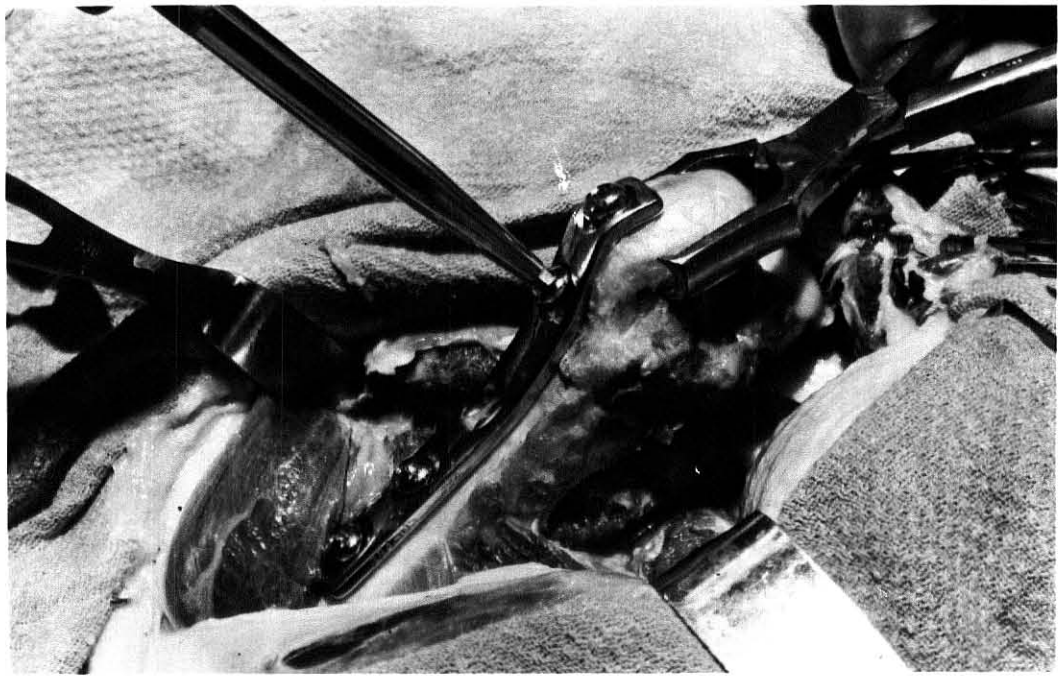


Fig. 26

Figure 27: A load guide was used to drill the hole in the most proximal portion of the recipient.

Figure 28: The graft was thusly fixed into position using the five hole DCP plate.



Fig. 27

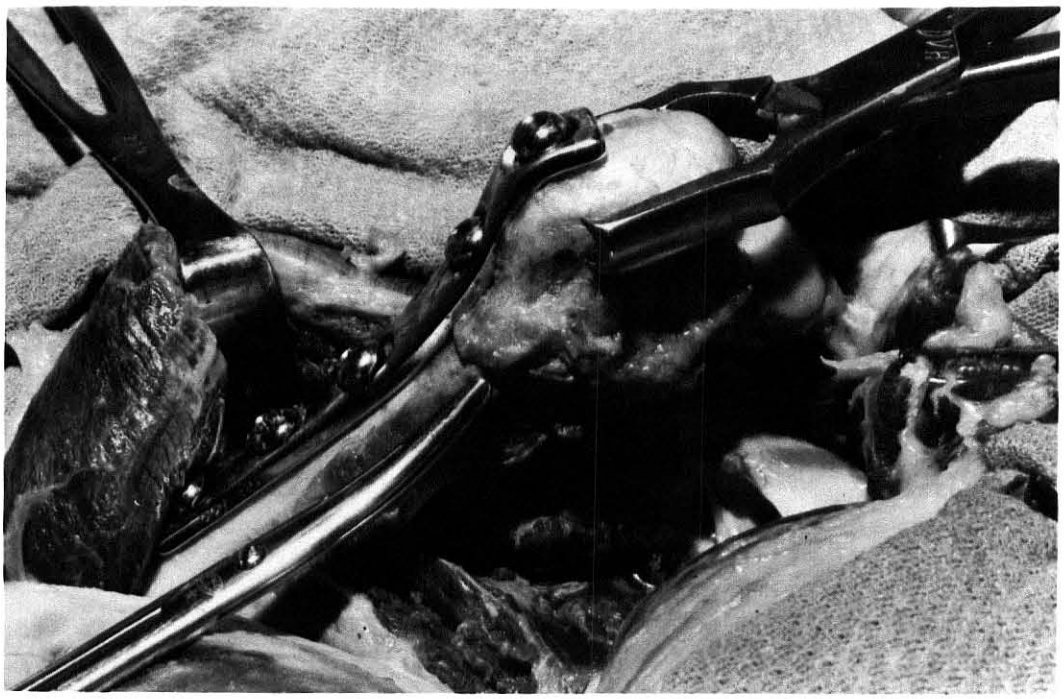


Fig. 28

Figure 29: Five #1 Chromic gut sutures were preplaced in the remaining attached portion of the joint capsule.

Figure 30: The femoral head was placed into the acetabulum and the preplaced sutures tied.

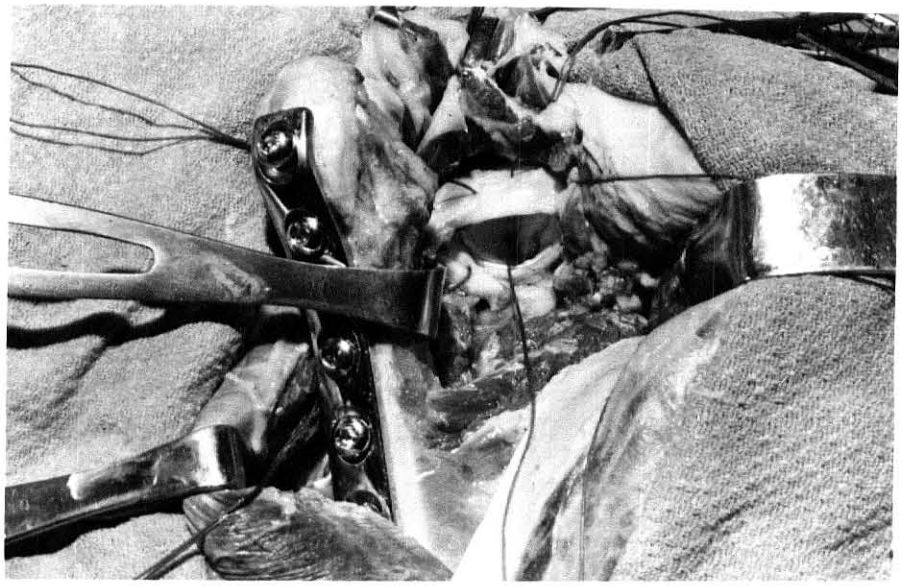


Fig. 29



Fig. 30

Figure 31: Using interrupted mattress sutures, the tendons of insertion of the internal and external obturators, gamelli and gluteals were placed in their normal anatomical positions.

Figure 32: The fascia of the posterior border of the vastus lateralis was sutured to the anterior border of the biceps femoris muscle.



Fig. 31

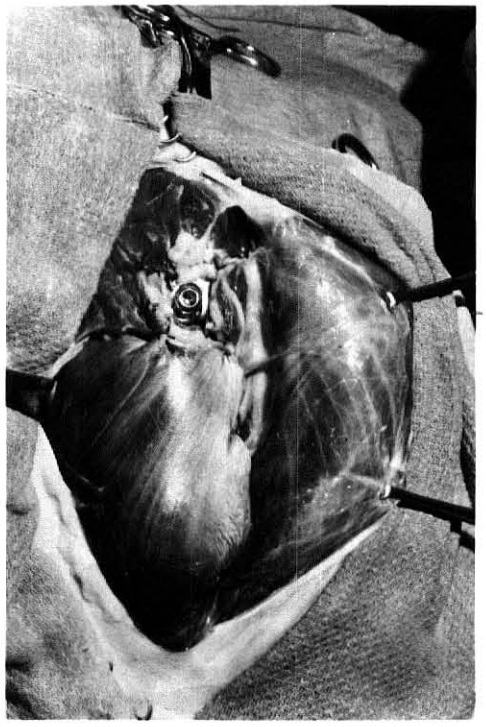


Fig. 32

Figure 33: The tensor fascia lata was sutured to the biceps femoris

Figure 34: The skin was apposed with 4.0 nylon



Fig. 33



Fig. 34

# RESULTS

## I. Radiographic Findings

On the immediate post operative films all transplants appeared to have been reduced and fixed adequately. The frozen grafts (Figure 35) appeared to have slightly less density than those of the other groups. Cortical apposition appeared quite good although in some cases either the graft was slightly larger than the recipient (27,12, 28), or the inverse was the case (9, 6). These differences in size were slight and were found not to significantly alter the rate or character of healing.

By the second post operative week all the femoral heads had luxated either dorsal or ventral to the acetabulum. A periosteal reaction was present in some cases on the medial and/or lateral cortex of the proximal portion of the recipient. Very early signs of demineralization were present in the regions of the greater trochanter and neck in fresh allografts (12, 27 and 28). The frozen allograft group did not show a significant change in density from previous films. In the autograft group, there was evidence of demineralization of the dorsal one-third of the neck and greater trochanter in grafts 3 and 8.

By the third week, a marked demineralization of the greater trochanter and dorsal one-half of the femoral neck was present in the frozen allograft (7)(Figure 36). The fresh allograft (4) appeared to have lost the least density at this time while the autograft (3) took an intermediate position. The autograft recipient had the greatest periosteal reaction on its medial proximal cortex. The periosteal reaction was less in the fresh allograft and absent in the frozen graft.

At four weeks, a slight decrease in density was noted in the region of the greater trochanter and dorsal one-third of the neck throughout

the autografts. In all groups, the periosteal reaction present along the proximal medial aspect of the recipient was slightly larger than previously noted, but appeared more quiescent. Very early evidence of callus formation in the autograft group could only be identified in graft 8. Greater evidence of attempts at callus formation was observed in the frozen allograft group (13, 15, 6). Frozen allograft 13 (Figure 37) had the most marked callus proliferation of all grafts at this time. Fresh allografts (27, 28 and 14) evidenced signs of early callus formation. A progressive demineralization of the head, neck and proximal shaft was seen in all frozen allografts with the exception of 9, which shows little change from that of previous weeks. In the fresh allograft group, demineralization of the neck, greater trochanter and proximal shaft was evident. In this group, 28 and 29 appeared to have shown the most demineralization.

At six weeks a progressing demineralization encompassed the head, neck and shaft of the autografts. This demineralization was most extensive in graft 21 (Figure 38). A radiolucent line separated the graft from the recipient in a number of cases (autograft 21; fresh allografts 27, 28 and 29; frozen allografts 15 and 6). Early evidence of callus formation was seen in all the grafts of the autograft and fresh allograft groups. In frozen allografts 9 and 6, there was no evidence of callus formation, and in grafts 18 and 15, only early signs were visible. Within the frozen allograft group, graft 13 had the largest external callus. The progressive demineralization involving the entire grafts of the fresh and frozen allografts continued. Among the three groups, demineralization was most marked in autograft 21 and frozen allograft 15. Comparing terminal dogs of this period (6, 12, 21), the autograft (21)

showed the most evidence of demineralization, and the fresh allograft (12), the least.

In all groups at eight weeks, the extent of demineralization continued. Of the autografts, graft 30 showed the most demineralization. The frozen allografts showed about the same amount of radiodensity as previously observed at six weeks. Overall, the autografts had maintained the most radiodensity at this time. The fresh allografts showed an increase in the amount of callus formation. A modest amount of callus formation was seen in all the grafts of the autograft group with the exception of 30. A modest indication of callus formation was also seen in frozen grafts (13, 18, and 15). Additionally, in frozen allograft 9 (Figure 39) there was a three millimeter cortical defect present on the medial cortex at the femur at the osteotomy site.

With regard to the nine week group, slightly more callus formation was evident in the frozen allograft (18) than was observed in the fresh graft (27). Healing appeared to have progressed most in the autograft (30). However, it was the autograft which showed the most homogenous appearance, had the thinnest cortex, and evidenced the greatest amount of pitting and articular degeneration. Also, a capital epiphyseal fracture had occurred here. The cortex of the frozen allograft evidenced the least amount of degenerative change. The fresh allograft appeared to have the most radiolucent character of this group.

Progressive demineralization continued into the tenth week. The rate of demineralization appeared to be slowest among the frozen allografts. An enlarging callus was visible in the fresh allograft group, which appeared to primarily originate from the proximal portion of the recipient. Frozen grafts 13 and 15 continued to show an increase callus

formation. Frozen graft 9 had no external callus formation and the deficit on its medial cortex had increased to four millimeters. A decreased density and thinning of the distal medial cortex could be seen in autograft 8 (Figure 40). In general, at this time, both the fresh and frozen grafts evidenced larger callus formation than was seen in the autograft group. However, in the autograft group, a small external callus was present around all grafts. A radiolucent line separated, to some degree, the graft and recipient in autograft 19, fresh allograft 28, and frozen grafts 15 and 9.

Among the twelve week graft group, the greatest amount of external callus appeared to be present in the fresh allograft. Demineralization was moderate in all the grafts of this group with the frozen allograft (9) evidencing the most change. The frozen allograft had a four millimeter radiolucent defect on its distal medial cortex and a small radiolucent area at the site of apposition between transplant and recipient. In the fresh allograft (14), a distinct radiolucent line separated the graft from the recipient. However, in the fresh allograft, cortical density was fairly good. In the autograft (8), screws one and two were in bone which was less radiodense than bones of other grafts. Although there was some decreased density involving the medial cortical bone of the autograft transplant, no radiolucent band was present at the site of implantation.

Comparing all grafts at twelve weeks, the autograft group appeared to have lost the least amount of its normal density, and the frozen allografts the most. The fresh allograft group evidenced the greatest amount of external callus formation of all three groups.

At fourteen weeks, the extent of demineralization progressed and

callus formation increased.

In the fifteen week group, the autograft had the least radiographic evidence of callus formation and the frozen allograft the most. In all grafts, a distinct radiolucent line separating the graft and recipient was apparent. All the grafts showed evidence of moderate demineralization. The distal medial border of the autograft appeared to be thinning near its transplant site. This thinning of the cortex was not noted in the other grafts.

Comparing the eighteen week group at sixteen and eighteen weeks, a radiolucent line separated the transplant from the recipient in the fresh allograft only. Healing at the union site appeared to be progressing satisfactorily in all three cases. The fresh allograft (Figure 4) and autograft (Figure 42) both appeared to have a much larger external callus than did that of the frozen allograft. Evidence of external callus formation in the frozen allograft was minimal. The difference in radiodensity of the three grafts was minimal. The head and neck of the frozen graft (Figure 43) showed the most degenerative change. The medial cortex of the frozen graft, and recipient proximal to the union site, showed the greatest loss in normal architectural appearance.

II. Gross and Microscopic Pathological Changes

A. Three Week Group:

In the three week group the femoral head had luxated dorsally to the acetabulum in all cases and was surrounded by a serous pocket. In the case of the fresh allograft (4) hemolytic staphylococcus was isolated from this pocket. Evidence of healing was apparent in the autograft (3) and frozen allograft (7), but little gross evidence

of healing had taken place in the fresh allograft.

Microscopic Findings

The cartilage of the entire femoral head of the frozen allograft had taken an eosinophilic characteristic. In the autograft, the outer two-thirds of the cartilage was eosinophilic and in the fresh allograft the outer one-third. The chondrocytes in the region were either dead or dying. The cartilage of the inner two-thirds of the fresh allograft and inner one-third of the autograft contained some cartilage cells in various stages of degeneration. In the autograft, the articular cartilage appeared to have splintered. Freezing artifacts were present in the frozen graft.

In all cases the subchondral bone of the femoral head was eosinophilic and contained empty lacunae devoid of osteocytes. No osteoblastic or osteoclastic activity was present in the Haversian systems or Volkmann's channels. The bone marrow spaces of the autograft and fresh allograft were occupied by RBC's and PMN's. Some early fibrous tissue infiltration was seen in the marrow of the autograft. In the autograft, the bone of the head appeared viable although 90% or more of the osteocytes had disappeared or had taken a basophilic staining property. The bone of both the fresh and frozen allografts was dead. No indication of vascular, osteocytic, or bone marrow activity could be identified in any of these grafts in the head of proximal shaft.

Near the union site in the autograft, a clear dividing line of the cortex was present between new bone formation and old transplanted bone. There was a seam of osteoid and bone on the

cortical surface close to the union site of the recipient and transplanted bone. The transplanted bone was separated from the newly formed bone by a resorption line. A resorption line was also present in the fresh allograft.

The fresh allograft showed the most external callus formation. The callus of the autograft was intermediate in size, and the frozen allograft the smallest. The callus was well attached to the transplant and recipient bone. A wedge of cartilage was present between the external callus and periosteal surface of the original bone in both the fresh allograft and autograft. In the fresh allograft, a bone plate was being formed transversely across the union site. In all grafts, the cortical portion of the graft was dead or dying. In both the autograft and fresh allograft a remodeling of the transplant and recipient bone was taking place. Osteoblastic and osteoclastic activity was present on the periosteal surface of the recipient and transplanted bone at the union site. The endosteal surface of the transplanted bone was occupied by a well formed endosteal callus in the autograft and fresh allograft and to a much lesser degree in the frozen allograft. Covering the endosteal surface of the fresh graft not seen in the autograft or frozen graft was a layer of osteoblasts which extended one-half of a 10x field. The bone marrow cavities of the transplants in this region contained fibrous tissue. The fibrous tissue was most abundant in the frozen graft.

B. Six Week Group:

Macroscopic Findings:

In all three grafts the femoral head had luxated. The arti-

cular surface of the femoral head of the autograft (21) was in part covered by connective tissue and appeared to have undergone some degenerative change. In the fresh allograft (12), a callus was forming at the site of apposition of the transplanted and recipient bone. In both the autograft and frozen allograft (6), little evidence of bony union was present. In the autograft, this site was surrounded by fibrous tissue containing a very dark serous exudate in which Beta hemolytic staphylococcus and non-hemolytic E. Coli were identified.

Microscopic Findings:

In the autograft and frozen allograft, the cartilage of the femoral head was nonviable. This complete destruction of the autograft cartilage was not present at three weeks. It appeared as an eosinophilic mass which was partly frayed and splintered. The cartilage cells of the fresh allograft were either nonviable or were degenerating. However, the extent of degeneration of the head of the fresh allograft was not as advanced as was the six week autograft. The subchondral bone of all the grafts had taken an eosinophilic stain. No living osteocytes were observed. In these three grafts, the bone marrow had taken an eosinophilic stain and no cellular or vascular activity was present. In both the autograft and fresh allograft the cortex of the femoral head was surrounded by an active fibrous tissue with a great deal of vascular activity. The ingrowth of fibrous tissue with a vascular core into the dead cortical bone of the head of the autograft had not been seen at three weeks. In both the autograft and fresh allograft some osteoclastic and osteoblastic activity

existed on the surface of this dead bone, indicating an attempt to remove bone and lay down new bone. However, in the frozen allograft, there was no fibrous or vascular activity on the cortex of the head. The cortical bone of all these grafts were nonviable. In all three cases the recipient bone was undergoing remodeling, but a normal bone marrow activity persisted.

At this time, basically the appearance of the autograft and fresh allograft was similiar. Both had a small periosteal callus. On the periosteal surface of the autograft there was a large amount of fibrous tissue containing a great deal of vascular activity. There was also fibrous tissue on the periosteal surface of the fresh allograft. Substantial osteoclastic activity was present at the distal end of the transplant in both cases. The frozen allograft differed in appearance from the other two in that it had a pronounced periosteal callus, very little remodeling had taken place in the recipient and a nonunion existed. It, like the fresh allograft, had a dense bone marrow plate separating the transplant and recipient.

C. Nine Week Group:

Macroscopic Findings:

Two of the femoral heads had luxated dorsal (18, 30) and one had luxated ventral to the acetabulum. The heads were surrounded by a pseudojoint. Degenerative change involved the articular cartilage of the femoral heads of the fresh allograft (27) and autograft (30). There was evidence of healing at the recipient site in all three grafts.

Microscopic Findings

In all three grafts, fibrous connective tissue covered the femoral head. In the autograft (30), for the first time, dead and dying cartilage was being replaced by a fine network of fibrous tissue containing blood vessels. This was also true in the frozen allograft (18) where fibrous tissue and blood vessels invaded the cortex of the head. The connective tissue of the fresh allograft (27) was much more mature than the other two. Here no vascular ingrowth into the head had occurred. The bone marrow of all three heads were either nonviable or were undergoing degenerative changes and had taken an eosinophilic stain. None of the grafts showed evidence of osteoblastic activity. However, osteoclastic activity was present. Proximal to the recipient site, the transplant cortical bone of all grafts were clearly nonviable and had taken an eosinophilic stain.

In the distal shaft of the autograft living osteocytes were present within cortical bone. The Haversian system of the autograft contained fibrous tissue and blood vessels. Osteoblastic and osteoclastic activity was present on the periosteal and endosteal surfaces of the transplanted bone. An excellent callus had formed which contained osteoblasts, osteocytes and new bone not previously seen. The endosteal callus of the transplant contained a mature, dense connective tissue with a good blood vessel supply. In the frozen allograft vascular activity persisted within the Haversian systems. Osteoblasts were being laid down on dead or dying trabeculae. A moderate periosteal bone marrow callus was present. In this same region osteoclastic activity was present on the periosteal

and endosteal surfaces of the fresh allograft. Here, there was fibrous tissue proliferation, but no osteoblastic activity, of the three grafts the fresh allograft had the poorer periosteal and endosteal callus.

D. Twelve Week Group:

Macroscopic Findings

The femoral head of all grafts in this group had luxated dorsal to the acetabulum and were contained within a pseudojoint. Grossly, there was very little degenerative change in evidence on the articular surface of the femoral heads. A fibrous tissue layer covered their surface and a callus was present bridging the graft to recipient in all cases.

Microscopic Findings

In all three grafts the cartilage and cortices of the femoral heads were nonviable. Their shafts were also nonviable. A fibrous tissue layer covered the heads of the grafts. In the autograft (8) a fine network of fibrous tissue and blood vessels invaded the cortex of the head. The cartilage of the frozen allograft (9) appeared as a homogenous eosinophilic mass. The frozen allograft transplant was young. Its epiphyseal plate appeared completely homogenous, eosinophilic, and contained a calcified cartilage matrix. No osteoblastic activity persisted. The metaphyseal bone of this graft was nonviable.

Osteoclastic activity was present on the periosteal surface of the distal shaft of the autograft. A very small callus existed between the recipient and transplanted bone which consisted primarily of fibrous tissue. Dense connective tissue filled the

bone marrow cavity of the transplant while the recipients' bone marrow was filled with a fine network of fibrous tissue. In the fresh allograft (14) the periosteal surface of its distal shaft was undergoing resorption. In both the fresh and frozen allografts there was a remodeling and resorption of the periosteal surfaces of the recipient bone. In the frozen allograft vascular and osteoblastic activity existed within some of the Haversian systems of the transplanted bone. A good periosteal and bone marrow callus existed in the fresh and frozen allografts and within their bone marrow cavities fibrous tissue was present.

E. Fifteen Week Group:

Macrosopic Findings:

The femoral heads of all three grafts had luxated dorsal to the acetabulum and were enclosed within a pseudojoint. Grossly, the articular cartilage of the auto graft and fresh allograft appeared to have undergone moderate degenerative change. Fibrous tissue covered the heads of all three grafts and a firm callus was present at each transplant site.

Microscopic Findings:

The articular cartilage of the heads of all grafts in this group were nonviable. In the autograft (19) and fresh allograft (29) degenerating cartilage was being replaced by connective tissue containing a vascular supply. The extent of the connective tissue and vascular ingrowth in the fresh allograft had been seen earlier. The bone of all the heads was nonviable. A fibrous tissue and blood vessel infiltration was present in the

bone marrow cavities of the heads in all grafts. Similiarly, there was an increased vascularity, osteoblastic activity and fibrous tissue invasion of the cortex of the femoral heads. The shafts of all grafts were nonviable.

A periosteal and endosteal callus was present in each case. The bone marrow cavities of the transplants contained fibrous tissue. In the autograft, a moderate endosteal callus existed. At the most distal end of this transplant osteoblastic activity was present. A dense fibrous tissue covered its periosteal surface. Here, a bone plate served as a transversely oriented callus which separated the transplant and recipient. Remodeling of the recipient was taking place both in the autograft and fresh allograft. In the fresh and frozen allografts, fibrous tissue, blood vessels, and active osteoclasts were present within the cortical bone of the distal shafts of the transplants.

F. Eighteen Week Group:

Macroscopic Findings:

All of the femoral heads had luxated dorsal to the acetabulum and lay within a pseudojoint. Degenerative change involving the articular cartilage of the femoral head were most marked in the autograft (5)(Figure 44) and frozen allograft (13). A fibrous connective tissue covered the femoral heads and a good callus had formed between the transplant and recipient in all three cases (Figure 45).

Microscopic Findings:

The articular cartilage (Figures 46, 47, 48) and bone of the femoral heads (Figures 49, 50) were nonviable. The cartilage

of the autograft was being invaded by a great deal more connective tissue than had been previously seen. Also in the autograft, osteoclastic activity was present (Figure 51) and in some areas osteoblasts had laid down bone around dead bone spicules. The femoral heads and necks of both the fresh and frozen allografts contained fibrous tissue (Figure 52), blood vessels and osteoclasts active in the removal of dead bone (Figure 53, 54). The bone marrow of the femoral heads were empty. The shafts of all the transplants were nonviable (Figures 55, 56). On their periosteal and cortical surfaces, fibrous tissue, blood vessels and osteoclastic activity was present (Figures 57, 58). The bone marrow cavities of the transplants contained fibrous tissue Figures 60, 61, 59). A good periosteal and endosteal callus had formed in the autograft. In the fresh allograft (28), a small external callus existed, a portion of which consisted of hyalin cartilage. A mixture of fibrous tissue and hyalin cartilage divided the transplant and recipient bone of the fresh allograft (Figure 62). In the frozen allograft a fibrous layer separated recipient and transplant. Here too (13), a good external callus existed.

III. Tetracycline Study:

At six weeks all the recipients had tetracycline labeling of their bone marrow cavities. None of the grafts were labeled. The recipient of the fresh allograft (12)(Figure 63) appeared to be the most uniformly labeled. In the autograft (21) and fresh allograft, a clear tetracycline labeled line marked the division between graft and recipient. In the frozen allograft (6) the tetracycline label

ended irregularly just distal to the original osteotomy site.

In the nine week fresh allograft (27)(Figure 64) there was a tetracycline label present in the distal two millimeter of the graft. The proximal six millimeter of the recipient and distal two millimeter of the fresh allograft showed a very high uptake of the label. In the frozen allograft (18) no label appeared in the transplant. However, here an increased fluorescent intensity was present in the proximal portion of the recipient at the osteotomy site, where a callus was being formed.

At the twelfth week the distal one centimeter of the autograft (8)(Figure 65) transplant had taken the tetracycline label. This was not the case with the fresh (14) and frozen (9) allografts. In both the fresh and frozen allografts the tetracycline label separated the labeled recipient from the nonlabeled transplant. An incresed uptake of the tetracycline label at the proximal portion of the recipient was evident in all grafts.

In the fifteen week group negligible labeling of the autograft transplant (19) was evident. In the fresh allograft (29) (Figure 66) and frozen allograft (15) the tetracycline label had been picked up by the distal three to four millimeters of the transplant. An increased fluorescent intensity appeared in the area of callus formation in these latter two casts.

In the eighteen week autograft (5) (Figure 67) an intense tetracycline fluorescence appeared in the distal three millimeter portion of the transplant and proximal portion of the recipient in the area of callus formation. In the frozen allograft (13) of this same time period the tetracycline label appeared as a linear strip one

millimeter distal to the second bone screw. It lined the endosteum and bone marrow cavity of the recipient.

Figure 35: Grafts of frozen allograft group appeared to be of slightly lesser density than those of the other groups (radiograft of frozen allograft 15).

Figure 36: A marked demineralization of the greater trochanter and dorsal one-half of the femoral neck was present in frozen allograft(7).

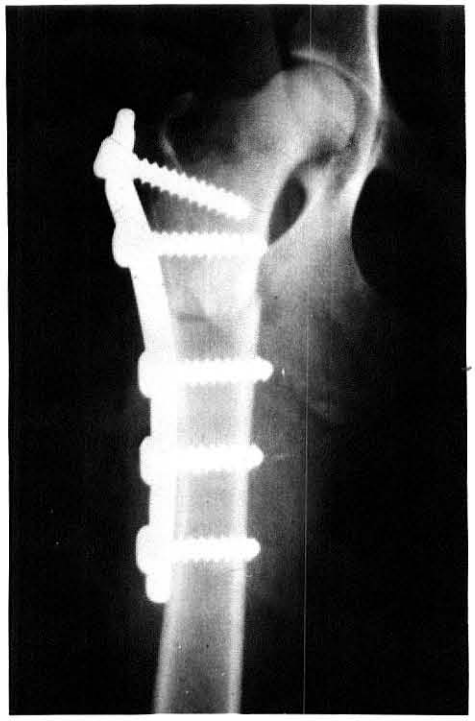


Fig. 35



Fig. 36

Figure 37: Frozen Allograft thirteen had the most prominent external callus of all grafts at four weeks. The periosteal reaction present along the proximal medial aspect of the recipient was slightly larger than previously noted, but appeared more quiescent.

Figure 38: The progressive demineralization which involved the head neck and shafts of the autografts was most extensive in graft 21.



Fig. 37

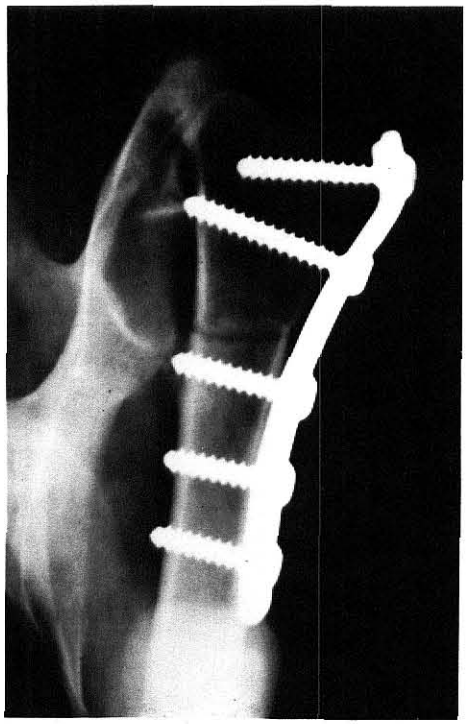


Fig. 38

Figure 39: Eight weeks post operatively a three millimeter defect was present on the medial cortex of the femur near the osteotomy site in frozen allograft 9. The greater trochanter, neck and shaft of the transplant showed a moderate degree of demineralization.

Figure 40: A decreased density and thinning of the distal medial cortex was seen in autograft 8, ten weeks following surgery.



Fig. 39

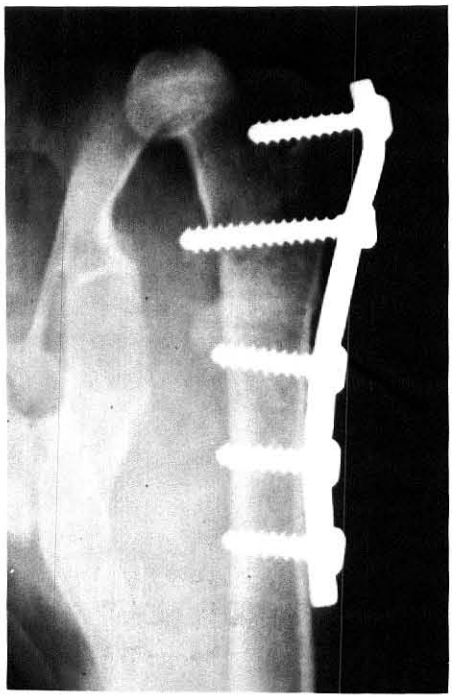


Fig. 40

Figure 41: Eighteen weeks post surgically fresh allograft 28 appeared to be undergoing a slow demineralization process. A moderate callus had formed at the union site.

Figure 42: By the eighteenth week autograft 5 had undergone a marked demineralization. The proximal portion of the marrow cavity of the recipient appeared less radiodense. A moderate callus was present surrounding the recipient site.



Fig. 41

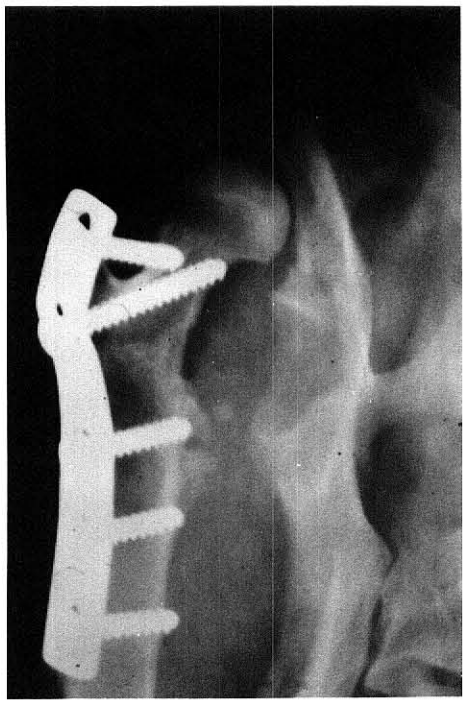


Fig. 42

Figure 43: The articular surface, as well as body of the head and neck of the transplant displayed signs of marked degeneration in frozen allograft 13. Sixteen weeks post operatively.

Figure 44: The articular cartilage of the femoral head in autograft had undergone a marked degenerative change.

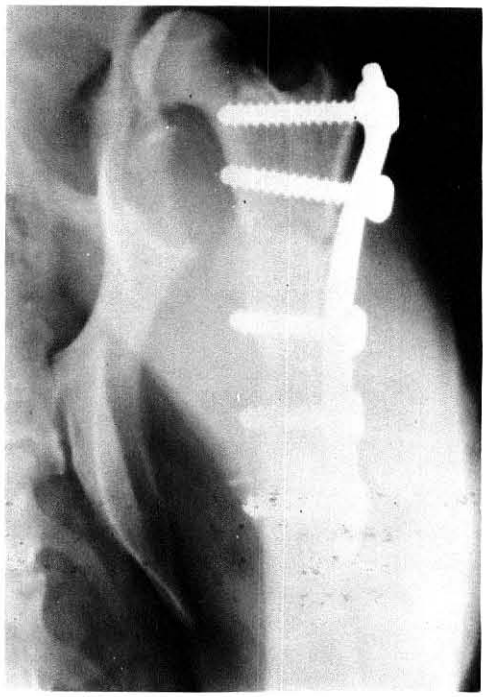


Fig. 43

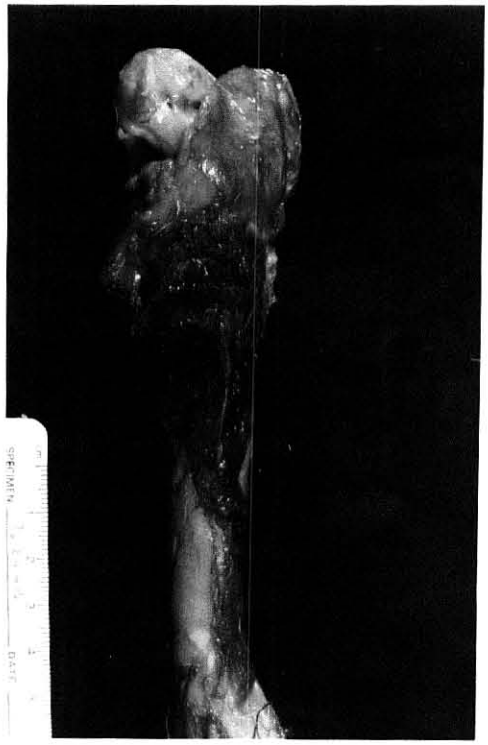


Fig. 44

Figure 45: A stable callus had formed between the graft and recipient in all three groups at eighteen weeks. Photograph taken of fresh allograft 28.

Figure 46: Photomicrograph of degenerating articular cartilage of the femoral head of fresh allograft 28. H+E, 120X.




Fig. 45

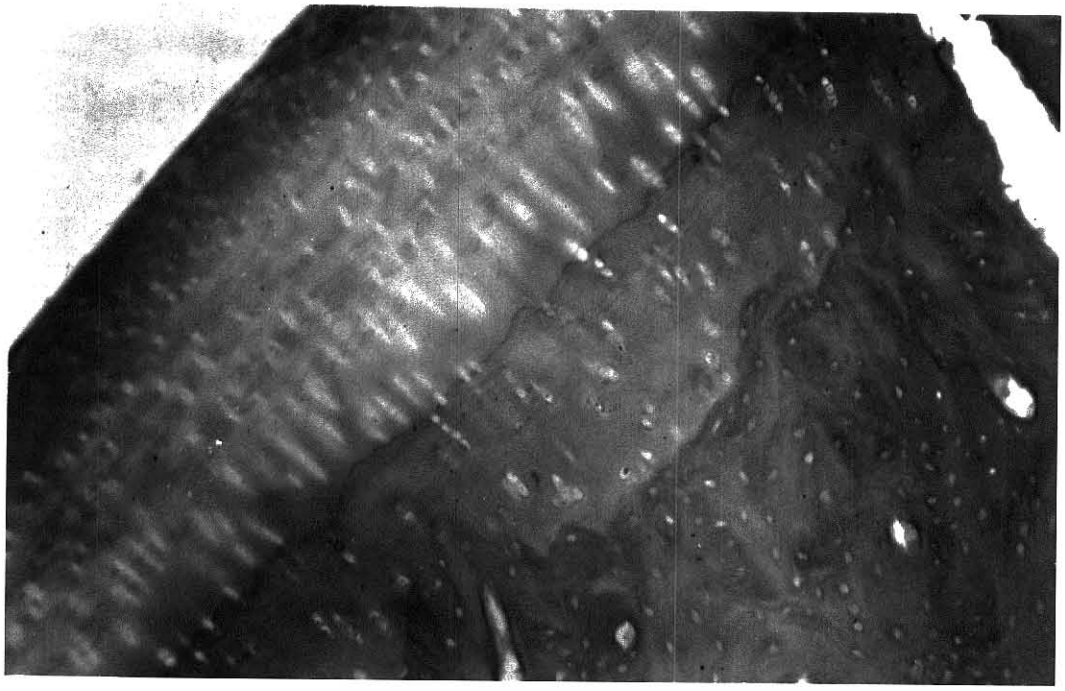


Fig. 46

Figure 47: Photomicrograph of dead and dying cartilage of the femoral head in autograft 5. H+E, 120X.

Figure 48: Photomicrograph depicting dead and dying cartilage of the articular surface of the femoral head in frozen allograft 13. H+E, 120X.

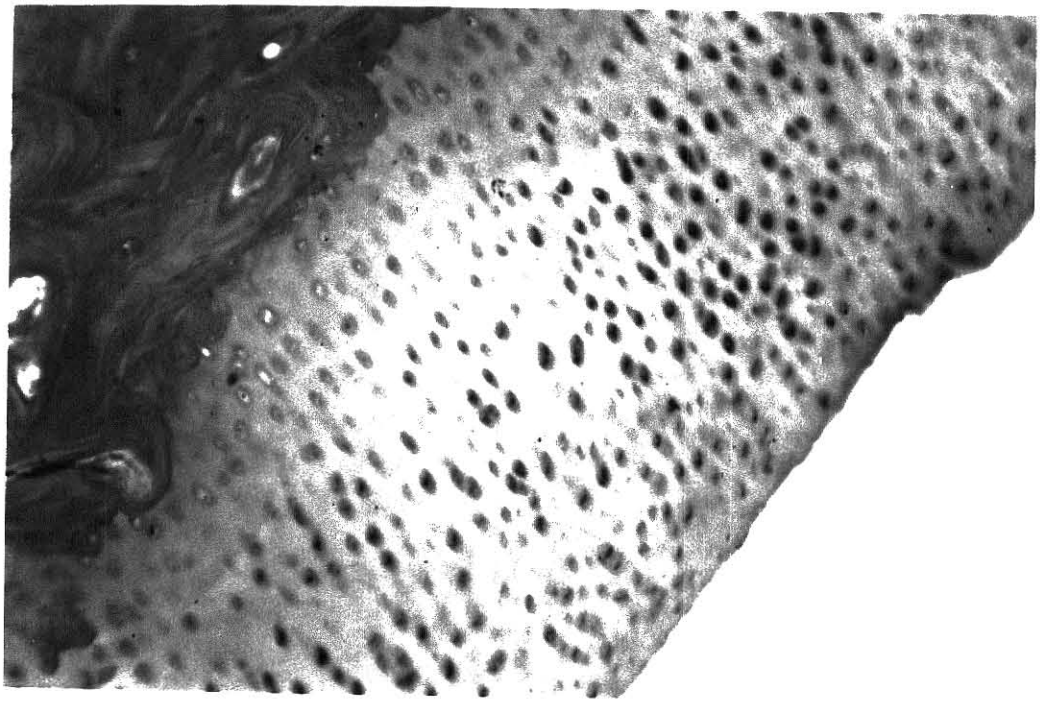


Fig. 47

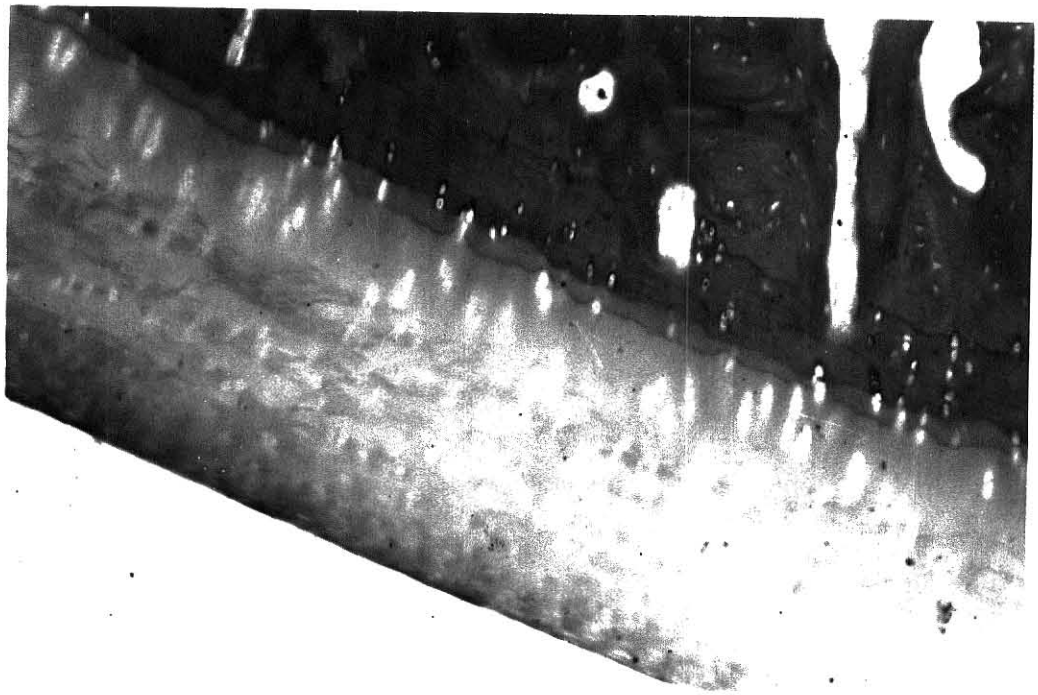


Fig. 48

Figure 49: Photomicrograph of nonviable bone in center of femoral head in fresh allograft 28. H+E, 120X.

Figure 50: Photomicrograph of nonviable tissue within the femur.

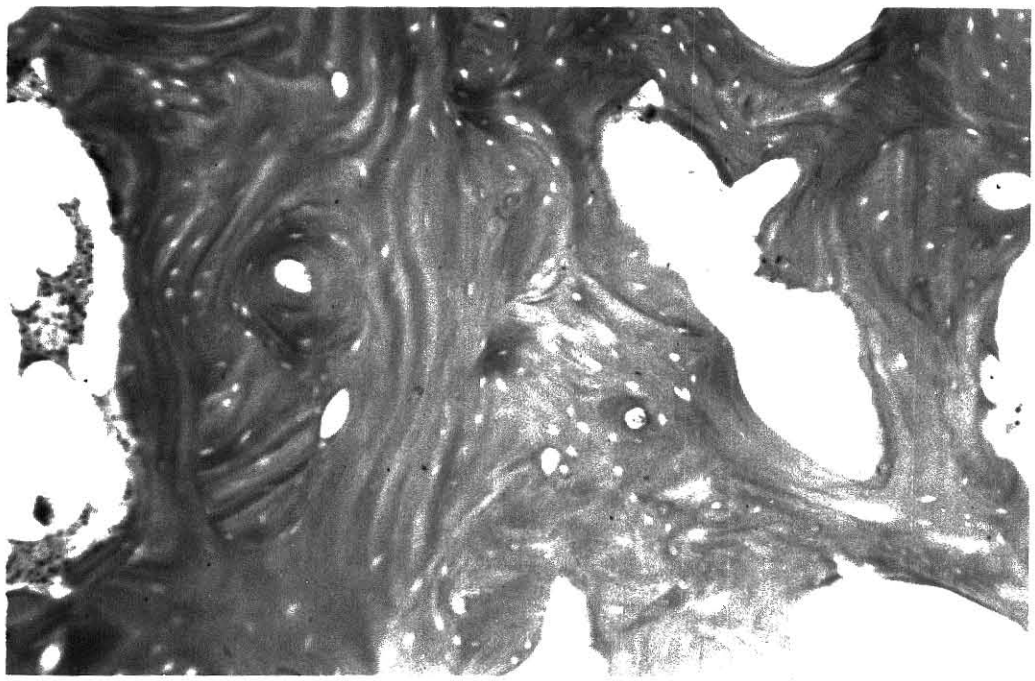


Fig. 49



Fig. 50

Figure 51: Photomicrograph of osteoclastic activity in the periostium of the femoral head in autograft 5. H+E, 120X.

Figure 52: Photomicrograph of the center of the transplanted femoral head showing a fibrous tissue invasion in autograft 5. H+E, 120X.

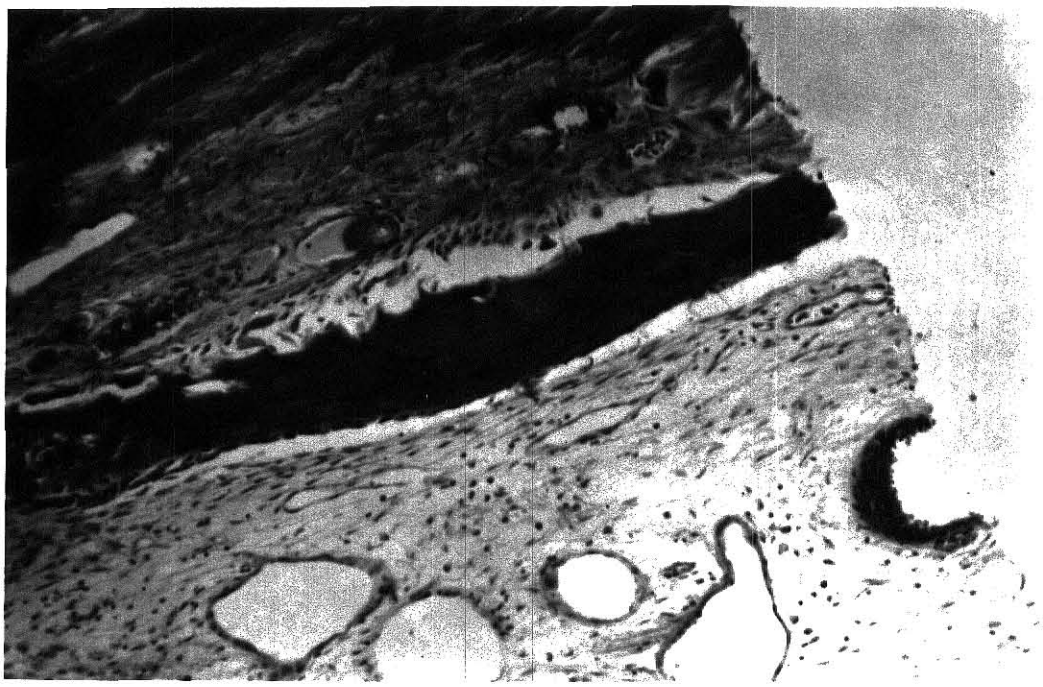


Fig. 51

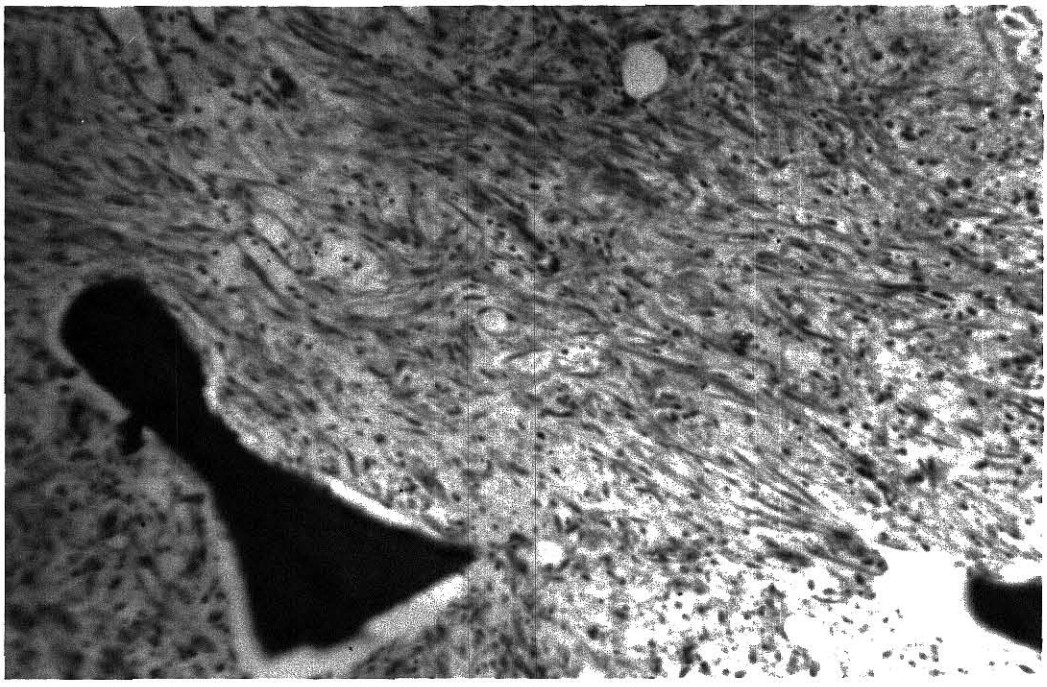


Fig. 52

Figure 53: Photomicrograph illustrating invasion of the periosteal surface of the femoral head by osteoclasts in fresh allograft 28. H+E, 120X.

Figure 54: Photomicrograph illustrating osteoclastic activity on the periosteal surface of the femoral head in frozen allograft 13. H+E, 120X.

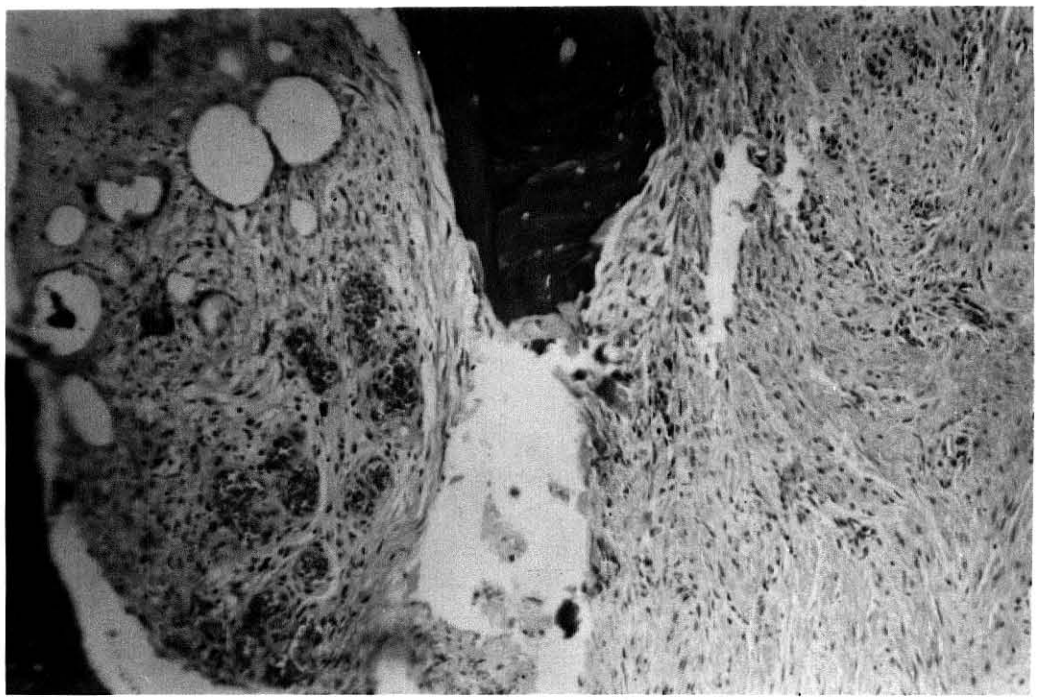


Fig. 53



Fig. 54

Figure 55: Photomicrograph showing the dead bone of the cortex of the transplant in fresh allograft 28. H+E, 120X.

Figure 56: Photomicrograph of the dead bone of the cortex of the transplant in autograft 5. H+E, 120X.



Fig. 55

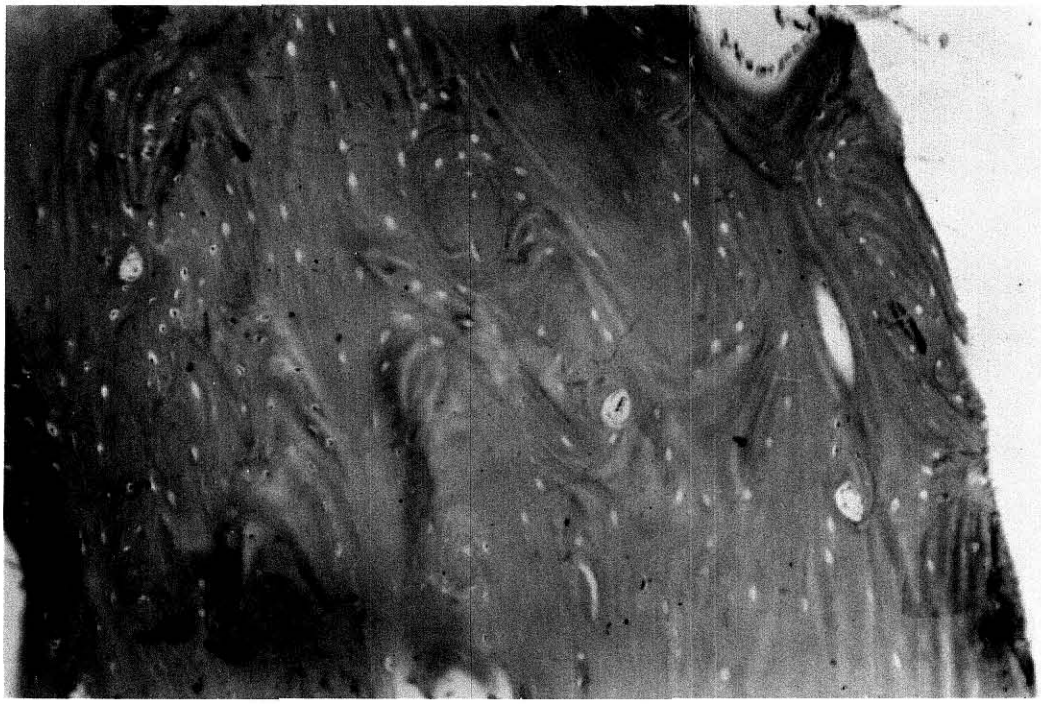


Fig. 55

Figure 57: Photomicrograph showing fibrous tissue, osteoblasts and bone on the periosteal surface of the distal portion of the transplant in frozen allograft 13. The remainder of the cortex was not viable. H+E, 120X.

Figure 58: Photomicrograph showing osteoblastic and osteoclastic activity in the periosteum of the transplant near the union site in autograft 5. H+E, 120X.

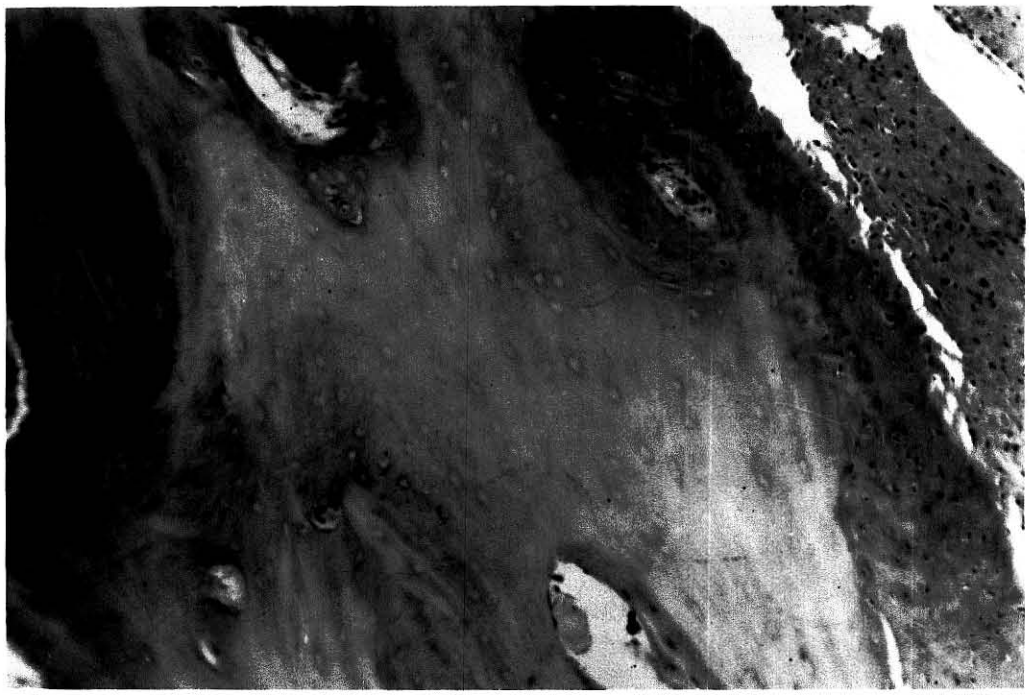


Fig. 57



Fig. 58

Figure 59: Photomicrograph showing the fibrous tissue composition of the bone marrow cavity of the transplant in autograft 5. H+E, 120X.

Figure 60: Photomicrograph showing the bone marrow cavities of both the transplant and recipient in frozen allograft 13. H+E, 120X.



Fig. 59

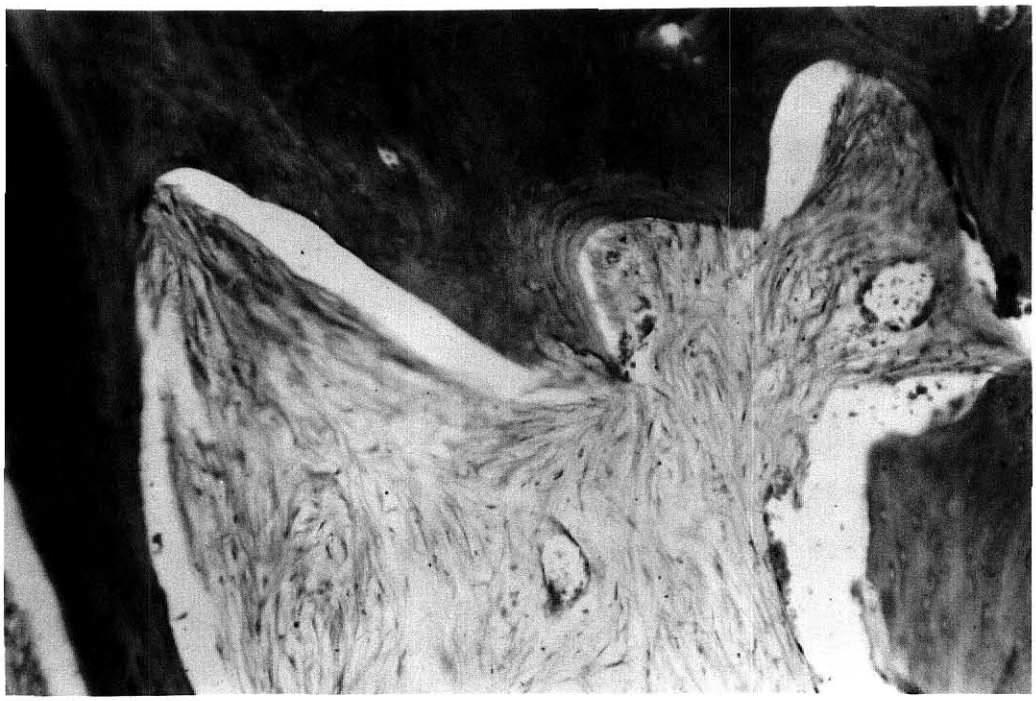


Fig. 60

Figure 61: Photomicrograph of fibrous tissue invasion of the bone marrow of the transplant in fresh allograft 28. H+E, 120X.

Figure 62: Photomicrograph at union site showing bone plug formed by recipient in fresh allograft 28. H+E, 120X.

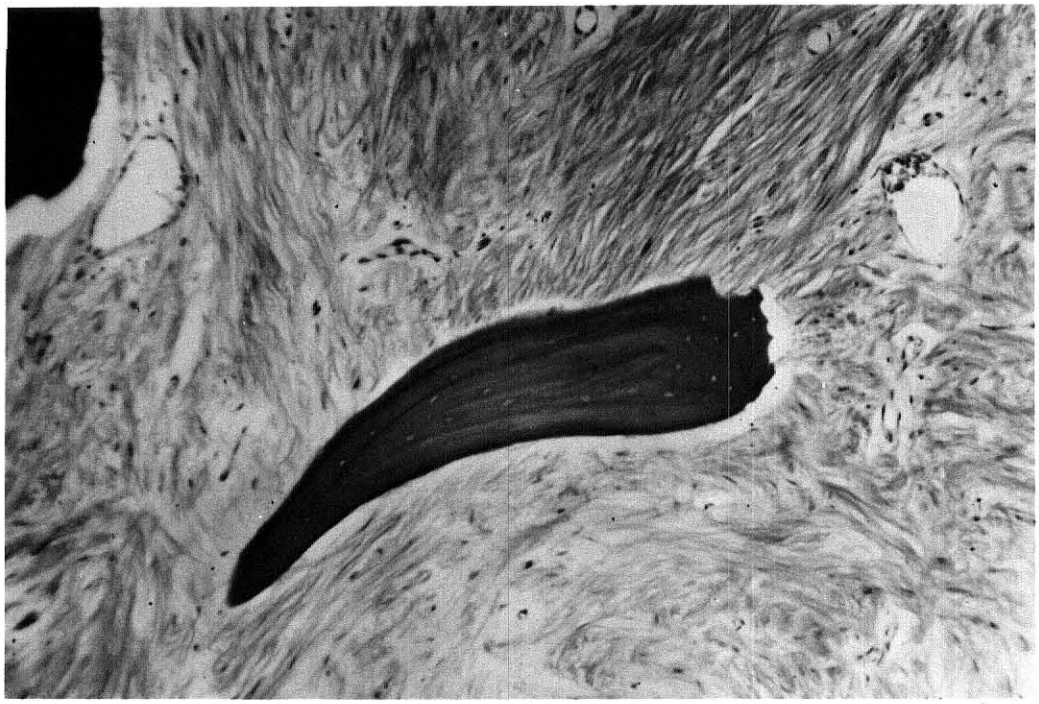


Fig. 61

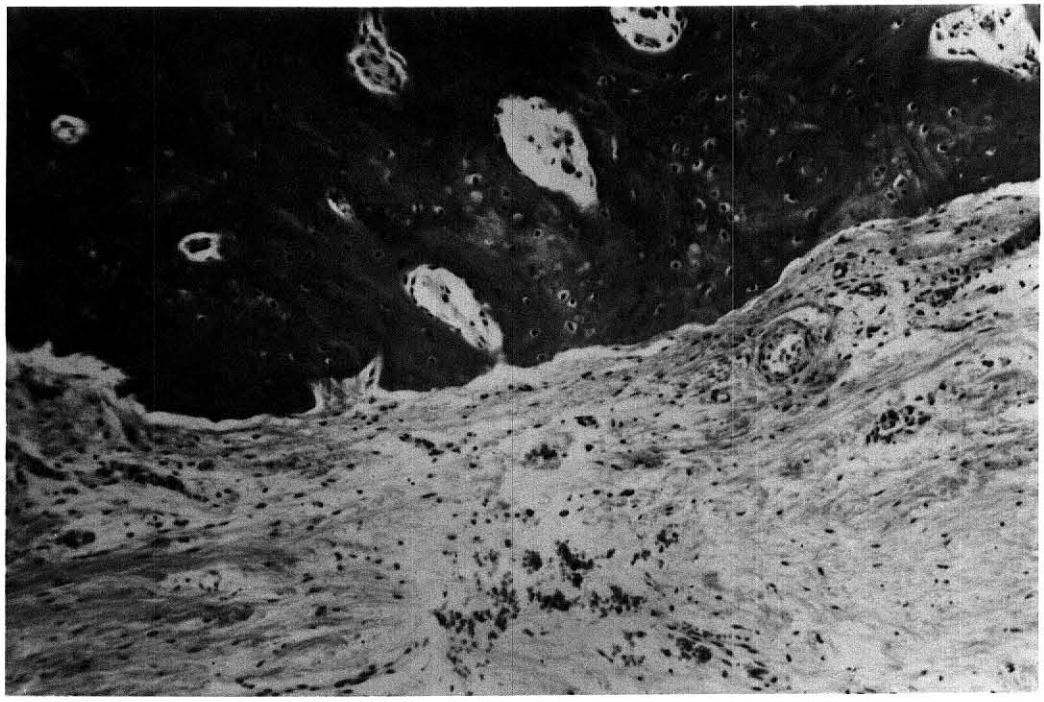


Fig. 62

Figure 63: At six weeks all the recipients had tetracycline labeling of their bone marrow cavities. None of the grafts were labeled. The bone marrow cavity of the recipient in fresh allograft 12 appeared the most uniformly labeled

Figure 64: At nine weeks there was a tetracycline label present in the distal two millimeters of the transplant in fresh allograft (27).

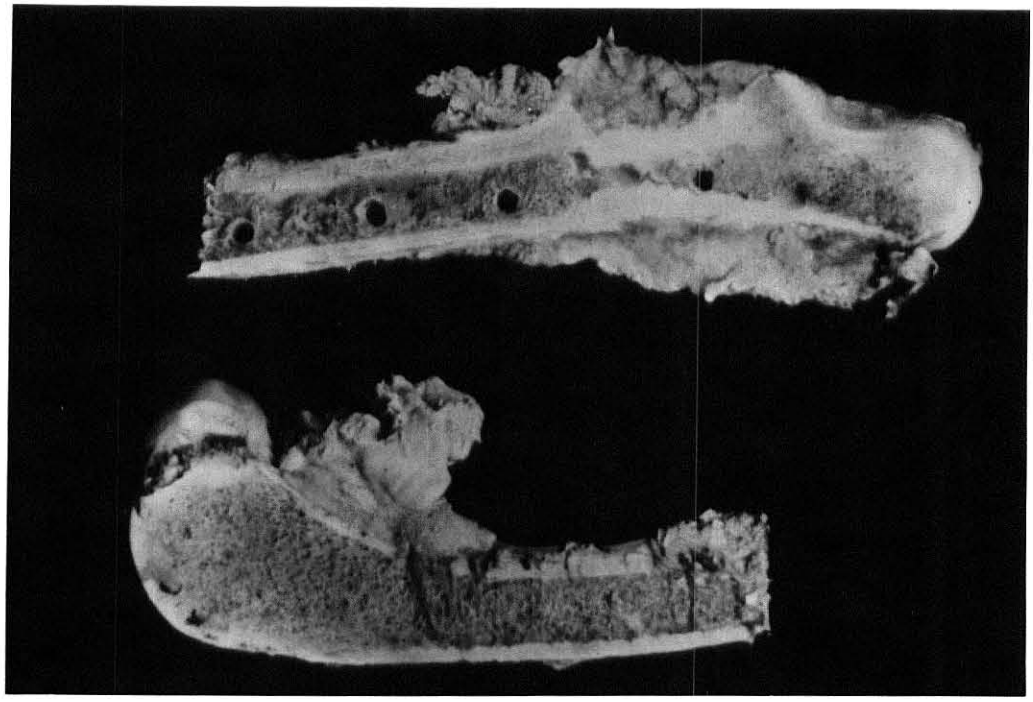


Fig. 63

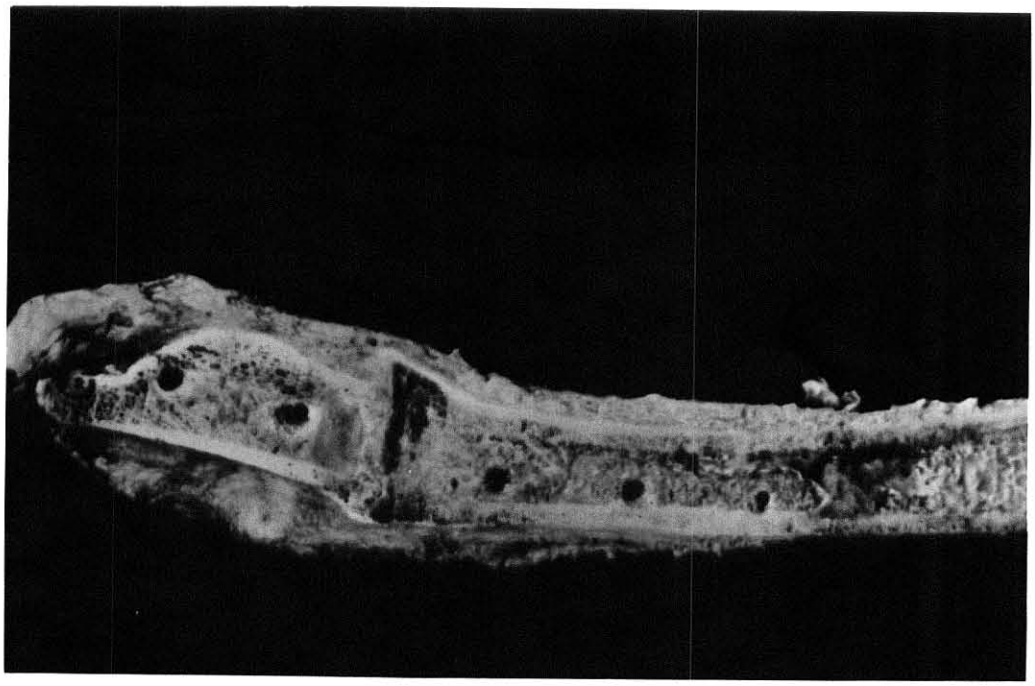


Fig. 64

Figure 65: The distal one centimeter of the transplant in autograft (8) had taken the tetracycline label twelve weeks following surgery.

Figure 66: A tetracycline label had been picked up by the distal 3-4 millimeters of the transplant of fresh allograft (29) by the fifteenth week.

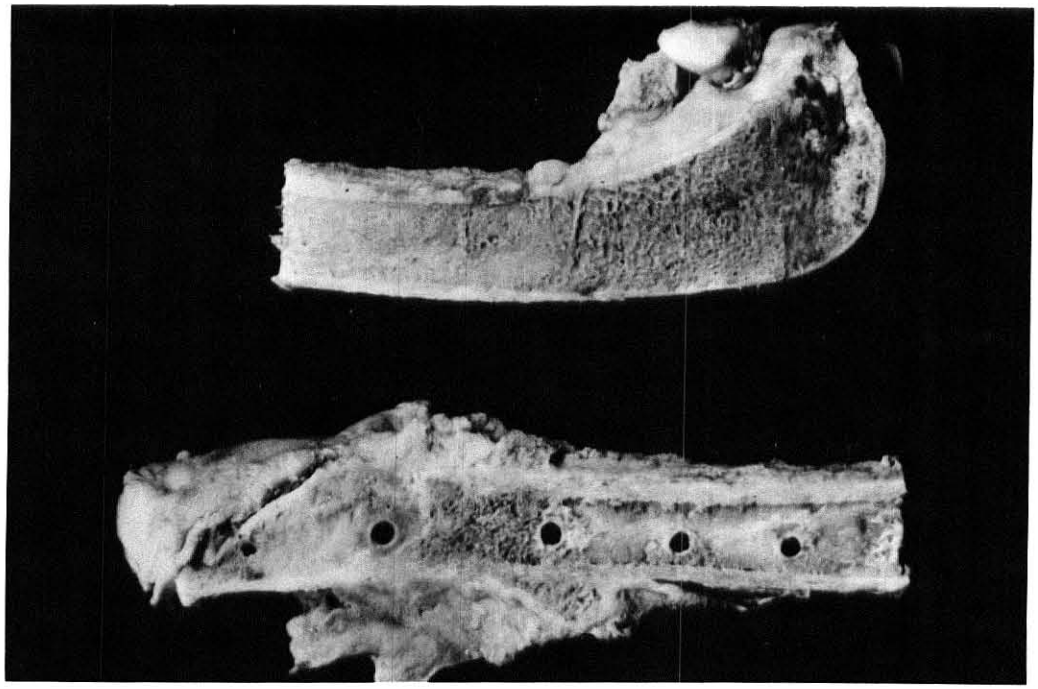


Fig. 65

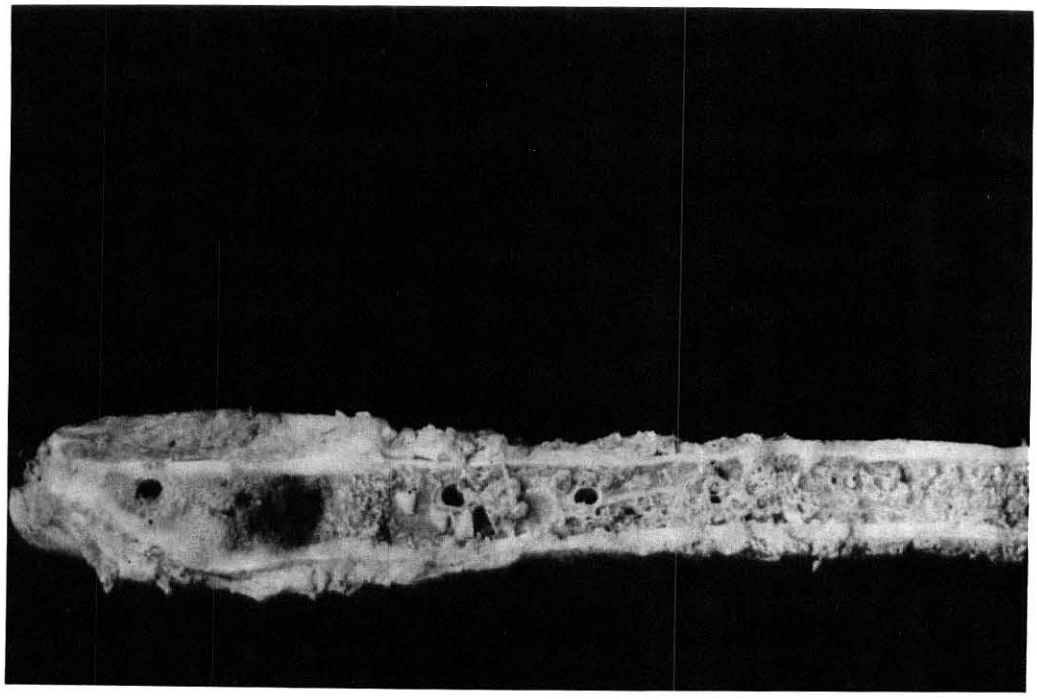


Fig. 66

Figure 67: An intense tetracycline flourescence appeared in the distal three millimeter portion of the transplant and proximal portion of the recipient in the area of new callus formation in autograft 5 eighteen weeks post surgically.

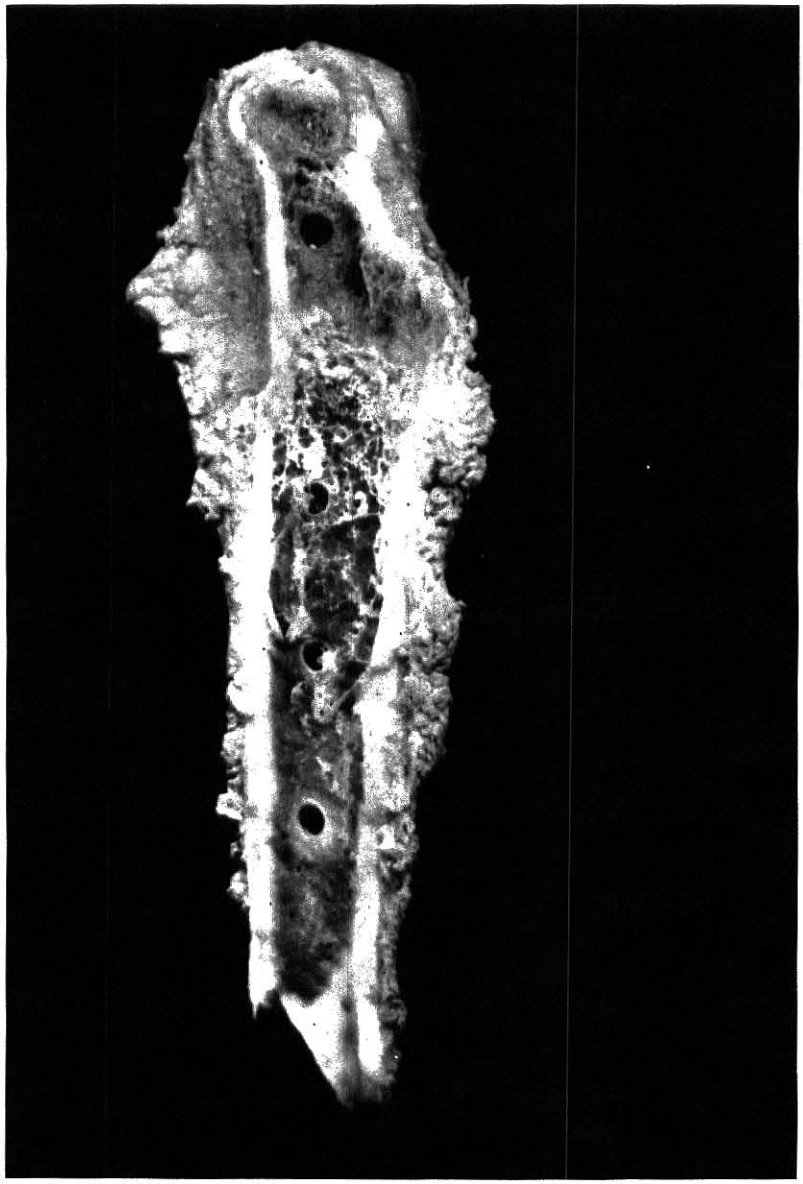


Fig. 67

## DISCUSSION

In 1956 Curran and Gibson (1956)(38) found they could not preserve adult cartilage at low tenperatures without loss of viability. Gibson (1957)(68 and (1965)(65), showed freezing did not preserve cartilage in a viable state. When whole cartilage was cooled to below $0^{\circ}$ centigrade the chondrocytes were placed in a saline solution containing 15 percent glycerol prior to cooling and they perished (Curran and Gibson 1965)(39). In 1960 Heyner (1960)(85), discovered that embryonic rat and mouse cartilage cells in rudimentary bones could survive cooling to $-79^{\circ}$centigrade after partial digestion of the matrix with trypsin and treatment with glycerol. Smith (1965)(154) isolated living chondrocytes from the hyalin and fibrocartilage of the adult mammals. He then froze the chondrocytes at $-79^{\circ}$ centigrade in a medium containing ten percent DMSO and was able to maintain the viability of the chondrocytes after freezing. The death of chondrocytes following freezing in this study is in agreement with these workers and further indicates that, thus far, it has not been possible to freeze cartilage and maintain its viability within an intact cartilage matrix.

Judet (1908)(96) demonstrated the fact that synovial fluid provided the nutrition for articular cartilage. Lemperg (1972)(108) showed that when articular cartilage fragments were transplanted onto a bearing surface, they retained their viability and continued to grow. From work with rats, Urist and Adams (1968)(161) demonstrated that the gliding surface of articular cartilage induced the formation of only fibrous tissue or hyalin cartilage. Seligman et al (1972)(150) performed whole knee transplantation in one hundred fifty-five dogs. His work involved the transplantation of autografts, frozen allografts, fro-

zen irradiated allografts and azathioprine treated frozen allografts. The articular cartilage in all groups except the autograft group were found to be nonviable. In the autograft group, a patchy survival was seen. The acellularity noted in the cartilage of the femoral head within six weeks in this paper was in agreement with Seligman's findings. Seligman found that fibroblasts from the cartilage-synovial junction grew over the acellur articular cartilage. The degenerated cartilage was invaded and replaced by cells primarily of a primitive mesenchmal type. This fibroblastic tissue underwent a metaplastic change into fibrocartilage. In all groups, dead cartilage was replaced by fibrocartilage, however, the rate at which this occurred differed. No circulating antibodies to cartilage were detected nor were cellbound cartilage antigen-antibody complexes.

Aho (1973)(2) studied the regeneration of orthotopic allogenic half-joints (without soft tissue) in twenty-three dogs which had been frozen at -60 degrees centigrade to -70 degrees centigrade. In his study, Aho also found that the cartilage surface of the transplant was first covered with a thin connective tissue layer. After several months this tissue underwent a metaplasia and new cartilage formation was seen. The connective tissue that covered the joint surface and that later developed into cartilage was thought to be a regenerative reaction by the host in which the organism attempted to protect the cartilage. Within six weeks after transplantation, the articular cartilage of all grafts in this study were nonviable. In this same time period a developing acellularity of the cartilage of the femoral head made its appearance. Fibrous tissue having a vascular core was seen to surround and later invade the femoral head. The invasion of fibrous tissue with a

vascular core was similiar to that which was seen in the reports of other researchers and probably indicated the initial phase in the development of fibro cartilage. Ottolenghi (1966)(127) reported that Scalietti and Casussio showed that the stimuli of normal function was required to preserve the integrity of articular cartilage. In the case of the experiment presented, all femoral heads had luxated as a result of failure to provide proper stabilization during the period of healing. Resultantly, the femoral heads were not resting in their normal anatomical location within the acetabulum. During the experimental period the involved limbs were also kept immobile and non-weight bearing. Ottolenghi (1966)(127) felt that the immobilization required for a massive bone transplant may favor degeneration and necrosis of the cartilage although it did help prevent the collapse of subchondral bone. The factors of immobilization and ectopic location should be given consideration in the evaluation of the data.

During the surgery, the extraosseous arterial supply to the proximal head of the femur was destroyed. This supply came from: 1. The synovial membrane plexes formed by the deep femoral, obturator, medial and lateral circumflex femoral, and to a small extent, caudal gluteal arteries; 2. The ligamentation teres arteries formed as articular branches of either the medial circumflex or obturator arteries; 3. The nutrient end branches of the medial and lateral circumflex femoral arteries (Fitzgerald(1961)(60). Resultantly the revascularization seen came primarily from the area of contact with the viable bone of the recipient, and the tissues which surrounded the head. Ottolenghi (1966)(127) found that large homografts initially underwent necrosis. Later, similiar to what was seen here, they were revascularized mainly from the

area of contact with healthy bone. He found the portions of the bone farthest from contact with living tissues were last to be replaced. The grafts in this study bear out Ottolenghi's findings in this respect.

Vaughan (1972)(163) reviewed the histological fate of transplanted bone. He pointed out that the process of repair and reorganization of the graft was via vascular connective tissue. Through transformation, the tissue became osteoblastic or osteoclastic. Subsequently the spongeosa and compactā reorganized. This reorganization was thought to take place by resorption and apposition. The rate of invasion and replacement of the graft was influenced to some extent by the physical nature of the bony element. Cancellous bone was replaced relatively rapidly because it had thin trabeculae and large channels which provided a ready entrance for vessels and allowed an extensive fluid contact. On the other hand, cortical bone was very dense and the invasion of vessels into the small Haversian canals slowed down rapid substitution. In the study presented using standard histopathologic techniques and tetracycline labeling studies a more rapid vascular invasion and replacement of the cancellous bone of the transplant was seen.

In Ottolenghi's (1966)(127) study, he mentioned that radiographically it appeared that the diaphysis of a graft had incorporated better than the epiphysis. However, histologically this was found not to be true. The decalcification of the epiphysis seen radiographically and interpreted as a degenerative sign represented a revascularization of this region. The radiographic evidence of demineralization involving an epiphysis reported by Ottolenghi, was also found in our study. Its presence correlated to some degree with the revascularization of a particular area. Radiographic evidence viewed in the light of current

knowledge concerning transplantation proved to be a valuable aid in the evaluation of each graft. Radiographic information concerning graft incorporation, callus formation, and bone and cartilage character were found to be closely alligned with histopathological findings.

Milch et al (1957)(119), Kokkanen and Slatis (1965)(102) and Tapp (1966)(156) have demonstrated the value of tetracycline for determinimg the viability of living osteocytes. It has been shown that only growing osteocytes can concentrate oxytetracycline. In my opinion this would be true of oseoblasts as well. Tetracycline activity was seen in the distal portions of the transplants of the nine week fresh allografts (27), the twelve week (8) and eighteen week autografts (5), and the fifteenth week fresh (29) and frozen (15) allografts. This study corresponded to the osteocytic activity seen in the histopathologic studies and correlated with the interpretation of incorporation of the graft by the host. No significant difference between the nature or rate of the autograft, fresh allograft and frozen allograft was found in this eighteen week period.

## CONCLUSION

Following freezing at -30° Centigrade for three weeks, the frozen allografts were found by standard histological techniques to be nonviable. By the sixth week following transplantation all grafts were nonviable. Formation of a callus at the recipient site appeared to be progressing by the third post-operative week. In the autograft and fresh allograft there was an ingrowth of fibrous tissue with a vascular core into the bone of the head at six weeks. This vascular ingrowth appeared in the frozen allograft by the ninth week. By the twelfth week an active remodeling of the distal portion of the transplant had taken place. It has been shown that in the first four months following transplantation of the proximal portion of the femur there was little difference between the nature or rate of healing of the autograft, fresh allograft and frozen allograft. Radiographic evaluation of healing following grafting appeared to be valuable for determinimg rate and nature of graft incorporation. The tetracycline labeling study substantiated standard histological analysis and interpretation.

Because of the slow rate of graft incorporate and lack of a direct vascular supply to the graft, it is unlikely using the technique outlined that the femoral head and neck would survive the prolonged incorporation process. However, it is probable that the shaft of the transplant would survive transplantation.

This study may be regarded as a pilot for the evaluation of transplantation of the proximal portion of the femur. The data obtained will serve as a basis for future studies. This data, along with the literature reviewed suggest that transplantation of the proximal shaft of the femur is a feasible procedure.

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# A P P E N D I X

TABLE I

INDIVIDUAL ANIMAL DATA

| GROUP | DOG NUMBER | AGE | WEIGHT(KG) | SEX | BREED | PERIOD EUTHANIZED FOLLOWING SURGERY IN WEEKS |
|---|---|---|---|---|---|---|
| Autograft | 3 | Mature | 21.8 | M(C) | Mixed Husky | 3 |
| Autograft | 21 | Mature | 21.8 | M | Laborador-Setter Cross | 6 |
| Autograft | 30 | Mature | 18.6 | M | Laborador-Setter Cross | 9 |
| Autograft | 8 | Mature | 20.0 | F | Laborador-Setter Cross | 12 |
| Autograft | 19 | Mature | 21.4 | F | Mixed Laborador | 15 |
| Autograft | 5 | Mature | 20.9 | F | Pointer | 18 |
| Fresh Allograft | 4 | Mature | 22.7 | M | Mixed Shepherd | 3 |
| Fresh Allograft | 12 | Mature | 17.3 | F | Mixed Shepherd | 6 |
| Fresh Allograft | 27 | Mature | 20.9 | M | Mixed Shepherd | 9 |
| Fresh Allograft | 14 | Mature | 17.7 | F | Mixed Shepherd | 12 |
| Fresh Allograft | 29 | Mature | 15.9 | F | Mixed Beagle | 15 |
| Fresh Allograft | 28 | Mature | 15.0 | M | Mixed Terrier | 18 |
| Frozen Allograft | 7 | Mature | 17.7 | M | Laborador Cross | 3 |
| Frozen Allograft | 6 | Mature | 23.6 | F | Irish Setter | 6 |
| Frozen Allograft | 18 | Mature | 18.6 | F | Laborador Cross | 9 |
| Frozen Allograft | 9 | Mature | 17.3 | M | Brittany Mixed | 12 |
| Frozen Allograft | 15 | Mature | 15.0 | F | Border Collie Cross | 15 |
| Frozen Allograft | 13 | Mature | 16.8 | M | Husky Cross | 18 |

I. Autograft Group:

A. Dog No. 3

1. Radiographic Interpretation

On the immediate post-operative films, the bone transplant had been seated properly in position and fixed by means of a five hole DCP plate. Two screws had been placed in the transplanted bone and three had been seated in the recipient site. Reduction and fixation appeared excellent.

On the two week post operative films, the right femur had luxated dorsal and anterior to the acetabulum. There was evidence of demineralization of the dorsal one-third of the neck and greater trochanter. Very slight, if any, demineralization of the head had taken place. There was some demineralization around the first bone screw. One and one-half centimeters distal to the recipient site, there was some soft tissue calcification which probably resulted from periosteal stripping during surgery.

The coxofemoral luxation persisted at three weeks. The demineralization involving the neck and greater trochanter were much more extensive at this time. A definite area of decreased density surrounded screw one. A periosteal reaction of the medial aspect of the recipient femur extended 4.5 centimeters distal to the transplanted site. Good reduction had been maintained. Radiographically, no callus was evident at the transplant site at this time.

2. Macroscopic Pathologic Changes

The femoral head had luxated dorsal and posterior to the

acetabulum. A large serous pocket was present ventral to the femoral head. Healing was taking place at the recipient site. All the screws had good holding power and were secure. The Gluteal muscles appeared to have undergone a great deal of scarring and fibrosis. Grossly, the articular cartilage of the femoral head appeared to be within normal limits.

3. Microscopic Pathologic Changes

The cartilage of the transplanted femoral head was very eosinophilic in its outer two-thirds. The inner one-third had taken a very basolphilic stain. The chondrocytes of the outer two-thirds were dead or dying. This was evidenced by the presence of empty lacunae and an eosinophilic cartilage matrix. The cartilage cells in this region had either disappeared or had shrunken, and were very eosinophilic. The cartilage of the inner one-third, closest to the bone, was more basophilic. In this region some cartilage cells still filled out their lacunae very well. However, here cartilage cells were also dying. The entire cartilage of the head of the femur seemed to have splintered and cracked. The subchondral bone of the head of the femur was uniformly eosinophilic. There was no vascular supply present in the Haversian or Volkmans canals. The marrow spaces were occupied by RBC's, PMN's and macrophages. There was some early fibrous tissue infiltration. The bone of the head was uniformly pink. Only a few osseous areas could be seen where osteocytes filled their bony cavities. Although the bone appeared viable, ninety per cent or more of the osteocytes had

disappeared or had taken a basophilic staining property. Empty cavities predominated.

The bone marrow surfaces of the femoral head showed neither the presence of osteoblastic nor osteoclastic activity. No cellular border was apparent. The locunae were principally empty and the bone marrow nonviable. The marrow had taken an eosenophilic stain. Most of the osteocytes seen were dead, and no vascular activity was present.

As one approached the union site between the graft and recipient bone, a clear dividing line on the cortex was present between new bone formation and old transplanted bone. There was a seam of osteoid and bone on the cortical surface close to the union site of the recipient and transplanted bone. The transplanted bone was separated from the newly formed bone by a dense basophilic line termed the reabsorption line. The newly formed bone on the periosteal surface was well vascularized and contained newly formed blood vessels. Viable osteocytes were seen in this location and osteoblastic and autoclastic activity was present. The cortical portion of the transplanted bone was dead or dying. This portion of the transplant was very eosinophilic and well separated from the recipient bone. The endosteal surface of the transplanted bone was occupied by a well formed endosteal callus composed of newly formed bone, blood vessels, osteoblasts and osteoclasts. Bone marrow activity was present both in the transplanted and recipient bone in this area.

At the union site on the outer surface of the bony cortex there was a marked callus formation. A cartilage wedge was being remodeled to bone. The callus was well attached to the transplanted bone, as well as to the recipient bone. An ongoing remodeling of the recipient and transplanted bone was taking place. Osteoblastic and osteoclastic activity was present on the periosteal surface of the recipient and transplanted bone. The cortical portion of the transplanted bone was nonviable. Here no vascular ingrowth had occurred.

There was a marked endosteal callus on the side of the transplant together with marked fibroplasia of the bone marrow of the transplanted bone. What was not occupied by callus was occupied with mature fibrous tissue having a sparse vascular supply. A fibrous tissue proliferation also involved the bone of the recipient.

B. Dog No. 21

1. Radiographic Interpretation

On the immediate post operative films fixation appeared excellent. Cortical apposition was good and the femoral head was located in its proper position within the acetabulum. Two weeks post operative the head has luxated ventral and posterior to the acetabulum. A small periosteal reaction one centimeter in length could be seen on the distal medial aspect of the transplant. By the fourth post operative week, a very slight decreased density was noted in the region of the greater trochanter and proximal one-third of the neck. No callus was in evidence at this time. On the sixth week

following surgery, there was extensive demineralization of the head, neck and shaft of the transplant. A very early indication of periosteal proliferation was present at the proximal medial aspect of the bone of the recipient. No lysis around the screws was seen, although the bone was demineralized. No callus was present. Healing appeared delayed. A radiolucent line separated the graft and recipient.

2. Macroscopic Pathologic Changes

A coxofemoral luxation had occurred. The articular surface of the femoral head was in part covered by layers of fibrous tissue. Early degenerative changes in the cartilage were present. A nonunion persisted at the site of contact between the transplant and recipient. This site was surrounded by serous fluid and was contained within a fibrous tissue envelope. Numerous beta hemolytic staph and nonhemolytic E. coli were isolated from this fluid.

3. Microscopic Pathologic Changes

The cartilage of the femoral head was totally nonviable. The complete destruction of the cartilage seen at this time was not present at three weeks. The cartilage now appeared as one uniform eosinophilic mass which was partly frayed and splintered. The subchondral bone had taken an eosinophilic stain. Here, no living osteocytes were observed. The bone marrow of the head had also taken an eosinophilic stain and appeared amorphous in character. No osteoblastic, osteoclastic or vascular activity was present.

The outer surface of the transplanted head was surrounded

by a layer of active fibrous tissue with a great deal of vascular activity. The capillaries present at this time had not appeared in three week autograft. There was also an ingrowth of fibrous tissue with a vascular core into the dead cortical bone of the head. This fibrous ingrowth was not seen at three weeks. Some osteoclastic activity was taking place on the periosteal surface of the transplant.

At the union site a minimal periosteal callus had formed. On the periosteal surface within the callus proper, a large amount of fibrous tissue containing a great deal of vascular activity was present. The transplanted bone was nonviable. No osteoblastic, osteoclastic, or vascular activity existed within the cortical bone. Within Volkmann's channels and the Haversian canals there was no cellular activity. At the distal end of the transplanted bone at the union site there was osteoclastic activity. Here, dead bone was being removed or resorbed.

The bone marrow of the transplanted bone consisted of a dense network of fibrous tissue containing some vessels. A dense fibrous tissue surrounded the dead bone. Some sequestras were present. The recipient bone, on the other hand, was viable and was being remodeled. Although fibrous tissue was found within its bone marrow cavity, normal bone marrow activity was present.

4. Tetracycline Label

The tetracycline label appeared in the proximal bone marrow of the recipient. No label appeared in the graft.

C. Dog No. 30

1. Radiographic Interpretation

On the immediate post operative films, graft placement and fixation appeared excellent. At two weeks, the femoral head had luxated dorsally. A periosteal reaction extended over the anterior, posterior, and medial aspects of the proximal portion of the recipient. There was very little evidence of demineralization in the transplant at this time. By the fourth week, early demineralization of the greater trochanter, neck and shaft was seen. At six weeks further demineralization of the greater trochanter and dorsal one-third of the neck had occurred. The head had taken on a more homogenous appearance. A periosteal reaction extended distally six centimeters along the posterior aspect of the proximal border of the recipient. By the eighth week, the entire transplant showed a marked degree of demineralization. The cortical bone of the transplant appeared very thin. Healing appeared to be progressing, however, there was no evidence of an external callus present. The osteotomy site could not be seen. An old periosteal reaction was present on the medial and posterior aspects of the recipient. At nine weeks, a capitoepiphyseal fracture had occurred. The entire transplant had continued to demineralize and there was overall homogenous appearance to the transplant. The cortices of the transplant were extremely thin. A marked demineralization and extensive pitting and degeneration of the articular surface of the head had taken place.

2. Macroscopic Pathologic Changes

A false joint had formed around the femoral head which was located dorsal and anterior to the acetabulum. A capital epiphyseal separation had occurred. The articular cartilage of the head appeared to be undergoing a degenerative change. The femoral neck had a small area of necrosis on its ventral border. A large callus bridged the union site. A sterile serous pocket had formed one centimeter distal to the union site.

3. Microscopic Pathologic Change

In the transplanted femoral head, dead and dying cartilage was being replaced by a fine network of fibrous tissue containing blood vessels. This replacement by fibrous tissue had not occurred previously. Within the marrow, the trabecular bone was either nonviable or was undergoing a degenerative change. The bone appeared to have an eosinophilic staining characteristic and was devoid of osteoblastic activity. A few pockets of PMN's were present. The periostium of the transplant had also been invaded by fibrous tissue containing vessels.

Near the recipient site, the transplanted cortical bone had taken an eosinophilic staining characteristic and was clearly nonviable. However, some living osteocytes were present within the cortical bone. Here, also, some osteoblastic and osteoclastic activity was present. Osteoid was being formed, and a new bone was being laid down. Bone resorption and deposition was also occurring on the periosteal and endosteal surfaces of the transplanted bone. An excellent

periosteal callus had formed between the transplanted and recipient bone. The callus contained active osteoblasts, osteocytes, vessels, and osteoid (new bone) not previously seen. An endosteal callus was also present composed of very mature dense connective tissue containing a good blood vessel supply.

The recipient bone appeared to have had a normal bone marrow and cortical appearance.

D. Dog No. 8

1. Radiographic Interpretation

On the immediate post operative films, transplant apposition and fixation appeared satisfactory. By the second post operative week, the femoral head had luxated dorsal to the acetabulum. The greater trochanter and dorsal one-third of the neck was beginning to show early signs of demineralization. A small periosteal reaction could be seen along the proximal medial aspect of the femur for a distance of four centimeters. At four weeks a decreasing radiodensity of the greater trochanter, neck and shaft of the transplant was observed. The previously described periosteal reaction had increased in thickness, but appeared to be somewhat quiescent. Healing appeared to be progressive at the osteotomy site. A line of demarcation between the graft and recipient bone was not visible. Very early evidence of callus formation could be identified. By the sixth week, a modest callus formation encompassed the most proximal end of the recipient bone at the union site. Little radiographic change was apparent by

the eighth post operative week. Further demineralization of the entire graft was seen by the tenth week. The cortical bone of the transplant appeared more radiolucent. A decreased density and thinning of the medial cortex was noted in the area between the second and third bone screws. A more extensive demineralization of the transplant could be seen by the twelfth post operative week. The bone around the first and second screws had retained little of its radiodensity. The medial cortex of the transplant one centimeter distal to the second screw appeared quite thin. The presence of large external callus could not be identified at this time radiographically.

2. Macroscopic Pathologic Changes

The femoral head was surrounded by a pseudojoint and was located posterior and dorsal to the acetabulum. A large amount of fibrous tissue surrounded the recipient site. Healing appeared to have been taking place. All the screws of the plate had very little holding capacity at this time. A small serous pocket was located anterior and medial to the second screw. Grossly, no degenerative changes involving the articular surface of the femoral head were visualized. Connective tissue filled the acetabulum.

3. Microscopic Pathologic Change

The acetabulum was filled with fibrous tissue although the superficial layers of the cartilage of the acetabulum was undergoing a degenerative change, the deeper layers of cartilage were viable and appeared normal.

The cartilage of the femoral head was degenerating and nonviable. A fibrous layer covered the head. The bone of the head was also nonviable and its bone marrow contained many PMN's. The cortex of the head was being invaded by a fine network of fibrous tissue and blood vessels.

Proximal to the recipient site, the transplanted bone was not viable. On its periosteal surface a great deal of osteoclastic activity could be seen. Fibrous tissue and pockets of PMN's could also be visualized here. The recipient bone was viable and had remodeled. Very little callus existed between the recipient and transplanted bone. The callus which did exist was composed primarily of fibrous tissue. Dense connective tissue filled the bone marrow of the transplant. The recipient's marrow cavity contained a fine network of fibrous tissue.

4. Tetracycline Label

The entire bone marrow of the recipient and distal one centimeter of the bone marrow of the graft took the tetracycline label. An incresed fluorescent intensity of the label was apparent in the area of the osteotomy and callus.

E. Dog No. 19:

1. Radiographic Interpretation

On the immediate post operative films, transplant apposition and fixation appeared excellent. By the second post operative week, the femoral head had luxated dorsal to the acetabulum. A periosteal reaction involved the medial, lateral, and to a lesser extent, the posterior border of

the proximal one-half of the recipient. Some periosteal reaction was also present on the medial border of the shaft of the transplant. At this time a very slight decrease in density in the area at the greater trochanter was seen. At four weeks, bone density appeared essentially unchanged. The periosteal reaction seen previously had increased in size, but appeared nore quiescent at this time. At six weeks, some early radiographic evidence of callus formation was becoming visible. The greater trochanter and adjacent bony region of the shaft appeared to be more demineralized than previously. Further bone demineralization was seen at eight weeks. At this time a fine radiodense line appeared in the bone marrow of the recipient at its site of apposition to the transplant. A small callus was forming at the union site. A thin radiolucent line separated the graft and recipient at ten weeks. The graft appeared moderately demineralized by the twelfth week. The radiolucency of the bone surrounding the first screw was more pronounced twelve weeks following surgery. The femoral head also showed a marked demineralization. A thinning of the medial cortex of the transplant was apparent. In the fourteenth and fifteenth week films, a three millimeter band of bone traversed the bone marrow cavity of the recipient, separating the marrow cavities of the transplant and recipient. At fifteen weeks post operative a very fine radiolucent line partially separated the host and graft.

2. Macroscopic Pathological Change

A false joint enclosed the dorsally luxated femoral head. Excellent healing had occurred with minimal callus formation. Rigid fixation was being provided by all but the second screw. Connective tissue filled the acetabulum. Fibrous connective tissue covered the degenerative cartilage of the femoral head.

3. Microscopic Pathological Change

The superficial layers of the cartilage of the acetabulum were undergoing a degenerative change. The bone of the head was not viable. The bone marrow cavity of the head was being invaded by blood vessels and fibrous tissue. On some of the surfaces of the femoral head layers of cartilage and viable osteoblasts were found. The cortex of the head was very vascular. Fibrous tissue and active osteoblasts were present within the bony cortex of the head. Along dead bone, specules osteoblastic activity was seen and some osteoid was being formed.

Proximal to the recipient site the transplanted bone was not viable. A moderate endosteal callus was present. The recipient bone was viable and had remodeled. A dense fibrous tissue ran along the periosteal surface of the transplanted bone. In the cortical bone of the transplant fibrous tissue and blood vessels were present. At the most distal end of the transplant some osteoblastic activity was present. At the most distal end of the transplant some osteoblastic activity was present. Only mature fibrous tissue

existed within the bone cavity of the transplant. Near the recipient site a large amount of fibrous tissue was present. A bone plate serving as a transversely oriented callus marked the separation between the recipient and transplanted bone. The marrow cavity of the recipient bone was normal.

4. Tetracycline Label

The bone marrow of the recipient had taken the tetracycline label. A transverse callus had formed at the osteotomy site. Negligible labeling of the graft had occurred.

F. Dog No. 5

1. Radiographic Interpretation

Immediately post operative appostion and fixation to the recipient site appeared satisfactory. By the second week, the femoral head had luxated dorsal to the acetaBulum. A very slight periosteal reaction could be seen along the proximal medial border of the recipient. By the fourth post operative week a very early indication of demineralization in the region of the femoral head and neck was evident. The periosteal reaction noted previously appeared nore quiescent. Early radiographic evidence of a callus formation was also visible. From the sixth through the tenth weeks, the transplant slowly became less radiodense. An external callus was forming primarily from the area of the recipient. The callus which was evident primarily at the proximal end of the recipient continued to slowly increase in size up to the eighteenth post operative week. During this same period a progressive demineralization of the

entire graft had taken place. By the eighteenth week, the entire graft showed a marked demineralization. The proximal portion of the marrow cavity of the recipient also appeared less radiodense. By the eighteenth week a more moderate size callus had formed.

2. Macroscopic Patholigical Changes

A pseudojoint had formed around the dorsally and slightly posteriorly luxated femoral head. Degenerative changes were apparent in the femoral head. A satisfactory union between the transplant and recipient bone had occurred with very little excessive callus formation. The acetabulum was shallow and had been filled with connective tissue.

3. Microscopic Pathologic Changes

The acetabulum was filled with connective tissue and the cartilage of its articular surface was being remodeled into bone.

Fibrous connective tissue had invaded the cartilage of the femoral head and was replacing it. There was a great deal more connective tissue present invading the head than had been seen previously. Islands of cartilage were visible in the head of the femur. The bone was nonviable. Much of the dead bone was being attacked by osteoclasts. There were areas which osteoblasts had laid down bone around dead bone specules. Some periosteal bone activity of the head was evident.

Proximal to the recipient site the transplanted bone was

not viable. Its cortical bone was being invaded by fibrous tissue, blood vessels, and numerous osteoclasts. The bone marrow cavity of the transplant contained fibrous tissue and blood vessels. The recipient bone had remodeled. In the recipient, a normal bone marrow cavity persisted. A good periosteal and endosteal callus had formed at the recipient site.

4. Tetracycline Label

The tetracycline label had been taken up by the distal three milimeter portion of the transplant and proximal portion of the recipient. In the region of callus formation fluorescence was intense.

II. Fresh Allograft Group

A. Dog No. 4

1. Radiographic Interpretation

Immediately post operative the graft had been fixed into position using a five hole DCP plate. Two screws had been placed in the transplant and three in the recipient bone. Bone apposition appeared excellent. The femoral head rested within the acetabulum. Screws four and five were well seated in the distal cortex but could have been two millimeters longer in order to insure a more secure stabilization.

Two weeks post operative, the femoral head had luxated dorsal and slightly anterior to the acetabulum. Cortical apposition of the transplant had been maintained. A very small area of dystrophic calcification was present on the

medial aspect of the femur approximately 1.5 centimeters distal to the transplant site. No demineralization of the head and neck was visualized.

Three weeks post operative the femoral head continued to remain luxated. A more extensive periosteal reaction was noted on the medial aspect of the proximal portion of the right femur. No radiographic evidence of healing was apparent at this time. Good fixation had been maintained by the bone plate. Very little, if any, loss in density of the head and greater trochanter could be visualized. There was much less demineralization apparent here than had been visualized in the three week autograft.

2. Macroscopic Pathologic Changes

The femoral head had luxated dorsal and anterior. A serous pocket containing henolytic Staphylococcus surrounded the femoral head. The plate fixation was secure. Little evidence of healing at the site was apparent. The acetabulum had been filled with connective tissue. There was evidence of fibrous of the gluteal muscles.

3. Microscopic Pathologic Changes

The cartilage of the outer one-third of the femoral head had taken on an eosinophilic staining character and contained dead and dying cartilage cells. The cartilage of its inner two-thirds still maintained its characteristic basophilic appearance, but contained cartilage cells in various stages of degeneration. There was not as much degenerative change here as was seen in the three week autograft

(3). The subchondral bone of the femoral head had undertaken an eosinophilic appearance and contained empty lacunae devoid of osteocytes. No osteoblastic or osteoclastic activity was present in the subchondral or periosteal bone of the femoral head. The bone of the femoral head was nonviable. Mature fibrous tissue lay next to dead and dying bone adjacent to the periosteal surface of the femoral head. PMN's and RBC's were present in the bone marrow cavity.

The union site was clearly identifiable. A large periosteal callus was present. A larger callus had formed here than was present in the three week autograft. A bone plate was forming across the union site. A wedge of cartilage lay between the external callus and the periosteal surface of the original bone. There was a dense resorption line between the external callus and the recipient bone. Superficially, the cortical bone of the recipient was being remodeled. A great deal of osteoclastic activity was seen. Although an external callus was present around the transplanted bone, no remodeling or vascular invasion had occurred. Similiar to the autograft, the cortical bone of the transplant was nonviable. Covering the endosteal aspect of the transplanted bone not seen in the three week autograft was a layer of osteoblasts which extended for one-half of a 10x field. A marked endosteal bone marrow callus of a limited length filled almost the entire marrow cavity of both the recipient and transplanted bone. Gaps between

bony trabeculae were occupied by a fine network of fibrous tissue.

B. Dog No. 12

1. Radiographic Interpretation

On the immediate post operative films, it was apparent that the diameter of the bone of the recipient was slightly smaller than that of the donor. Bone apposition never-the-less was good and fixation was excellent. At two weeks, an anterior dorsal coxofemoral luxation was present. A periosteal proliferation extended four centimeters along the proximal portion of the medial face of the recipient. A small periosteal proliferation was seen on the medial surface of the transplant. Very little demineralization of the greater trochanter and neck was seen. By the fourth week, the greater trochanter and dorsal one-third of the neck had begun to show signs of demineralization. At six weeks, a continued demineralization of this region was apparent. A loss of the normal architectural quality of the transplant was evident, as well as a more homogenous appearance to the bone. No external callus could be identified. The extent of demineralization and loss of bone density was not nearly as extensive as seen in the autograft (21) of this same period.

2. Macroscopic Pathologic Changes

A pseudojoint encompassed the femoral head which was seated dorsal and posterior to the acetabulum. A callus was forming at the site of apposition of the transplanted

and recipient bone. Compared with earlier cases, very little fibrosis of the gluteal muscle was seen.

3. Microscopic Pathologic Changes

The cartilage cells of the femoral head were either nonviable or were degenerating. This degeneration was not as extensive as that seen in the six week autograft. The cartilage matrix and ground substance had taken an eosinophilic stain. The bony trabeculae within the bone marrow had an eosinophilic appearance and was not viable. No cellular or vascular activity was present. The cortex of the femoral head was surrounded by a fibrous tissue cover similiar to that seen in the six week autograft. Some osteoclastic and osteoblastic activity existed on the surface of the dead bone indicating an attempt to remove bone and lay down new bone. This latter activity had also been seen in the six week autograft.

There was a small periosteal callus formed at the site of apposition of recipient and transplanted bone. The bone marrow cavity of the transplant contained dense fibrous tissue. The transplanted bone at this site was nonviable. There was fibrous tissue activity on the periosteal surface of the region. The recipient bone was undergoing a remodeling. Here, osteoblastic and osteoclastic activity was present. The bone marrow of the recipient had a normal vascular appearance and appeared to be active. At the union site an endosteal or bone marrow callus transversed the bone marrow cavity like a plate attempting to shut off

the bone marrow cavity between the transplant and the original. Basically these changes were consistant with the findings of the six week autograft.

4. Tetracycline Label

The tetracycline label appeared throughout the bone marrow of the recipient. At the osteotomy site a clear line of demarcation between the graft and tetracycline labeled was present. No label appeared in the graft.

C. Dog No. 27

1. Radiographic Interpretation

On the immediate post operative films it was apparent that the transplant was of a slightly larger diameter than than the recipient. Never-the-less, apposition and fixation was good. Screw number four should have been two millimeters longer. By the second week the femoral head had luxated ventral and posterior to the acetabulum. Very early signs of demineralization of the transplant were seen at this time. By the fourth post operative week, little more demineralization appeared to have taken place. On the posterior cortex of the transplant and recipient the beginning of very early attempt at union could be seen. At six weeks, the entire transplant appeared to have an increased radiolucency. The cortex of the transplant also appeared to be thinner than was previously observed. On the eighth week films further attempt by the recipient bone to bridge the transplant were evident. By the ninth week, the cortex of the transplant was extremely thin. The transplant had

an overall demineralized appearance. A line separating the transplant from the recipient was still present. Some evidence of an attempt by the recipient to bridge this gap persisted. More bone demineralization was present in this graft (27) than was seen in autograft (30) of this same period of time. The osteotomy site which was clearly present in this graft could not be visualized in the autograft (30) of this time period.

2. Macroscopic Pathologic Changes

The femoral head luxated posterior and ventral to the acetabulum and ventral to the tuber ischii. The head was encompassed by a false joint and covered by a fibrous tissue layer. The articular cartilage of the femoral head appeared to be undergoing degeneration. At the recipient site, healwas occurring. Screws one and two which were seated in the graft were loose, but no indication of infection was found. The acetabulum was shallow and had been filled with connective tissue.

3. Microscopic Pathologic Changes

A much denser and more mature connective tissue was present over the degenerating cartilage of the head than was seen in the autograft of this group. The bone marrow of the head was nonviable. There was a lot of fibrous tissue and osteoclastic activity in the periosteal bone of the head. No deposition of newly formed osteoid or bone could be seen. No vascular ingrowth into the head had occurred.

At the recipient site the transplanted bone was non-

viable and its bone marrow had been replaced by fibrous tissue. The endostium of the transplanted bone was bone was being attacked at the surface by osteoclasts attempting to absorb bone. On its periosteal surface pockets of pus were present. Here, there was a good deal of osteoclastic activity and a proliferation of fibrous tissue. However, there was no osteoblastic activity and neither bone nor osteoid had been deposited. A very poor periosteal and endosteal callus resulted. Of the three grafts seen at this time, the fresh allograft showed the poorest callus.

4. Tetracycline Label

At nine weeks the entire bone marrow cavity of the recipient had taken the tetracycline label. The label also appeared in the callus and in the distal two millimeters of the bone marrow of the graft. The proximal six millimeters of the recipient and distal two millimeters of the graft appeared to be more heavily labeled with tetracycline than other portions of bone.

D. Dog No. 14

1. Radiographic Interpretation

Immediately post operative cortical apposition of the transplant and recipient, as well as fixation appeared excellent. At two weeks, a dorsal and slightly anterior luxation of the femoral head was present. An extensive periosteal reaction was seen on all aspects of the recipient, as well as on the distal medial border of the transplant. By the fourth week, a thickened but more quiescent perios-

tium was visible. The distal metaphysis and epiphysis of the recipient had an extremely mottled appearance which might have been indicative of an infection. Some early attempts at callus formation were seen at this time. By the sixth week, the demineralization which was just beginning to present itself at four weeks was visible. The demineralization involved the greater trochanter, and femoral neck and shaft. The bone marrow cavity of the recipient near the transplant site also appeared more radiolucent. In the eighth and tenth week films, some demineralization of the transplant was seen, along with an attempt on the part of the recipient to bridge the osteotomy site. By the tenth post operative week, the callus had increased in size. Demineralization of the graft was occurring, but at a very slow pace. The autograft in this time group (8) showed greater transplant demineralization, a thinner cortex and less external callus formation than was seen in this graft.

2. Macroscopic Pathologic Changes

The femoral head had luxated dorsally and was surrounded by a pseudojoint. A serous pocket one by two centimeters was located along the proximal shaft of the femur just distal and medial to the femoral head. The articular cartilage of the head grossly showed very little degenerative change, however, because of its connective tissue covering its surface was difficult to evaluate. A good callus had formed at the recipient site. The acetabulum was shallow

and was covered by fibrous connective tissue.

3. Microscopic Pathologic Changes

The superficial layer of cartilage of the acetabulum had been replaced by fibrous connective tissue. A mature connective tissue filled the acetabulum.

The femoral head was nonvaible and its cortex was covered by fibrous tissue. At the recipient site the transplanted bone was nonviable and its periosteal surface was undergoing resorption. On the periosteal surface of the recipient remodeling and resorption was occurring. Here, a small seam of periosteal callus had completely ossified or remodeled. A good union existed between transplant and recipient bone. A good endosteal callus was present. Fibrous tissue filled the bone marrow cavity of the bone transplant. The endosteal surface of the transplanted bone contained a layer of osteoblasts which were actively forming bone.

4. Tetracycline Label

The bone marrow of the recipient was labeled with tetracycline. At the osteotomy site a clear line separating labeled and non-labeled bone was apparent. There was no tetracycline label in the transplant.

E. Dog No. 29

1. Radiographic Interpretation

Fixation and appostion of the transplant appeared satisfactory on the immediate post operative films. By the second week following surgery, the femoral head had luxated dorsally. A periosteal reaction along the medial aspect

of the shaft of the transplant and medial proximal one-half of the shaft of the recipient was present. At four weeks there was evidence of the loss of mineralization from the shaft of the graft. The periostium appeared to be more quiescent. Early attempts at callus formation seen at four weeks continued to be present at six weeks. A continued increase in the amount of external callus formation was seen up through the fifteenth week. In this same period, the entire graft showed a slowly decreasing radiodensity. A radiolucent line separated the graft and recipient at fifteen weeks.

2. Macroscopic Pathologic Changes

The left femoral head had luxated dorsal to the acetabulum and a pseudojoint had formed around the head. Some degenerative changes were present on the surface of the head. A good union between the transplant and recipient bone had formed. Near the recipient site the cortex of the transplant appeared thin. Proximal to the osteotomy site, a dark circular band surrounded the cortex of the transplant. The first screw seated within the transplant had become loose. The acetabulum was shallow and had been filled in by connective tissue.

3. Microscopic Pathologic Changes

A dense mature fibrous connective tissue was found adhered to the remaining cartilage of the acetabulum. On the articular surface of the acetabulum, cartilage was being remodeled into bone.

Fibrous tissue was in the process of replacing degenerating nonviable cartilage of the femoral head. This ingrowth of fibrous tissue and vessels was not seen in earlier cases. More fibrous tissue ingrowth alone was present here than was seen on all earlier cases. The bone of the head was also nonviable. Its cortical surface contained fibrous tissue, blood vessels, some osteoblasts and numerous osteoclasts. The cortical surface of the shaft of the transplant was also nonviable. Fibrous tissue and blood vessels invaded the cortex of the transplanted as well as the bone marrow. Osteoclastic activity existed within the cortical bone of the transplant. The recipient bone was undergoing remodeling. Its bone marrow cavity contained fibrous tissue. A good periosteal and endosteal callus had formed at the union site.

4. Tetracycline Label

The bone marrow of the recipient and distal three millimeters of the bone marrow of the graft appeared to have taken the tetracycline label. An increased fluorescent intensity of the callus was apparent.

F. Dog No. 28

1. Radiographic Interpretation

Apposition and fixation of the transplant appeared satisfactory immediately following surgery. The shaft of the transplant was of a slightly larger diameter than was the recipient. By the second week, the femoral head had luxated dorsal to the acetabulum, and by the fourth week, a

dimineralization of the neck, greater trochanter and shaft of the transplant was apparent. Also, at this time an external callus was developing primarily at the proximal end of the recipient. At six weeks, a radiolucent line could be identified between the transplant and recipient. From the sixth through the tenth post operative week this radiolucent line persisted. Throughout this same time period at the union site, the callus had become progressively larger. A modest demineralization of the graft was seen. From the twelfth through the eighteenth week, a moderate callus continued to form around the union site. The radiolucent line noted previously appeared to be filling in during this time interval and little change in the radiodensity of the graft had taken place.

2. Macroscopic Pathological Changes

A dorsal coxofemoral luxation had occurred. The articular surface of the femoral head was covered by connective tissue and the femoral head lay within a pseudojoint. A good callus was present bridging transplant and recipient. Screws one and two had lost most of their holding power. A fibrous tissue filled the acetabulum.

3. Microscopic Pathologic Changes

The cartilage of the acetabulum was eroded and had been invaded by a dense layer of connective tissue.

The cartilage of bone of the femoral head were nonviable. Fibrous tissue having a vascular core invaded the femoral head and neck. Here too, were osteoclasts

active in the removal of dead bone similiar to that seen in the frozen allograft of this same post operative period.

Near the recipient site fibrous and vascular tissue invaded the periosteal surface of the transplant. Osteoclastic activity was present within the bone of the transplant. A small external callus had formed between the transplanted and recipient bone, a portion of which consisted of hyalin cartilage. A mixture of fibrous tissue and hyalin cartilage was located between the transplant and recipient bone at their union site. The bone marrow cavity of the transplant was filled with a mature dense connective tissue. The marrow cavity of the recipient was filled with a dense internal callus which consisted partially of hyalin cartilage.

III. Frozen Allograft Group

A. Dog No. 7

1. Radiographic Interpretation

Immediately post operative, the transplant had been fixed onto the recipient site using a five hole DCP plate. Cortical apposition appeared excellent. The femoral head was seated within the acetabulum. It was apparent that the transplanted bone was not as well mineralized as were the autograft and fresh allografts of this same time period. Areas of modest demineralization were observed in the greater trochanter, femoral neck and shaft. The lack of bone density was especially noticeable around the first bone screw. On the two week post operative films, the

femoral head had luxated dorsally. The greater trochanter, neck and shaft had undergone further demineralization. Three weeks post operative the femoral head continued to remain luxated. A marked demineralization of the greater trochanter and dorsal one-half of the femoral neck was evident. Screw one was seated in bone which had lost a good percentage of its radiographic density. A progressive demineralization of the ventral one-half of the neck and shaft of the transplant was taking place. The demineralization was much more extensive than that of the three week autograft (3).

2. Macroscopic Pathologic Changes

The femoral head had luxated dorsal and slightly anterior to the acetabulum and was enclosed within a pseudojoint. Early evidence of bridging existed at the appositional site. The surgical site was free of any evidence of infection. The gluteal muscles had a more normal appearance than others of this three group.

3. Microscopic Pathologic Changes

The cartilage of the articular surface of the femoral head had taken a faintly eosinophilic stain and was barely discernable on the slide. Freezing artifacts (bubbles, water and ice clefts) were present. The bone of the head appeared to have a very deep eosinophilic staining quality. No viable osteocytes or cartilage cells were present, nor was there any bone marrow or vascular activity here. A layer of fibrous tissue covered portions of the periosteal

surface of the head. The shaft of the transplant was non-viable and lacked the presence of vascular osteocytic or bone marrow activity.

Approaching the union site, a dense network of fibrous tissue was present on the inner surface of the transplant. This fibrous connective tissue was much more dense than that which had been seen in both the three week autograft and allograft. At the union site a modest callus was present. There was also a small periosteal callus. However, less callus formation was seen here than was observed in the other two of this three week group. Near the recipient site the marrow cavity of the recipient contained a fine network of fibrous tissue.

B. Dog No. 6

1. Radiographic Interpretation

On the immediate post operative films, the entire graft appeared to have less radiographic density than was seen in the films of the autograft and fresh allograft groups. The transplanted bone was of a slightly smaller diameter than the recipient. Resultantly, although fixation was excellent cortical apposition was achieved on only approximately two-thirds of the circumference of the recipient. Resultantly, the medial cortex of the transplant was displaced approximately five millimeters lateral to the medial cortex of the recipient. By the second week a dorsal coxofemoral luxation had occurred. A periosteal reaction extended along the medial and lateral cortices of the recipient.

This reaction extended from the most proximal portion of the recipient distally 4.5 centimeters along its medial border and 6.5 centimeters on its lateral border. Four weeks post operatively this periosteal reaction was more pronounced. At this time early signs of healing and callus formation were noted. Some demineralization of the head, neck, and shaft was observed. At six weeks, the transplant appeared to have undergone an extensive demineralization. This could be seen in the area of the femoral neck and shaft, as well as in the region of the greater trochanter. A less pronounced demineralization of the head and its articular surface was observed. Radiographically, no callus formation was evident. Compared to the other two dogs euthanized in this time period the autograft (21) appeared to have undergone the most demineralization and the fresh allograft (12) the least. In this regard the frozen allograft filled an intermediate position.

2. Macroscopic Pathologic Changes

The femoral head had luxated dorsal and posterior to the acetabulum. Grossly, pathology of the femoral head could not be identified. There was a nonunion present at the recipient site. No pockets of infection or draining tracts were identified.

3. Microscopic Pathologic Changes

The cartilage and bone of the femoral head were nonviable. Freezing artifacts (bubbles and cracks) were

present. Unlike the autograft and fresh allograft there was no fibrous or vascular activity present on the cortex of the head.

There was a nonunion of the recipient and transplant at this time. However, a pronounced periosteal callus was forming and pockets of cartilage were identified within this callus. A large endosteal callus was also forming. Fibrous tissue traversed the union site. Bone remodeling was taking place primarily in the proximal portion of the recipient. A dense bone plate separated the bone marrow cavity of the recipient from the transplant.

4. Tetracycline Label

The tetracycline label appeared in the bone marrow of the recipient up to a point just distal to the osteotomy site. No label appeared in the transplant.

C. Dog No. 18

1. Radiographic Interpretation

On the immediate post operative films, cortical apposition and fixation appeared excellent. By the second post operative week the femoral head had luxated ventral and posterior to the acetabulum. A very slight increased demineralization of the femoral head and neck could be seen. A mild periosteal reaction extended along the proximal portion of the medial and lateral borders of the recipient. By the fourth week, the femoral neck appeared much less radiodense and the original osteotomy site was still visible. At six weeks, further graft demineralization was evident. At this

time very early attempts at union were seen. Little change in the radiodensity of the graft occurred at eight weeks. A fine radiolucent line separated the graft and recipient at nine weeks and there was added evidence of early callus formation. Although there had been a continued loss in the radiodensity in the transplant, the normal architecture of the transplant was still visible. The osteotomy site was barely discernable at this time.

2. Macroscopic Pathologic Changes

The femoral head had luxated dorsal and posterior to the acetabulum and was surrounded by a pseudojoint. Grossly, no evidence of necrosis involving the head and neck could be seen. A fibrous tissue layer covered a large portion of the articular surface of the femoral head. Satisfactory healing at the site of apposition of transplant and recipient appeared to be taking place. All screws of the plate had maintained their proper holding capacity. The acetabulum had been filled with fibrous connective tissue.

3. Microscopic Pathologic Changes

No freezing artifacts were present within the femoral head. The cartilage and bone of the head was nonviable. The cartilage had no cellular detail and was covered by a thin layer of fibrous tissue. The bone of the head had taken an eosinophilic stain. Fibrous tissue and blood vessels had penetrated into the cortex of the head. Here, osteoclastic, but no osteoblastic activity was present.

The shaft of the transplant was nonviable. In the

Haversian system of the transplant there was vascular activity. Osteoblasts were being laid down on dead and dying trabeculae. A moderate periosteal and bone marrow callus was present. A fine connective tissue filled the bone marrow cavity of the transplant. There was no clear separation between the bone marrow cavities of the transplant and recipient.

4. Tetracycline Label

The entire bone marrow cavity of the recipient had taken the tetracycline label. The fluorescent label appeared to be increased in intensity of the proximal portion of the recipient at the osteotomy site where a callus was being formed.

D. Dog No. 9

1. Radiographic Interpretation

The immediate post operative films showed that the graft had been seated and fixed well to its recipient. The diameter of the shaft of the transplant was slightly smaller than that of the recipient. By the second week, the femoral head had luxated dorsal and posterior to the acetabulum. A mild periosteal reaction extended along the proximal five centimeters of the medial border of the recipient. Little radiographic change was apparent at four weeks except for a slight increase in the extent of periosteal reaction. At six weeks an early demineralization of the greater trochanter and neck was seen. There was no evidence of an external callus formation. At eight weeks a moderate amount of

demineralization was seen to involve the greater trochanter, neck and shaft of the transplant. The bone marrow of the recipient proximal to the osteotomy site appeared more radiolucent. A three millimeter cortical defect appeared on the medial border of the femur at the osteotomy site. A radiolucent area separated the transplant from the recipient. At ten weeks the cortical defect was approximately one millimeter wider and the radiolucency separating the transplant and recipient appeared more prominent. By the twelfth week, the graft appeared to have undergone further demineralization. Prominent radiographic evidence of callus formation was not apparent.

2. Macroscopic Pathological Changes

The femoral head luxated dorsal to the acetabulum and lay within a pseudojoint. Grossly, the articular cartilage of the head had a normal appearance. The transplant and recipient bone appeared to be healing with minimal callus formation. Fibrous connective tissue filled the acetabulum.

3. Microscopic Pathologic Changes

Superficially, the cartilage of the acetabulum showed some evidence of degeneration. The entire acetabulum was filled with a dense layer of fibrous tissue.

The cartilage of the femoral head appeared to be one homogenous eosinophilic staining mass. The bone of the head was nonviable. No osteoblastic, osteoclastic or vascular activity was present. The epiphyseal plate was homogenous in appearance and had an eosinophilic staining quality.

It contained a calcified cartilage matrix. In the metaphyseal region the bone of the cortex and medulla was nonviable and devoid of cellular activity.

At the recipient site fibrous tissue surrounded the periosteal surface of the transplanted bone. Some of the Haversian systems within the transplanted bone contained fibrous tissue, vessels and small amounts of osteoblastic activity. A good periosteal and bone marrow callus existed. The recipient bone was viable and was undergoing remodeling. The bone marrow cavity of the transplanted bone contained fibrous tissue. There was also some fibrous tissue located in the bone marrow cavity of the recipient bone. However, the marrow cavity of the recipient was normal.

4. Tetracycline Label

The tetracycline label has been taken up by the bone marrow of the recipient. The proximal one centimeter of the recipient showed an increased flucrescence was also apparent in one segment of the developing callus. The graft had taken no tetracycline label.

E. Dog No. 15

1. Radiographic Interpretation

On the immediate post operative films, the graft could be seen to be of slightly less diameter than the recipient. Apposition and fixation was satisfactory. By the second week the femoral head had luxated dorsal to the acetabulum and a periostitis appeared on the medial aspect of the proximal one-half of the recipient. By the fourth post oper-

ative week this periosteal reaction was quiescent. A very slight decreased density involving the proximal one-third of the shaft and a portion of the neck could be seen. Early attempts at callus formation were present at the most proximal end of the recipient. An increased radiolucency of the entire graft and a continued increase in the amount of callus formation was taking place through the fifteenth week following surgery. A radiolucent line separated the graft and recipient from the sixth to the fifteenth post operative week.

2. Macroscopic Pathologic Changes

The femoral head had luxated dorsal to the acetabulum and lay within a pseudojoint. A firm callus had formed in the region of contact between transplant and recipient. Grossly, no abnormalities of the articular cartilage of the femoral head were identified. No draining tracts or areas of abscessation were present. The diameter of the transplant was only three quarters of that of the recipient bone. Resultantly, there had not been circumferential cortical apposition of the bone segments. Screw one was loose and the second screw had decreased holding power.

3. Microscopic Pathologic Changes

There were focal areas of degeneration involving the articular cartilage of the acetabulum. The entire articular surface of the acetabulum had been filled with a dense connective tissue. There was no cellular reaction present and the subchondral bone appeared healthy and viable.

The articular cartilage of the femoral head was nonviable. It had lost its cellular detail and normal architectural appearance. The outer two-thirds of cartilage had taken an eosinophilic staining quality and the inner one-third a slightly basophilic characteristic. The bone of the femoral head also was nonviable and had taken a bright eosinophilic stain. Its bone marrow cavity was filled with mature connective tissue and many blood vessels. This was especially apparent in the periferal and central regions of the marrow cavity. In the marrow of the epiphysis many RBC's were seen. No bone marrow activity was present. Within this marrow cavity there were many empty spaces and no fat cells. The periferal bone of the femoral head was being invaded by fibrous tissue and blood vessels. Osteoclastic activity was present within the periosteal layer of the femoral head. Osteoblasts were in the process of forming new bone on bony spicules. Haversian canals and Volkmann's canals were filled with fibrous tissue and blood vessels.

The shaft of the transplant was nonviable. Fibrous tissue, blood vessels and osteoclasts invaded its distal cortex. Here, the bone marrow cavity was filled with fibrous tissue and blood vessels. At the recipient site a very active and healthy periosteal callus had formed. A wedge of hyalin cartilage lay beneath this callus. A large amount of well formed new bone had been deposited at the union site. At the distal end of the transplant and proximal recipient there was osteoclastic activity. The bone marrow of the recipient

was normal. A fibrous tissue layer separated the transplant and recipient.

4. Tetracycline Label

The bone marrow of the recipient had taken up the tetracycline label as did the distal four millimeters of the bone marrow cavity of the graft. An intense fluorescence was displayed in the area of callus formation surrounding the osteotomy site.

E. Dog No. 13

1. Radiographic Interpretation

Immediately following surgery and transplant apposition a fixation was satisfactory. By the second week the femoral head had luxated dorsal to the acetabulum and a periosteal reaction had appeared on the proximal one-third of the shaft of the recipient. Signs of early demineralization of the transplant in the area of the greater trochanter and proximal shaft were evident four weeks following surgery. By this time the periosteal reaction previously observed had enlarged but appeared more quiescent. A callus proliferation appeared around the pecimal end of the recipient. By the sixth week the external callus had reached its maximum size. From the eighth through the fourteenth week following surgery, a progressive and marked graft demineralization was seen. During this time the distal medial cortex of the transplant and proximal medial cortex of the recipient beween the second and third screws began losing its normal architectual appearance. By the sixteenth week, the arti-

cular surface of the head, body and neck displayed marked degenerative signs.

3. Microscopic Pathologic Changes

A thick fibrous tissue layer covered the surface of the acetabulum. The cartilage of the acetabulum had degenerated and only a small portion of the cartilage still remained viable.

The cartilage cells of the head of the femur were nonviable and the articular cartilage had degenerated. The bone of the head had taken an eosinophilic staining character. Lacunae were either empty or occupied by nonviable osteocytes. The bone marrow cavity of the head was empty. On the perifery of the head and neck fibrous tissue invaded Haversian and Volkmann's canals. There was also a great deal of blood vessel activity in this region. On the periosteal surface of the head osteoclasts were removing dead bone and some osteoid was being laid down.

Near the recipient site the transplanted bone was nonviable. At the perifery of the cortex, bone was being invaded by blood vessels and fibrous tissue. A good external callus bridged the recipient and transplant. The bone marrow cavity of the transplant was filled with a dense fibrous tissue. Normal bone marrow activity persisted in the recipient.

4. Tetracycline Label

A very modest linear tetracycline label was present in

the distal two millimeter portion of the graft transversing the bone cavity. The tetracycline label appeared to line the endosteum and adjacent bone marrow in the region between the second and third screws.

# WHOLE BONE TRANSPLANTATION OF THE PROXIMAL PORTION OF THE FEMUR USING A SELF-COMPRESSING PLATE

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AN ABSTRACT OF A MASTER'S THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1977

Even though some clinical transplantations have been performed involving whole bone of the proximal portion of the femur, little scientific research had been devoted to this area. An eighteen week study was conducted involving eighteen dogs. These dogs were divided into three groups. One received autografts, a second received fresh allografts and a third group received grafts which had been frozen at -30° centigrade for three weeks prior to transplantation. The grafts were fixed into position using DCP plates. During the experimental period the dogs were given tetracycline and radiographed at two week intervals. One dog in each group was euthanized every three weeks and the femurs studied by standard histo-pathological techniques, as well as fluorescent studies.

The frozen allografts were nonviable at three weeks. By the sixth week all grafts were found to be nonviable. The formation of a callus at the recipient site was evident by the third post operative week. In the autograft and fresh allograft at six weeks, and in the frozen allograft by nine weeks a fibrous tissue invasion with a vascular core into the bone of the head was seen. By the twelfth week remodeling of a distal portion of the transplant had occurred. It was shown that in the first four months following transplantation there was little difference between the nature or rate of healing in the three types of grafts. Tetracycline labeling and radiographic analysis supported these findings. Radiographic evidence appeared to be of value for determining rate and nature of graft incorporation.

Using a slow freeze and rapid thaw technique it was not possible to maintain chondrocyte viability. The fibrous tissue invasion of the degenerated cartilage was thought to be an early stage in the formation

of fibro cartilage.

Because of the slow rate of graft incorporation and lack of direct vascular supply to the graft, it is unlikely using the technique outlined that the femoral head and neck would survive the prolonged incorporation process. However, it is feasible that a whole bone shaft graft would survive transplantation.