Combination of a Cortical Allograft with a Cancellous Autograft in the Canine Tibia

by

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INTRODUCTION

Bone grafts have been used for many years in human medicine and have recently gained acceptance in Veterinary Medicine.

The preferred material for bone grafting is autogenous bone. Sometimes the need for grafting material exceeds the amount of autogenous bone that can be retrieved from the patient. This is especially true with large cortical defecits due to severe communition from trauma or surgical excision of a tumor etc.

Allogenous bone has been studied for many years as a supplement or replacement to autogenous bone. It has been utilized in the fresh state, frozen, freeze-dried, decalcified, deproteinized, boiled, autoclaved and other ways in order to determine such properties of allografts such as 1) vascularization capabilities, 2) cell survival, 3) induction properties, and 4) immunological rejection.

Ultimately, it has been shown that allografts will be incorporated into the host skeleton but there are differences of opinion as to the structural stability and to the completeness of the graft incorporation.

One of the main problems with fresh allogenous bone, whether it be cancellous or cortical, is the immunological rejection which destroys any vascularization and new bone formation after approximately ten to fourteen days. There is then a delay of several weeks before revascularization becomes subtantial. This is apparently due to the cellular content of the bone.

There has been work done that shows that a combination of allogenous bone freed of its own marrow, and autogenous red

marrow will stimulate more new bone than either component alone and there appears to be less of a problem with rejection.[10,11,12]

There have been clinical cases in animals where allogenous tubular cortical grafts have been used for defecits in the mid-shaft of long bones with success.[62] Others have used this same procedure and augmented the autologous cortical graft with autogenous cancellous bone, which has a high red marrow content, and holes in the cortical segment to allow revascularization, with success.[21] There is no experimental evidence to show whether or not this is an advantage to incorporation of the graft.

In this study we will determine if autogenous cancellous bone, gently packed into the medullary canal of a four centementer fresh allogenous cortical graft, placed in the canine midshaft tibia will indeed, aid in the incorporation of that graft into the host skeleton. A similar allograft of cortical bone without autogenous cancellous bone will be placed in the opposite tibia of the same dog as a control.

Literature Review

Bone grafts have been studied for many years. The number of articles written on the subject is tremendous. Duhamel, in 1742, reported on the osteogenic properties of the periosteum.[15] Von Haller, however, in 1763, felt that the periosteum was mainly for support of blood vessels.[15] These two views were a controversy until 1842 when Flourens conclusively showed that periosteum is osteogenic and is the chief agent in the healing of bone defects.[15] Axhausen's work in 1907 and 1908 showed that periosteum has a high degree of survival and osteogenic activity in autografts,

markedly less in allografts and practically none in heterografts. Since then, many papers have contributed to the knowledge of bone grafting, but Axhausen's principles are still largely accepted today.[15]

In human medicine, grafts have been used for: 1) an aid in the healing of delayed and non-union fractures; 2) to promote healing in the arthrodesis of joints; 3) fusion of growth plate cartilage; 4) filling cavities in bone; 5) replacement of bone and joint loss; and 6) augmentation of skeletal deficiencies of the forehead, nose, maxilla and mandible.[12] In Veterinary Medicine, bone grafts have been used for basically the same procedures. Recent work has also indicated the use of autologous cancellous bone in cases of osteomyelitis.

For purposes of clarity, the following terms will be used throughout the paper: autologous or autogenous are the terms used for a graft in which the donor is also the recipient; isograft or isogenic, meaning a graft between individuals of closely inbred strains; allograft or allogenic which denotes a graft between genetically dissimilar members of the same species; and xenograft or xenogeneic, meaning a graft between different species.[63]

Bone grafts aid in the healing process by providing: 1) bone cells for osteogenesis; 2) bone induction agents that stimulate the host bed; 3) structural support; 4) a framework for vascular invasion; and 5) protection of new vascular buds and bone formation.[12] Cancellous bone provides all of the above except structural support and is probably the most common type of bone graft utilized. Cortical bone provides all of the above, but

its cellular content is much less than that of cancellous bone. For osteogenic purposes, cancellous bone is vastly superior to cortical bone because: 1) the endosteal surface of cancellous bone is larger; 2) it contains red marrow, which in itself is osteogenic; and 3) the sponge like matrix of cancellous bone is usually readily invaded by living cells.[12]

The preferred material for use as a graft is autogenous cancellous bone. Two disadvantages of cancellous bone which have been reported are: 1) it requires an additional surgical site for its procurement, and 2) with large bone deficits there may not be enough cancellous bone available.[37] The most common area to obtain cancellous bone is the wing of the ilium because of its high red marrow content. Other areas commonly used to procure cancellous bone are the proximal tibia and humerus. Autogenous cortical grafts have been obtained from ribs and strips of tibia to be used to fill bone defects or be applies as onlay grafts for additional support.[55]

Because of the lack of autogenous bone available for large bony defecits, much work has been done utilizing allogenous bone, either in the fresh state or preserved in some other manner. Some of the first attempts to use free bone grafts clinically were in the nineteenth century. Phillip von Walther used an autogenous transplant in 1820 and Macewen was the first to report on the successful use of allogenous bone in 1878.[15] After the experimental works by Ollier (1862), Radzimowski (1881), Jakimowitsch (1881), Bonome (1885), Saltykow (1900), Sultan (1902) and Axhausen (1907, 1909), the transplantation of bone was performed in an increasing

number of human patients.[15] In the begining of the twentieth century there was a great increase in the use of bone transplants, but mainly with autogenous bone. Albee, (1919) alone reported on over 1,600 successful cases of autogenous transplantations.[12,15] It was not until the introduction of the bone bank in 1942, however, that the use of allogenous bone became more common.[1,29]

When considering bone grafting, four biological mechanisms come into play; 1) vascular and cellular invasion, 2) osteogenic conduction, 3) immunological rejection, and 4) remodelling.[12]

Bone grafts are incorporated into the host skeleton by resorption of the old matrix and apposition of new bone, as stated by Axhausen.[15] The term "creeping substitution" is commonly used to describe resorption and apposition but is actually erroneus. The term was originally used by Barth and Marchand in the late eighteen hundreds to describe a process of replacement by budlike masses of new bone invading the old without previous resorption of the old bone.[15]

The two most important factors essential for the healing of bone are vascularization and stabilization.[48] Vessels can invade an osseous graft only when the shearing motion between compliant soft tissues and non-compliant hard surfaces or between two non-compliant hard surfaces is largely eliminated.[4] Since the 1930's the advance of metallurgy reduced the need for the bone graft to supply internal fixation as well as osteogenesis.[12] This added considerably to the stability of the grafts.

The vascularization of the canine tibia has been studied extensively because it has been a good model for healing in

relation to vascular supply because in one end of the tibia, the proximal metaphysis, there is one of the richest vascular areas of the long bones and the other end, the distal epiphysis, is one of the poorest in vascular supply.[57] In 1906, Delkenskamp first published his investigations on the vascular pattern in healing fractures of the dog's tibia. He felt that the main factor responsible for the vascular profusion was the nutrient artery at the center of the healing mechanism. Kolodny however, in 1923, disagreed. Using the radius of a dog, he showed that the union of a fracture depends mainly on the blood supply of the periosteal callus. There is still some controversy today.[57]

The vasculature of the canine tibia and its response to fracture was probably best described by Rhinelander in 1974.[48] There are three main sources of blood to the canine tibia: 1) the principle nutrient artery, which divides into an ascending and descending medullary artery. These two branches subdivide into arterioles that supply all areas of the endosteal surface and provide the major afferent blood supply to all of the diaphyseal cortex; 2) the metaphyseal arteries, which are multiple, have terminal branches which enter the proximal and distal extremities of the medullary cavity to anastomose with the terminal branches of the ascending and descending medullary arteries, and 3) the periosteal arteries, which enter the cortex only at fascial or ligamentous attachments. Beneath the muscle bellies, where the periosteum is very thin and tenous, no functional periosteal arterioles have been observed. Where the periosteal arterioles

are present, they supply the outer one-third of the cortex and anastamose with the medullary arterioles. The inner two-thirds of the cortex, where periosteal vessels are present, is supplied by the endosteal vessels as is the entire thickness of cortex in areas where periosteal vessels are not present.

The venous circulation of the tibia consists of large emissary veins and a vena comitans of the nutrient artery which drain the medullary contents exclusively, the cortical venous channels which drain the deeper portions of the cortex into the periosteal venules, and the periosteal capillaries which are in continuity with the cortical capillaries of the superficial cortical lamellae.

All the blood vessels of the tibia and other bones convey blood in a centrifugal pattern, that is from the medulla to the periosteum. The ultimate vascular channels connecting the arteries and veins are the Haversian and Volkman canals. There are no true capillary beds because there is no diffusion, they simply convey nutrients to the osteocytes through the canaliculi.

The blood vessels of the Haversian canals are the only longitudinal vascular channels through the normal cortex. The interconnected Haversian canals do not appear to extend longitudinally for more than one to two millimeters before terminating, therefore a medullary arteriole can supply only a very short segment of cortex proximally and distally.

When the bone is fractured, all three main blood supplies are enhanced, but the medullary and metaphyseal arteries remain dominant. The metaphyseal arteries can sustain the medullary

arterial supply from both ends of a long bone due to the anastomoses that are present. The periosteal arteries which enter the cortex in areas of ligamentous attachment are enhanced somewhat but do not add greatly to the medullary supply because the anastomoses are within the cortex and cannot expand. Macnab and de Hass[33] contradict this by saying that the periosteal arterioles penetrate the cortex to re-establish the medullary supply if the periosteum is intact.

The healing fracture also receives blood from a fourth source which Rhinelander has termed the extraosseous blood supply. This arises from torn blood vessels in the vicinity of the fracture site and is responsible for the external hematoma and periosteal callus formation. This source of blood is present until the medullary supply is re-established.

Rhinelander felt that the first osseus union to develop was the endosteal side, Trueta however, disagrees.[57] He feels that the periosteal callus, supplied with blood from the surrounding tissue, is by far the most important and bony union will occur much quicker than if rigid fixation is achieved by means of compression with a metal plate and external callus is not allowed to form. If the periosteal callus is suppressed, bony union will occur endosteally but takes twice as long (6 mo. compared to 3 mo.) to heal.[57]

The revascularization of bone grafts has been commented on in many articles. Marchand (1901) seems to have been the first to study vessels in transplanted bone.[56] Gallie and Robertson (1919) saw new capillaries in the opened Haversian canals two

days after transplantation, but Bertini (1926) did not observe the first signs of revascularization until the fifth day.[56] Most of the recent articles have compared the vascular invasion of the autograft and allograft. Chase and Herndon (1955) stated in their review article that following a massive viable bone graft, healing takes place by the same process as in autogenous grafts, but the rate of the healing process is slower. The revascularization in the allograft occured as rapidly as in the autograft, but the process of resorption and apposition was delyaed.[15] Sieffert (1955), however, with a study on defects in rabbit ulnas filled with either autogenous or allogenous bone, felt that the autogenous bone was rapidly invaded while the allogenous bone was revascularized more slowly. He also found that small transplanted fragments were more rapidly surrounded, invaded and replaced by new bone than larger fragments. Stringa (1957) supported this with his study on cancellous grafts in rabbits. He found that an autogenous cancellous graft five millimeters thick may be completely vascularized in twenty to twenty five days whereas the allogenous cancellous graft of the same size showed a revascularization of only about three-fifths of its thickness in thirty to thirty-five days. Both the autogenous and allogenous grafts were surrounded by a capillary network with the autogenous graft being invaded immediately and entirely and the allogenous graft invaded only after ten to fifteen days and only in parts of the graft.[56] Anderson (1961), studying transplants in the anterior chamber of the rat's eye also felt that the allograft had a delay in incorporation.

Some investigators in the late fifties, believed that an antigen-antibody reaction of the delayed inflammatory type played a role in reducing the vascularization of the allografts, while others stated that the allografts were revascularized just as rapidly as the autografts.[27]

Kingma and Hampe (1964) did a study in rabbits on iliac grafts and found that the autogenous and allogenous grafts were both revascularized and replaced but at different rates. They determined that the formation of new bone was slower with allografts, that this new bone was slower in making contact with the graft, the vascular penetration was slower and less dense and the eventual replacement of the allograft was slower.[32]

In 1965, Deleu and Trueta reported on a study of grafts in the anterior chamber of the rat's eye. They stated that the best graft was that which was most rapidly and permanently vascularized. Not only was the biological affinity between the graft and the bed important, but the structural facilities offered by the graft for the penetration of vessels were also very important. They felt that the best graft was one that was rich in blood vessels and caused little reaction, namely, autogenous cancellous bone. They suggested that the rapid revascularization of the autogenous cancellous graft was due to the direct anastomosis of the vessels of the host with the vessels of the graft.[17]

The readiness of autogenous implants to become incorporated into the host skeleton is believed by some authors to be caused by the early penetration of the graft by blood vessels from the

host. The delayed incorporation of allografts was thought to be the result of an antigen-antibody which disturbs the initial vascularization. This has been stated by numerous authors.[12,17,27,42,46]

Another controversy which has been debated over the years is whether cells in the bone survive the transplantation process. Campbell et.al (1953) using histological examination found that osteogenic cells and osteocytes survived in the fresh autogenous grafts and the osteogenic activity of these cells was found to take part in the incorporation of the graft. They also noted a foreign body reaction consisting of a dense fibrous tissue between the graft and the invading osteogenic tissue from the host in all grafts of preserved and fresh allogenous bone.[13]

Anderson's study in rats previously referred to, showed that cellular elements of both autogenous and allogenous grafts survived and proliferated in the anterior chamber of the eye. The new bone produced by the allogenous bone died after two weeks. He felt that at least in the early stages after transplantation, new bone is produced by surviving transplanted cells.[1]

Ray and Sabet (1963) used tritiated thymidine as a cellular label to show that when the femora of inbred, newborn mice were transplanted as isografts, both the cells of the graft and of the host contributed to the new bone formation in the first two weeks. In the case of allografts, the new bone formation in this time interval is limited and is derived primarily from the graft and not the host.[47]

Arora and Laskin (1964) used a specific chromatin mass in the cells of female rabbits to determine cell viability. Using

isografts of highly inbred rabbits they found that a major portion of the graft survived the transplantation procedure and subsequently contributed to the reparative process.[2]

Nishimuar et. al. (1962) felt that there was no active participation of the graft in the osteogenic activity even if the osteocytes did survive.[17]

Pappas and Beisaw (1968) discussed four variables which could affect the incorporation of the graft: 1) donor graft osteocytes and osteoblasts within 0.2 mm of the graft periphery may survive and form new bone; 2) donor and host cells may modulate and differentiate to become osteoblasts; 3) host cells may be induced to become osteoblasts; and 4) small round cells may assume a negative role in the incorporation of fresh allografts. These round cells appeared at one to two weeks and did not appear to singly inhibit bone formation but generally contributed to delay in osteogenesis. Histologic findings related to fresh allografts, included the marked, persistent round cell response, delayed and dimished vascular invasion and an edematous cellular perivascular infitration. These characteristics have been identified with the transplant immune response and are likely to be the cause for the delayed incorporation of the fresh allografts.[42]

Puranen (1966) showed that fresh autografts of bone had a more intense uptake of tetracycline than autografts which had been exposed to air for one hour or placed in saline for three hours. He suggested that the greater osteogenic power of fresh autografts was due to survival of bone producing cells.[5]

Bohn et. al. (1968) used rabbits to show that new bone forma-

tion in fresh autografts could be demonstrated even during the first four days after transplantation and indicated that osteogenic cells from the fresh autografts continue their activity under favorable conditions.[5]

Graft rejection is another subject which has received much attention. The instruments of transplantation immunity in terms of graft rejection, are antibodies carried by cells of the lymphoid series, namely small lymphocytes.[12] Infiltration of these cells occurs in the one to two week period following transplantation.[6,10,12,42,46] This inflammatory response is variable in amount and seen primarily around newly forming bone on the periosteal and endosteal surfaces.[12]

Bonfiglio and Jeter (1972) did studies on ulnar cortical allografts in rabbits. New bone was produced during the first two weeks but by the third week the newly formed bone frequently underwent necrosis. This necrosis and absorption of the new bone formed in the early phase of allograft repair at the time of maximum inflammatory cell response suggests a host foreign tissue recognition. When a second allograft, from the same donor, was given to the host, the reaction was increased and prolonged over the reaction of the first graft. The timing and character of the reaction plus the inability to detect serum-bound antibodies by any of the usual serologic tests conforms to a delayed hypersensitivity response and is frequently termed a second set reaction.[6]

Several investigators have found that freezing the allograft will produce a less intense reaction.[6,12] Some studies have

shown freeze-drying of the allograft to reduce antigencity and some have not.[9]

Goldberg and Lance (1972) did a study in rabbits and utilized an immunosuppressive drug (azothioprine) to alleviate the graft rejection. They found that the allografts in the treated animals were revascularized and incorporated very similarly to the autografts. The untreated rabbits showed the typical signs of graft rejection.[24]

It has been recognized that new bone formation in bone implants occurs only in the presence of vascular invasion.[17] Cell action is required to unite a bone graft with the hose, to increase or decrease the mass of the graft and to precipitate the rejection phenomenon.[4] Heiple et.al. (1963) described the incorporation of an autogenous cancellous graft into the defecit in a dog's ulna: 1) organization and replacement of the fibrin clot formed at the proximal and distal seams between the host bone and the graft; 2) formation of callus-internal callus within marrow canal of host was the first to form, then progressively larger masses of external osteochondral or bony callus formed on the host bone and graft and finally a bridging callus formed; 3) a union between the host bone and the graft; 4) invasion of dead marrow of graft by a fine fibered embryonic connective tissue which carried blood vessels and cells with phagocytic, osteoclastic and osteoblastic potentialities into the graft; 5) resorption of dead bone trabeculae of the graft, appositional new-bone formation and eventual replacement by new tracebuale, and 6) removal of the external callus and formation of new cortical compact bone.[27]

Enneking et. al.[19] (1975) described the revascularization and incorporation of a autogenous cortical graft. The repair is internally be resorption and apposition of living bone for dead bone which is externally reinforced by an enveloping callus that is produced either by surviving elements of the transplant or by the surrounding host cells. As resorption excavates a Haversian canal, the cavity is enlarged until its margin has reached the periphery of the osteon. At this point resorption ceased as if the cement line demarcating the periphery of the osteon from the surrounding lamellae formed a barrier to further resorption. Old necrotic Haversian systems were enlarged by osteoclasis which accompanied the invading buds of vascular granulation tissue. This remodeling produced the early porosity seen in grafts about six weeks post-transplantation and lasted about six months.[12,19]

Many factors govern the rate of repair of bone transplants. Those which have been described are: 1) the size of the transplant; 2) its porosity; 3) the amount of exposed surface of the graft; 4) the immunological compatibility of the transplanted cells; 5) the vascularity of the recipient bed; 6) the degree of immobilization of the transplant; 7) the age of the recipient and 8) the metabolic state of the host.[20]

Intact periosteum seems to hinder revascularization of underlying bone in cortical grafts and periosteal-free grafts usually revascularize along necrotic Haversian canals.[12] Segmental cortical grafts usually die after transplantation and are revascularized from the periosteal surface and by vessels growing into the medullary canal from both ends.[12]

Bone grafts, whether autogenous or allogenous, which are very small (<2.8 mm x 1 mm) will usually incite a foreign body reaction and are resorbed.[1]

Grafts which have an increase porosity, such as those which have been decalcified with 0.6 mm hydrochloric acid, are readily revascularized and populated by mesenchymal cells. It appeared that empty marrow, Haversian canals, lacunar spaces and lattice-like matrix offered channals for instant permeability to the reparative mesenchymal cells.[58]

In skeletal sites, both allogenous cortical and cancellous grafts are revascularized and incorporated more slowly than their autogenous counterparts.[12] Revascularization studies by intra-arterial dye perfusion has shown that allogenous bone revascularizes slower than autogenous bone.[24] The revascularization of the allografts generally had no detectable difference than the autografts until about the eight post-operative day when the inflammatory response would obliterate the vascular bed. This could be generally attributed to the slower incorporation of allografts.[4,12,46] Ultimately, however, after three to twelve months there is little difference between the incorporation of allografts and autografts.[12]

Freezing and freeze-drying allografts have been found to reduce their antigenecity.[4,12] Burchardt et.al. (1978), showed in a study on dogs, that the majority of fresh segmental, allogenous, cortical grafts were repaired incompletely and failed to re-establish satisfactory skeletal continuity as did freeze-dried allografts.[9] Freeze-dried bone has several advantages over frozen bone: 1) it

causes minimum protein denaturation; 2) constancy of its physical state; 3) faster rate of incorporation into the host; and 4) ease of handling because it could be stored at room temperature.[12]

Boiling and autoclaving of bone grafts has been shown to be very detrimental.[12] It causes coagulation of the soft tissues in the Haversian canals, which impairs revascularization and it denatures the collagen which retards the resorption of the dead bone.[12]

Merthiolate preserved allografts have been shown to be not as effective as frozen or freeze-dried grafts.

The inductive power of a bone graft to produce new bone is another area which has received considerable attention. In 1934, Levandar presented a hypothesis that an extractable chemical was present in transplanted bone that induced the host bone or primitive connective tissue to produce new bone.[34] In 1947, Lacroix termed the inducing factor osteogenin.[33] In 1956, Axhausen described two schools of thought: 1) specialized cells, either those from the periosteum or those lining the bone trabeculae are the only antecedents of the tissue of accretion, that is, the bone forming tissue of the repair process; and 2) any connective tissue cell, where properly stimulated, transforms into osteogenic tissue.[3] Axhausen felt that the recipient bed, when stimulated by an inductor substance in the bone transplant, was able to elaborate bone. In a suitable environment, connective tissue cells can differentiate further into bone-forming tissue.[3] Axhausen's paper claimed that specific cells, specialized for the production of bone are present in the soft tissue components

of bone. Under the activating influence of necrotic bone tissue, these cells differentiate into typical osteoblasts within several days and begin to form new bone.[3]

With this idea of induction it can be seen that the value of a bone graft, in addition to support, depends on: 1) the characteristic and amount of repairative tissue it can stimulate the host to form; and 2) the production of new bone by the graft itself.[2]

The first cells proliferating around and in the graft are regarded by most authors as young mesenchymal cells or fibroblasts whose multiplication and differentiation Carrel and Baker (1962) attributed to proteins and "split" products from dying cells.[17]

In Burwell's study in rats (1964), which dealt with composite allogenous cortical bone and autogenous red marrow, he felt that there is some evidence that a specific stimulus to osteoblastic differentiation is present in living marrow and is liberated by the necrosis of the marrow.[10] The cellular population of normal red marrow suggests strongly that the primitive cell in red marrow which becomes a bone forming cell is derived from the littoral (reticular) cell lining its vascular sinusoids.[10] The majority of the new bone formed by this composite of fresh, allogenous cortical bone and autogenous red marrow has its source in the red marrow and is due, most probably, to the development of an inductive system leading to the differentiation of these primitive wandering cells from the vascular sinusoids into osteoblasts. It was found that this composite graft formed more new bone than did either component when transplanted seperately.[7,10]

In a later paper, Burwell (1966) stated that the maximum degree of osteogenesis about a transplant of bank bone could be achieved by the combination of a treated allogenous graft, to reduce antigenecity, of high inductive potential such as a frozen or freeze-dried graft and a connective tissue of high osteogenic potential such as a cancellous bone or red marrow.[11] He also found that the inductive potential of bone tissue is reduced by such treatment as boiling or removing the organic component of bone.[11]

Allografts form new bone in two phases. Early phase bone occurs in the first two weeks and is derived from the graft and then dies. After about one month, some grafts form a late phase bone which is interpreted as an inductive affect of bone on the tissues at the site of grafting.[1,12]

Urist (1968) stated that the working hypothesis in regards to induction is that ground substance of competent mesenchymal cells combine with an inductive substrate to produce a bone induction principle.[57] Present evidence suggests that this bone induction principle is a highly complex protein bound macromolecular entity which acts as a extrinsic control mechanism for differentiation of competent mesenchymal cells into bone producing cells.[22,28,60]

These competent mesenchymal cells, as already stated, generally arise from perivascular cells associated with proliferating blood vessels, reticular elements of bone marrow, trabecular surfaces and the cambium layer of the periosteum.[12,22,48] Burwell

termed these as osteoprogenitor cells which can divide and give rise to osteoblasts, osteoclasts and osteocytes.[12]

Narang and Wells (1971) added a second hypothesis for induction.[37] They discussed that the diffusion of electrons and not the macromolecules, causes the mesenchymal cells to undergo cytodifferentiation and form new osseus tissue.

Several papers have shown that decalcified bone still possesses a high degree of induction, whereas other methods of preservation of bone have been shown to reduce the inductive potential.[28,37,38,58]

Materials and Methods

Twelve mature mixed breed dogs were used for this study. The sex of these dogs were all male, due to availability of male dogs rather than a preference of male over fmale. The maturity of the dogs was determined by closure of epiphyseal plates radiographically.

Pre-operative radiographs of each tibia were taken to determine: 1) epiphyseal plate closure, 2) length and diameter of tibia and 3) to rule out any previous lesions or other abnormalities.

The dogs were matched in pairs according to the length and diameter of the tibial shaft as closely as possible for the surgical procedure.

The dogs were studied for periods of five weeks, nine weeks, thirteen weeks, and seventeen weeks. The dogs were kept in 3' x 6' kennels and fed a commercial dog food with no additives throughout the study period.

Progress of the grafts was monitored throughout the experimental study with radiography and radionuclide bone scanning.

Radiographic examination of the grafts was performed to monitor healing. Each dog had bilateral tibial radiographs - one day and one week post-operatively. A posterior-anterior and a lateral view were taken each time. The tibias were then radiographed at two week intervals until termination.

Starting at one week post-operatively, bone scans were done on each dog at two week intervals until termination, to determine

areas of osteogenesis in the allograft segments. Technetium 99 (Tc 99) tagged to Medronate Sodium*+ was the agent used.

The studies were done with a General Electric Radicamera II, containing a crystal thirteen inches in diameter and three-fourths of an inch thick and containing nineteen photomultiplier tubes. A standard low energy, paralle hole collimator with a relative sensitivity of 110% was used. The studies were recorded on hard copy negative 8" x 10" film. The information was also stored on a General Electric MedStor II computer with a Nuclear Data (ND 812) Board.

The dose of the Medronate Sodium Tc 99 was one millicurrie per ten pounds of body weight. The agent was injected through a cephalic vein and the recordings were taken two to three hours post-injection. The dogs were anesthetized with Thiamylal Sodium** for the procedure. Two views, an anterior and a lateral, were taken for each view at a density setting of 6.5.

Data was retrieved from both the MedStor computer and densitometer readings from the film.

Four regions of interest were studied with the computer. The center of the graft was visually determined. The first region of interest was taken eight pixcels below the center of the graft. The second region of interest was in the center of the graft. The third region of interest was eight pixcels above the center

*Osteolite - New England Nuclear
+TechneScan MDP Kit Mallincroft Nuclear
**Surital - Parke-Davis

of the graft and the fourth region of interest was eight pixcels above the third region of interest. Each field of interest contained ten pixcels.

The densitometer readings were taken with a Model 301 X-Rite Densitometer with a three millimeter aperture. The background density was nulled prior to the readings. The points were taken from the stifle joint to the tarsal joint at five millimeter intervals. Two readings were taken at each level and the numbers averaged to get the final densitometer reading at each level.

Only the lateral views were utilized to obtain the data from both the computer and densitometer.

Tetracyclines were administered to nine of the twelve dogs to label any new bone formation. Dogs #4, #9, #10, #11, and #12 were given oral tetracycline* at a dose of 25 mg/kg once daily for five days starting eleven days prior to euthanasia. Dog #3 was given 25 mg/kg of oral tetracycline for four days beginning seven days prior to euthanasia. Dogs #5, #6, and #8 were given oxytetracycline+ intravenously at a rate of 25 mg/kg for three days, starting seven days prior to euthanaisa. Dogs #1, #2, and #7 were not given tetracyclines.

At the termination of each study the dogs were euthanitized** and necropsied. The entire tibia was removed from each leg and the soft tissues were removed grossly with scissors and scalpels.

*Tetracycline Hydrochloride - 100 mg capsules, Richlyn Laboratories Inc.
+Liquamycin Injectable - 50 mg/mk, Pfizer Inc.
**T-61 Euthanasia Solution, National Laboratories

The legs were split longitudinally with a band saw in a sagittal plane through the screws holes. Photographs were taken of the whole and split specimen. Then using an ultraviolet light source to flouresce the tetracycline labeling, additional photographs were taken. The legs were then placed in 10% buffered neutral formalin until histopathological examination could be done.

Before histopathological examination, the bones were again sliced longitudinally with a band saw approximately three to four millimeters thick. The bones were than decalcified with Rapid Bone Decalcifier* until soft enough to be cut with a razor blade.

Five sections of the host tibia and graft were then prepared for histopathological study. The sections began three centimeters above the graft and were cut at two centimeter intervals through the graft and ending three centimeters distal to the graft. Section number one contained two centimeters of proximal host tibia, section two contained two centimeters at the proximal graft-host junction, section three contained two centimeters at the distal graft-host junction and section five contained two centimeters of the distal host tibia.

The sections were then embedded in paraffin, sliced on a microtome and stained with hematoxylin and eosin.

*RDO-Dupage Kinetic Laboratories

Surgical Procedure

Each pair of dogs was operated on simultaneously. An intravenous catheter was placed in a cephalic vein. Induction was accomplished with thiamylal sodium (Surital), the dogs were incubated and anesthesia was maintained on fluothane (Halothane) and oxygen. Both rear legs and hind-quarters were clipped with a number 40 Oster blade. Both shoulders were also clipped. The dogs were given one million units of Na Penicillin intravenously pre-operatively. A drip of LRS was maintained throughout the procedure at a rate of 10cc/lb/hr. The dogs were placed in dorsal recumbency. The front legs were tied back along the body in order to expose the shoulder joints. The rear legs were hung from an IV stand prior to scrubbing. The legs and shoulders were prepared for surgery by scrubbing with Betadine soap and an alcohol rinse. Betadine solution was sprayed on the surgical sites following the scrub.

With the first four sets of dogs, both legs and shoulder areas were all draped off simultaneously and both legs were operated on without stopping. Due to prolonged surgical times and some post-operative infections, the last two sets of dogs had each leg draped off and operated on individually under the same anesthesia.

The left leg was always operated first. The approach to the tibia and placement of the cortical graft was the same in both the left and right legs.

An antero-medial incision was made over the tibia from the

tibial plateau to the tarsus. If possible, the medial saphenous vein was left intact. In about half the dogs, the vein needed to be ligated and transected to allow application of the plate. The periosteum was incised along the medial aspect of the leg and elevated off the midshaft. A template was then used to cut the cortical segment from each leg. The template was designed to take the segments all the same distance from the tibia-tarsal joint. The joint was identified with a 25 ga. needle. The four centimeter segments were taken distal to the nutrient foramen in order to disturb the medullary blood supply as little as possible. The osteotomies were performed with an oscillating saw with a saline lavage to help prevent excessive heat build up.

Prior to completing the osteotomies through the lateral cortex, an eight hole, 4.5 mm, Dynamic Compression Plate* was bent to conform to the shape of the medial cortex with the two center holes of the plate centered at the graft site. The plate was then held in place with bone clamps and the two center-holes in the segment to be grafted were drilled with the buttress guide and tapped. The osteotomy cuts were then completed. The medullary canal was cleaned of its marrow contents with a currette and flushed with sterile saline. The cortical segment from each dog was then placed in the opposite dog making these segments cortical allografts. The plate was attached to the graft and then placed on the tibia and held in place with bone clamps. A ronguer was used to remove any spurs at the ends of the graft or host cortical shaft. Also a segment of fibula was removed

*Synthes

with the ronguer to make up for the loss of length of the tibia due to the osteotomy cuts and allow compression of the graft. The remaining holes in each plate were then drilled and tapped and 4.5 mm cortical screws inserted. The first holes on either side of the graft were drilled using the load guide in order to get compression of the graft and the rest of the holes were drilled using a neutral guide.

The fascia was closed with catgut in a simple continous pattern as was the subcutaneous tissue. The skin was closed with nylon suture material in either a ford interlocking pattern of a simple interrupted pattern.

The cortical grafts in the right legs were handled in the same manner, but before each graft was placed in the host, autogenous cancellous bone retrieved from the greater tubercle of the humerus was gently packed in the medullary canal of the graft.

The cancellous bone was obtained by making a small incision over the greater tubercle of the right humerus. A 4.5 mm drill bit was used to enter the medullary area. A small currette was used to scoop the cancellous chips out and placed on a blood soaked sponge. Within a few minutes the cancellous bone was then gently packed into the medullary canal of the cortical allograft. The cortical grafts were then immediately placed in the tibias. Occasionally, cancellous bone had to be obtained from the left greater tubercle to get a quantity sufficient to fill the medullary canal of the cortical allograft. The incisions over the greater tubercles were closed with a simple interupted catgut suture in the subcutaneous tissues and nylon suture in the skin.

The remaining procedure on the right legs was identical to the left legs.

Post-operatively the dogs were placed in light support bandages for four to seven days with periodic changes.

Skin sutures were removed in ten to fourteen days.

RESULTS

Table No. 1 lists the numbers of the dogs for this study.

All the dogs were lame but weight bearing on both rear legs one day post-operatively and all progressed to full weight bearing on both rear legs within five to seven days. Dogs #3, #4, and #7 developed draining tracts at the distal end of the incisions between ten to fourteen days post-operatively. Staphyloccocus aureus was cultured from the legs. The dogs did not respond to antibiotic therapy and developed severe osteomyelitis in either both (#3) or one (#4, #7) leg. These dogs were eliminated from the study. Dog #1 became acutely lame in the left rear leg on the sixth post-operative day. Radiographs revealed an oblique spiral fracture of the proximal tibial shaft above the graft. The dog was euthanized and submitted for histopathological study but eliminated from the bone scan study. Dogs #5 and #9 both had small areas of wound dehiscence and the distal end of the incisions in both tibias which appeared about the seventh post-operative day. These wounds healed within two weeks by second intention healing. Dog #10 had a small area of dehiscence and the proximal end of the left tibial incision which also healed within two weeks by second intention healing. Dog #6 had a slight amount of serous discharge from the incisions for the first couple of days but healed with no further complications. Dogs #1, #2, #8, #11, and #12 all healed without complications.

Dog #2 died on anesthetic induction three weeks post-operatively for the bone scanning. The dog was necropsied and

used for histopathological study and a three week bone scan study.

Dogs #5 and #6 were terminated five weeks post-operatively.

Dog #8 was terminated nine weeks post-operatively.

Dogs #9 and #10 were terminated at thirteen weeks post-operatively.

Dogs #11 and #12 were terminated at seventeen weeks.

Table No. 2 lists the studies which were performed on each dog and evaluated.

Table No.1

Dog I.D. Number	Study Number
436	1
423	2
268	3
419	4
3780	5
4080	6
327	7
281	8
430	9
437	10
320	11
418	12

Table No. 2

	Radiographic	Bone Scan	Tetracycline Labeling	Histopathology
#1	-	-	-	+
#2	+	+	-	+
#3	-	-	-	-
#4	-	-	-	-
#5	+	+	+	+
#6	+	+	+	+
#7	-	-	-	-
#8	+	+	+	+
#9	+	+	+	+
#10	+	+	+	+
#11	+	+	+	+
#12	+	+	+	+
TOTAL	8	8	7	9

Radiographic Interpretations

Post-operative - 8 dogs

The post-operative radiographs revealed all the grafts to be well aligned with the host tibias and all the screws appeared tight although the grafts were put in under compression, most of the graft-host junctions show a small gap with the maximum gap observed less than two millimeters. (Fig. 1)

One week radiographs - 8 dogs

Two dogs (#2, #12) showed a slight periosteal reaction on the host tibias of the right legs. Three dogs (#2, #5, #12) showed areas of decreased densities in the medullary canal of the graft in the left legs. Three dogs (#5, #6, #9) showed an overall increase in the density of the medullary canals of the grafts in the right legs.

Three week radiographs - 8 dogs

All the dogs began showing varying signs of periosteal reactions along the shaft of the host tibias with very little reaction over the grafts themselves. Four of the dogs (#2, #5, #6, #12) continued to show small areas of decreased densities in the medullary canals of the grafts in the left legs. (Fig. 1)

Five weeks radiographs - 7 dogs

The periosteal reaction along the host tibial segments was slightly more pronounced in all the dogs except #11 which showed a marked periosteal proliferation along the host tibia of both legs. Again the grafts in all dogs showed minimal periosteal

reaction except for dog #8 which had a moderate amount. Three dogs (#5, #8, #11) were showing callus formation attempting to bridge both graft-host junctions of both legs. Dogs #9 and #10 showed callus begining at both graft-host junctions in the left leg while Dog #9 showed callus formation at the proximal graft-host junction only in the right leg and Dog #10 showed callus formation at the distal graft-host junction of the right rear leg. Dog #12 showed begining callus formation at the proximal graft-host junction of the right leg and the distal graft-host junction of the left leg. Dog #6 showed some callus formation at the proximal graft-host junction of the left leg. Dog #8 was also showing an area lysis between the plate and the cortex of the graft.

Seven week radiographs - 5 dogs

The graft-host junctions of all the remaining dogs are bridged with callus at this point. The left distal graft-host junction of dog #12 is healing with minimal callus formation. Dog #8 continued to show a line of lysis under the plate over the graft. (Fig. 2) The periosteal reaction along the entire shaft of both legs in Dog #11 is more marked than all the other dogs. It was difficult to see the graft-host junctions in the left leg of Dog #11 due to the amount of periosteal reaction and callus formation. The periosteal reaction in the rest of the dogs was becoming smoother and less reactive.

Nine week radiographs - 5 dogs

All of the graft-host junctions in both legs of all dogs appeared to be healing with callus remodeling in various stages.

There were areas of lysis at the graft-host interfaces in several of the legs. (Fig. 2,3) The periosteal reaction in the right leg of Dog #11 is greatly reduced from the previous study but the periosteal reaction in the left leg still appeared active. The plates in several of the dogs were being incorporated into the new bone formation of the periosteum and callus.

Eleven week radiographs - 4 dogs

Dogs #9, #10, #12 showed the graft-host junctions to be very nearly healed with callus remodeling continuing. Dog #11 showed the right leg to be healing well with the callus remodelling and the periosteal reaction reduced. The left leg of dog #11 still showed a lot of periosteal reaction along the entire shaft.

Thirteen week radiographs - 4 dogs

The right leg in Dog #9 showed both graft-host junctions to be healed and the left leg very nearly healed. Dog #10 showed the grafts to be very nearly healed with the callus remodeling. Dog #11 showed the right graft to be healed with the callus remodeling and the periosteum non-reactive. The left leg showed the periosteum to be less reactive and smoothing out. The graft-host junctions appeared healed. Dog #12 showed the graft to be healed in the right leg and the left graft healed at the proximal graft-host junction and the distal graft-host junction almost healed. The callus formation was minimal.

Fifteen week radiographs - 2 dogs

Dog #11 showed both graft-host junctions in the right leg to be healed and the left graft also healed with less periosteal

reaction than previously. Dog #12 showed all the graft-host junctions to be healed except for the distal graft-host junction of the left leg which still showed some decreased density at the interface. (Fig.3)

Seventeen week radiographs - 2 dogs

Dog #11 showed all the graft-host junctions to be healed with the periosteal reaction on the left leg continuing to become smoother. (Fig. 4 and 5) Dog #12 showed the graft-host junction to be healed with the decreased density at the distal graft-host junction of the left leg to be less noticeable.

Technetium 99 Bone Scan Results

Problems with computer acquisition and retrieval led to loss of approximately one-half of all the studies, therefore the bone scans were evaluated from the densitometer reading only. The higher densitometer reading coincides with increased uptake of the medronate sodium - Tc 99 radionuclide agent and therefore an increase in osteoblastic activity or vascularity.

The densitometer readings from the bi-weekly studies were so variable from one study to the next that a statistical analysis of the results comparing the right tibia to the left tibia could not be accomplished.

Generally, the proximal host tibia was slightly less active than the distal host tibia. The graft-host junctions were the most active areas of the legs. The right legs were generally more active in the proximal graft-host junction and the left legs were more active in the distal graft-host junction. This was especially true in the longer term studies (Dogs #10, #11, and #12). Dog #9 showed the left proximal graft-host junction to be more active than the right proximal graft-host junction and the right distal graft-host junction. The shorter term studies (Dogs #2, #5, #6, and #8) were generally more active in the right graft area than the left graft area.

The longer term studies were also showing a smaller degree of difference in activity in the entire leg between the right and left tibias. (Figs. 6,7,8,and 9)

The more detailed descriptions and graphs can be found in the Appendix.

Gross Results

After soft tissue removal the legs were examined grossly, the plates were removed and then the bones were split longitudinally in a saggital plane through the screw holes.

Right Legs

All the legs showed some amount of new bone along the bone plate with the least amount being on the short term studies and the most being on the longer term studies. Dog #11 showed the most new bone production over the proximal end of the plate. (Fig. 10) Very little new bone had formed over any of the grafts or the distal host tibia. All of the screws were tight. After the plates were removed the grafted bone could be easily seen in all the legs because of the whiter color of the cortex. Dog #8 had a thin layer of yellowish necrotic tissue under the plate over the grafted segment. (Fig. 11)

In the shorter term studies up to nine weeks (Dogs #2, #5, #6, and #8), the actual osteotomy line between the host and the graft could be visualized at both ends of the graft. In the longer studies (Dogs #9, #10, #11, and #12) the osteotomy line was not visible. Dogs #2, #5, and #6 were unstable after the plate was removed but did not fall apart. Dogs #8, #9, #10, and #12 were all stable after plate removal. After the bones were split, the medullary canal was examined. All of the graft medullary canals were filled with a red cancellous bone which was uniform in distribution and continuous with the host medullary canal. (Fig. 12) Dogs #2 and #8 had some cartilaginous tissue at the graft-host

junctions in the medullary canal which looked like internal callus formation. Very little evidence of any external callus was seen at the graft-host junctions on any of the legs.

Left Legs

All of the legs showed some new bone formation along the plates. The majority of new bone was over the proximal end of the plate. The longer term studies generally had more new bone than the shorter term studies. Dog #11 showed the most new bone formation and it covered the entire length of the plate. (Fig. 14) All of the screws were tight except for the ghree screws in the proximal host tibia of Dog #11. They were secure but not tight like the other screws. After the plates were removed the grafted segments could easily be identified in all dogs because of the whiter color of the cortex. Dog #11 had a small amount of yellowish necrotic tissue under the plate at the distal end of the grafted segment. (Fig. 13) The legs were very unstable in Dogs #2, #5, and #6. The remaining legs were stable. The osteotomy lines were easily seen in Dogs #2, #5, #6, and #8. The osteotomy lines could not be seen in Dogs #9, #10, #11, and #12. On the split sections the medullary canal of the host tibia was generally filled with a red cancellous bone. The grafted segments contained a yellowish, cystic, necrotic fatty tissue consistently between the screws. (Fig.13) The proximal end of the graft above the first screw and the distal end below the sceond screw contained a red cancellous marrow continous with the host marrow in Dogs #2, #8, #9, #10, #11 and #12.

Dogs #5 and #6 did not have any cancellous bone in any of the graft medullary canal. Dog #5 had some fibrous tissue at the proximal end of the graft which filled the medullary canal. There was only minimal evidence of external callus formation in any of the legs.

Tetracycline Labeling Results

The results of the tetracycline labeling were obtained by gross examination of the longitudinally split tibia with an ultraviolet light. Dogs #5, #6, #8, #9, #11, and #12 were the only dogs evaluated. Dogs #1 and #2 were not given tetracyclines due to an unexpected early death. Dog #10 was given the tetracycline but due to a technical error, was unable to be evaluated.

All of the bones were showing various amounts of labeling in the periosteum along the host shaft and the graft. The cortices of the graft varied from a greenish tinge to a starch white. There was only minimal labeling in the cortices of any of the grafts except Dog #8 which was extensively labeled throughout the graft cortex of the right leg and light labeling of the cortex of the graft in the left leg.

The medullary canals of the right legs generally showed labeling throughout the host and graft medullary canals except for Dog #6 which did not show any labeling in the middle two centimeters of the graft. The medullary canals in the left tibias were consistently labeled in the host areas of the tibia and usually several millimeters into the medullary canal of the graft. None of the medullary canals were labeled in the middle two centimeters of the grafts.

The more detailed results may be found in the Appendix.

Histopathology Results

All of the grafts studied, except in Dog #1, were showing signs of healing ranging from callus formation in the three week study (Dog #2) to bony union with remodeling of the cortex.

There was varying amounts of periosteal new bone on all of the tibias ranging from very thin layers to several millimeters thick. This was usually seen only on the lateral cortex, as the plate was applied over the medial cortex.

The grafts were often covered with layers of fibrous connective tissue. This layer of connective tissue usually had an infiltration of small round cells, namely lymphocytes and macrophages. There was usually a heavier infiltration of cells in the shorter term studies and only a few or no cells in the longer term studies.

The grafts in the right tibias, which were packed with autogenous cancellous bone, did not show any bone cell activity in the trabecula in the medullary canals of the studies of five weeks or less but did show considerable bone cell activity in the trabecula of the longer term studies.(Figs. 15 & 19) Red marrow activity had been re-established in the long term studies as evidenced by the presence of heamopoitic cells in the medullary canal.

The grafts in the left tibias all had fibrous connective tissue in the medullary canals. There was usually some trabecular bone and red marrow activity in the proximal and distal ends of the grafts to the screw holes. Only Dogs #8 and #11 showed

any new trabecular bone inside the screw holes in the graft but the very center of the medullary canal was still filled with conncetive tissue.

The cortices of the grafts were generally more active in the right legs than in the left legs. The five week studies showed complete bridging of the graft-host junctions in the right tibias and even a bony union at the distal graft-host junction in Dog #6. (Fig. 18) The graft-host junctions in the left tibias were not completely bridged by callus. The rest of the long term studies did show bony union of the grafts in both legs or at least a bridging callus. The right legs generally showed a heavier internal callus than the left legs. The graft cortices did not show any new blood vessels in either leg in the five week studies. In Dogs #8, #9, #10 and #12 however, there were considerably more new blood vessels and cellular activity in the cortices of the grafts in the right tibias than in the cortices of the grafts in the left legs. Dog #11 showed about equal activity in the cortices of both grafts.

Individual dog evaluation of the histopathological studies can be found in the Appendix.

Discussion

The research on bone grafts for the most part has been done in laboratory animals, such as rats and rabbits, and in dogs. These studies have been conducted on cancellous and cortical bone as autografts, allografts, and xeno-grafts. These studies were also usually done at non-physiological sites such as ectopic muscles, the anterior chamber of the eye and defects in the wing of the ilium where the every day stresses which are applied to bone are not present. The size of the bone graft studied was usually small. Only a few studies have been done in which a large graft was placed in a full weight bearing bone in a physiologic situation. For these reasons, this study was conducted using fairly large dogs (50-55 lbs.) with a fairly large graft (4 cm) that would be more comparable to an actual clinical case. The idea of using a full cortical graft to re-establish the continuity of the diaphysis of a long bone damaged by trauma, surgical excision or other reasons, has been used before but little scientific data has been published.

The tibia was chosen as the bone for the study because of the ease of accessibility for a bilateral procedure and the fact that the blood supply of the canine tibia has been studied in depth.

In a clinical case where an animal has suffered a severe comminuted fracture to a long bone, reconstruction can be accomplished but usually with much anguish and prolonged surgery time. Also in these cases, because of the comminution, the plate may be subjected to severe cycling stressed because of the lack of support

of the opposite cortex and the length of the bone may be shortened. In these cases, the use of a cortical allograft could provide more support, shorten surgical time, and maintain bone length.

It has been shown in previous studies that allogenous bone is eventually incorporated into the host skeleton but at a slower rate than autogenous bone. This prolonged incorporation rate would require the support of the plate for a longer period of time which could add additional stress to the plate.

The purpose of this study was to determine if the addition of autogenous cancellous bone to the medullary canal of a fresh allogenous cortical graft will aid in faster incorporation into the host skeleton than a fresh allogenous cortical bone used alone.

Dogs #3, #4, and #7 developed severe infections in one or both legs which was attributed to wound dehiscence from self-mutilation. These dogs were eliminated from the study. Infection is one of the most serious complications of bone grafts and the strictest aseptic technique should be utilized.

In his studies on the vascularization of the canine tibia, Rhinelander has very adequately demonstrated that the main blood supply of the long bone is its medullary supply. The periosteal vessels will help with the external callus formation in the event of a fracture but do not contribute greatly to the re-establishment of the medullary blood supply. The medullary blood supply must be re-established across the fracture site before healing can occur. The endosteal callus, according to Rhinelander is always the first to accomplish bony union after the blood supply has been

re-established. It is known that the ultimate blood channels of cortical bone are the Haversian systems and their interconnecting Volkman canals. Microangiography has shown that these channels receive blood from the endosteal surface of the cortex and move in a centrifugal pattern toward the periosteal surface and that these vascular channels never extend in a longitudinal direction for more than one to two millimteres from the point of entry of the endosteal vessel. Therefore in the mature animal there is virtually no longitudinal blood supply in the cortex.

It is obvious then that the medullary vascular supply would be of utmost importance in the incorporation and revascularization of a full cortical shaft graft. It would be necessary for the nutrient and metaphyseal arteries to establish a network of vessels across a void created by a full cortical shaft graft in order for the entire length of the graft to become vascularized. This would be true for any length of graft over a few millimters long.

The medullary canal of a mature diaphyseal shaft of a long bone, from which the majority of full cortical grafts would be taken, is generally filled with fatty marrow tissue and very little trabecular bone.

There are a couple of reasons why a cortical graft containing fatty marrow tissue would not be conducive to re-vascularization. Fatty tissue may not provide adequate support for the ingrowth of new vessels into the medullary canal of the graft and in long cortical segments, the marrow content, devoid of its blood

supply, would become necrotic and could become a barrier to complete revascularization.

Numerous authors have discussed the antigenecity and immunologic response which has been associated with allogenous bone grafts. Burwell has indicated that the antigenic portion of a bone graft mainly resides in its cellular components. It has been shown that bone, freed of its marrow content is much less antigenic and grafts which have been frozen or greeze-dried produce less of an immunologic response.

A fresh cortical allograft which still contains its marrow content should therefore stimulate an immunologic response from its host. The immunologic response to foreigh grafts has been shown to destroy any new vascularization. This vascularization will eventually be re-established after the immunologically reaction has subsided and has been shown to become active again six to eight weeks after the transplantation.

It would seem logical then, to remove the marrow content in a fresh cortical allograft to reduce this antigenic response. This would however, leave little tissue in the medullary canal to support the ingrowth of new vessels. This is where the autogenous cancellous graft would play a vital role in the revascularization of the cortical allograft.

Cancellous grafts have been shown to be advantageous to bone healing in several ways, two of which are: cells for osteogenesis and a framework for support and protection for the ingrowth of new vessels. The cells provided by the cancellous

graft come from two sources. The first is from osteoblasts and osteocytes which survive the transplantation process and continue to produce new bone. The second source of cells comes from the marrow content of the cancellous bone which contains undifferentiated mesenchymal cells. These cells have been shown to be capable of producing new bone when induced by the host. This induction factor, which has not been identified, has been theorized to work in several ways.

These theories are: 1) that living functional cells of the graft survive the transplantation and induce the unspecialized mesenchymal cells of the host pool to differentiate by diffusion or cell contact; 2) that a complex group of protein bound macromolecules diffuses from the matrix of the graft and interacts with the mesechymal cells; and 3) that a charge develops on the surface of the graft by hemodynamic or mechanical forces which create an electrical potential which stimulated the mesenchymal cells.[7,21,36,59]

Burwell, in his studies in rats has shown that allogenous cortical bone, freed of its own marrow and combined with autogenous marrow is capable of producing more new bone than either component alone which supports some sort of inductive mechanism.[10]

Packing the medullary canal of a cortical allograft, which has been cleaned of its marrow content, with autogenous cancellous bone should then provide: 1) support and protection for the ingrowth of new vessels to re-establish the medullary blood supply; 2) provide cells for osteogenesis, 3) provide induction factor; and 4) reduce the antigenecity of the allograft by removing the majority of its cellular content.

The periosteal fibrous tissue surrounding both grafts was infiltrated with lymphocytes. This is the normal immune rejection response to be expected. The reaction in this study was not severe and was the same around both the right and left grafts. A severe immune reaction would not be expected as the cellular content of cortical bone is very low. The most antigenic portion of these grafts was their marrow contents and this was removed prior to placing the grafts in the host.

Radiographically, no major differences could be detected between the healing of the grafts in the right and left legs. In the healing fracture there should be a bridging callus over the fracture gap with eventual remodelling of the callus to normal cortical bone. Stabilization of a fracture site or an osteotomy site with a metal plate and screws should reduce the amount of callus formation. This would be expected to occur in the healing of the graft-host junctions.

In this study, the amount of callus formation that was visualized radiographically was not extensive except in a couple of instances. The callus formation was usually seen begining with the three week post-operative radiographs in both the right and left legs. By five to seven weeks the callus had generally bridged the graft-host junctions in both the right and left leg of all the dogs. The remodeling of the callus continued in the long term studies throughout the entire study period. An increase in porosity was seen at the graft-host junctions after nine weeks post-operatively and was seen until the dogs were euthanized. The radiographic events seen

in this study followed the normal route of healing bone with no significant difference noted between the right and left leg. It has been shown that bony union will occur between live cortex and dead cortex. As the callus is formed, new bone would be laid down in apposition to the dead bone and remodeling of the callus would begin. The dead cortex in the callus area would be invaded by vascular tissue and remodeling would occur as in fracture healing. Therefore we would not expect to see a radiographic difference at the graft-host junctions between the right and left grafts. If these studies were extended for longer periods we would expect to see an increase in porosity of the graft cortices as resorption of old bone and apposition of new bone progresses.

Some of the dogs showed a marked periosteal reaction along the entire shaft of the tibia. In most probability this reaction could be attributed to the amount of damage to the periosteum at the time of surgery. The periosteum was incised and elevated off the tibia before the graft was removed. Although the periosteum was not intentially elevated off the host tibia above and below the graft site it occured in almost every case. Also damage to the periosteum occured when bone clamps were used to hold the bone plate in place. This periosteal reaction over the graft itself would mask the graft-host junctions in some of the cases so that accurate determination of callus formation, healing and graft density could not be evaluated. The best example of this was Dog #11 which had a marked periosteal reaction which began three weeks post-operatively. The right leg was

extremely active and by seven weeks it was difficult to see the graft-host junction, yet on histopathological studies of those bonew revealed cartilage present in the osteomy sites while the radiographs showed a solid bony union. (Figs. 4 and 5) Therefore because of the periosteal reactions present on nearly all the legs the grafts could not be evaluated for definite results.

This periosteal reaction was also responsible for the inability to use the bone scans as an indicator for graft incorporation. Uptake of the Technetium 99 tagged to Medronate sodium appears to be related to osteogenic activity and to skeletal blood perfusion. The deposition in the skeleton is bilaterally symmetrical with increased activity in the metaphyseal areas of the long bone as compared to the diaphysis. Localized areas of abnormal accumulation of the radiopharmaceutical may be seen with primary skeletal malignancies, metastatic malignancies to the bone, acute or chronic osteomyelitis, arthritides, recent fractures, areas of ectopic clacification, Paget's disease, regional migratory osteoporosis, areas of aseptic necrosis and, in general, any pathological situation in which there is increased osteogenic activity or localized increased osseous blood perfusion. Localized areas of decreased accumulation of the radiopharmaceutical may be noted in areas of bone to which blood flow has been interrupted.[41]

The uptake of the radiopharmaceutical by the graft over the study period then should be an indication of revascularization of

the graft and new osteogenic activity. The marked decrease in uptake of the agent in the region of the graft is the one week post-operative bone scan is associated with the loss of blood supply to the graft. (Figs. 6 and 7) Over the study period, the degree of the decreased uptake of the agent became less. In the longer term studies it could be seen that the amount of uptake of the agent in the area of the graft was approaching the uptake of the agent of the host tibia. (Figs. 8 and 9)

In studying the graphs made from the densitometer reading, it can be seen that the proximal host tibia consistently had slightly lower densitometer readings than the distal host tibias. A higher densitometer reading is associated with a higher uptake of the radiopharmaceutical agent by the bone. The proximal tibia is probably not lower in activity than the distal tibial as the graphs suggest. In taking the bone scans, lateral views of the tibias were taken. The energy particles omitted by Technetium 99 are low energy, therefore do not penetrate soft tissues well. When lateral views are taken, the anterior tibialis muscle is between the bone and the crystal, whereas in the distal tibia there is virtually no muscle mass to absorb the particles. This then could account for the higher densitometer readings in the distal host tibia.

As was stated earlier, the periosteal reaction could interfere with the bone scan results. The periosteum is stimulated to produce new bone after it is damaged. Therefore it should take up the Technetium 99 - Medronate Sodium agent very readily. The periosteal

reaction over the graft sites could mask the actual activity of the graft itself. This was evident in several of the studies, especially Dog #11.

The callus formation is high in osteoblastic activity so therefore should take up the agent very readily also. This is evident in the graphs by the high densitometer readings at each graft-host junction. The overlapping of callus formation on the graft could also mask the actual graft activity. This was most evident in Dog #12 which had an extremely high peak at the distal graft-host junction of the left leg which was associated with a moderate external callus and lysis at the graft host junction radiographically. (Fig. 3)

Because of the individual biological variations amoung animals, it is very difficult to compare the incorporation of the grafts in one dog to the incorporation of the grafts in another dog and the densitometer readings from one dog cannot be compared to the densitometer readings of the other dogs. It can be seen from this study that this even holds true between the right and left tibias of each individual dog. The amount of periosteal reaction and periosteal new bone is related to the amount of damage to the periosteum at the time of surgery in each leg and how that individual animal responds to such damage. If little periosteal reaction was seen radiographically then the densitometer readings were usually lower than in the dogs which radiographically showed a more marked periosteal reaction. Again this holds true for each leg in a dog. Therefore because of the wide range of densitometer

readings recorded, a statistical analysis of uptake of the radiopharmaceutical agent by each graft was not feasible.

The use of tetracyclines to label bone has been noted for many years. The tetracyclines are bound to calcium ions as the new osteoid is mineralized. At the dosage rate of twenty-five milligrams per kilogram of body weight, only new bone is labeled whereas at higher doses such as fifty milligrams per kilogram are used virtually all bone is labelled.

With the first sets of dogs that were given tetracyclines, the oral route of administration was used over a five day period and ending about one week before euthanasia. The amount of labelling visualized by flourescing with an ultraviolet light was very difficult to see. Therefore with the last sets of dogs the tetracyclines were given at the same dosage rate, (25 mg/kg) but with the intravenous route and given only over a three day period. The labelling visualized by flouresence was much better.

The labelling seen in the right grafts was better visualized than the labelling in the left grafts in all of the dogs. The medullary canal showed consistent labelling across the graft in the right leg of all the dogs except Dog #6. The medullary canal in the center of the left grafts did not show any labelling on any of the dogs.

In the long term studies, over nine weeks, the cortices of the grafts in the right leg generally showed more flouresence than the cortices of the grafts in the left legs.

This increase in labelling in the right grafts is a good indication of more new bone formation in the right grafts than the left grafts.

Gross examination of the longitudinally split specimens always showed the right tibias to contain a completely filled medullary canal of a red cancellous bone material which was continous from the host tibia across the entire four centimeters of the graft. (Fig.12) The medullary canal material of the grafts was the same consistency and color as the host medullary canal contents. The left grafts however, were consistently filled with a yellowish, necrotic, fatty material in the center two centimeters of the medullary canal. (Fig. 13) Some of the left legs had re-established a red cancellous marrow up to the screw holes in the graft but never into the center of the graft. These results would indicated that the vascular supply to the cancellous bone placed in the medullary canal of the right graft had been re-established. If it had not been re-established then one would expect the center of the graft medullary canal to be filled with necrotic tissue as in the left grafts.

The screws traversing the medullary canal of the graft may be thought to be hindering the revascularization of the graft. Studies have shown that blood vessels can pass around the screws in the medullary canal. This study could support that fact. Histologically, the center of the medullary canals of the right grafts consistently showed live trabecular bone, blood vessels and heamopoietec activity in the nine weeks studies and longer. The fact that there was no bone cell activity in the bony trabecula

in the short term studies was expected because of the loss of blood supply to the center of the grafts.

The left grafts all showed some re-establishment of trabecular bone and heamopoietec activity in the ends of the graft medullary canal with Dog #8 showing a small amount of marrow activity inside the center of the medullary cavity passed the screws. It could be assumed then, that the entire medullary canal would eventually be re-established with good cancellous marrow, but for the time periods studied, it can be shown that the center of the medullary canals of the left grafts were consistently filled with necrotic debris or connective tissue.

Histopathologically, after nine weeks, the cortices of the right grafts began showing new osteons. The new osteons could be seen throughout the length of the cortex of the graft and usually consisted of a blood vessels surrounded by osteoblasts lining the sinus. (Fig. 21) Occasionally new areas of osteoid could be seen in conjunction with these new blood vessels. The cortices of the left grafts would usually show new blood vessels near the ends of the graft where remodeling of the bony union was taking place. The center of the graft cortices in the left leg would show some new blood vessel after nine weeks but was usually much less than the corresponding right graft.

The increased porosity at the graft-host junctions radiographically was visualized histopathologically by the increased number of vascular sinusoids in the cortex on both sides of the graft-host junction.

The histopathological studies of the grafts in this experiment would indicate that the presence of autogenous cancellous bone in the medullary canal of a cortical allograft does indeed aid in the re-establishment of the medullary blood supply and consequently the revascularization of the graft cortices.

Summary and Conclusions

Twelve mature dogs were used to study the combination of an autogenous cancellous graft and an allogenous cortical graft in the tibia. A four centimeter cortical allograft was inserted into a four centimeter defecit in the midshaft tibia in each leg. The left leg received the cortical allograft alone and the right leg received the combination cortical allograft and cancellous autograft. The dogs were studied over various periods up to seventeen weeks post-operative. The progress of the grafts were monitored with bi-weekly radiographs and bone scans with Technetium 99. The end results were studied with Tetracycline labeling of the bones, gross pathology and histopathological studies.

The results of the radiographic exam and the bone scans were such that any definite differentiation between the right and left grafts could not be determined. However, the gross pathology, tetracycline labeling and histopathological studies all indicate that the right graft was more active in regards to revascularization and osteogenic activity than the left grafts.

We know from previous studies that the allografts will eventually be incorporated into the host skeleton but it may take up to several years for large grafts. It was the purpose of this study to determine if the autogenous cancellous bone packed into the medullary canal of an allogenous cortical graft was beneficial to the re-vascularization and incorporation of the grafts, in hopes that the incorporation period could be shortened. The results from this short study show that the autogenous cancellous bone is beneficial to re-vascularization.

There are numerous other studies needed to further study this type of grafting procedure. Longer term studies, sequential fluorochrome labeling and use of preserved cortical allografts in combination with autogenous cancellous bone are just a few of these studies. Drilling holes in the cortex of the cortical graft to aid in revascularization has been tried clinically but no data has shown whether this is of any value. Microangiography or arterial dye perfusions would also add greatly to study of the revascularization of full cortical allografts.

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APPENDIX

I. Technetium 99 Bone Scan Results

Because of problems with retrieving data from the computer only the densitometer readings were evaluated. The higher the densitometer reading recorded coincides with a higher uptake of the Technetium. The graphs of the densitometer readings are on the following pages.

The results in Dog #1 consisted of one technetium study one week post-operative. The right tibia showed higher activity in the proximal host tibia but lower activity in the graft. The distal host tibias of both legs were approximately the same.

Dog #2's evaluation was from one technetium study three weeks post-operatively. The proximal and distal portions of both tibias were of approximately equal activity with the graft of the right leg showing higher activity than the left graft.

Dog #5 was evaluated with three technetium studies over a five week period. The one week study showed both tibias to be of approximately equal activity throughout the leg. The three week study showed the proximal and distal tibia of the left leg to be slightly more active than the right leg and the graft of the right leg was about twice as dense as the left graft. The five week study showed the left proximal tibia to be considerably more active than the right proximal tibia. The right graft was slightly more active than the left graft except for the distal graft-host junction which showed the left leg to be more active than the right. The distal portions of both tibias were approximately equal.

Dog #6 was evaluated with three technetium studies over a five week period. The one week study showed the right leg to be slightly more active than the left leg in both the host and graft areas. The three week study showed both legs to be of approximately the same activity in both the host and graft areas. The five week study showed the left proximal tibia to be more active near the proximal graft host junction. The right graft was more active than the left graft. The distal host tibias were of approximately equal activity.

Dog #8 was evaluated with five technetium studies over a nine week period. The one week study showed the left tibia to be more active than the right tibia in the proximal and distal host sections but less active than the right tibia in the grafted area. The three week study showed the proximal tibias of both legs to be approximately equal. The left graft was more active than the right graft and the right distal tibia was slightly more active than the left distal tibia. The five week study showed the right proximal tibia and the right graft to be more active than the corresponding left leg segments except at the graft-host junctions which were slightly more active in the left legs. The distal tibial fragments of both legs were of approximately equal activity. The seven week study was more active in the right proximal tibia than the left proximal tibia. The right graft was more active than the left proximal tibia. The right graft was more active than the left graft in the proximal half of the graft and less active in the right leg in the distal half of the graft. The left distal tibia was more active than the right distal tibia. The

nine week study showed the right tibia to be more active in the proximal area and graft areas than the left tibia and the distal tibial segments were approximately of equal activity.

Dog #9 was evaluated with seven technetium studies over a thirteen week period. All seven technetium studies showed the activity of both tibias to be of approximately equal activity over the entire host tibia and graft.

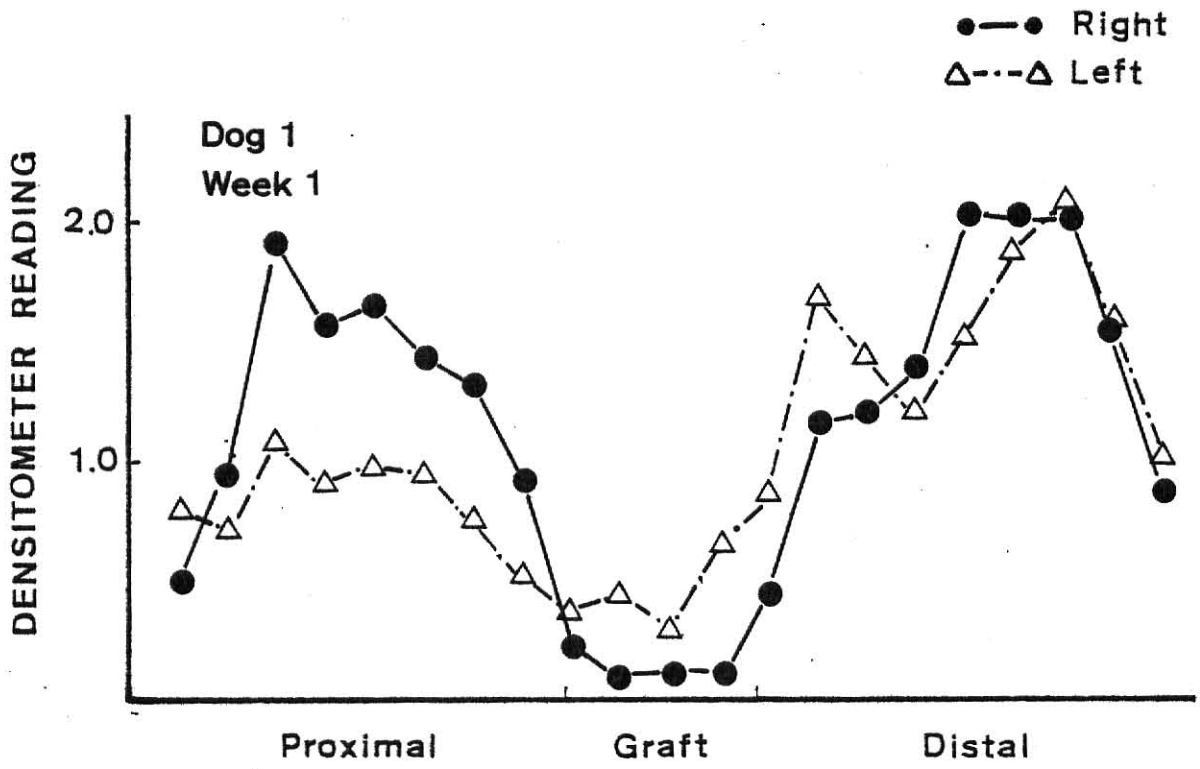
Dog #10 was evaluated with seven technetium studies over a thirteen week period. The activity of both host tibias and grafts were of approximately equal activity in all seven studies.

Dog #11 was evaluated with nine technetium studies over a seventeen week period. The first three studies showed the host tibia and grafts to be of approximately equal activity in both legs. The last six studies showed the proximal tibias of both legs to be of approximately equal activity except at the proximal graft-host junction where the right leg was considerably more active. The left tibial graft was consistently higher than the right tibial graft in the last six studies. The distal host tibias were of approximately equal activity.

Dog #12 was evaluated with eight technetium studies over a seventeen week period. The three week and five week studies showed the right proximal tibia and graft to be slightly more active than the left proximal tibia and graft with both distal tibias being of approximately equal activity. The remaining studies showed the proximal and distal host tibias of both legs to be approximately equal with the right proximal graft more active than the left proximal graft and the left distal graft more active than the right distal graft.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.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Left
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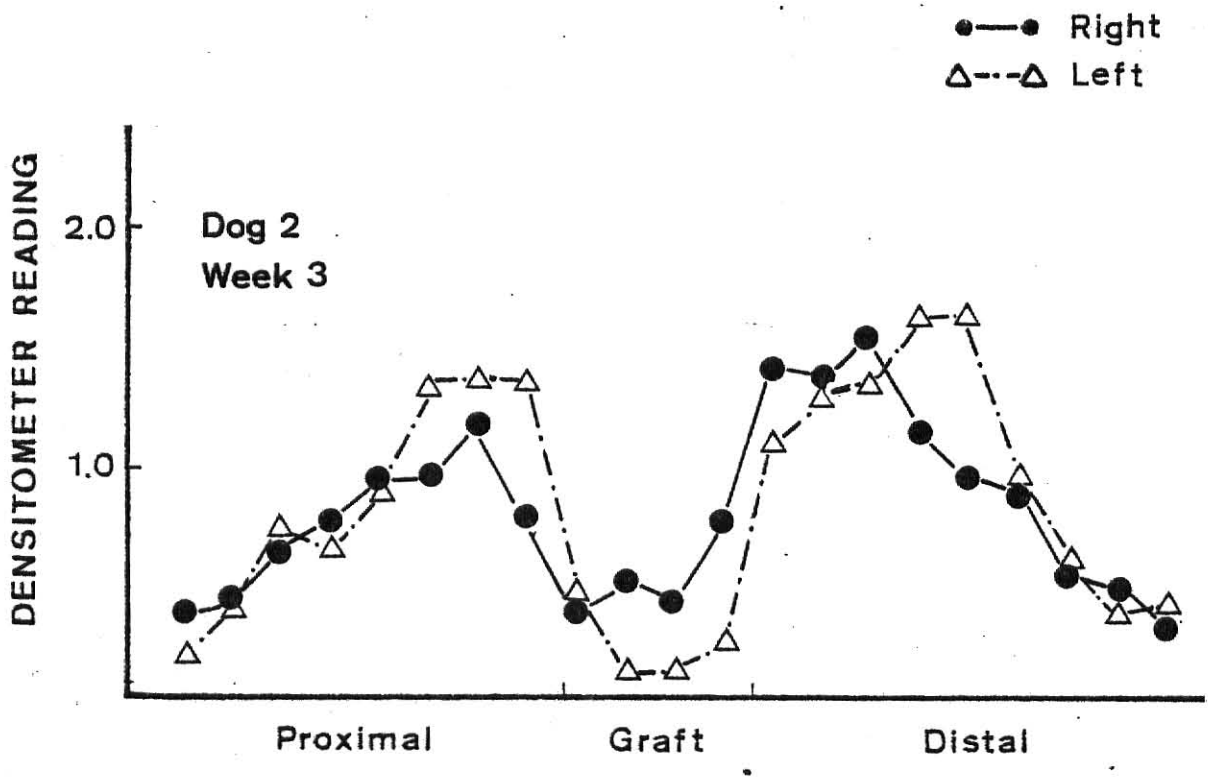
Week 1
DENSITOMETER READING
2.0
1.0
Proximal
Graft
Distal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Left
Dog 2
Week 3
DENSITOMETER READING
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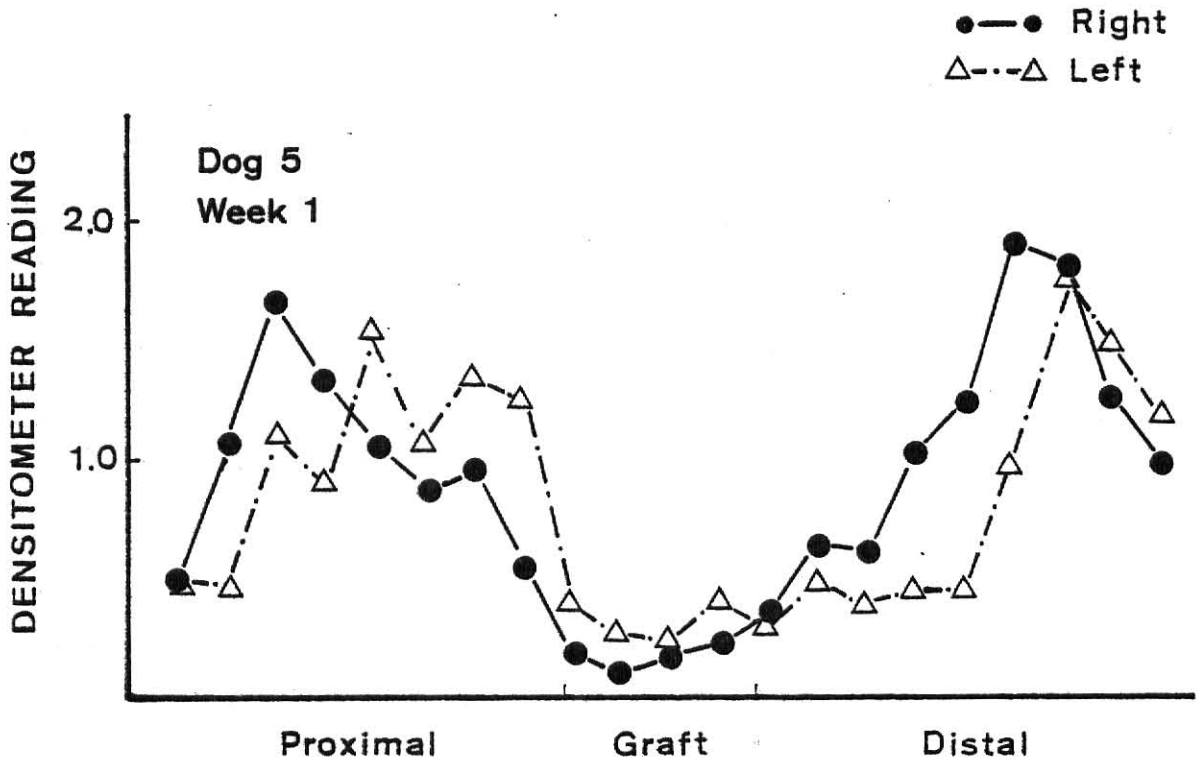
1.0
Proximal
Graft
Distal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Week 1
DENSITOMETER READING
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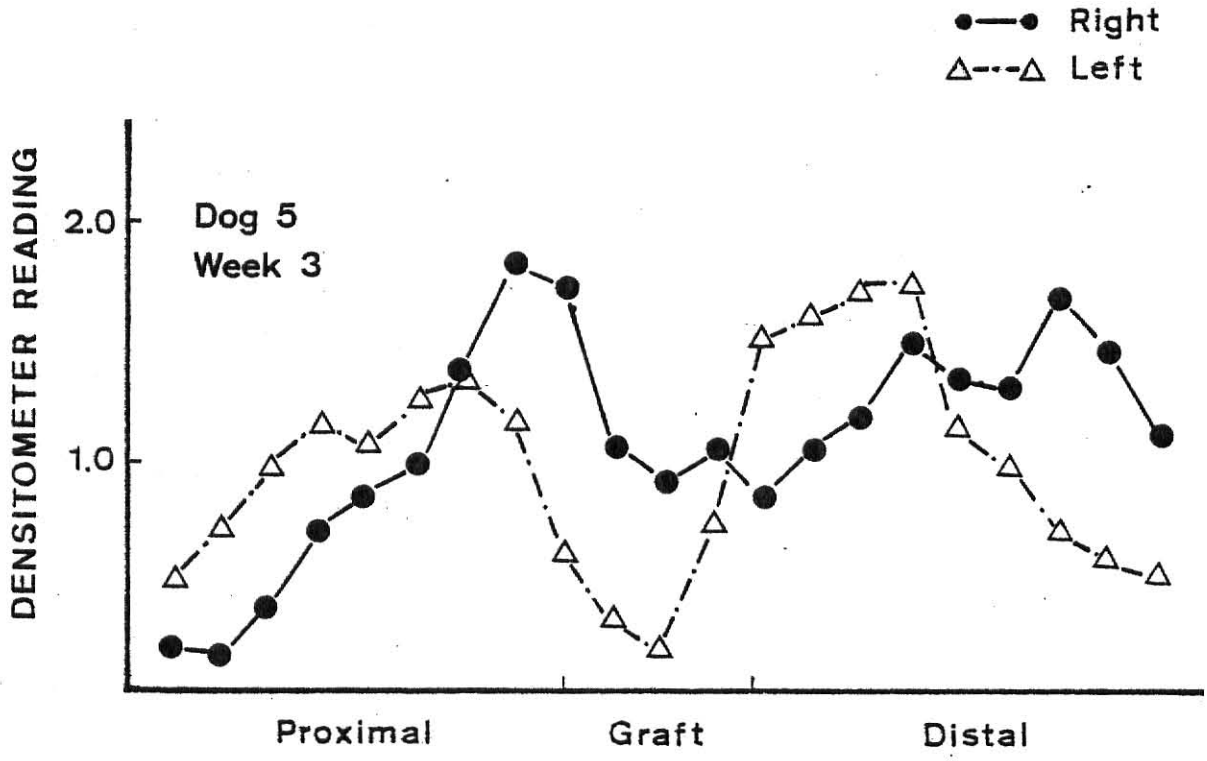
Proximal
Graft
Distal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Left
Dog 5
Week 3
DENSITOMETER READING
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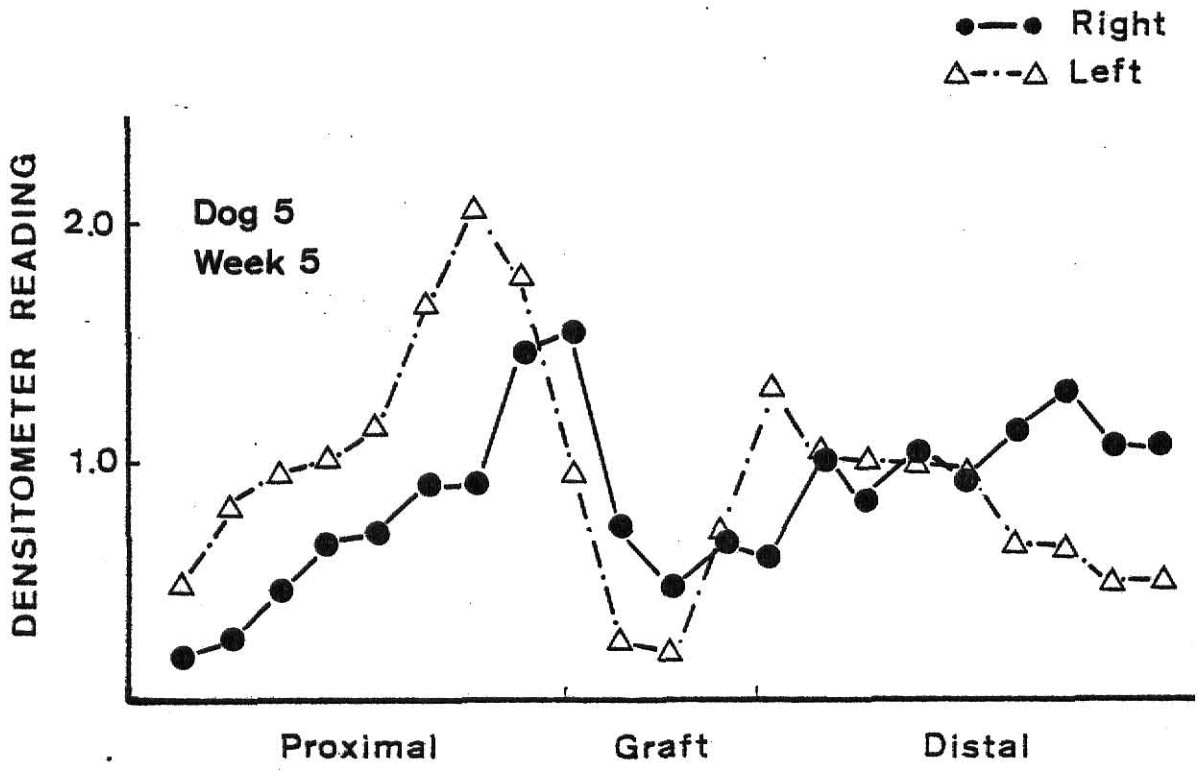
1.0
Proximal
Graft
Distal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Left
DENSITOMETER READING
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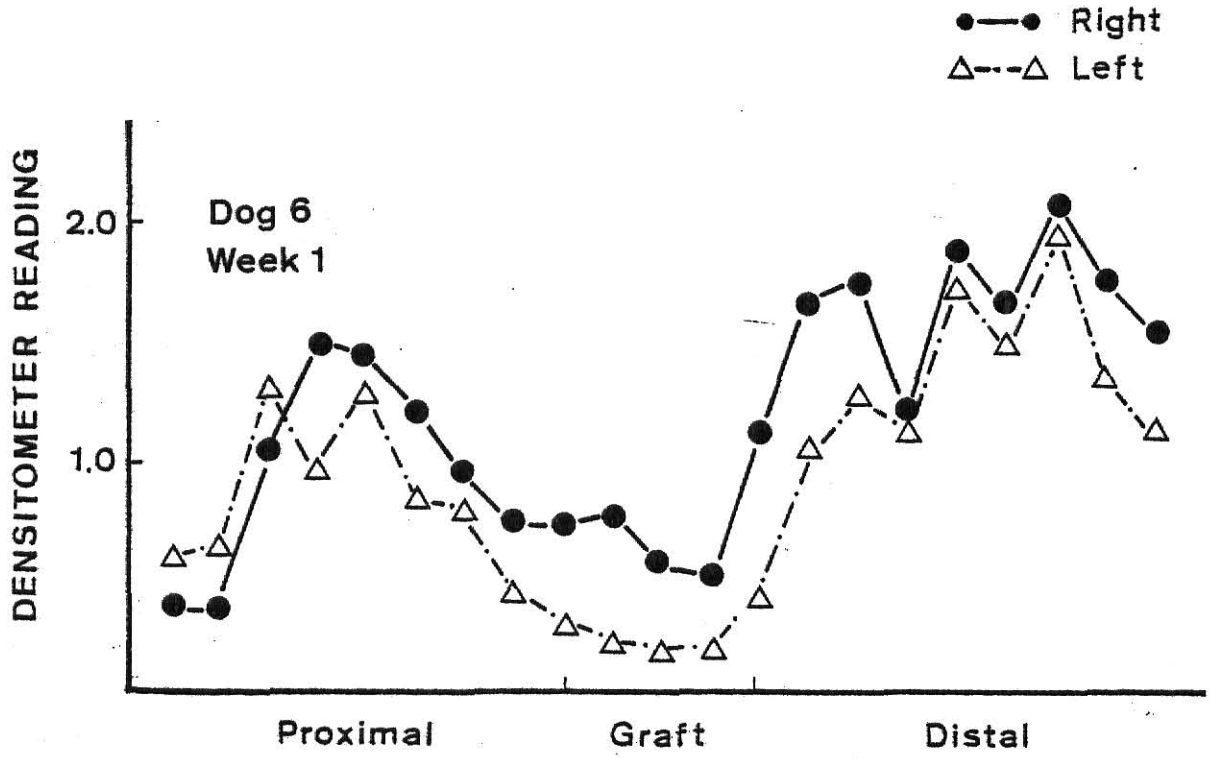
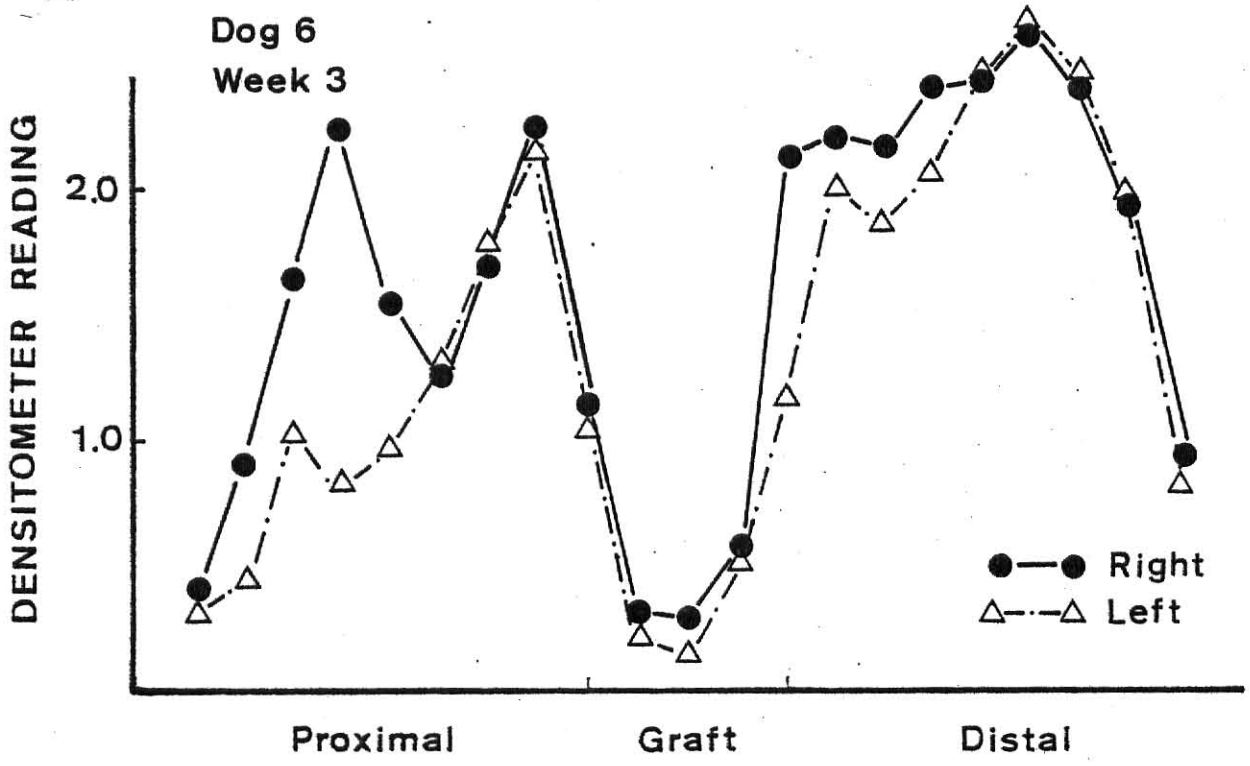
1.0
Dog 5
Week 5
Proximal
Graft
Distal


Right
Left
DENSITOMETER READING
2.0
1.0
Dog 6
Week 1
Proximal
Graft
Distal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Week 3
DENSITOMETER READING
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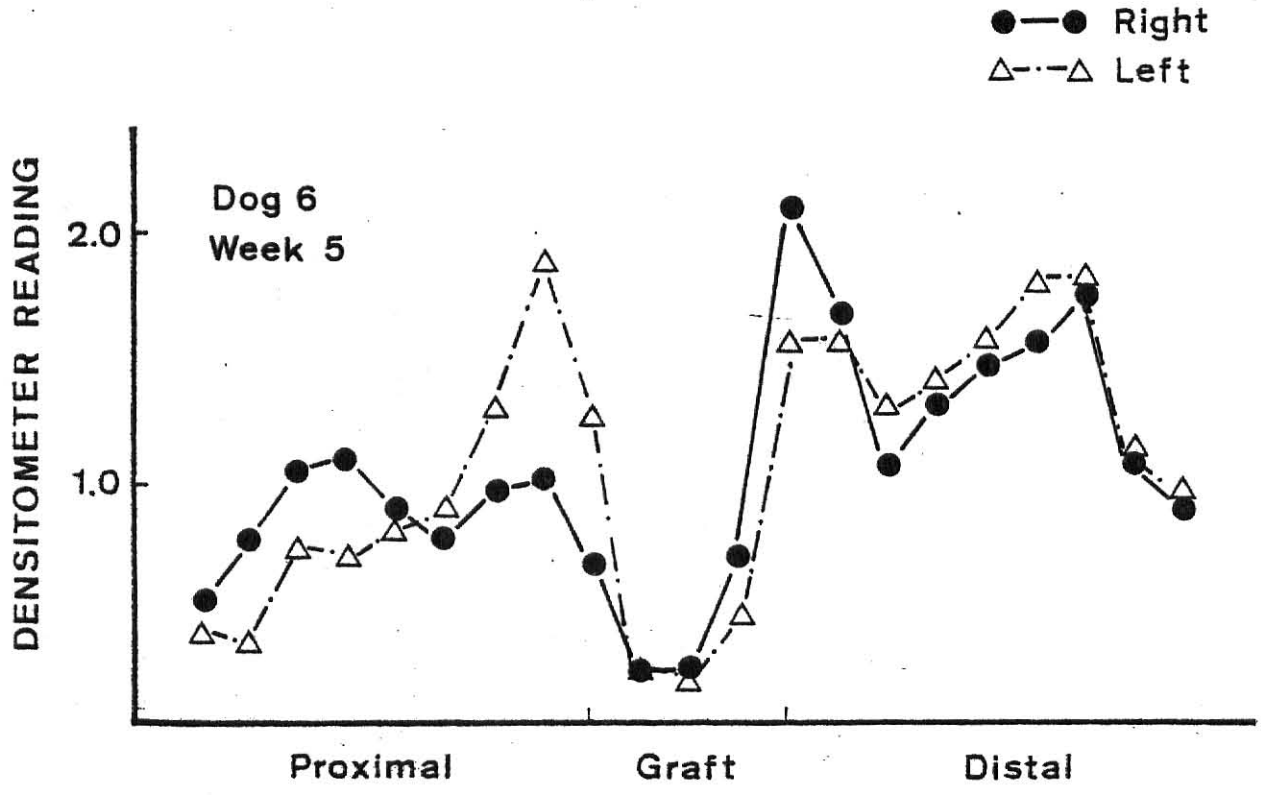
1.0
Right
Left
Proximal
Graft
Distal


Right
Left
Dog 6
Week 5
DENSITOMETER READING
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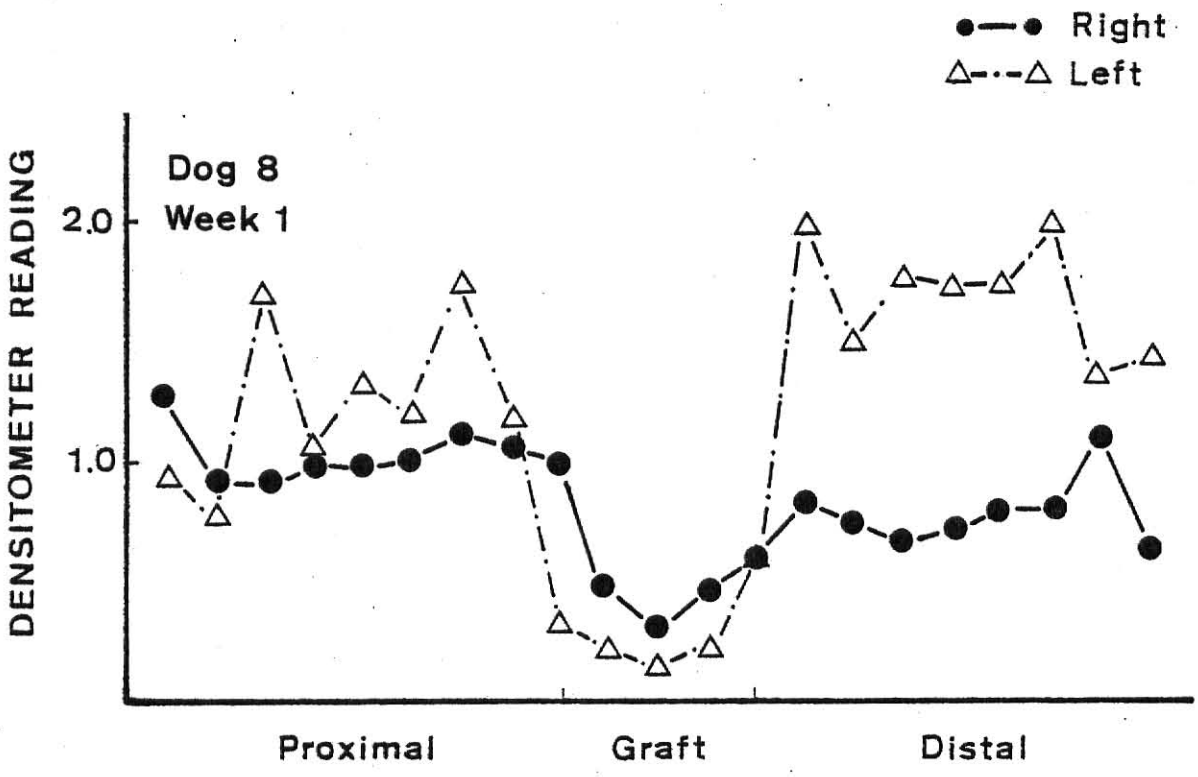
1.0
Proximal
Graft
Distal


Right
Left
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Week 1
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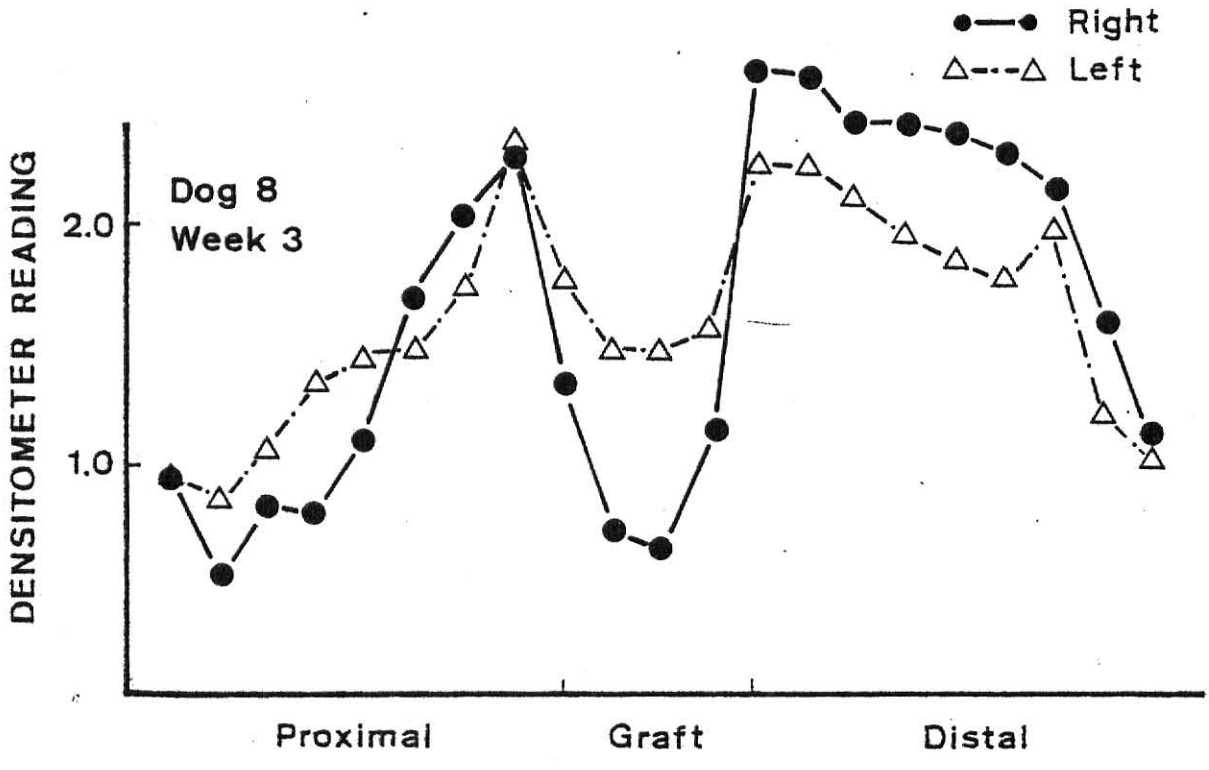
Graft
Distal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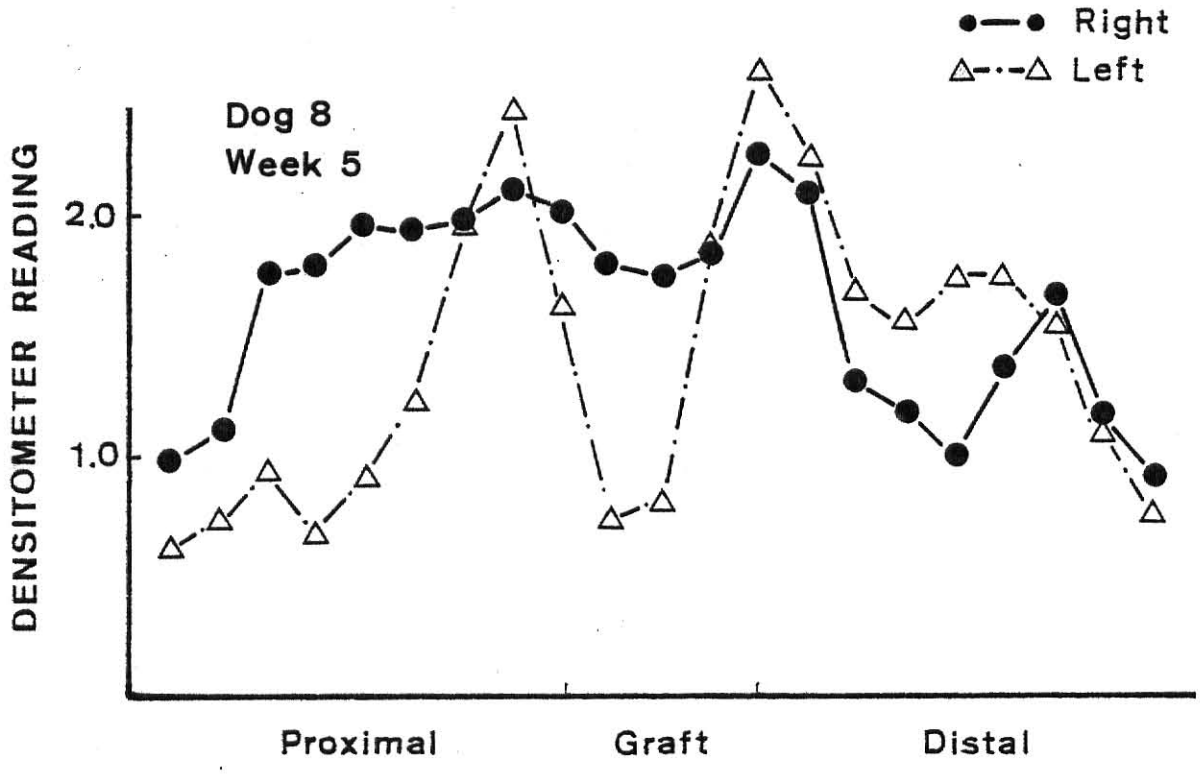
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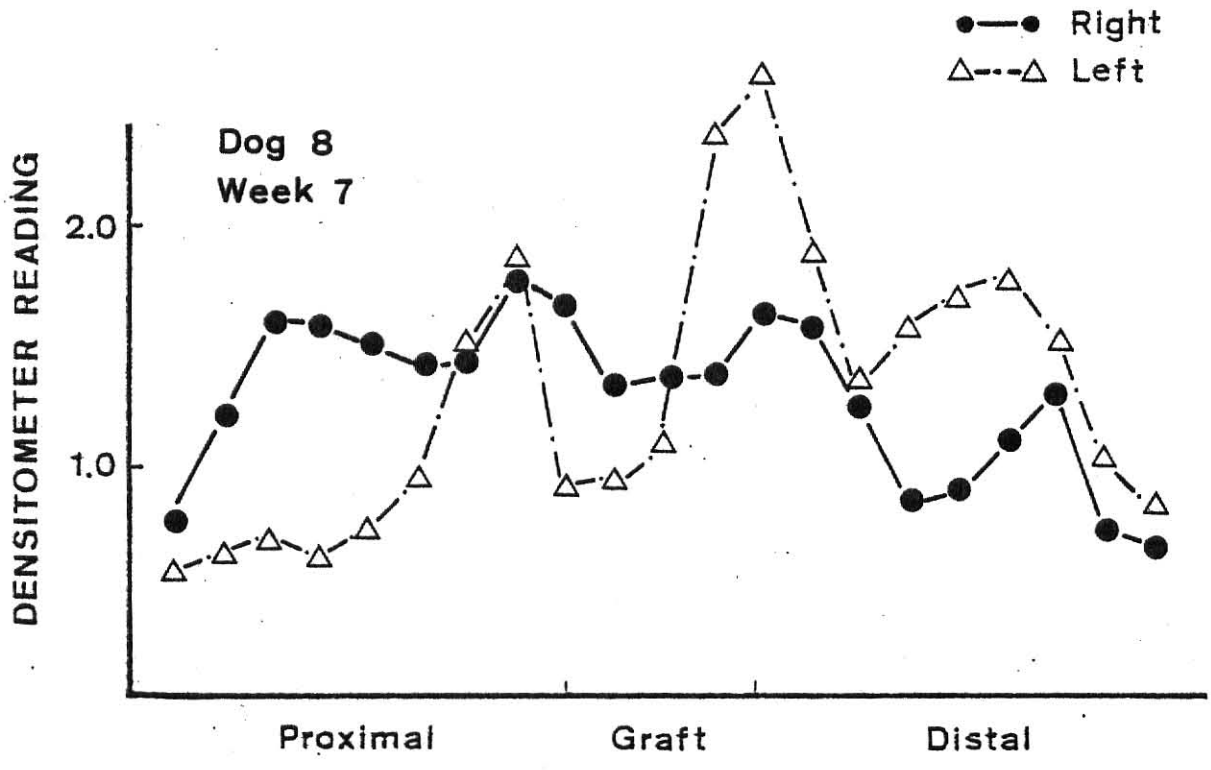
Distal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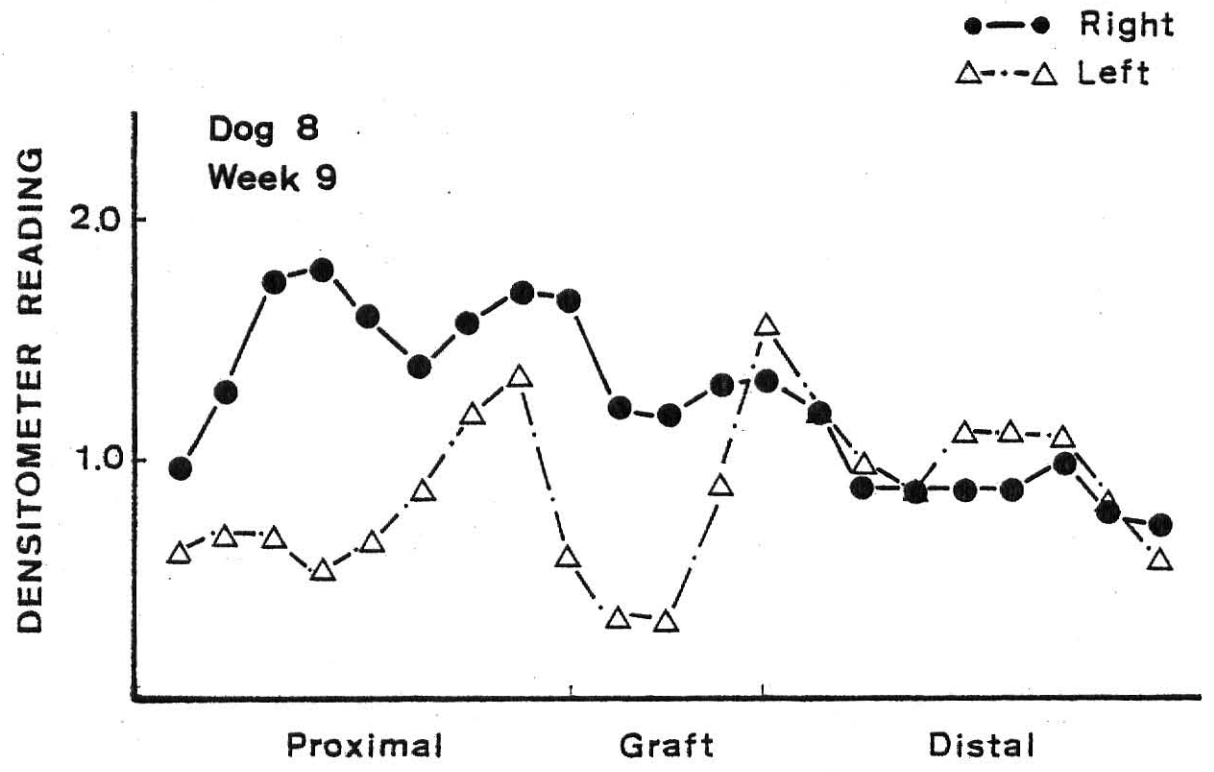
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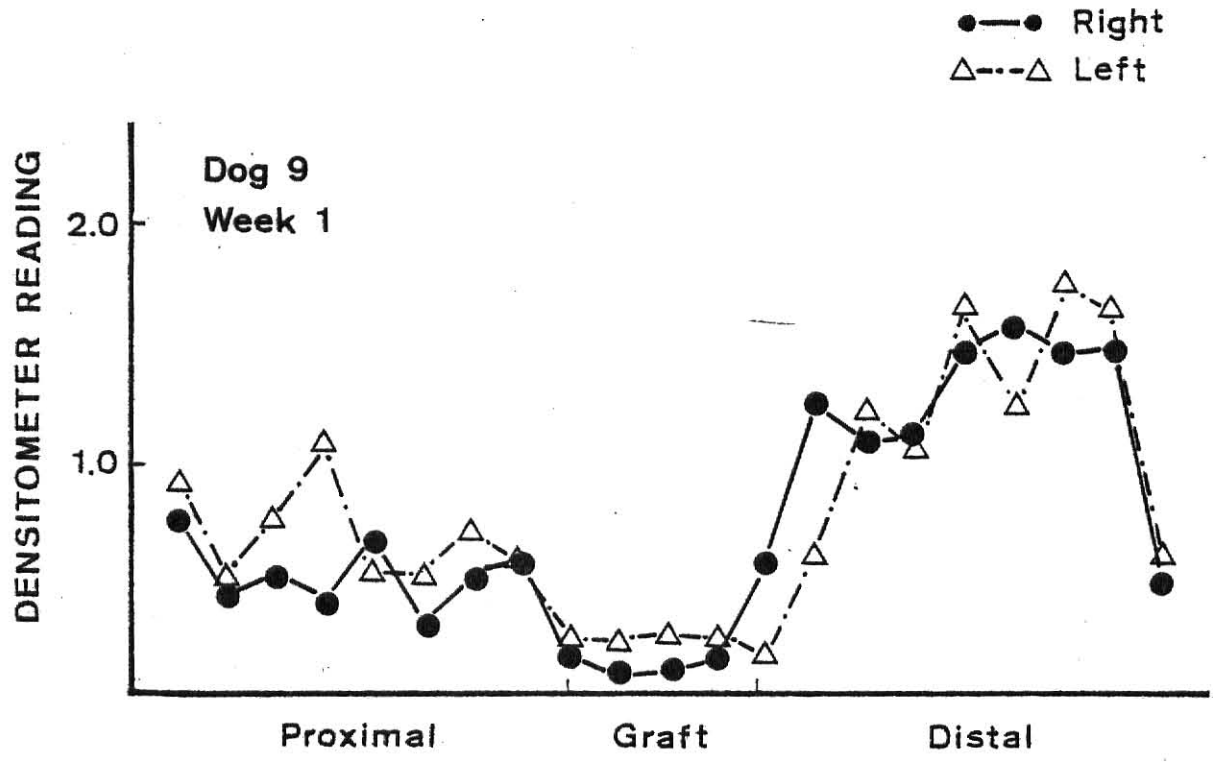
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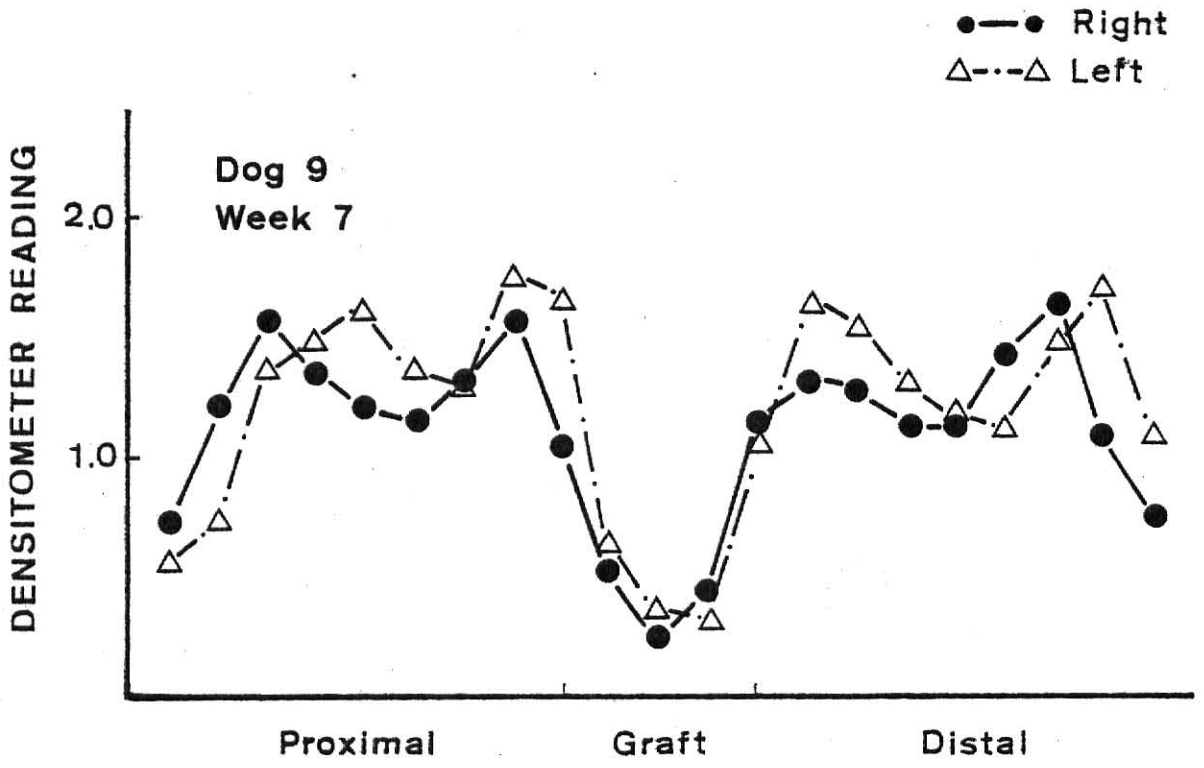
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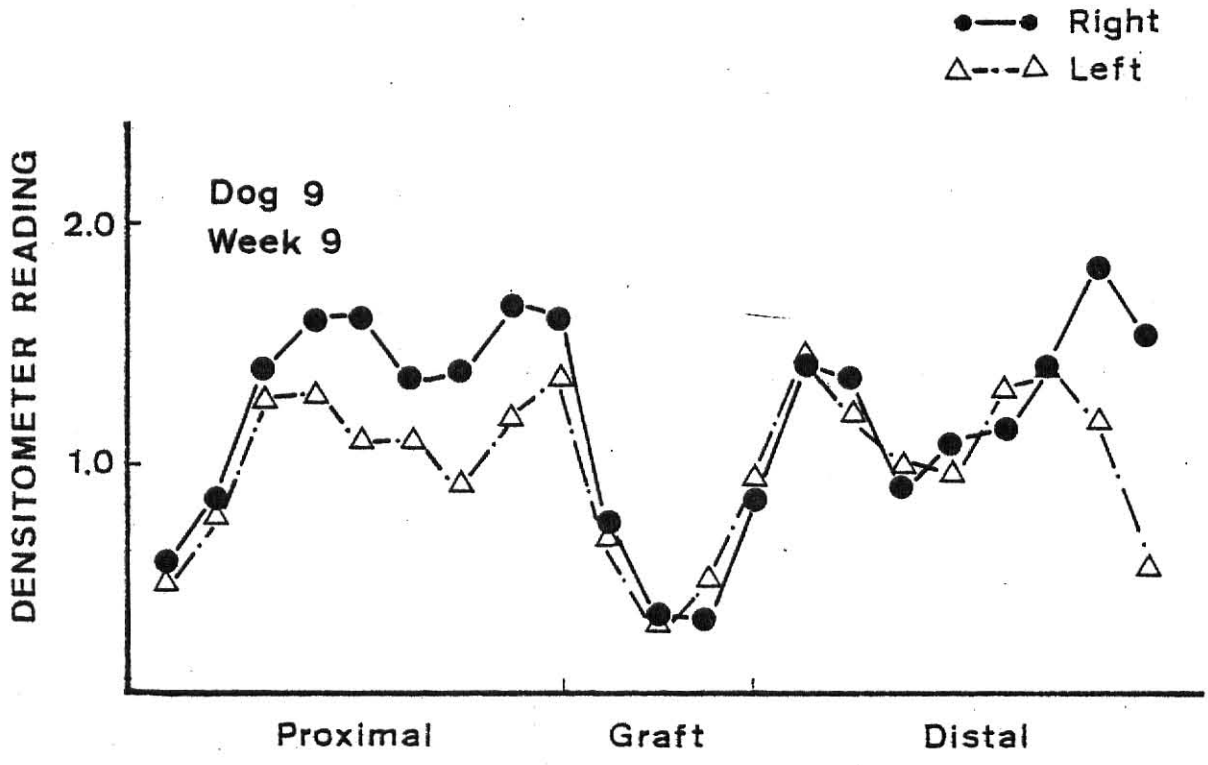
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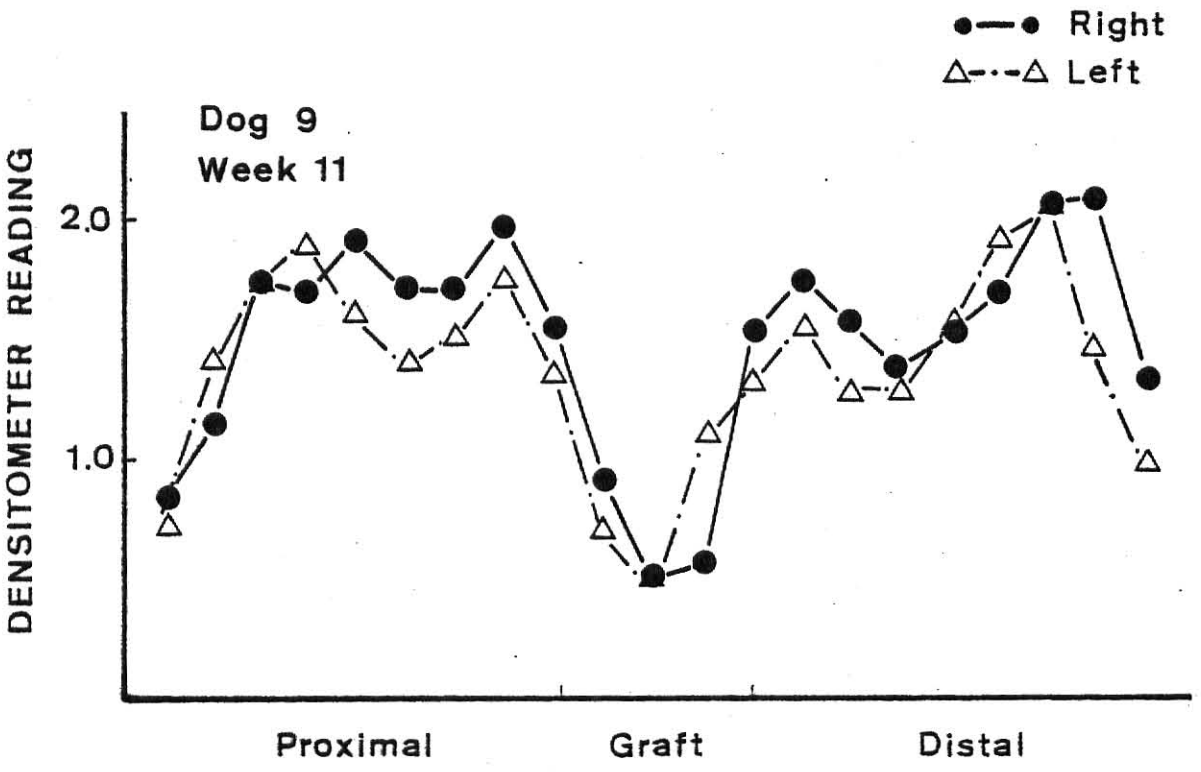
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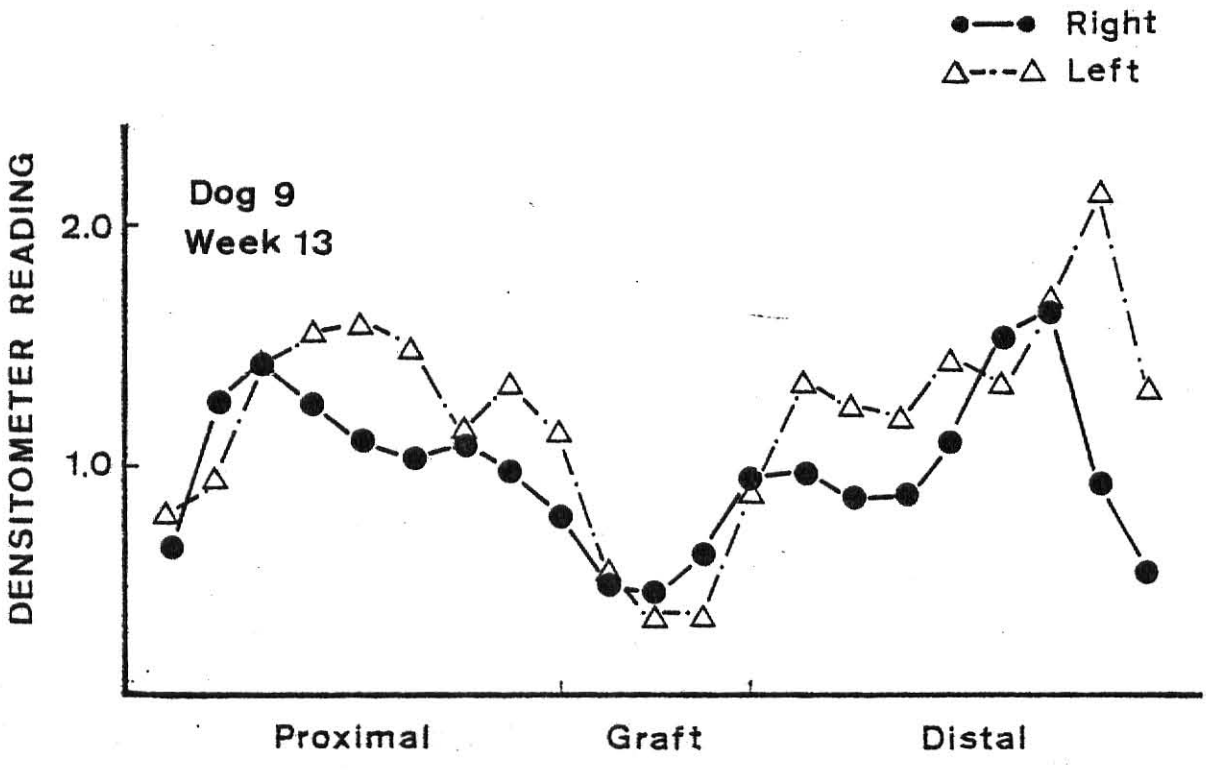
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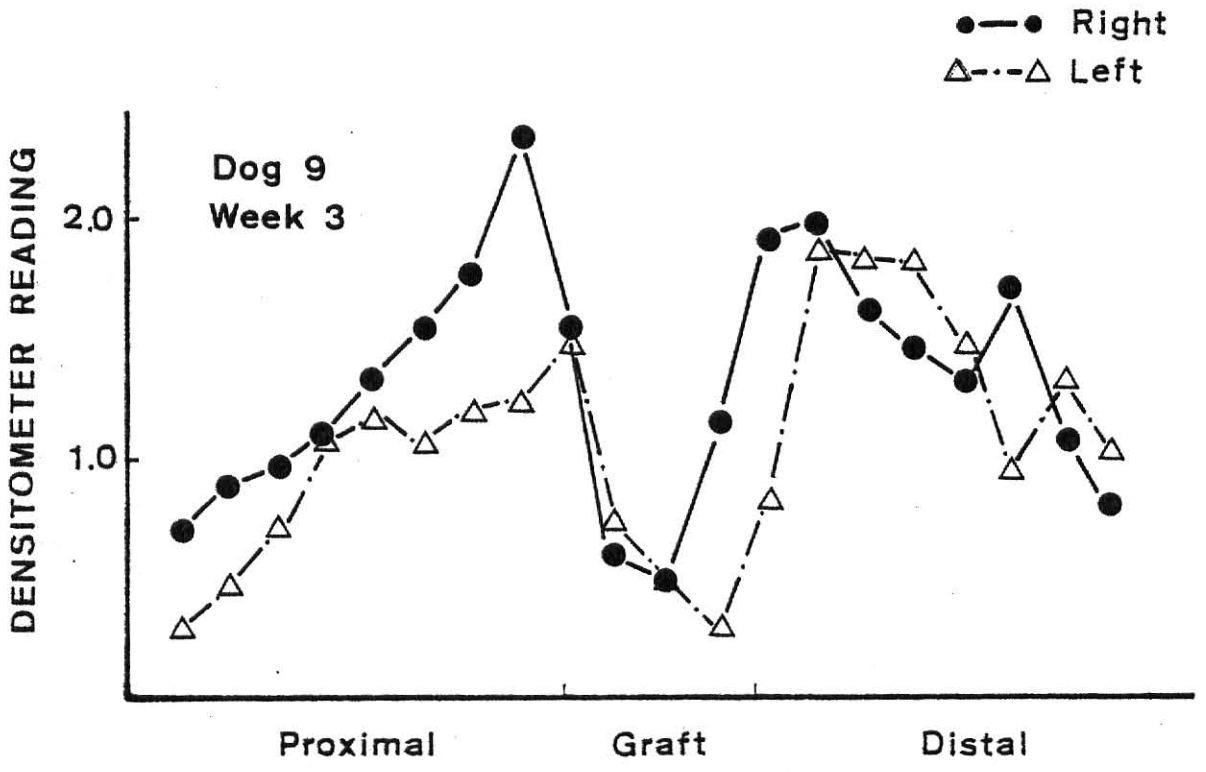
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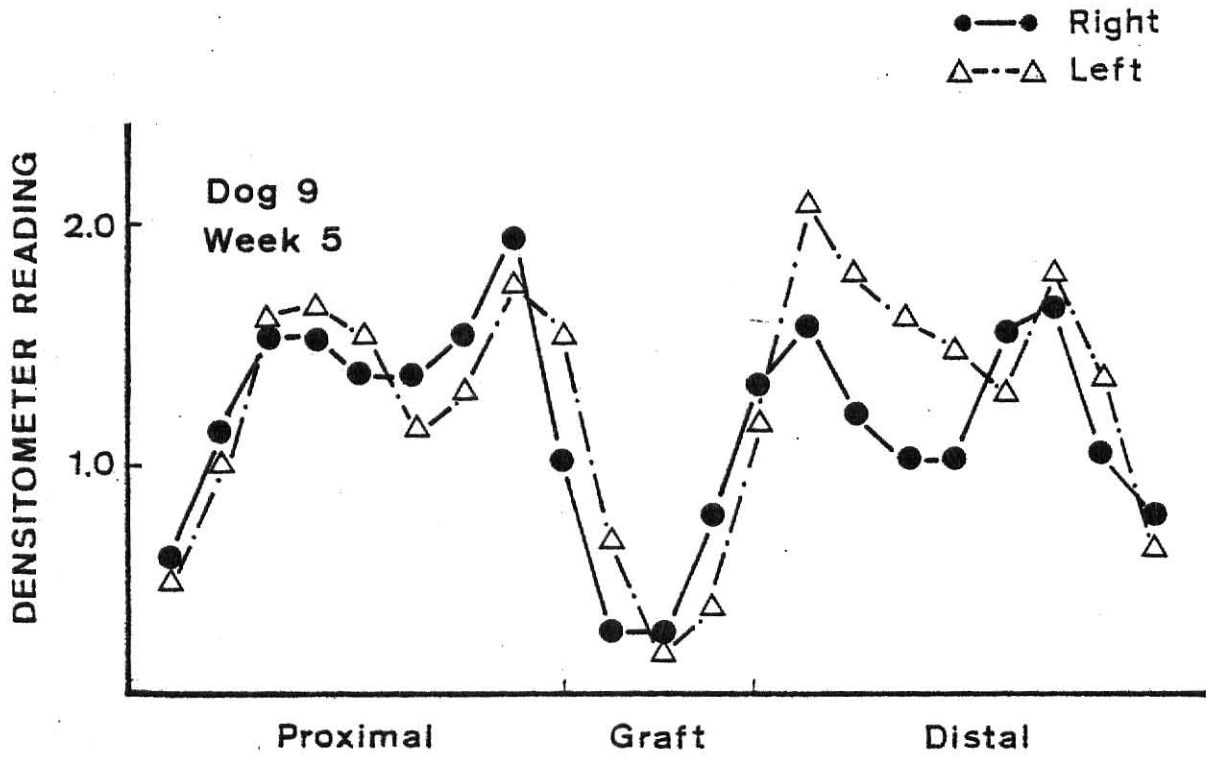
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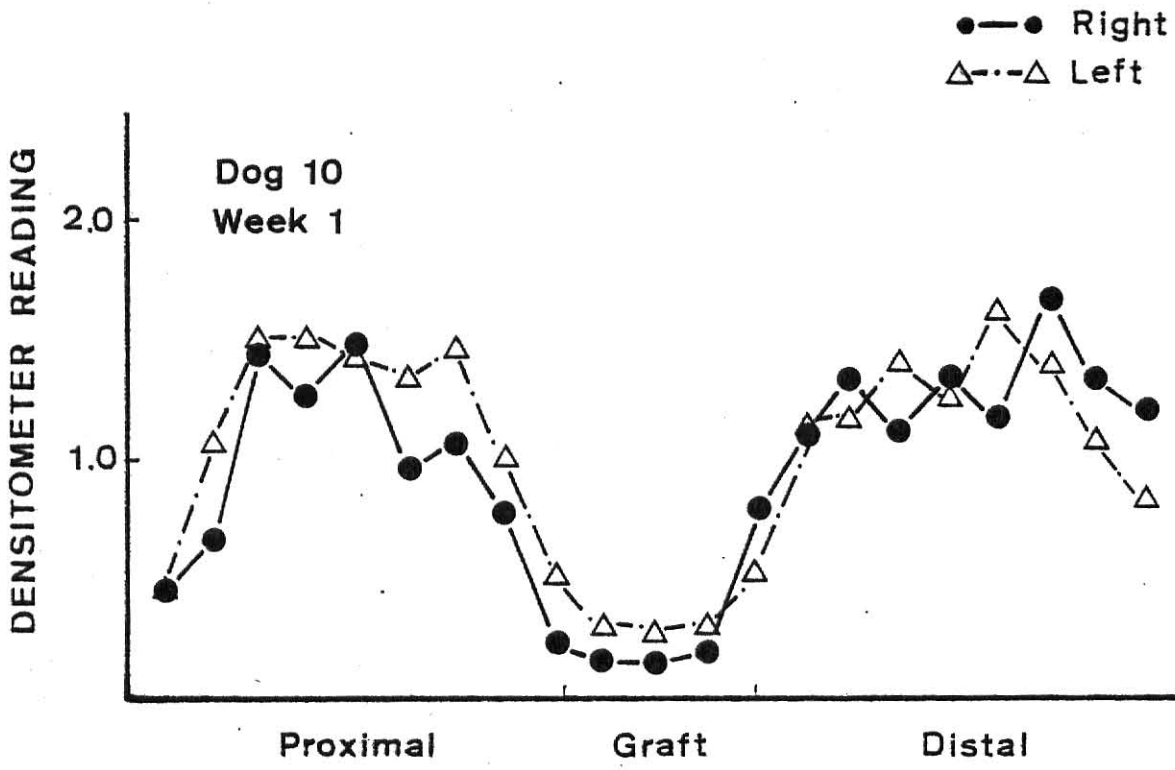
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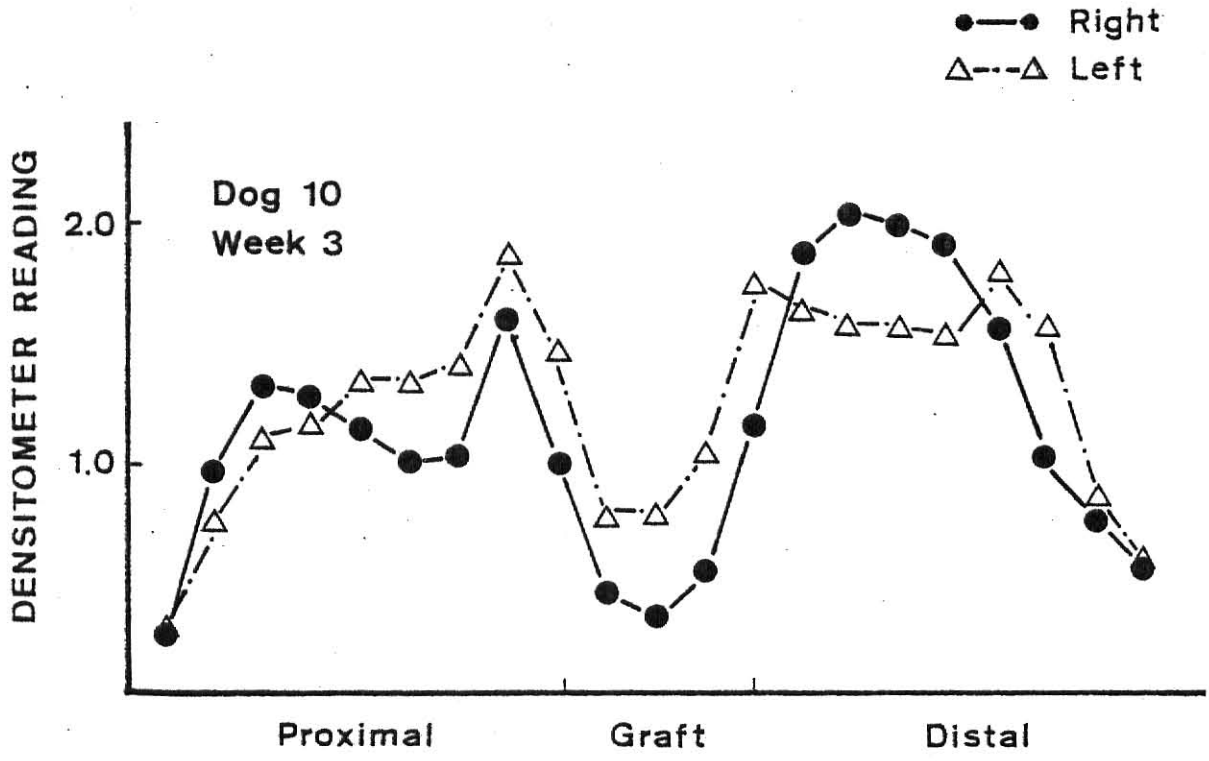
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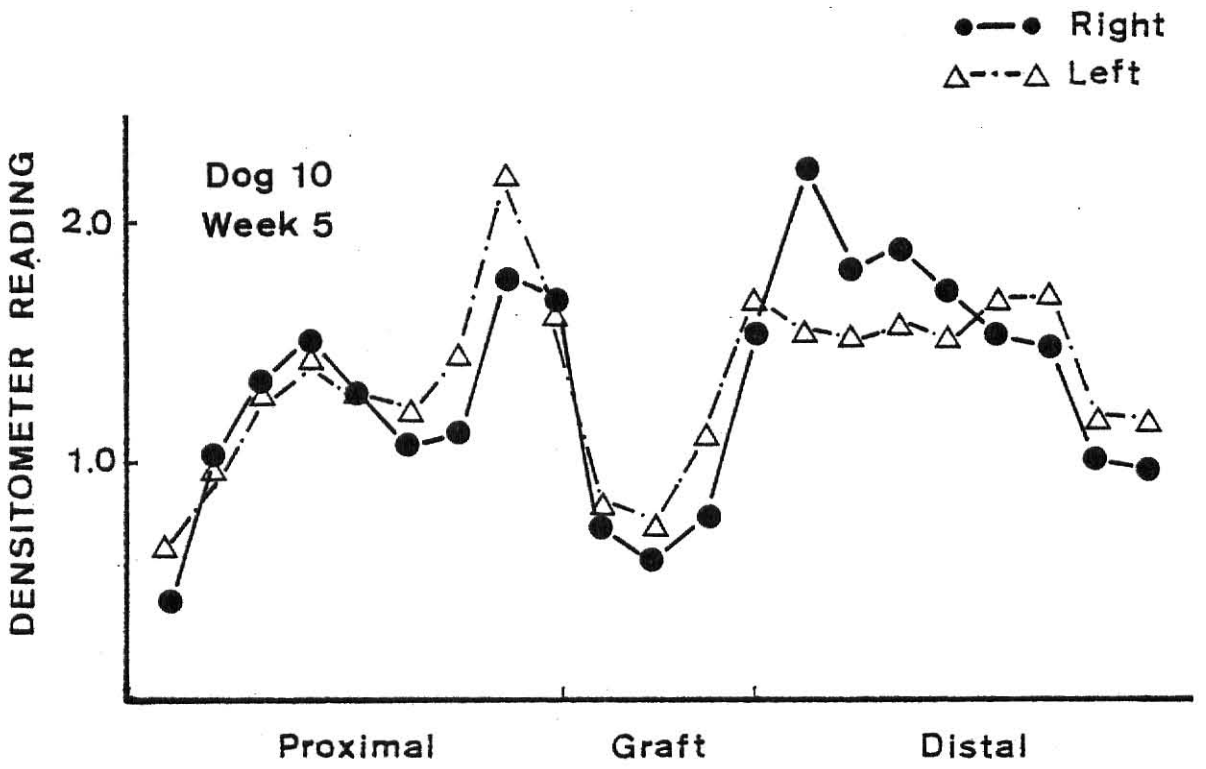
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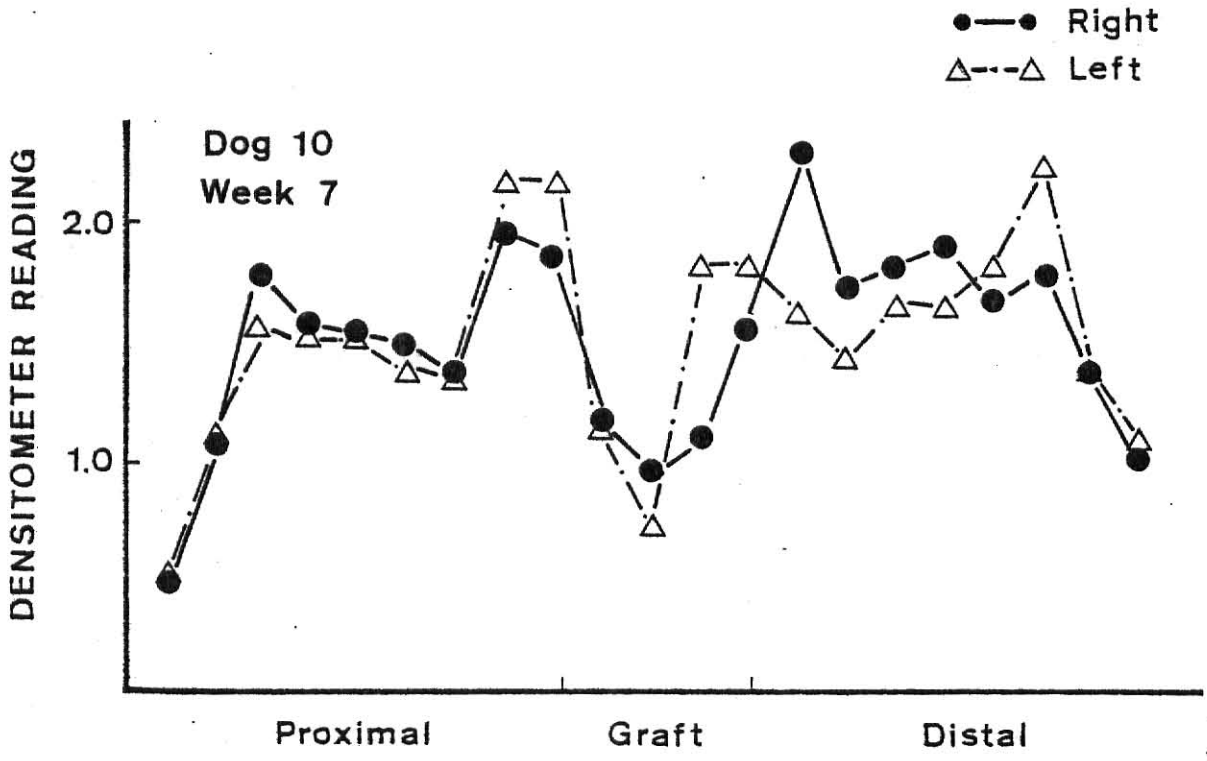
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Graft
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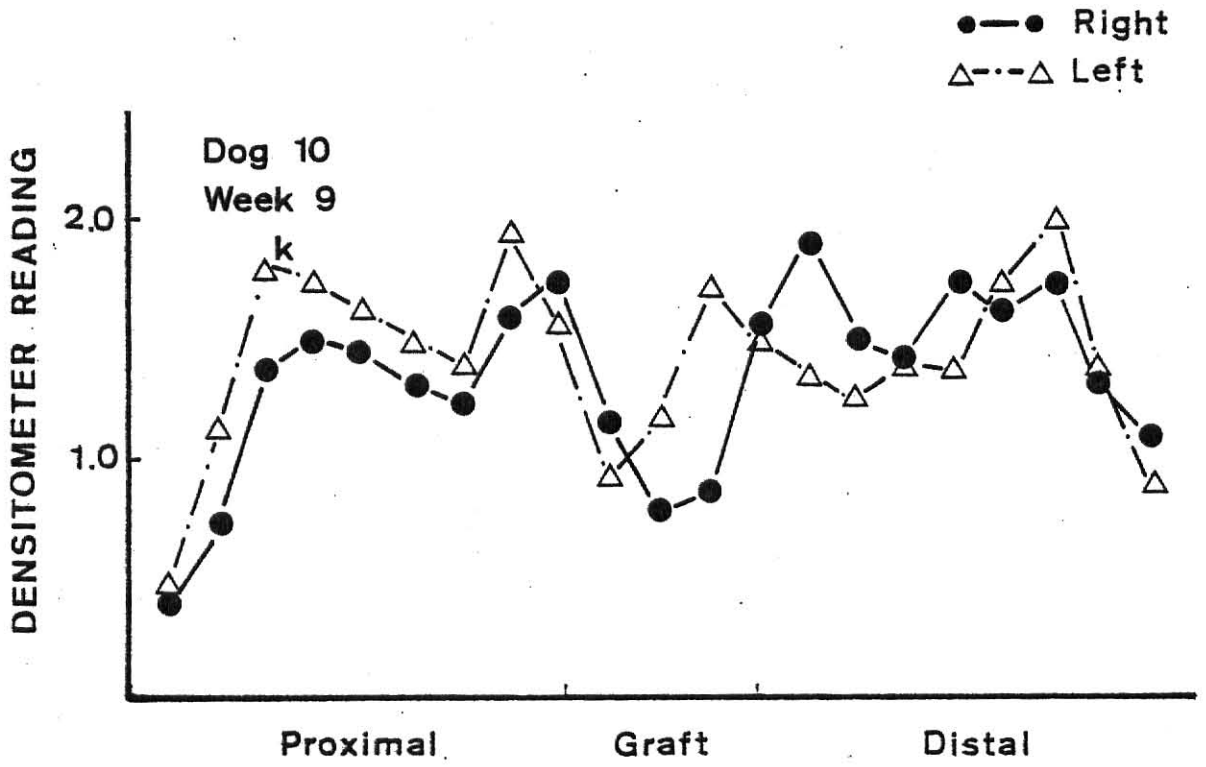
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Week 9
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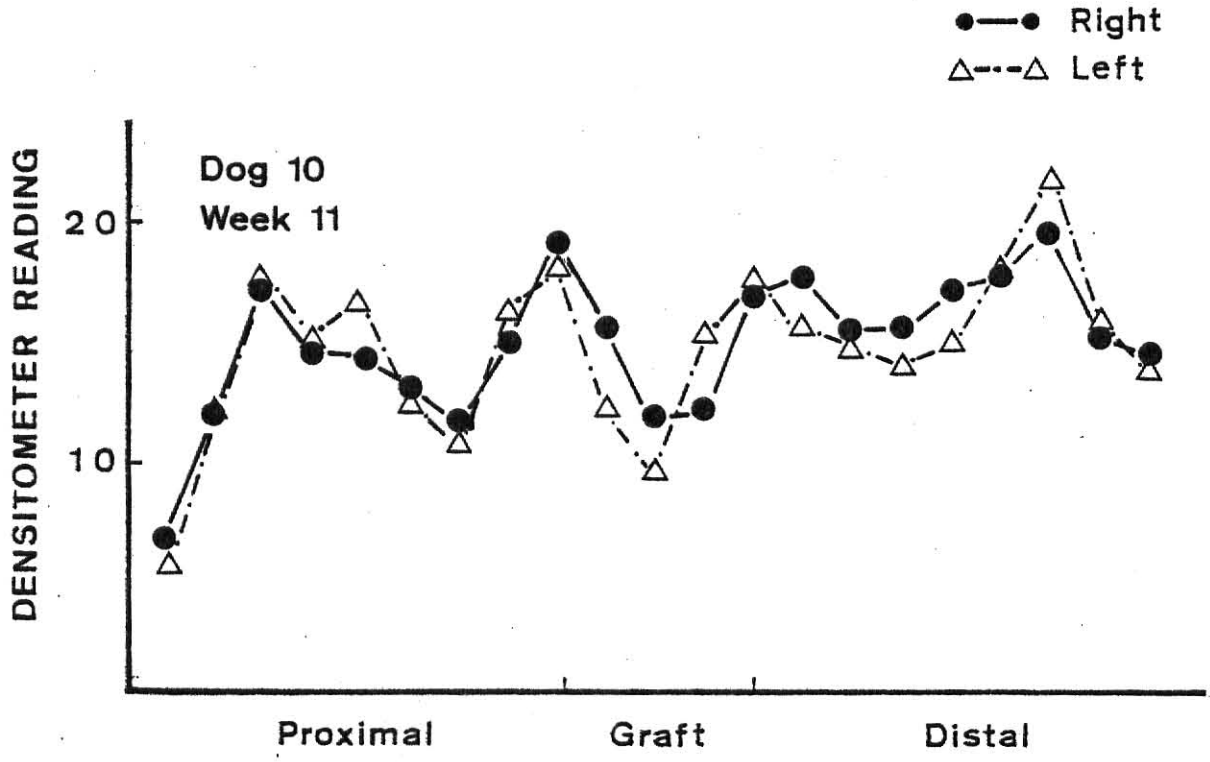
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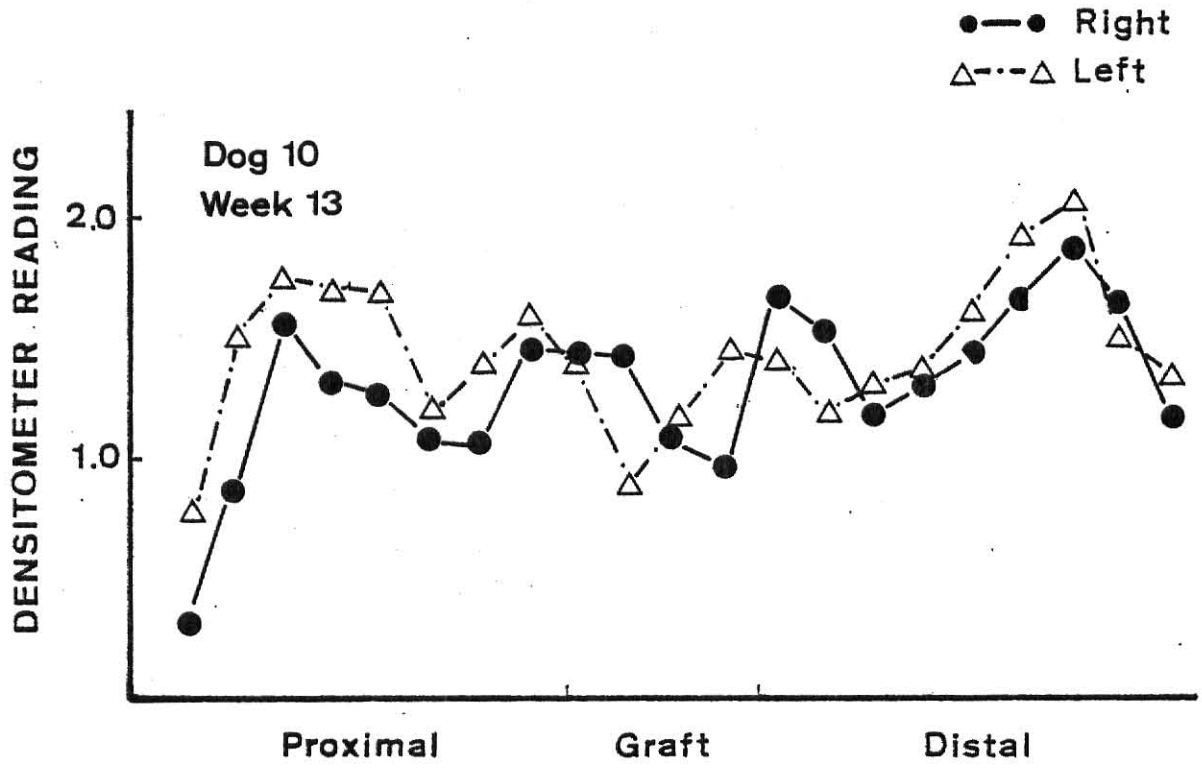
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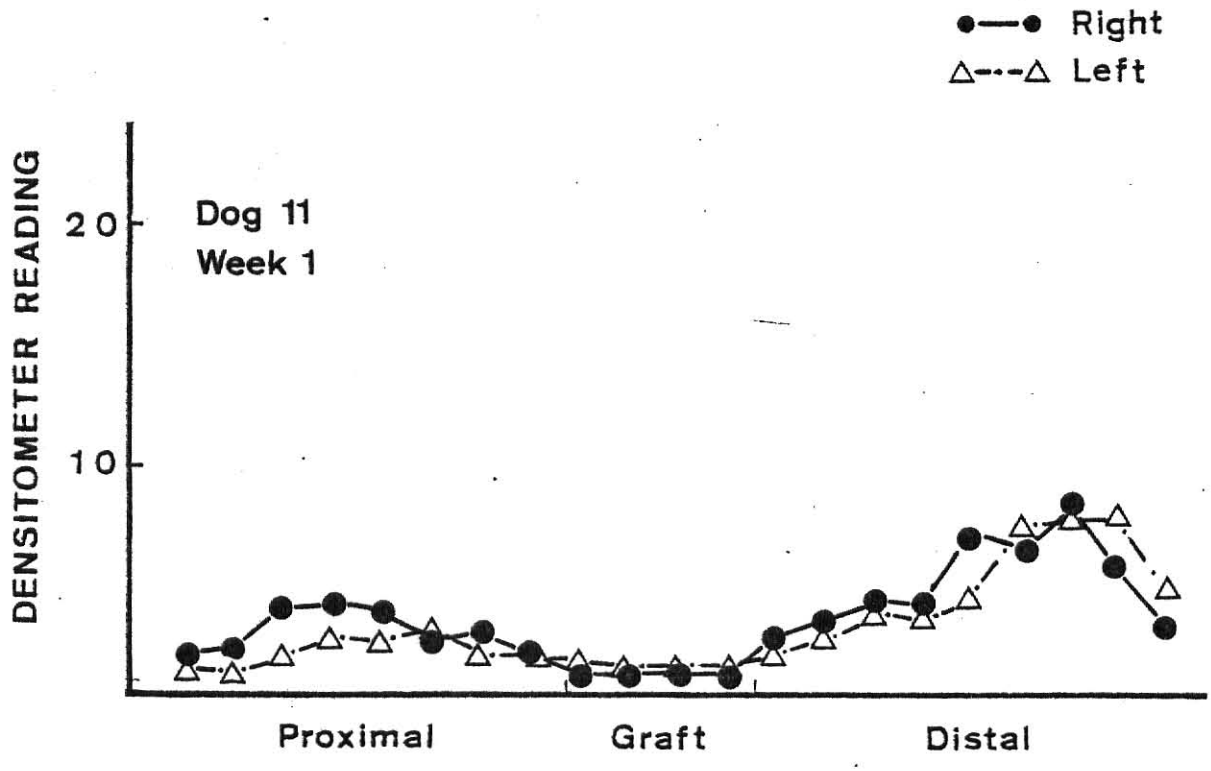
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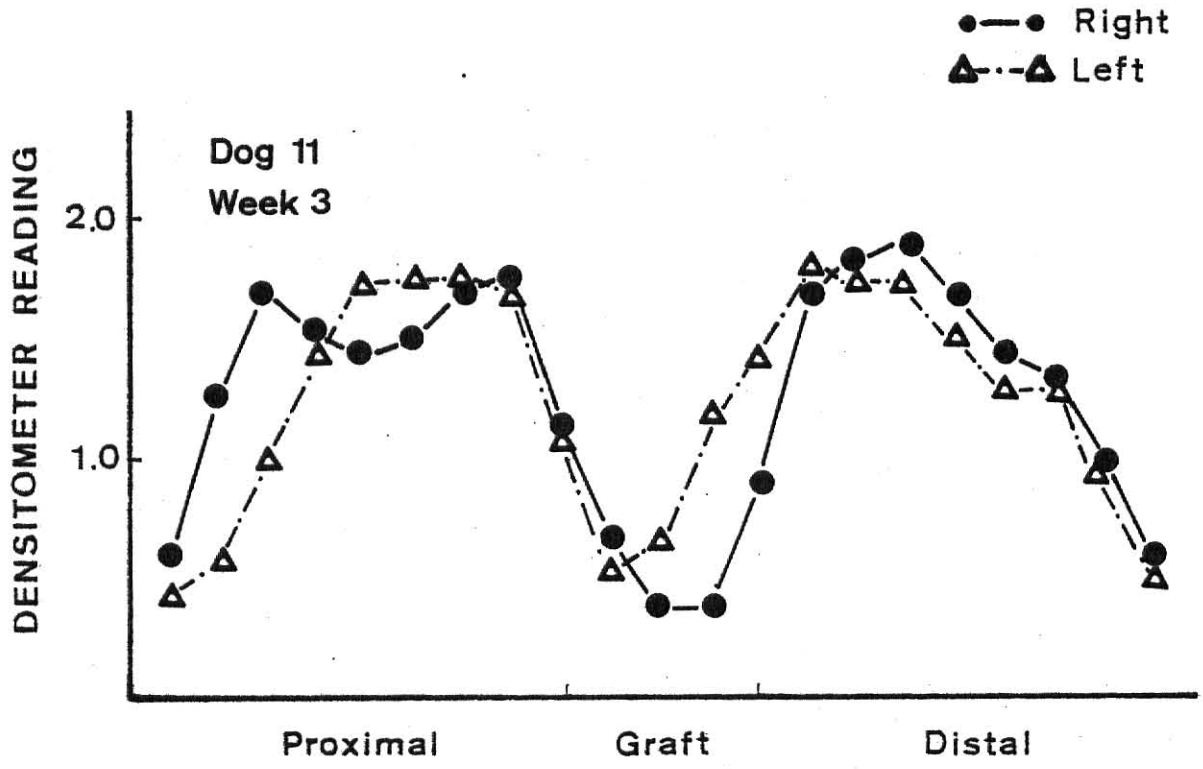
Graft
Distal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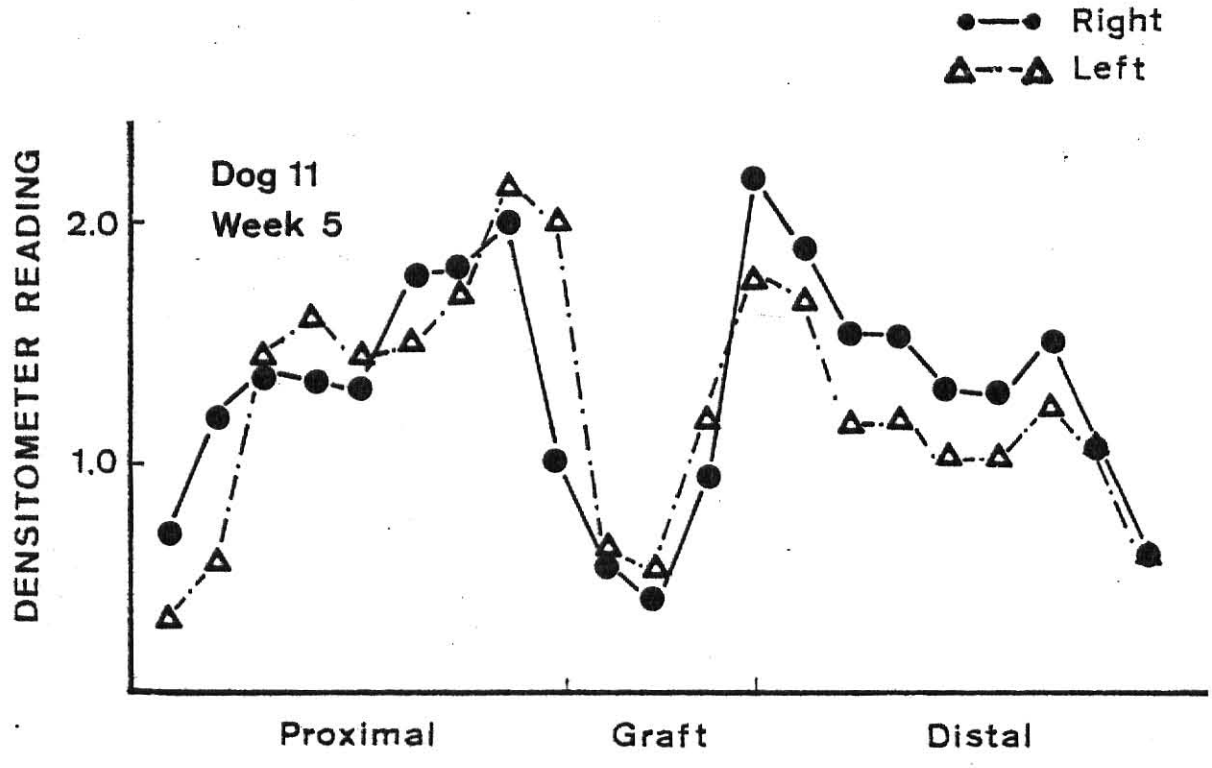
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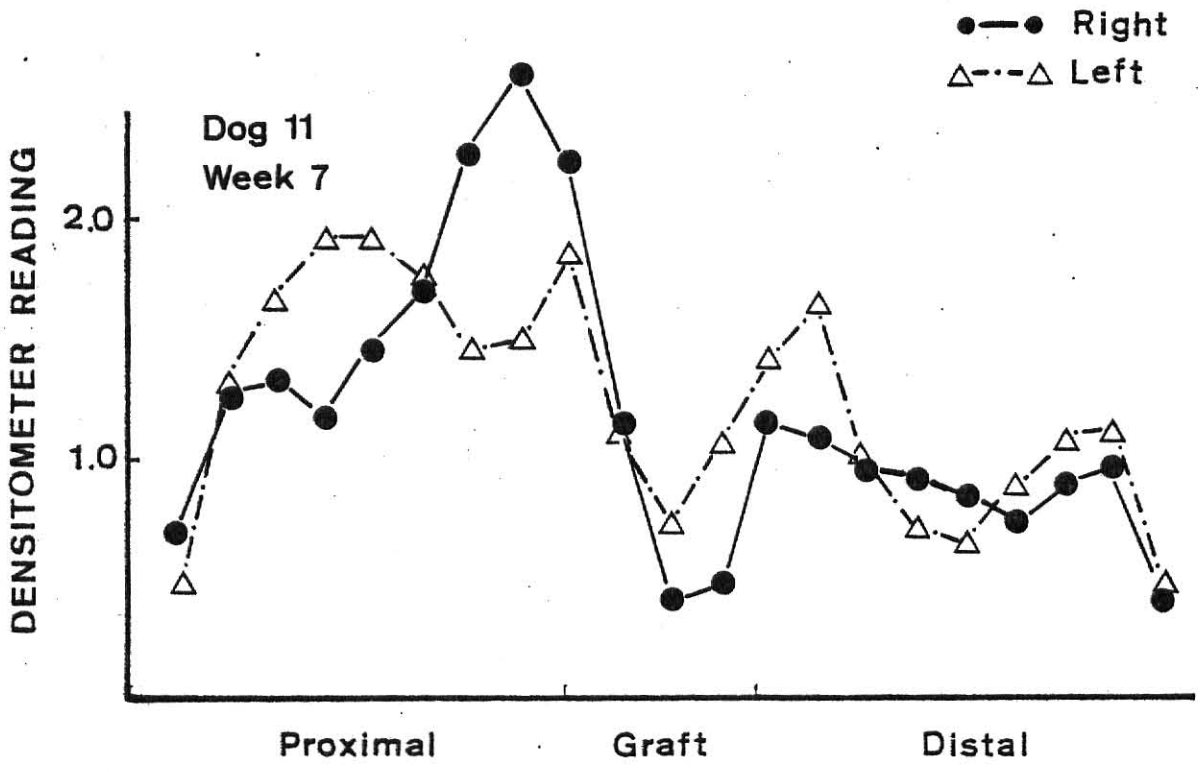
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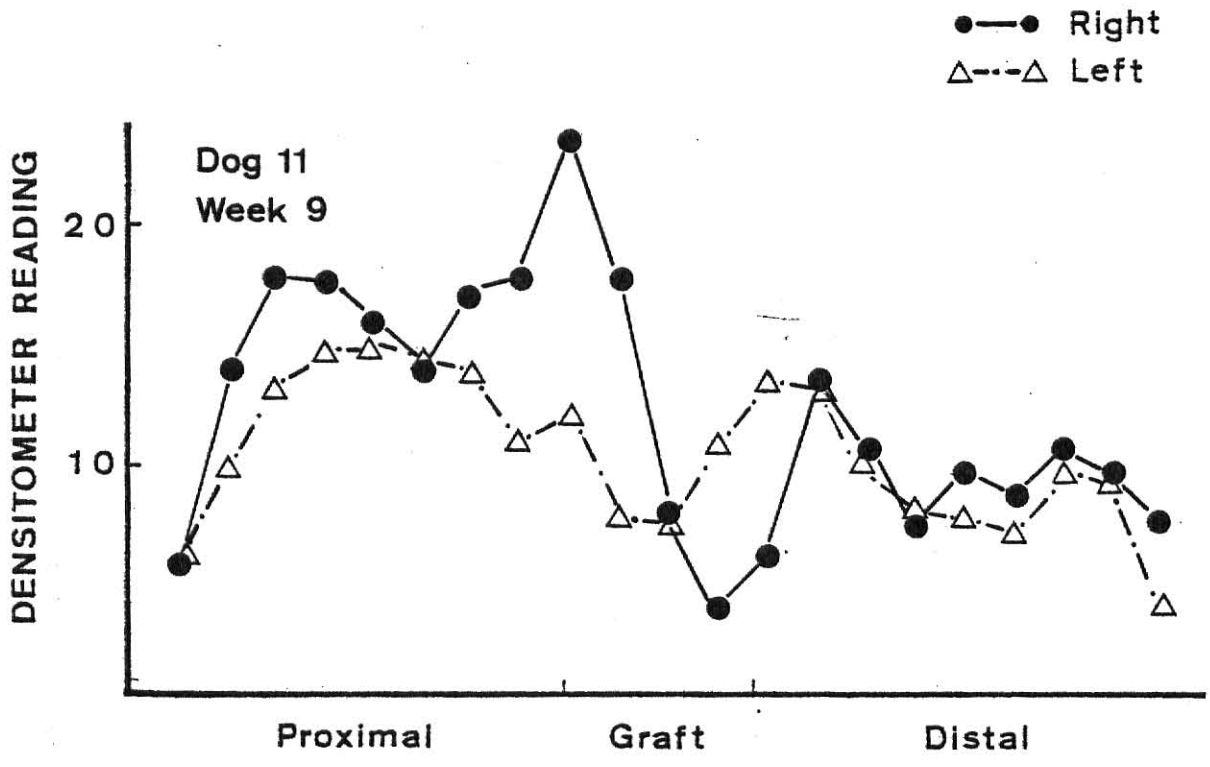
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Graft
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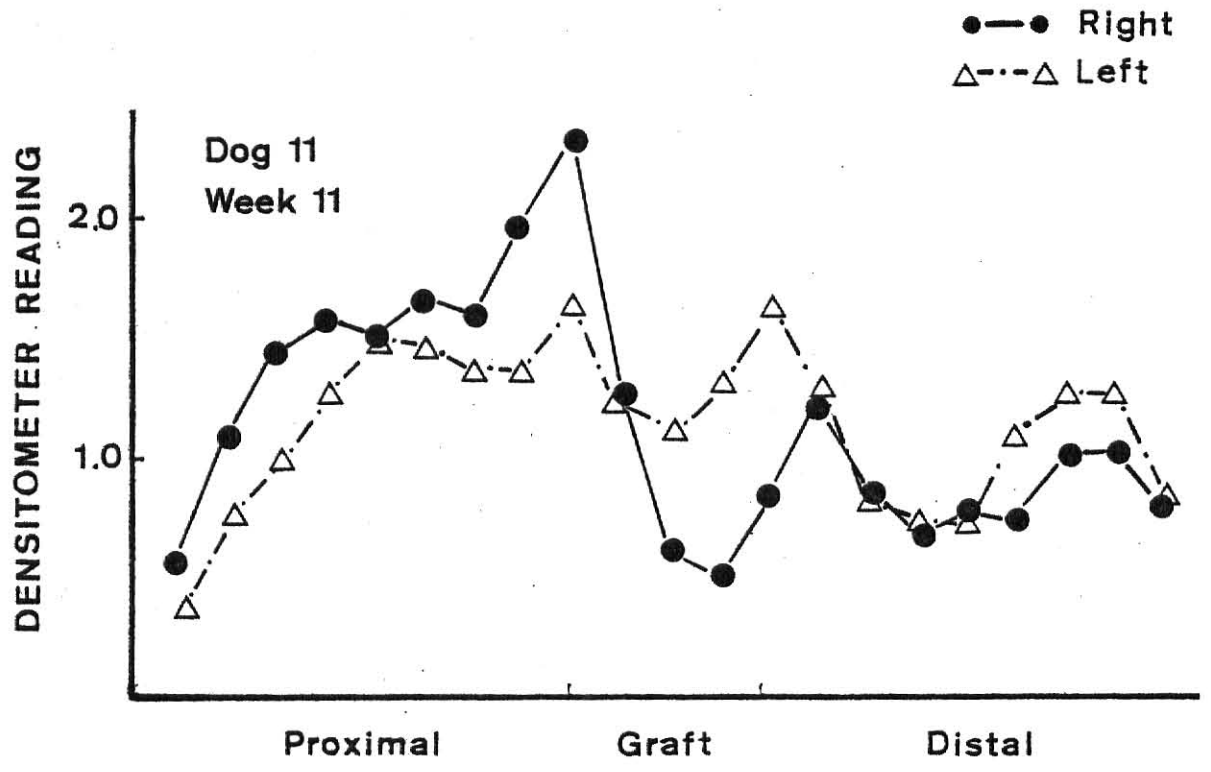
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Graft
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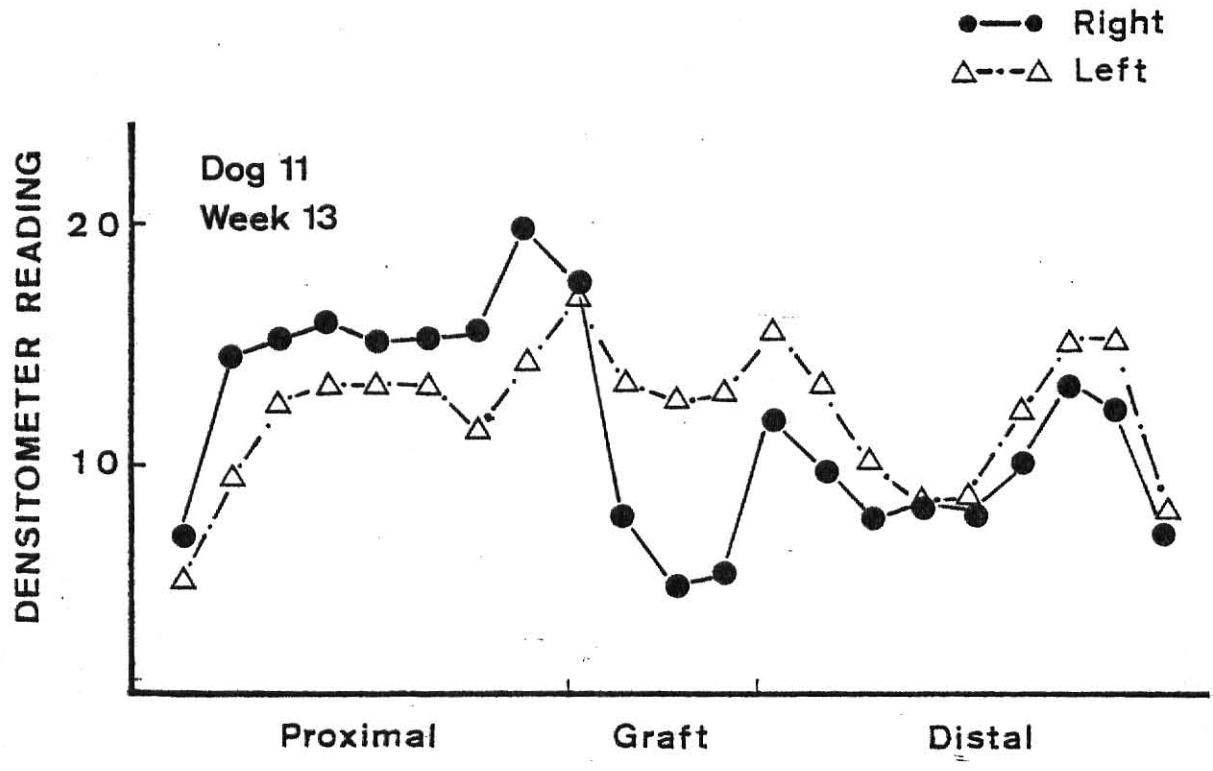
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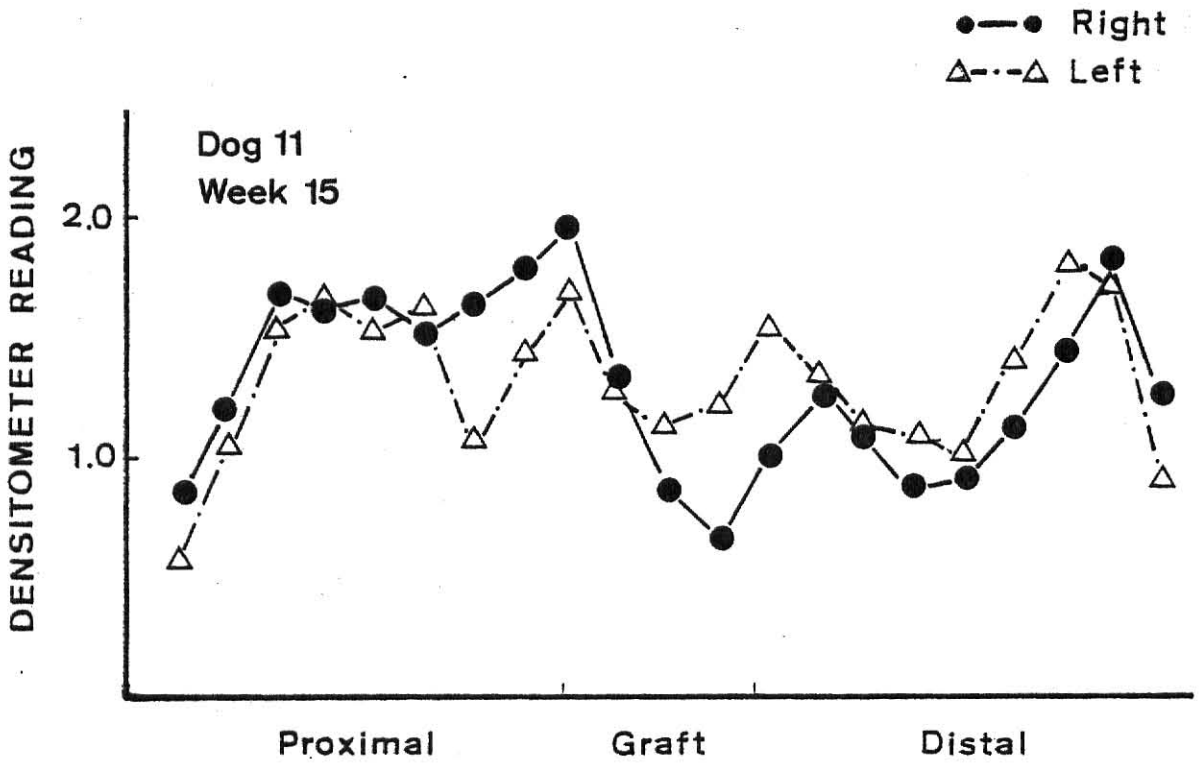
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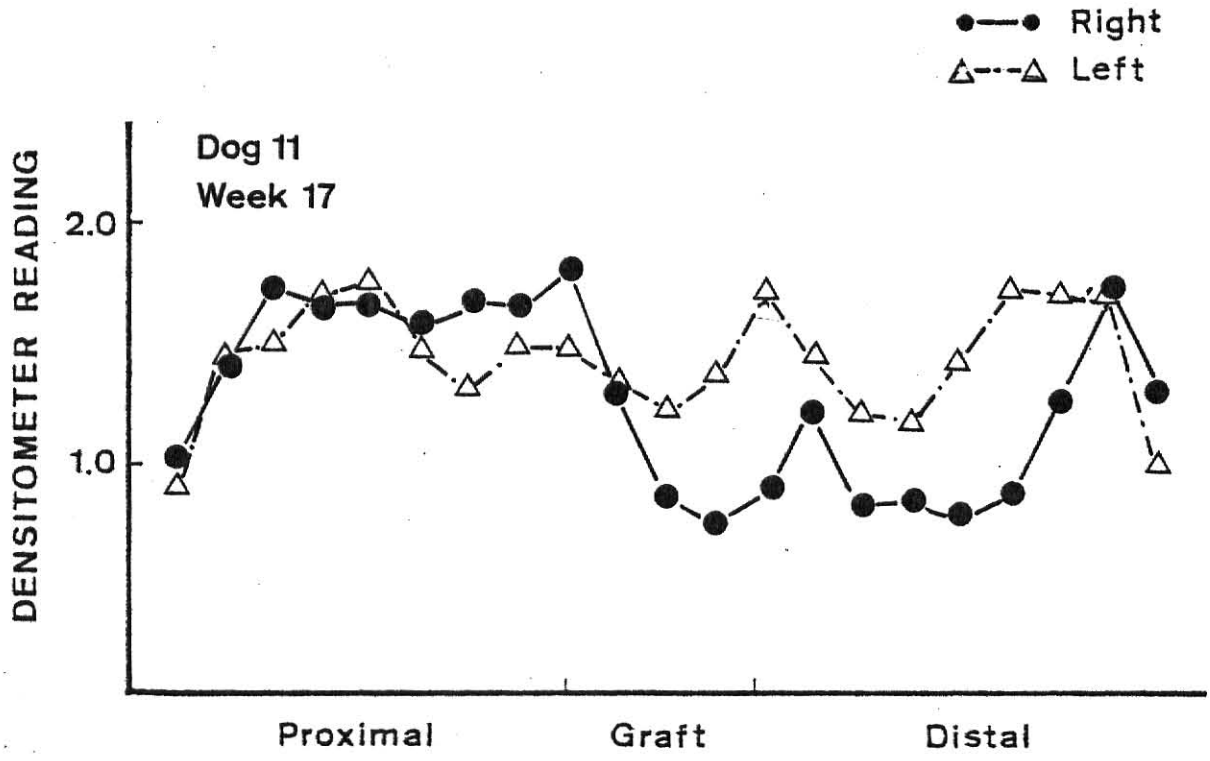
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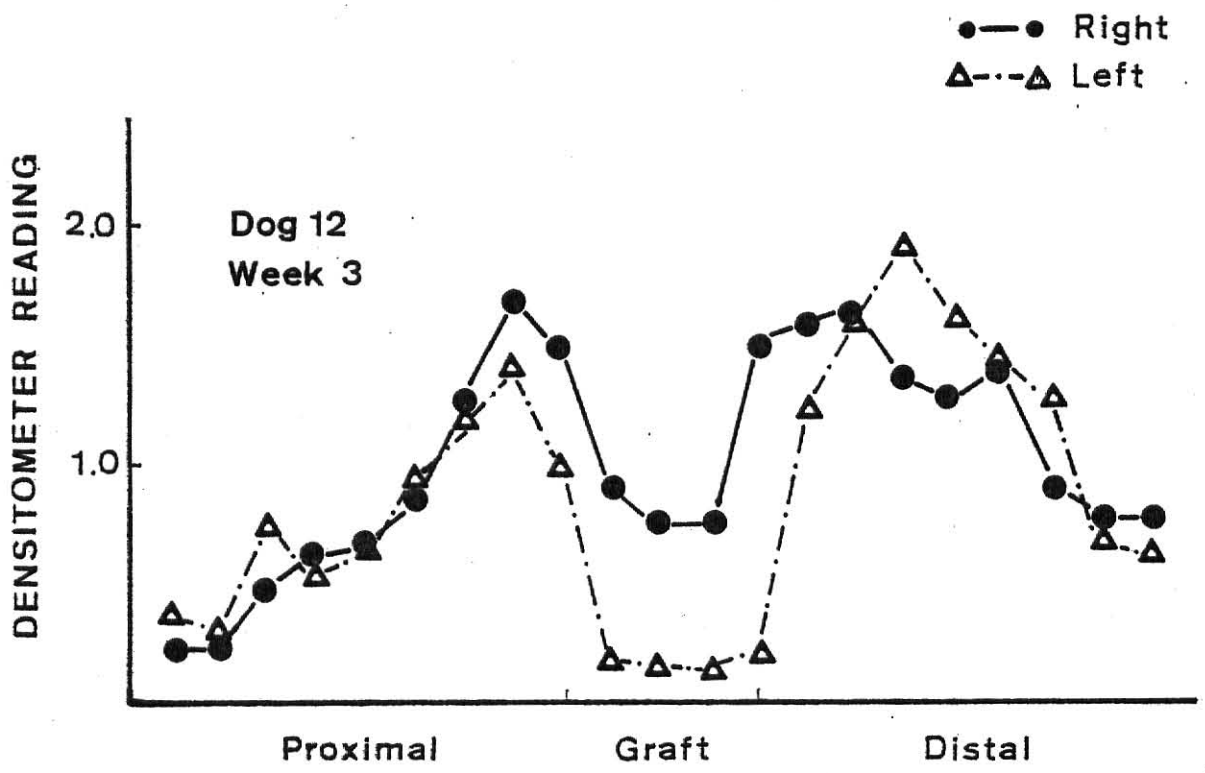
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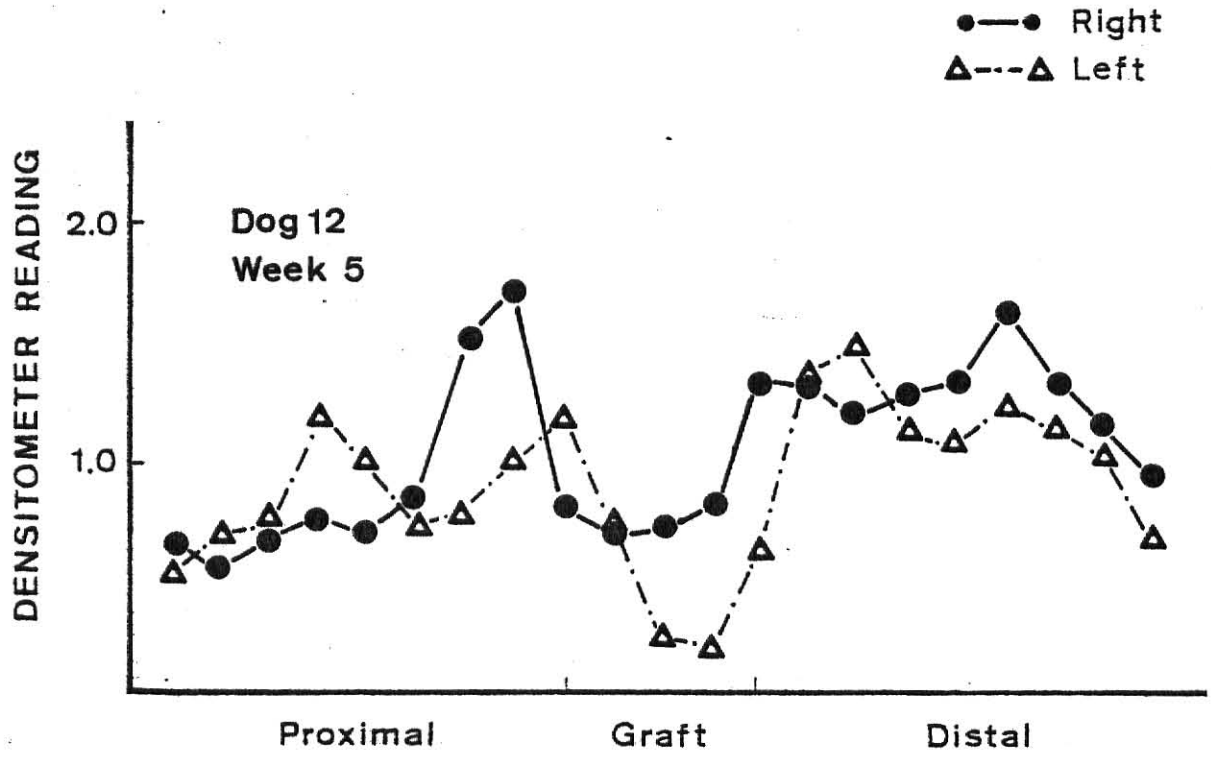
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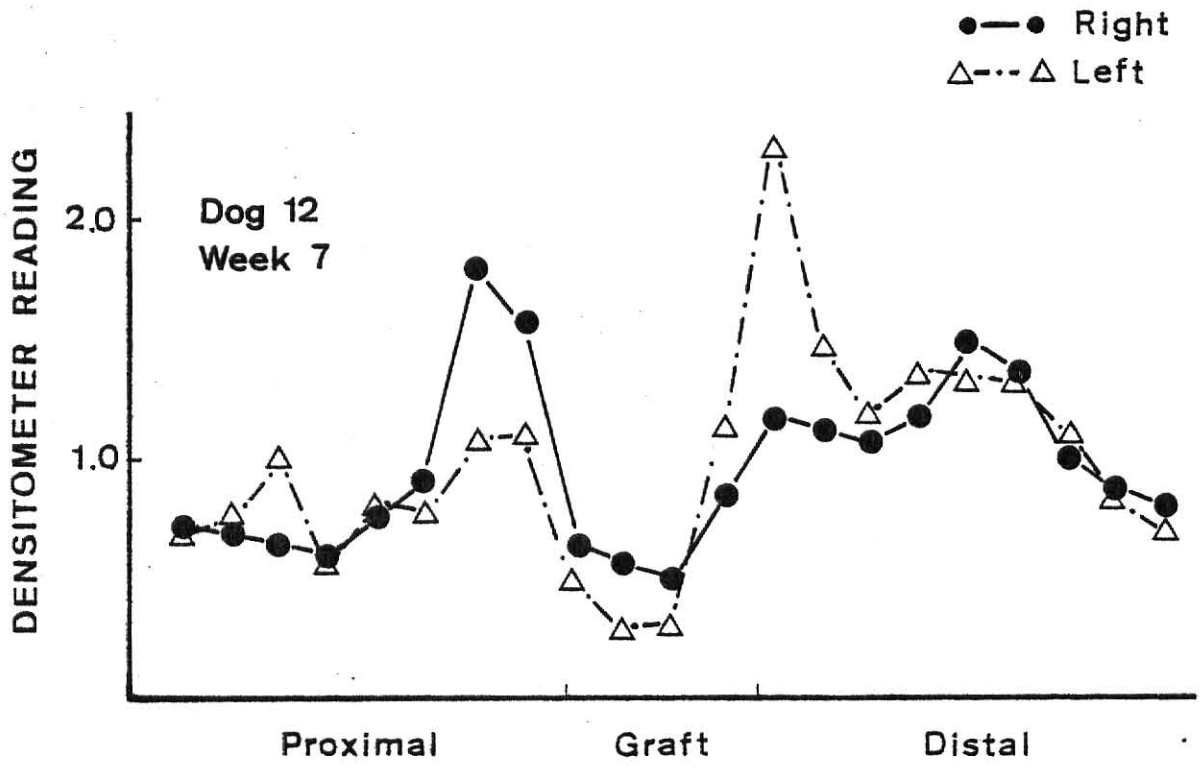
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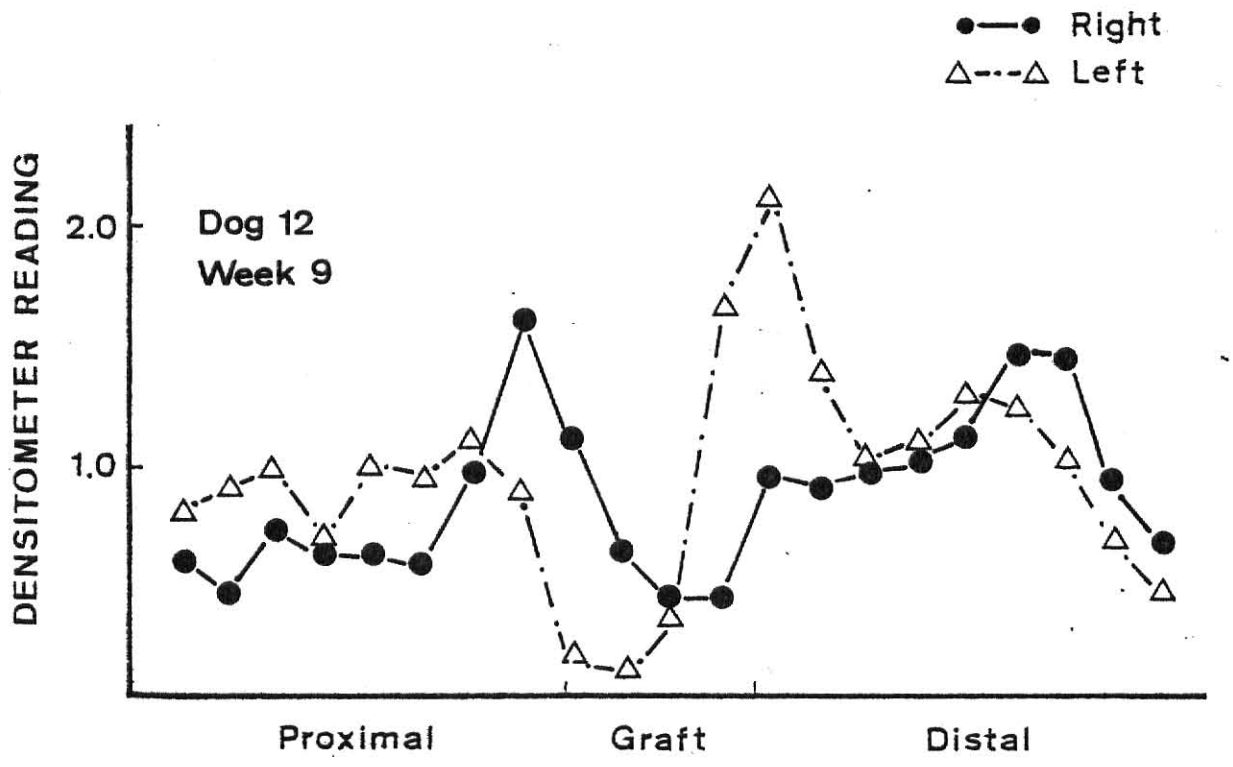
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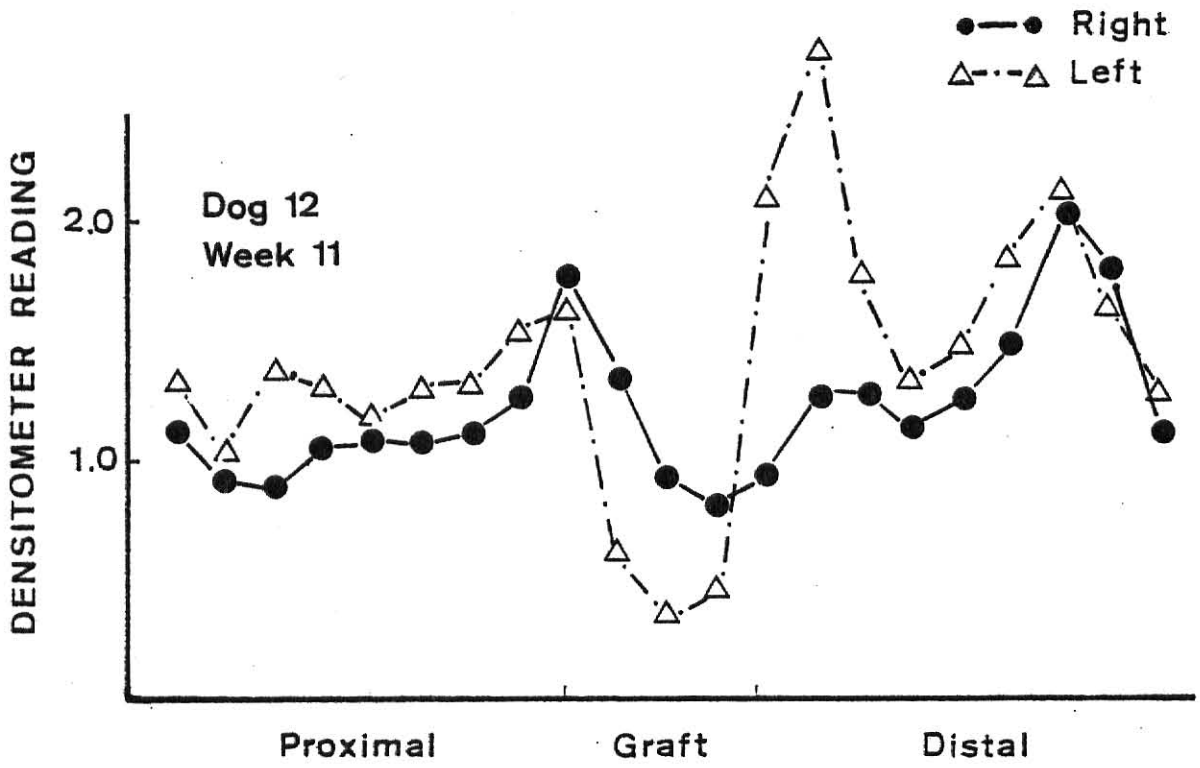
Distal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Week 11
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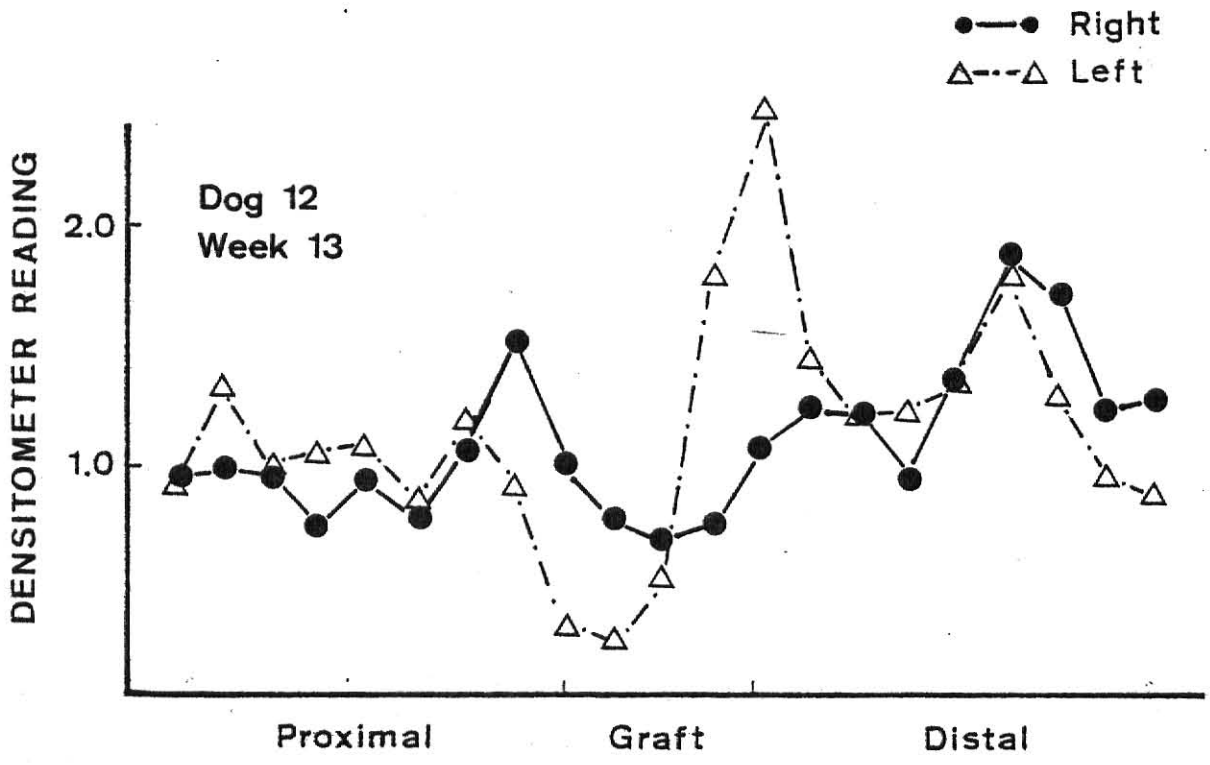
Graft
Distal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Week 13
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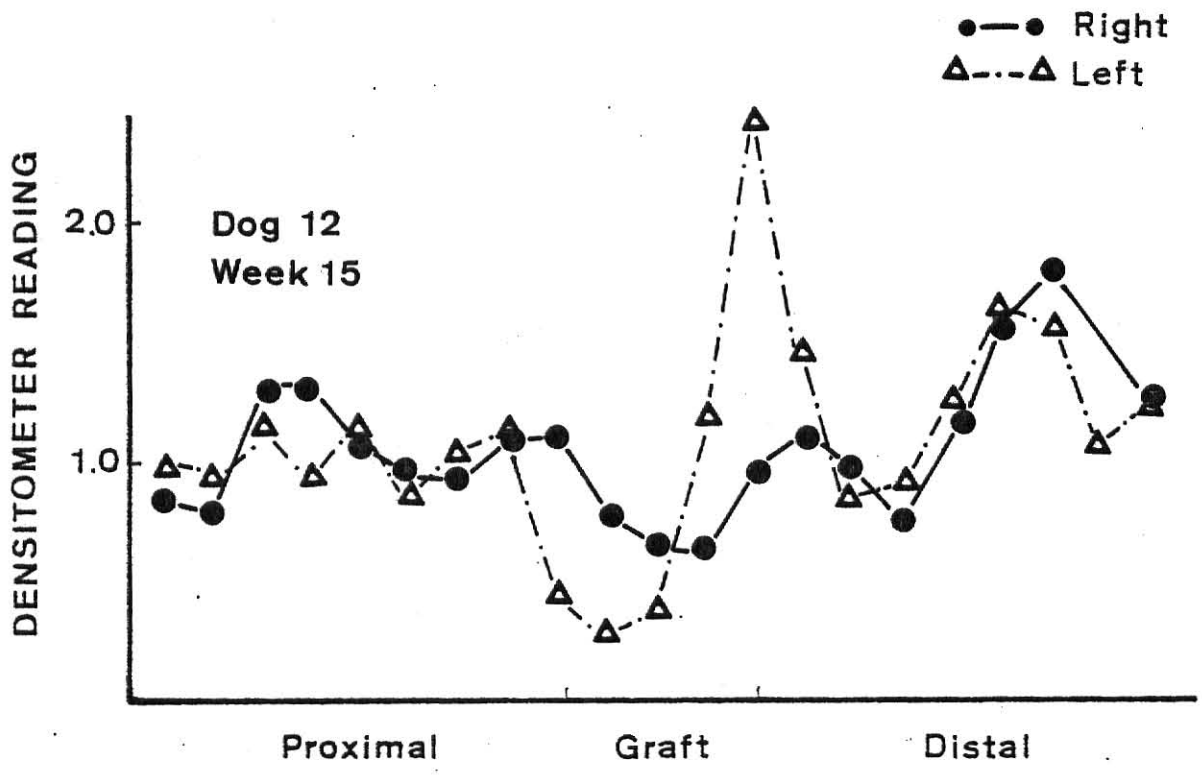
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Left
DENSITOMETER READING
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Dog 12
Week 15
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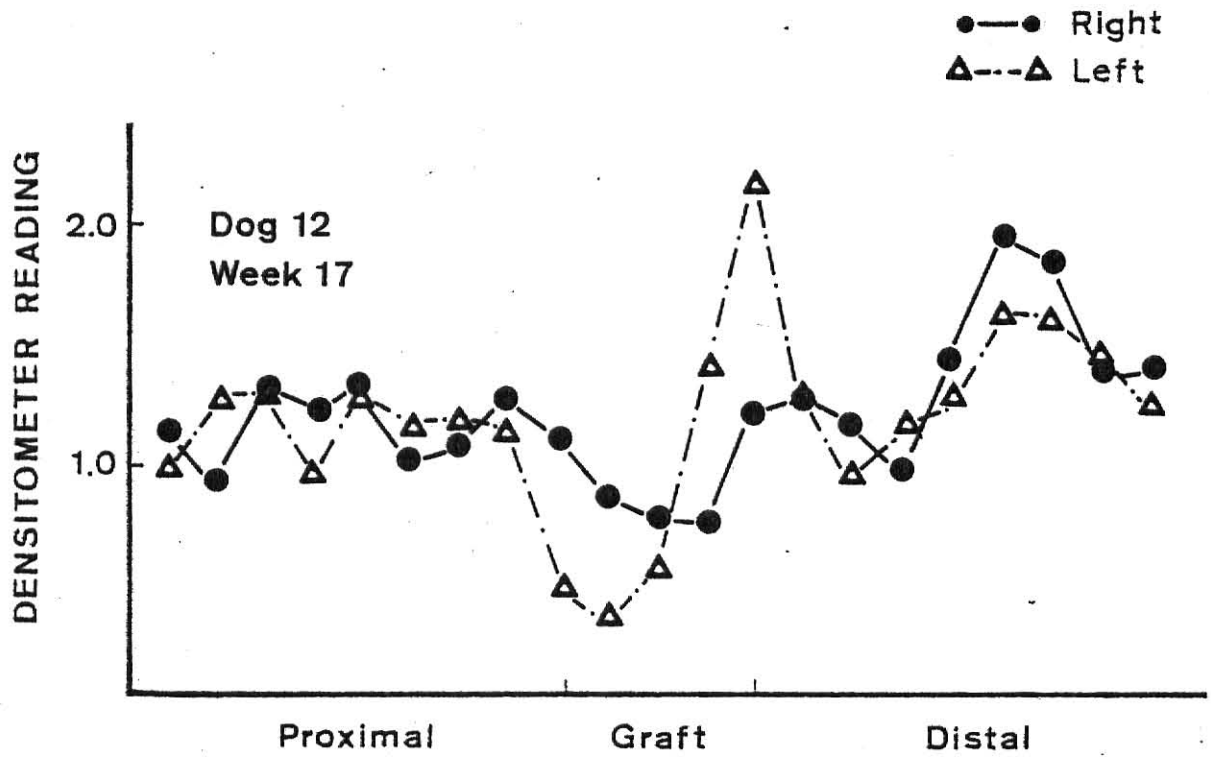
Graft
Distal


Right
Left
DENSITOMETER READING
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1.0
Dog 12
Week 17
Proximal
Graft
Distal

II. Tetracycline Labeling Results

The results of the tetracycline labeling were determined by gross examination of the longitudinally split sections of tibia with an ultraviolet light. Dog #5, #6, #8, #9, #11 and #12 were evaluated.

Dog #5 showed considerable periosteal labeling on both tibias. The host cortices and medullary canals of both legs were also heavily labeled. The cortices of the grafts in both legs were not labelled. The medullary canal of the right graft was labeled lightly throughout while the medullary canal of the left graft was only labeled at the proximal graf-host junction. There was a very thin line of labeled bone at all the graft-host interfaces in the cortex.

Dog #6 showed minimal labeling in the periosteum of both legs but good labeling of the host medullary canals in both legs. The host cortices in both legs showed only light labeling. The medullary canal of the right graft was labeled only in the proximal and distal one centimeter with the center showing no labeling. The medullary canal of the left graft was labeled in only the proximal three to four millimeters. There was a thin line of labeled bone at the proximal and distal graft-host cortical interface in the right leg and at the proximal graft-host cortical interface of the left leg. The distal graft-host cortical interface did not label.

Dog #8 showed minimal periosteal labeling in both legs but heavy cortical and medullary canal labeling in the host tibias of

both legs. The cortices of the right graft showed heavy labeling throughout the length of the graft as was the medullary canal. The cortices of the left graft showed light labeling throughout and the medullary canal showed light labeling in only the proximal and distal one centimeters of the graft. The center of the left graft was not labeled.

Dog #9 showed slight periosteal labeling in both legs with minimal cortical and medullary labeling in both host tibias. There was no evidence of labeling in the cortices of the right graft except at the screw holes and the medullary canal was labeled lightly. The cortices of the left graft were very white and showed no evidence of labeling. The proximal one centimeter of the medullary canal of the left graft showed very light labeling.

Dog #11 showed light periosteal labeling in both legs. The host cortices of both tibias showed some labeling. The medullary canal of the right tibia was labeled consistently throughout the host and the graft. There was some light labeling of the cortices at the proximal three to four millimeters of the graft. The medullary canal of the left tibia showed light labeling in the host bone which was continous with both the proximal and distal one centimeters of the graft. The center of the graft medullary canal was not labeled. The medial cortex of the graft was very white while the lateral cortex of the graft was labeled lightly.

Dog #12 showed no periosteal labeling in either leg. The cortices of both host tibias showed minimal labeling. The medullary canal of the right leg was moderately labeled consistently through

the host and graft. The cortices of the graft did not appear labeled except for a thin line at the graft-host interfaces. The medullary canal of the left tibia was labeled lightly in the host bone and extending into the graft medullary canal for about one centimeter from either end. The center part of the medullary canal of the left graft was not labeled. The cortices of the graft did not appear to be labeled except for a thin line at the distal graft-host interface.

III. Histopathology Results

Dog #1 - One week post-operative

The host bone appears as normal cortical and trabecular bone with some periosteal new bone. No evidence of external callus formation at this time but internal callus is forming. The trabecular bone in the medullary canal of the right graft does not appear viable. The cortex of the right graft does not appear viable either. There were no blood vessels of osteocytes in the lacunae. The cortex of the left graft was the same. The medullary canal of the left graft was full of red blood cells and no evidence of bony cells. There was an infiltration of round cells - lymphocytes, macrophages and plasma cells - and fibrous connective tissue on the periosteal surfaces of the grafts.

Dog #2 - Three week post-operative

There was considerable new bone periosteally on the right tibia but this was not bridging the graft-host junction. A good internal callus was being formed at both graft-host junctions. There was some new woven bone in the gap between the graft and host proximally. The trabecular bone in the medullary canal of the graft was not showing any bone cell activity. (Fig. 15) The cortex of the graft did not appear to have any blood vessels or osteocytes in the lacunae.

The proximal left graft-host junction did not show any callus formation but the distal graft-host junction did have begining of an external and internal callus. The graft was surrounded by a layer of fibrous tissue. The graft cortex did not appear viable.

The medullary canal of the graft was filled with connective tissue and debris. (Fig. 16)

Dog #5 - Five week post-operative

The right graft-host junctions were bridged by both an internal and external callus. There was a lot of periosteal newbone present which contained some cartilage cells. The cortex of the graft was not showing any activity. Most of the trabecular bone in the medullary canal of the right graft appeared inactive but some of the chips showed osteoblastic and osteoclastic activity. There was no evidence of any endosteal new bone present. Bone marrow had been re-established in the medullary canal of the graft, as megakeryocytes and other heamopoietic cells were seen.

The graft-host junctions in the left leg were not bridged by callus. There was some new woven bone in the gaps at the graft-host junctions but the gap was not bridged. (Fig 17) There was a layer of fibrous connective tissue around the graft cortex which was infiltrated with round cells. The cortices of the graft was filled with connective tissue and red blood cells.

Dog #6 - Five week post-operative

The right host tibia had some periosteal new bone. The graft-host junctions were both bridged internally with callus. An external callus with some cartilage cells was attempting to bridge the proximal graft-host junction. The gap at the distal graft-host junction was completely filled with new bone on one side. (Fig 18) The cortex of the graft appeared inactive and was surrounded by fibrous connective tissue. The medullary canal

of the graft had a lot of dead trabecular bone but there was considerable bone cell activity throughout the graft medullary canal. There was some heamopoietic tissue present also.

The host tibia of the left leg appeared as normal cortical bone. The proximal graft-host junction was almost bridged by an external callus. The distal graft-host junction was bridged by an internal callus. There was some periosteal new bone along the graft but it was mainly surrounded by connective tissue. Some trabecular bone did extend into the distal end of the graft medullary canal but it was mainly filled with connective tissue. The cortices of the graft were inactive.

Dog #8 - Nine weeks post-operative

The graft in the right tibia was being incorporated into the host skeleton. Both graft-host junctions had a bony union which was begining to remodel. The graft was surrounded by layers of connective tissue, round cells and necrotic soft tissue (plate side only). The cortex of the graft did contain new blood vessels. The bony trabeculae in the medullary canal of the graft was active. There could also be seen blood vessels and heamopoietic cells in the medullary canal. (Fig. 19)

The left graft was also being incorporated into the host tibia. The proximal graft-host junction had a bony union which was begining to remodel and the distal graft-host junction was bridged by an internal and external callus. The graft was surrounded by fibrous connective tissue. There were some blood vessels seen at the very ends of the graft cortices but not throughout the length of the graft.

New trabecular bone and red marrow were invading each end of the graft but the center two centimeters of the graft medullary canal was still filled with connective tissue.

Dog #9 - Thirteen weeks post-operative

The right graft was becoming well incorporated into the host. Both graft-host junctions had bony union and were remodeling. Very little internal and external callus was present. The graft was surrounded by connective tissue and some round cells. The cortices of the graft were surrounded by connective tissue and some round cells. The cortices of the graft contained many new blood vessels and new osteoblasts could be seen along with the blood vessels. The medullary canal of the graft contained active trabecular bone and heamopoietic cells.

The left graft was not as well incorporated as the right graft. There was some necrotic bone in the gap between graft and host posximally but there was a bridging of internal callus. The graft was surrounded by connective tissue. The cortices of the graft did contain an occasional new blood vessel. The medullary canal of the graft was filled with connective tissue and some necrotic bone chips.

Dog #10 - Thirteen weeks post-operative

The right graft was being incorporated rather well into the host tibia. The graft-host junction has good bony union which was begining to remodel with new Haversian systems. Callus formation is almost completely gone. The graft was surrounded by connective tissue which contained a few round cells. The cortices of the

graft contained many new blood vessels and new osteoglastic activity. (Fig. 20) The medullary canal of the graft contained active bone trabeculae, some connective tissue and some small chips of necrotic bone. There was little bone marrow activity.

The left graft had bony union with the host tibia at both ends. There was little internal or external callus. The graft was surrounded by a layer of connective tissue with numberous round cells. There was some new bone outside the layer of connective tissue but this could very well be the fibula. There were a few blood vessels visible in the graft cortex. (Fig. 21) The medullary canal of the graft was filled with connective tissue.

Dog #11 - Seventeen week post-operative

The right graft was showing extensive remodeling throughout its length. The proximal graft-host junction had some cartilaginous cells present but bony union was present through the internal and external callus. The distal graft-host junction had a bony union undergoing remodeling. The graft cortices showed numerous blood vessels and osteoblastic activity. There was remodeling on both the endosteal and periosteal new bone, connective tissue and some necrotic bone on the medial side of the cortex near the plate. The medullary canal of the graft contained active bone trabecular and fatty marrow.

The left tibia had considerable periosteal new bone over the host tibia and the graft. The graft-host junctions were remodeling very actively. There was some necrotic bone periosteally which was surrounded by polymorphonuclear cells. The cortex of the graft

was very active with new blood vessels. The medullary canal contained a lot of connective tissue but some bone trabeculae were present.

Dog #12 - Seventeen weeks post-operative

The graft in the right tibia was united with the host tibia and both graft-host junctions were remodeling. (Fig. 22) The graft was surrounded by a layer of fibrous connective tissue with a few round cells. There was little external or internal callus present. The graft cortices contained new blood vessels and bone cells. The medullary canal had bony trabeculae which did not appear to be very active; the osteoblasts were present but very little new osteoid seen. There was some heamopoietic tissue present.

The left graft was being incorporated inot the host tibia with remodeling occuring at both graft-host junctions. The graft was surrounded by fibrous connective tissue. The cortex of the graft contained few blood vessels. The bone trabeculae in the marrow cavity of the host was continous into the graft for about one centimeter in each end. The center of the graft medullary canal was filled with connective tissue. (Fig. 23)

Fig. 1 - Dog #5 One week post-operative

Left Right

Fig. 2 - Dog #8 Nine weeks post-operative

Left Right

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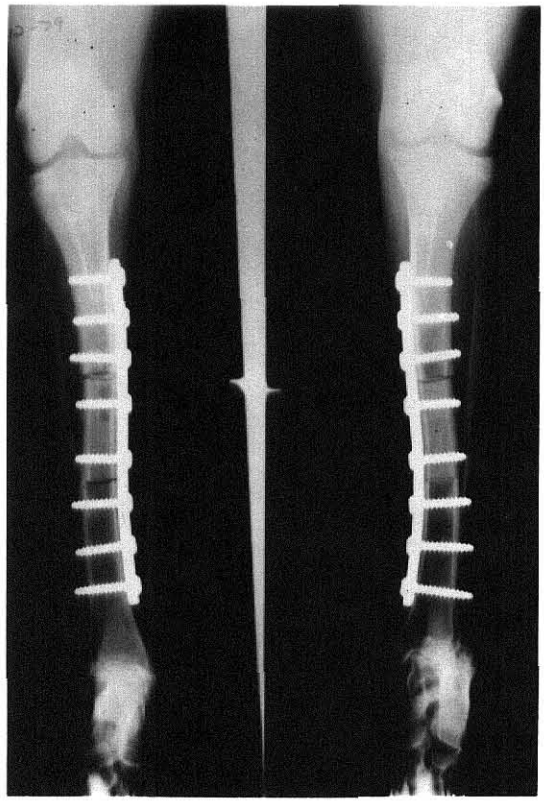

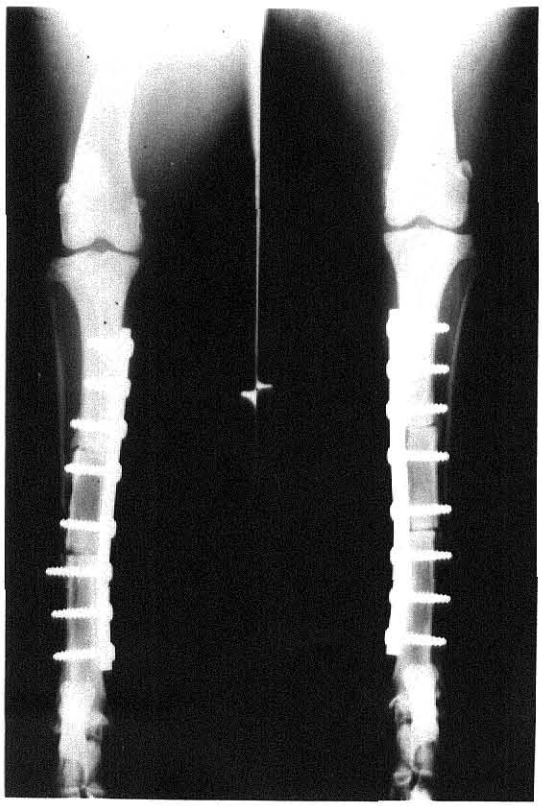

Fig. 3 - Dog #12 Seven weeks post-operative

Left AP Left Lateral

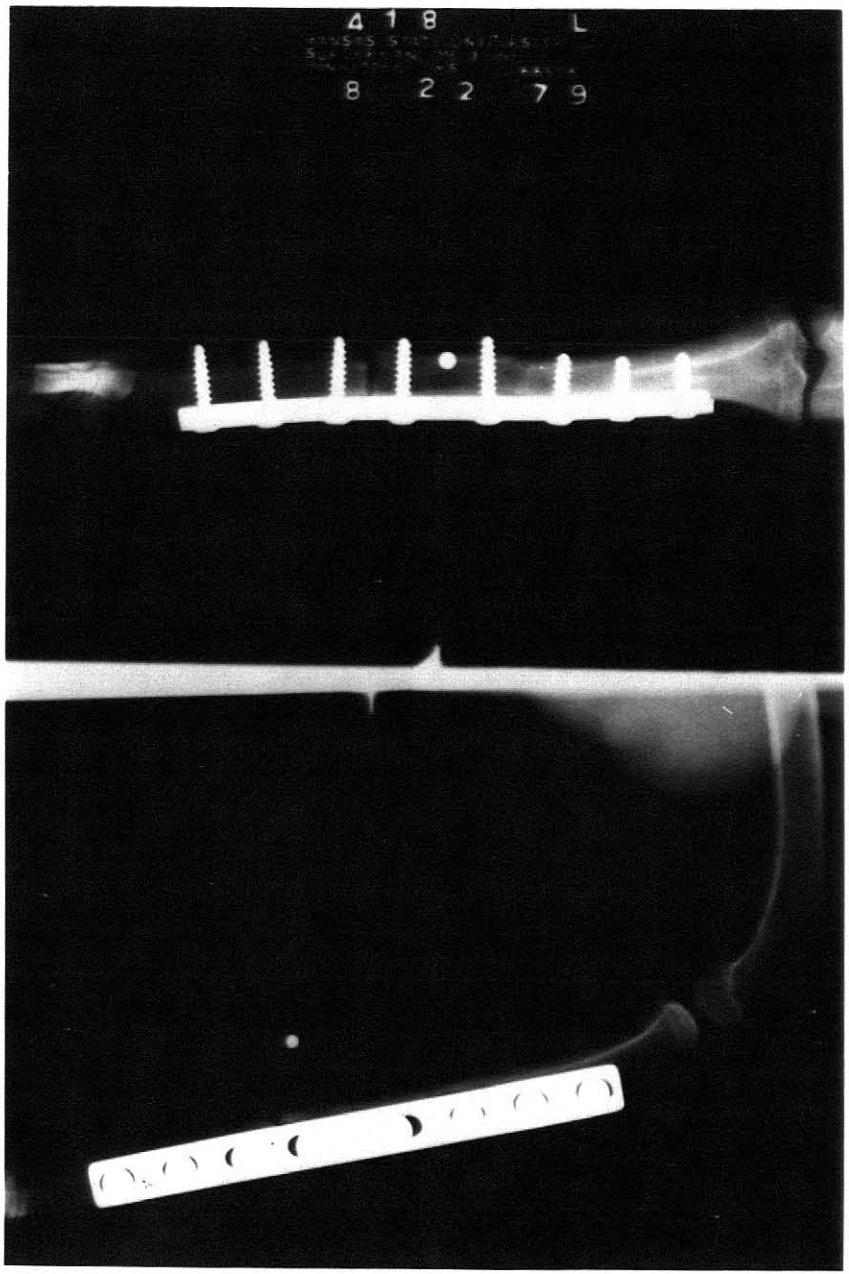
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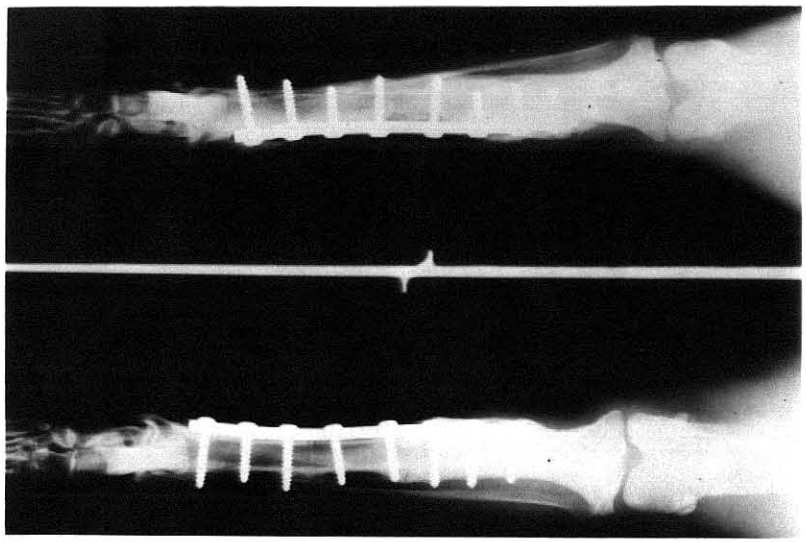
Fig. 4 - Dog #11 Seventeen weeks post-operative

Left Right

Fig. 5 - Dog #11 Seventeen weeks post-operative

Left Right



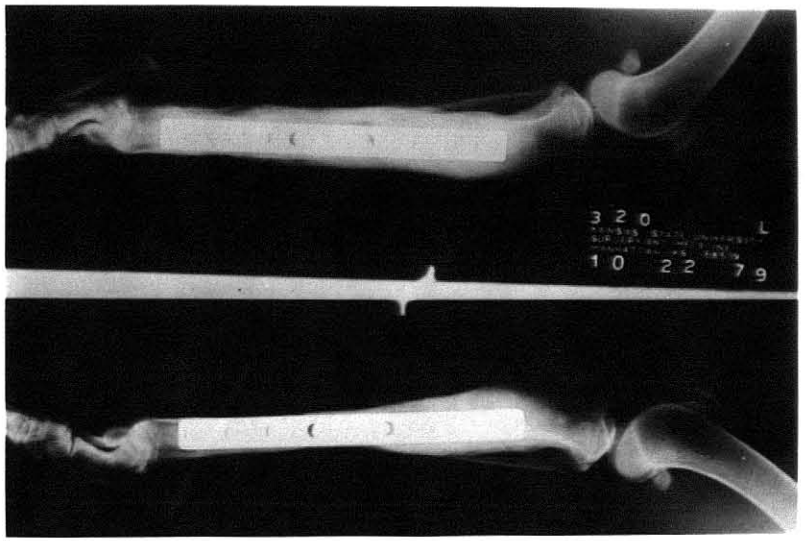

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Fig. 6 - Dog #9 Tc 99 Bone Scan
One week post-operative Right

Fig. 7 - Dog #9 Tc 99 Bone Scan
One week post-operative Left

ILLEGIBLE DOCUMENT

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Fig. 8 - Dog #9 Tc 99 Bone Scan
Thirteen weeks post-operative Right

Fig. 9 - Dog #10 Tc 99 Bone Scan
Thirteen weeks post-operative Left

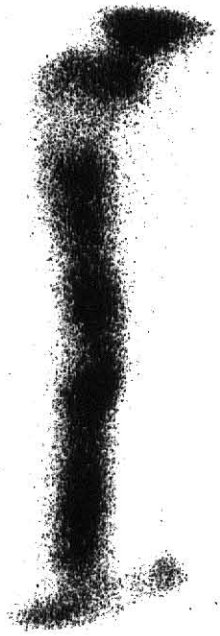
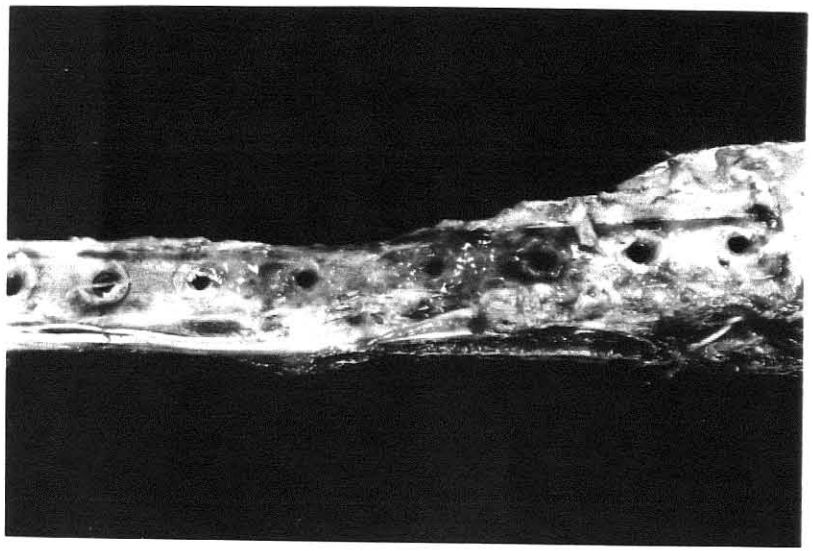


Fig. 10 - Dog #11 Right Tibia

Fig. 11 - Dog #8 Right Tibia



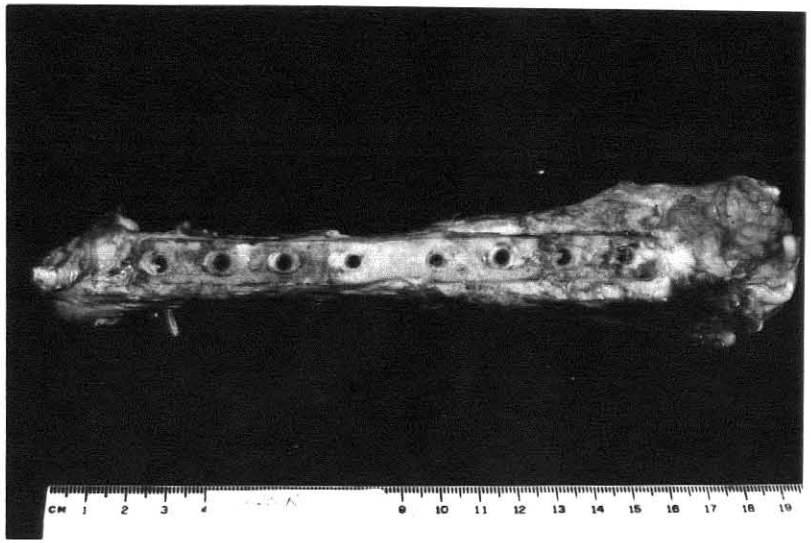

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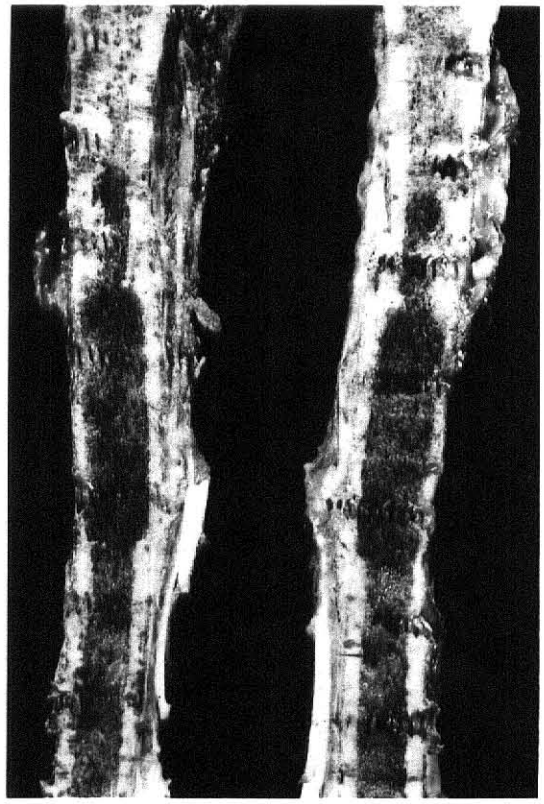
Fig. 12 - Dog #8 Right Tibia

Fig. 13 - Dog #8 Left Tibia



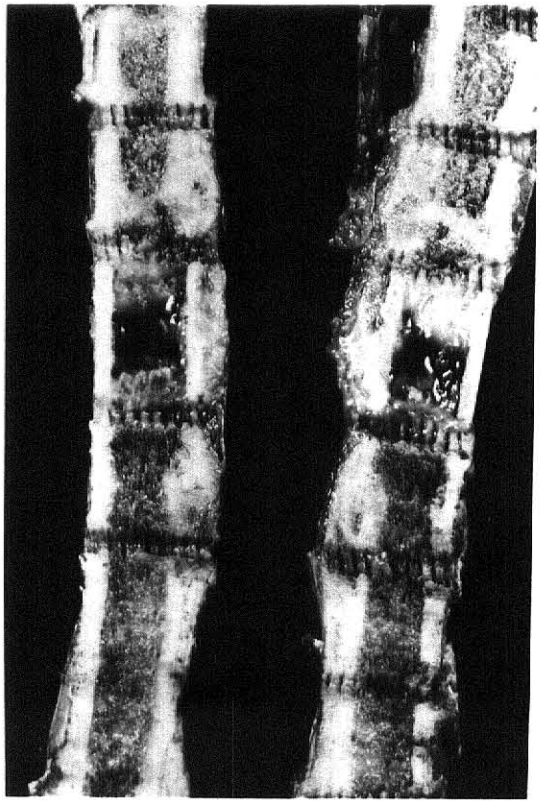
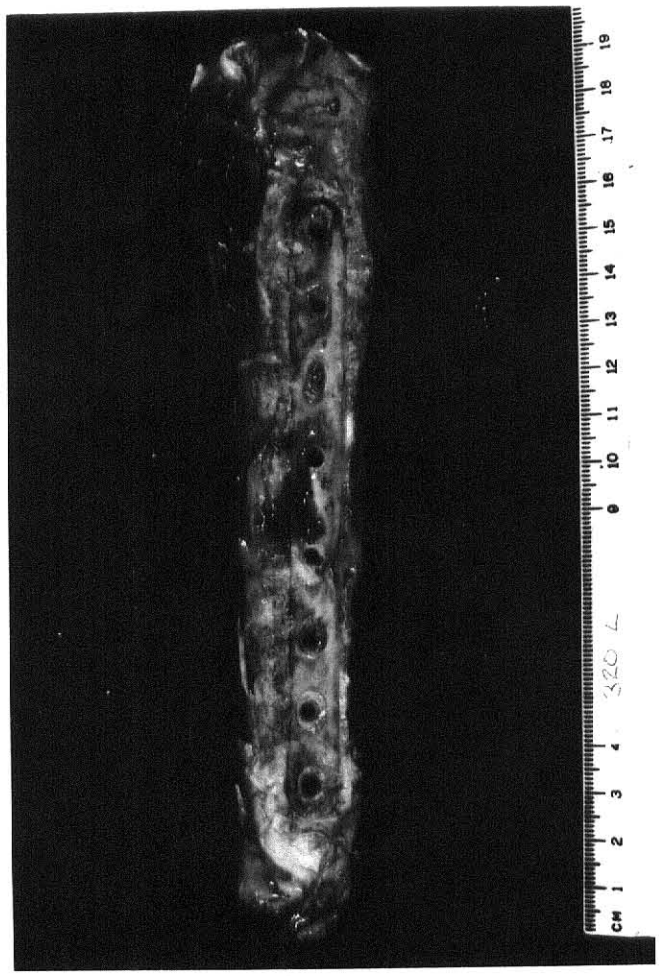


Fig. 14 - Dog #11 Left Tibia

Fig. 15 - Dog #2 Right Graft -- Medullary Canal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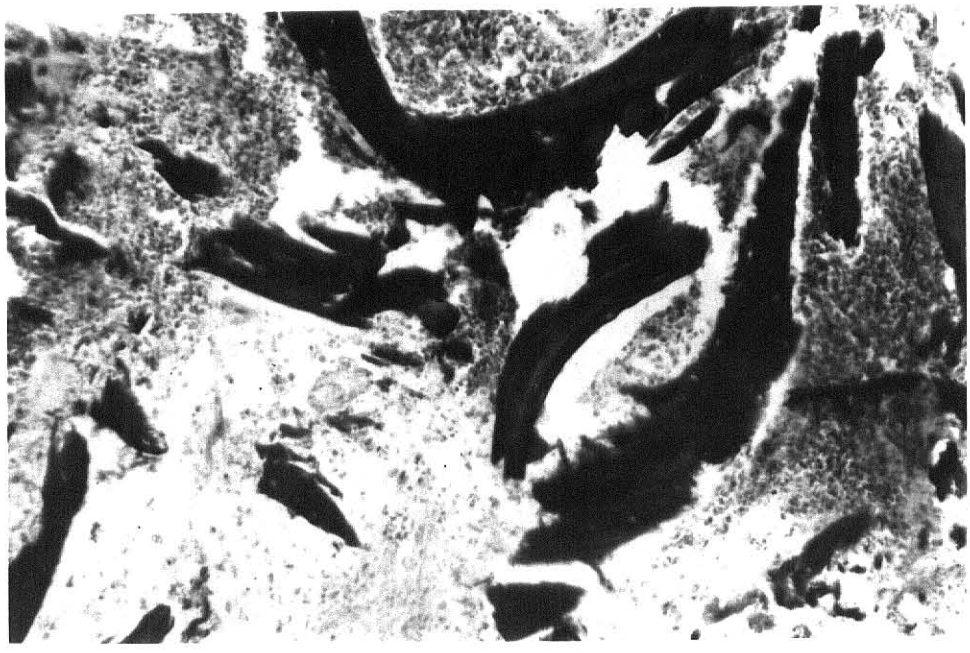


Fig. 16 - Dog #2 Left Graft -- Medullary Canal

Fig. 17 - Dog #5 Left Graft -- Distal Graft-Host Junction

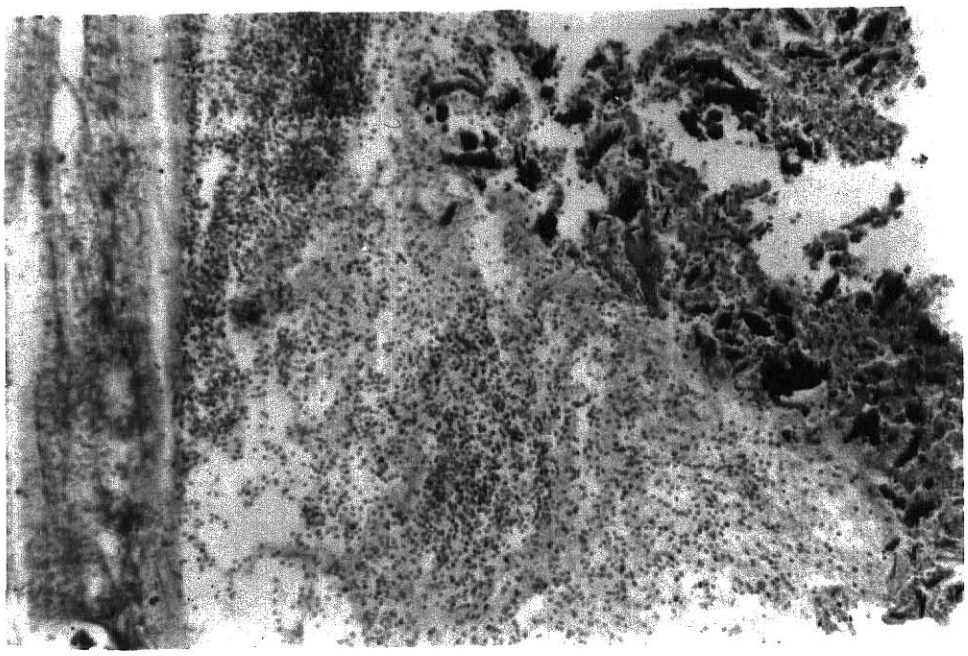
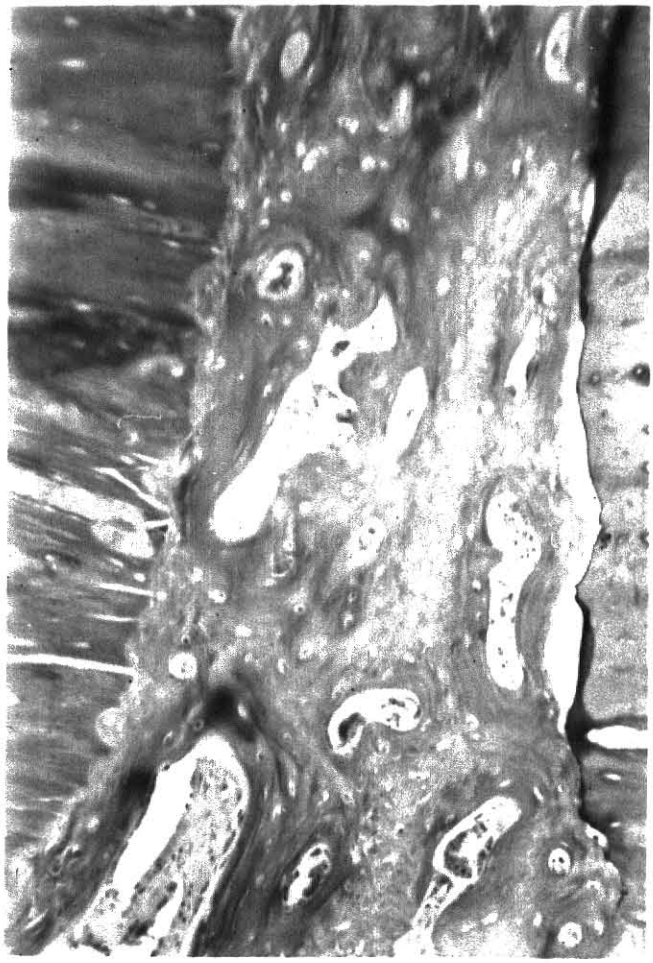


Fig 18 - Dog #6 Right Graft -- Distal Graft-Host Junction

Fig. 19 - Dog #8 Right Graft -- Medullary Canal



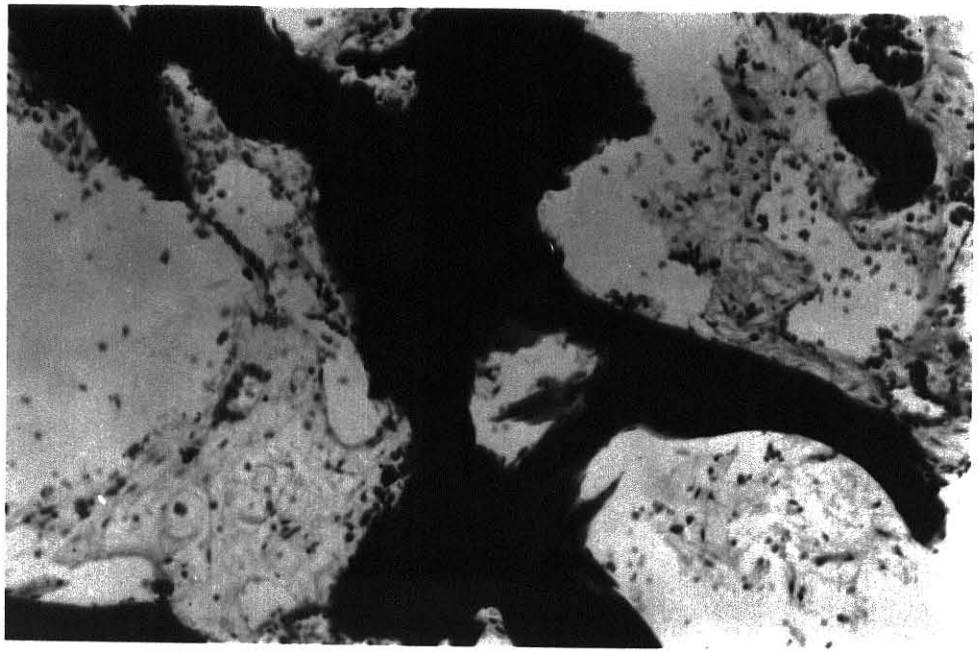


Fig. 20 - Dog #10 Right Graft -- Cortex

Fig. 21 - Dog #10 Left Graft -- Cortex

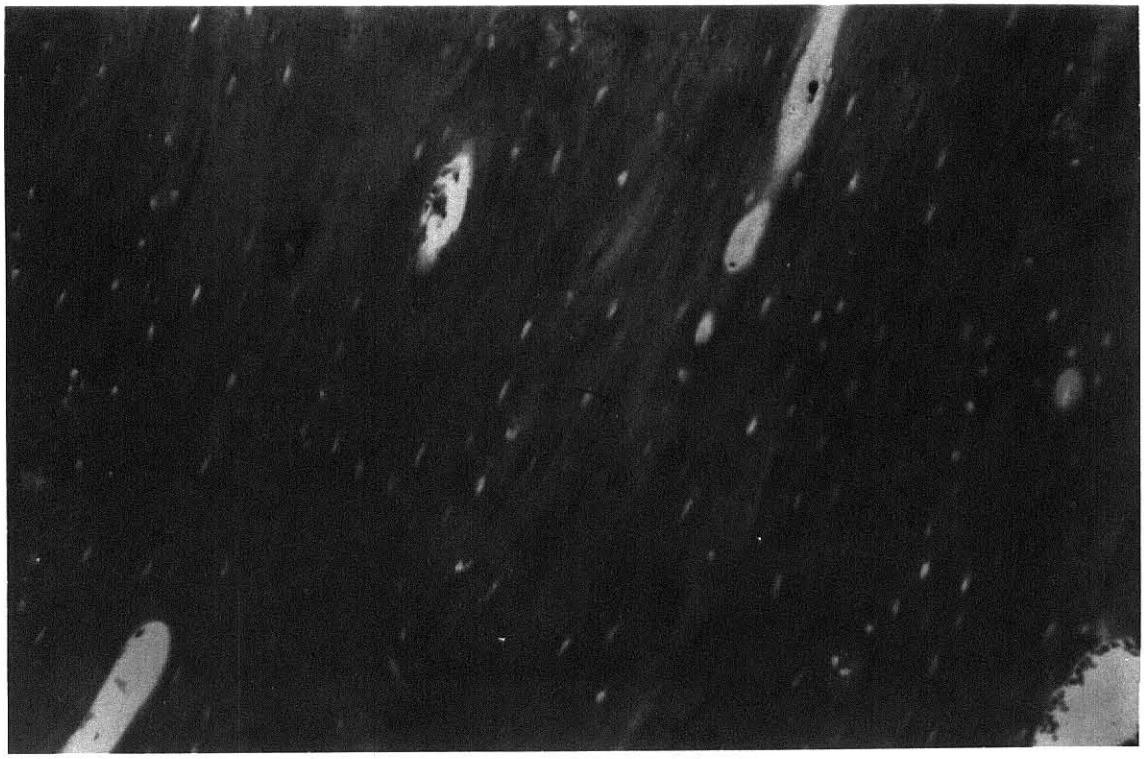
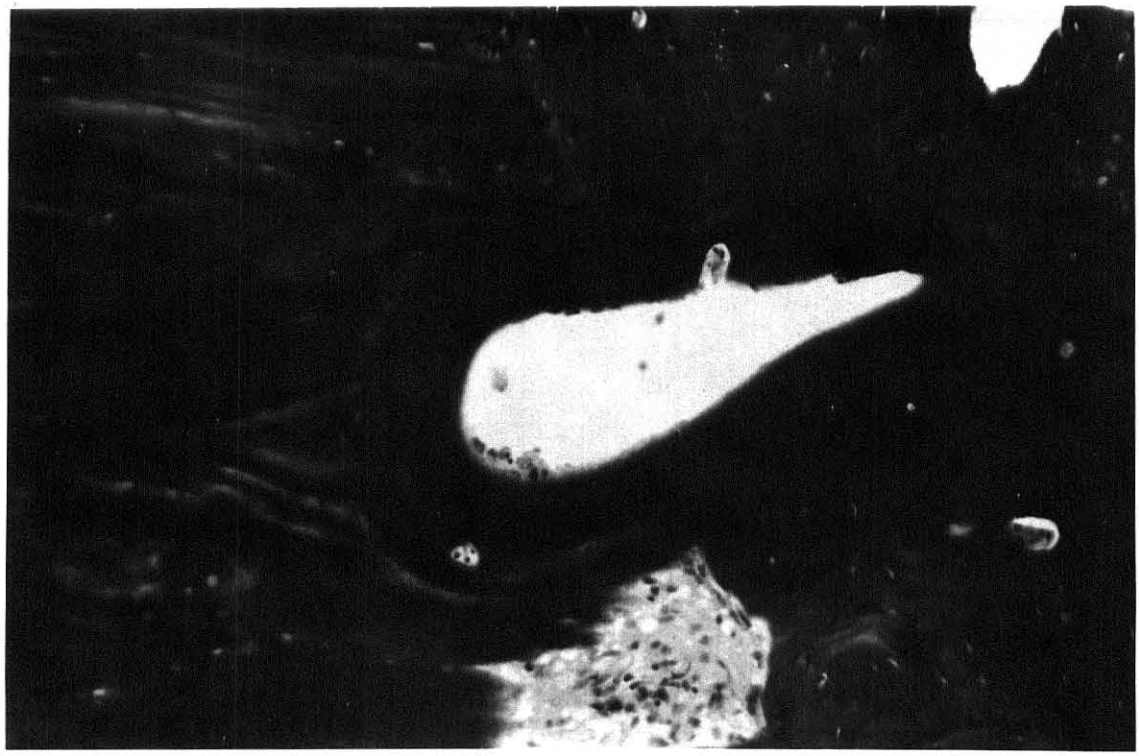
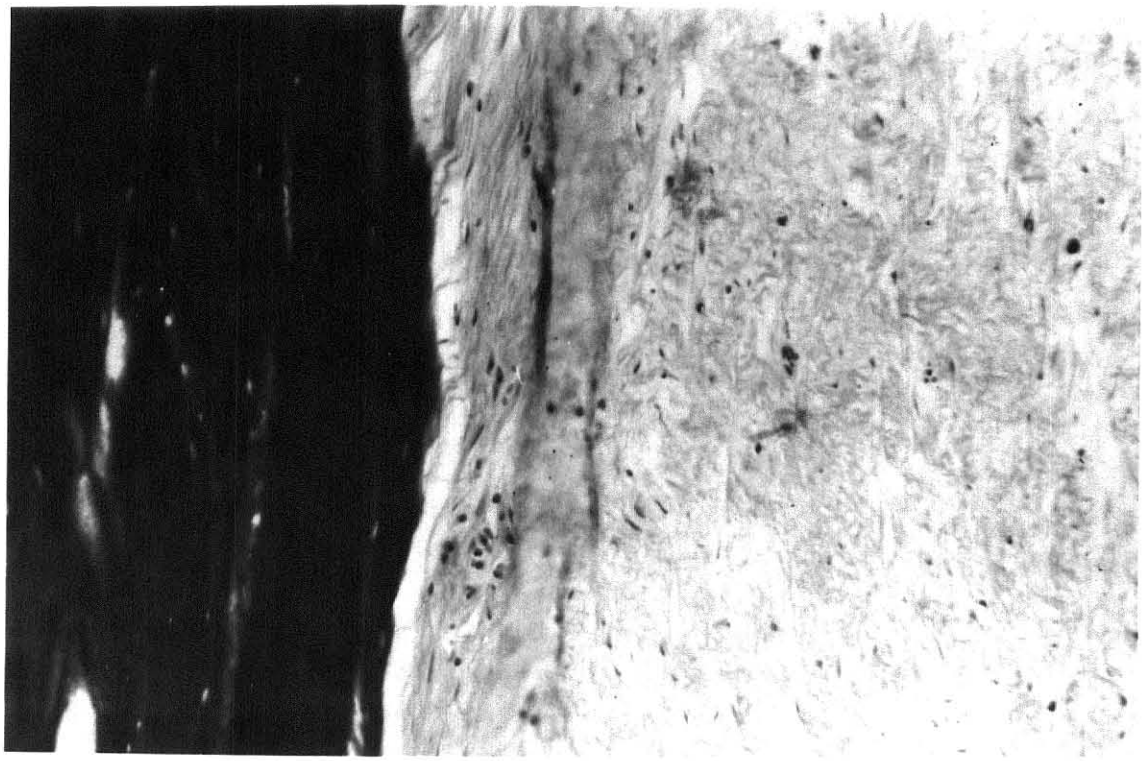


Fig. 22 - Dog #12 Right Tibia -- Distal Graft-Host Junction

Fig. 23 - Dog #12 Left Graft -- Medullary Canal and Cortex





Combination of a Cortical Allograft with a Cancellous Autograft in the Canine Tibia

by

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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
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1980

Bone grafts have been used and studied for many years in human medicine and have recently gained acceptance in Veterinary Medicine. Autogenous cancellous bone is the preferred material for grafting but occasionally the need arises, where additional support is required. In these cases, cortical grafts from animals of the same species can be used. Some of the problems associated with the use of these allografts are: 1) graft rejection, 2) slow revascularization, and 3) slow incorporation into the host skeleton.

In this study, four centimeter, fresh, cortical allografts were placed in four centimeter deficits in the mid shaft of both tibias in twelve mature canines weighing approximately twenty to twenty-five kilograms. The graft in the left tibia was reamed of its medullary content before being placed in the leg. The graft in the right tibia was reamed of its marrow content and then gently packed with autogenous cancellous bone from the greater tubercle of the humerus of the host dog. The grafts were secured in place with a Dynamic Compression Plate* and screws. The dogs were studied over periods ranging from three weeks to seventeen weeks. Progress of the grafts was monitored with bi-weekly radiographic examination and radionuclide bone scanning with Technetium 99 - Medronate Sodium complex+ Final studies on the grafts were done with tetracycline labeling, gross pathology and histopathology.

* Synthes
+ Osteolite - New England Nuclear
+ Technescan MDP Kit - Mallincroft Nuclear

The results of the radiographic exams and radionuclide bone scans did not show conclusive evidence that the graft in the right tibia containing the autogenous cancellous bone was being incorporated into the host skeleton any faster than the graft in the left tibia which did not contain any autogenous cancellous bone. The gross pathology showed the medullary canals of the grafts in the right legs to be filled with a red cancellous bone whereas the grafts in the left legs were filled with a fatty, necrotic tissue. The tetracycline labelling and the histopathological studies did show that the graft in the right tibias had more osteogenic activity and new vasculature than the grafts in the left tibias.

In conclusion, autogenous cancellous bone packed into the medullary canal of a four centimeter, fresh cortical allograft in the mid shaft tibia is beneficial to the incorporation of that graft.