THE OSTEOGENIC POTENTIAL OF THE TEMPORALIS MYOPERIOSTEAL FLAP: AN EXPERIMENTAL STUDY

by
James M. Stone

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science

Department of Surgery
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ABSTRACT

The potential for periosteum to produce new bone has intrigued researchers for the last three hundred years.

Until the middle of this century, most documented accounts or experimental reports were anecdotal with little attempt made to advance the understanding of periosteal behavior. The last four decades have witnessed a dramatic increase in the critical investigation of the osteogenic role of free periosteal grafts, composite periosteal grafts and pedicled osteoperiosteal flaps. These latter researches, however have been limited primarily to the young animal with an activated periosteum.

This thesis was designed to study the osteogenic potential of a vascularized periosteal graft transferred from a distance using the temporalis muscle as a vascular carrier (a myoperiosteal flap). The procedures were performed in male, mature New Zealand white rabbits beyond the epiphyseal growth stage. After confirming that a temporalis muscle did provide a vascular supply to its underlying periosteum, standardized defects were made in the posterior border of the rabbit's mandible. The defects were covered with either a temporalis myoperiosteal flap, a free autogenous tibial periosteal graft, a tibial periosteal graft transplanted to temporalis muscle and subsequently transferred as a tibial graft-myoperiosteal flap or a Vitallium (Tm) mesh overlaid with a temporalis myoperiosteal flap. All animals were allowed full use of their mandibles after surgery, with sacrifice at 2 weeks, 5

weeks, 9 weeks and 12 weeks after operation. The surgical specimens were evaluated radiographically to determine relative-quantities and patterns of new bone formation. Histologic sections were subsequently made to evaluate the quality of new bone formation.

Overall, evidence suggested that tibial graft-myoperiosteal flaps produced significantly more new bone followed by the temporalis myoperiosteal flap, the free autogenous tibial periosteal grafts and the Vitallium (Tm) mesh-myoperiosteal flap reconstructions. The amount of new bone reached a maximum (in some cases 100% healing) by 5 weeks, followed by a subsequent decline.

The bone produced in all cases was intramembranous with interspersed cartilage islands, subsequently maturing to a mature trabecular pattern. Occasional areas of marrow were seen within the healing defects.

By contrast, a mandibular defect stripped of surrounding periosteum healed poorly; primarily by endosteal remodelling and resorption of the defect margins. No periosteal regeneration was seen at the defect margins.

REVIEW OF THE LITERATURE

THE EARLY INVESTIGATORS: TO 1867

The first bone graft was indirectly reported by J. van Meek'ren in 1668. He described a record of the Reverend Englebert Sloot of Slooterdijk based on information from a missionary in Russia, John Kraanwinkel. Part of a dog's skull was apparently transplanted into the cranial defect of a soldier and although the graft healed, the church ordered its removal on pain of excommunication (van Meek'ren, 1668).

Havers published the first comprehensive account of osseous structure in 1692. He felt that periosteum had no bone forming function but was only a vascularized, limiting membrane (Hey Groves, 1917).

In 1741, Cheselden, an English surgeon anatomist, described the periosteum as a highly vascular membrane, covering all bone surfaces and supplying at least part of the osseous blood supply.

Cheselden's pupil, John Belchier, accidently discovered the principle of vital staining, that is, a developing tissue can permanently take up a substance, retaining that substance's distinctive colour. One evening in 1736, Belchier was served a joint of pork, the bone of which possessed a brick-red colour. On enquiry, he learned that his host occasionally mixed madder root in the meal given to his pigs. Sometime later Belchier fed madder to his own chickens, noting that their bones became stained the same red colour. He reported his findings to the Royal Society in 1736 (Belchier, 1736; Keith, 1918a).

Duhamel had read Belchier's work and repeated his madder-feeding experiments. Duhamel discovered that the bones of young, actively growing animals stained more extensively and deeply than bones of older animals. He further discovered that alternating madder-stained diets with normal diets would produce red and white rings in cortical bone. He realized that as bone was stained red only during the period of madder-feeding, osseous tissue must be laid down in layers or as lamellar growth. He placed silver rings under the diaphyseal periosteum of growing bones, finding after several weeks that the rings had become covered with bone. He suggested the bone could have only come from the periosteum (Smith, 1915; Keith, 1918a). Duhamel coined the term "cambial layer" to describe the osteogenic function of the deeper, cellular periosteum. He further observed that periosteum thickened and laid down external callus during the repair of fractures (Hey Groves, 1917). In presenting his findings on the nature of periosteum to the Academy of Science in 1742 and 1743, Duhamel began a controversy that continued for well over two hundred years.

Haller, an English surgeon, rejected these theories. After injecting fluid into large vessels of experimental animals he subsequently found dye in the smaller vessels of cartilage and periosteum. He regarded periosteum as nothing more than a vascular covering for bone, the arteries within being the true osteogenic organs. One of Haller's pupils, John Hunter, was strongly influenced by the vascular theory of osteogenesis. By 1754 he had begun a study of jaws and teeth, finding his earlier teaching inadequate to explain how an infant's jaw could grow to accommodate a full adult dentition. Hunter reasoned that the

jaws (and indeed all bones) must grow and remodel through deposition and absorption of bone. He repeated Duhamel's madder-feeding experiments and studied the healing of fractures concluding that arteries were the main osteogenic factors, periosteum being only one of many arterial carriers (Keith 1918a, 1919).

In 1768, another English surgeon, Charles White, performed an upper humeral resection on a 14 year old boy. Four months later, the humerus had largely regenerated. He believed that the arterial supply was the cause of regeneration of the resected tissue (Keith, 1918b).

John Howship (1810) subjected the developing bones of infants and animals as well as healing fractures to microscopic examination. He described both bone and cartilage as arising from a substance secreted by arteries on the deep surface of periosteum. He described healing fractures of rabbit femurs, both grossly and microscopically. He found the periosteal arterial supply about the fracture abundant by 5 days although no subperiosteal bone had formed. By 9 days the periosteum had produced a distinct callus, having the histological appearance of cartilage. Ossification of the cartilage was present by 15 days, while periosteum appeared to have reverted to its resting state by 32 days and the subperiosteal callus was almost completely ossified. Observations of healing fractures in humans yielded similar conclusions: periosteum in the region of injury produced new bone. Howship believed coagulated blood in the periosteum of the fracture site provided the stimulus for subperiosteal bone production. With a more severe fracture or increased movement of fragments, the amount of subperiosteal callus was

increased. Howship also concluded that newly formed subperiosteal reparative callus contained a higher content of organic matrix than the original bone tissue (Howship, 1818).

James Paget (1839) conducted further studies on the ability of growing bones to take up madder. Paget believed that madder combined with the mineral components of bone in the circulation to be deposited on the surface of growing bone as a coloured ring. Paget deduced that bones of older animals underwent remodelling through deposition and resorption although more slowly than young animals, supporting the concept that periosteum was the source of new bone.

James Syme (1840) described the case of a young girl suffering from osteomyelitis of her tibia. He was forced to amputate the leg. On dissecting the limb, he found an incomplete subperiosteal shell of new bone surrounding the necrotic shaft. Syme felt this sufficient proof of the bone producing ability of periosteum. He performed a series of experiments on dogs, resecting a part of one radius including the periosteum and a similar resection on the other radius leaving periosteum intact. Six weeks later, he found a gap still existed where both periosteum and bone had been removed while the opposite side had united with formation of a vigorous subperiosteal callus. His researches convinced him that bone must be formed from periosteum.

John Goodsir originally believed Hunter's doctrines that bone was formed by arteries and removed by lymphatics. He studied bone growth using the more sophisticated microscopic techniques that had become available by

the 1840's concluding that bone was formed by cells rather than arteries and these cells could both form and resorb bone. He felt periosteum produced new bone only if small bone particles, containing osteogenic cells, were adherent to it (Keith, 1918b, 1919).

In 1842, a French researcher, Marie Jean-Pierre Flourens confirmed Duhamel's conclusions that periosteum produced bone. Flourens also observed periosteum forming new bone over an infected necrotic shaft of long bone, agreeing with Hunter that bone was continually remodelled throughout life (Keith, 1918c).

Ollier (1867) was the first to make a systematic examination of the osteogenic function of periosteum. He originally felt periosteum had no bone-forming properties and designed his earlier experiments to test this hypothesis. His microscopic examination of periosteum led him to describe it as a layered structure. He noted that the fibrous outer layer became more cellular in its deeper aspects the cells eventually developing intimate contact with bone. These observations suggested to him that the deep layer of osteoblasts might function as a powerful osteogenic organ. He found osteoblasts to be abdundant in growing bone, and scarcer in adults although easily stimulated to activity by injury or irritation (Keith, 1919).

Ollier's experiments were performed on young cats, dogs and rabbits, with publication of results in 1867. He showed that free autogenous periosteal grafts transplanted into soft tissues produced bone more readily in young animals than old, while grafts from long bones were

bones were more successful than grafts from flat bones (Keith, 1918c) (Hey Groves, 1917, quoted Ollier as describing periosteal grafts from both flat bones and long bones in young animals doing equally well).

Ollier believed that a freshly transplanted autogenous or homologous bone graft with its periosteum intact retained viability as the periosteum rapidly revascularized from the host tissues (Peer, 1955). Free periosteal grafts seemed to be capable of producing new bone, though of lesser quality while the donor site regenerated new periosteum. Scraping the bone surface, however, would decrease the ability of new periosteum to form.

Ollier created flaps of tibial periosteum, wrapped them around muscle and found they produced bone. If the deep periosteal surface was scraped, however, no bone would form around the flap. The scrapings, however, implanted in the thigh did produce bone nodules. He discovered that bone created by a periosteal graft resorbed if placed in a soft tissue bed. He repeated and expanded Syme's experiments to conclude that subperiosteally resected bones or joints in young animals would regenerate while removal of the periosteum prevented new osseous tissue from forming.

Ollier's results are remarkable as he did not use the microscope to examine his experimental findings, nor did he have the benefit of radiographic examination (Hey Groves, 1917). He worked under septic conditions, suppuration resulting from many of his experiments. He used this factor to his advantage, discovering that massive infection killed

periosteal grafts (Keith, 1919) while a mild infection did not stop bone formation (Hey Groves, 1917).

By the late nineteenth century, two firmly opposed concepts of periosteal function had arisen. The supporters of Duhamel believed periosteum was vital for growth and repair of bone. Those embracing Haller's teachings were convinced that periosteum was a simple limiting and covering membrane of bone with no osteogenic potential.

PERIOSTEUM AS AN OSTEOGENIC TISSUE: 1881-1924

Radzimowsky (1881) performed a series of experiments showing that all bone transplanted with its periosteum died while the periosteum survived to produce new subperiosteal bone on the surface of the graft.

Bonome experimented with rats, demonstrating that bone near a fracture site died while the overlying periosteum replaced the dead bone (Bonome, 1885).

Truehart (1885) described a successful case of periosteal grafting in 1885. A 22 year old seaman suffered the loss of 3 1/2 inches from his left clavicle through a gunshot wound. Nine weeks after the injury, Truehart dressed the wound with periosteal grafts taken from the scapula and legs of a dog. This operation was repeated twice over the next two months, with new bone uniting the clavicular fragments five months after the beginning of treatment. No comment was made on the use of the shoulder or arm after healing.

Cushing (1899) described a treatment for osteomyelitis, utilizing the osteogenic properties of periosteum. He recommended removing the sequestered bone when the cambial layer of periosteum was at its maximum thickness, just before new subperiosteal bone was formed. The time for this procedure was judged by taking repeated biopsies of the infected periosteum until the correct stage of thickness was seen.

He presented the case of a sixteen year old male with osteomyelitis of the left tibia. The sequestrum extended from one epiphysis to the other and was removed sixty-five days after admission. The cut periosteal edges were sewn together as a tube.

Forty-nine days after this procedure new subperiosteal bone of normal density was visible on the radiograph while two years after surgery the bone had regenerated sufficiently for the patient to walk unaided.

Nichols (1904) argued that subperiosteal bone deposited as a shell around bone made necrotic by osteomyelitis came from osteoblasts in the deep layer of periosteum. He carried out a number of procedures similar to Cushing's, subperiosteally removing sequestered bone and suturing the cut periosteal ends to form a flat cylinder. He described almost complete regeneration of the left femur in a four year old boy nine months after sequestrectomy. Among his other cases was an eighteen year old male with mandibular osteomyelitis. Six months after removal of the necrotic left mandibular body, the periosteum had regenerated the lost bone except for the alveolar process. No reports of regeneration in adults were reported by either Cushing or Nichols.

Axhausen's experimental work (1908) on bone grafting suggested that a transplanted bone graft died, while its covering periosteum survived. Provided the periosteum could establish rapid vascular connections with the host blood supply, it would produce new bone from its inner surface. Axhausen also felt bone was produced by grafted marrow cells and cells brought into the graft by the host vessels. He reported that

the cut edge of periosteum produced the most bone, recommending that grafted periosteum be incised to increase the amount of subperiosteal osteogenesis.

Axhausen observed that a bone graft without periosteum was inferior to one covered with periosteum, and would produce bone only if remnants of cambial periosteum (and endosteum) remained on its surface. Axhausen felt that heterografts would survive but were inferior to autogenous tissue.

Janeway (1910) presented a comprehensive review of bone grafting, including one of his own cases: the transplantation of a periosteally covered tibial fragment transplanted into the resected ulna of a 42 year old woman. Two months after surgery the arm was reported useful for washing clothes. Janeway concluded (without the benefit of histological examination in a controlled experiment) that the cortical bone died while periosteum (and marrow) survived to generate new bone.

Carter (1911) described the use of autogenous bone grafts for the repair of depressed nasal fractures. While he accepted periosteum as a bone forming tissue, he removed it before inserting his grafts as he felt bare bone was more adequately nourished by host tissues. Several years later (Carter, 1923) he modified his technique to include periosteum, taking advantage of its osteogenic properties.

Hibbs (1911) described an operation for knee arthrodesis. He removed periosteum and cartilage from the femur, patella and tibia; subsequently

approximating the bones. Originally he left the superior patellar ligament and periosteum intact to provide a blood supply. He discontinued using this pedicle, and instead placed the patellar periosteum directly over the three bones as a free periosteal graft. He presented a series of patients, thirteen to seventeen years old who underwent this procedure. In all cases but one, subperiosteal bone was deposited, producing bone union between four to seven months.

Carrel (1912) took periosteum from living and recently sacrificed dogs, and stored it in plasma or Ringer's solution at 38^{o}C. He found periosteum incubated in plasma showed evidence of growth for up to ten days. After one to three days of incubation both sets of grafts were transplanted into different dogs, the periosteum incubated in plasma produced more bone. He described storing periosteal grafts at temperatures from -1^{o}C to 7^{o}C for up to 48 hours. The grafts were subsequently transplanted and produced new bone. Unfortunately, details of microscopic changes were not given in the article.

Bowlby (1911) described two cases of blunt trauma to the arms of 20 and 21 year old individuals sufficient to cause a subperiosteal hematoma but not strong enough to fracture the bone. In both patients swelling the size of an egg developed, while radiographs showed evidence of subperiosteal bone growth. Both swellings decreased to normal by three weeks after injury. He concluded that tearing of periosteum with the formation of a subperiosteal hematoma was sufficient to initiate bone formation.

Haas (1913) described periosteum as having three layers, a fibrous outer layer containing blood vessels, a fibro-elastic central layer containing lymph spaces and an inner osteogenic layer next to bone. The inner layer was rich in osteoblasts and contained a large number of blood vessels travelling between the cortex and marrow. These features were most prominent in growing bones; while in the adult, the osteogenic layer for the most part was not present.

Haas performed subperiosteal rib resections on young cats, dogs, and rabbits. He discovered that islands of bone (and occasionally cartilage) formed between the rib stumps only when periosteum was present, while the amount of bone formed was increased by the presence of a blood clot in the defect (Haas 1912, 1913). He noted bone forming at the angle between the resected rib space and bone stump as a constant finding. He interpreted this as further proof of periosteal ability to produce bone (Haas, 1913).

Haas believed bone and cartilage formation were closely related, speculating that osteoblasts could become chondroblasts or vice versa. To support this he demonstrated the change of perichondrium to periosteum during endochondral bone formation. In a series of experiments on rabbits, he removed adjoining costal cartilage and rib segments both subchondrally and subperiosteally. Both cartilage and bone regenerated although the periosteum occasionally produced cartilage-like tissue which subsequently appeared to change into bone.

Haas felt the osteogenic property of periosteum depended on an intact inner cellular layer, closely adherent to bone (Haas, 1914).

McWilliams (1912) originally believed periosteum had a questionable role in osteogenesis. Two years later, based on clinical and experimental evidence he had changed his views (McWilliams, 1914a). Only 48% of his human bone grafts without periosteum survived while 87% with a periosteal covering did survive. He was convinced periosteum was capable of osteogenesis in many cases, providing it was carefully elevated.

He used dogs in his experiments, resecting segments of ribs. In most cases, the defects completely regenerated after 6 to 8 months if the segments were resected subperiosteally, while little bone regenerated if periosteum was taken with the bone (McWilliams, 1914b).

Murphy (1912a, 1912b, 1913) believed the periosteal grafts and flaps were osteogenic provided the deep osteoblastic layer was included. Based on both clinical and experimental studies, he felt that periosteum transplanted into soft tissue as autogenous free grafts or flaps would produce bone which persisted. Homografts of these tissues would produce bone that resorbed, and heterografts never produced permanent bone. Periosteum-covered autogenous bone grafts transplanted into soft tissue died and resorbed. In very young persons, however, the graft would increase in size from periosteal bone formation. While the periosteum did form new bone, he felt it acted as a barrier to the ingrowth of blood vessels, especially in older persons. Murphy discovered that autogenous periosteal grafts contacting bone would produce the most new bone at the angle of attachment.

Albee (1914) included periosteum in his bone grafts. He felt periosteum aided in giving an early blood supply to the transplanted bone grafts through capillary anastamoses with the host tissues. He felt periosteum was osteogenic, providing the osteoblastic layer was included with the graft (Albee, 1913). He primarily used autogenous bone grafts from the tibia, the grafts containing periosteum, cortex, endosteum and marrow. He felt those tissues should match as closely as possible the tissues of the graft bed for a successful transplant. He incised the periosteum on his grafts hoping to release osteogenic cells as well as allowing blood to pass more freely into the graft.

In 1912, MacEwen produced an extensive monograph denying the role of periosteum as an osteogenic tissue. Mayer and Wehner (1914) conducted a series of experiments, similar to MacEwan's. They felt that periosteum was an osteogenic tissue providing the subperiosteal layer of osteoblasts was left intact. They transplanted a strip of tibial periosteum into the thigh muscle of young dogs, trying to keep the osteoblastic layer with the graft. By 14 days bone was seen to form from this layer of periosteum. They resected ribs in six rabbits leaving a periosteal strip on the pleural surface only. All animals regenerated bone (passing through an intermediate cartilaginous stage) from the retained periosteum after 14 days.

Using 17 rabbits "of varying age" and 6 "young" dogs, metal or glass caps were fastened subperiosteally into a groove on the anterior tibial surface such that the cap margin formed a seal with the bone. The space bounded by the cap interior and bone surface was left alone. The bone

surface was scraped to remove as much residual periosteum as possible, before placing the cap. In 9 of 23 procedures periostum could not be found growing under the cap margins. No bone formation was demonstrable as having arisen from the cortical surface of bone under the cap. Periosteum had regenerated outside the cap, however, covering it and occasionally imbedding the cap with new subperiosteal bone. This process was evident in both rabbits and dogs up to 55 days after surgery. The cortical surface seemed to be undergoing various degrees of resorption, presumably from cellular elements of the Haversian system, 32 days or longer after surgery. No further details were given relating animal species, age or degree of subperiosteal bone formation. In 12 experiments periosteum was seen growing under the cap to produce new bone, always 2 weeks or more after surgery. The periosteal tissue was always in contact with the cortical Haversian systems.

Mayer and Wehner autogenously transplanted both tibial and metatarsal bone into the thigh muscles of young rabbits. Although periosteum was removed from the tibial grafts and the cortical surface filed, fragments of periosteum remained and were capable of producing new bone. Periosteum on the metatarsal grafts remained viable, producing new bone by 6 days. In all of their experiments periosteum from young animals produced a more lively osteogenesis than periosteum from older animals. Their primary conclusion: periosteum does survive and should be included with all bone grafts, especially in young patients.

Phemister (1914) created ulnar defects of 1 1/2 inches in dogs of unspecified age, leaving the periosteum intact in some, and removing it

in others. He observed that bone grafts containing periosteum and endosteum would unite at the recipient site much more rapidly than cortical bone alone. He also fractured a number of his transplants, observing subperiosteal repair around the fractured graft. He believed that periosteum was strongly osteogenic and survived in bone grafts (together with endosteum and bone cells from Haversian canals) to actively unite the graft with the recipient bed. As subperiosteal bone formation appeared in all cases to be stimulated by damaged underlying bone, Phemister concluded that periosteum should always be included with a bone graft while conversely, bone must be added to cavities formed through subperiosteal resection (except in young persons where growing periosteum would produce adequate bone on its own).

Gill worked with adult dogs, autogenously transplanting the second metatarsal bone from one front paw to the other. All dogs were sacrificed between 7 to 8 1/2 months postoperatively. While he did not mention whether periosteum was included in the grafts, he described five of the eleven transplants as having normal macroscopic appearance, function and histology. The periosteum around the transplants was a well stained, definite tissue.

He claimed that all connective tissues in thin bone initially developed from ingrowing periosteal buds. Even after periosteum was stripped from bone, osteoblasts remained on the cortical surface to produce new bone. He concluded that periosteum must be included with bone transplants, especially in older animals both to supply osteoblasts necessary for bone repair as well as providing an early blood supply through vascular

connections with the surrounding tissue. He felt that entire long bones with their joints and covered with periosteum could be successfully transplanted autogenously. The graft would then functionally adapt to its new environment.

Smith reviewed the literature to 1915, and noted that many authors arrived at completely different results from similar experiments. He questioned whether all investigators were including the same tissues in their definition of periosteum. He described his own view of periosteum. He felt periosteum covered all bone surfaces, except at joints, providing a rich blood supply to the cortical bone. Some of these vessels passed through cortex to anastamose with the nutrient artery. He also noted Sharpey's fibres rising from periosteum and entering cortical bone of periosteal origin. He described three layers of tissue, an outer fibrous, vascular layer, a middle fibroelastic layer and an inner "periosseous" layer. This inner layer was closely adherent to bone, rich in blood vessels and osteoblasts during growth. This layer formed subperiosteal bone in young animals and although decreasing to a very thin, sparsely populated layer in adults, it could be reactivated if irritated.

Smith mentioned a number of his experiments in passing, only to conclude that periosteum stripped rapidly in young animals would produce bone more frequently while slower meticulous stripping in older animals would give a higher percentage of success.

He summarized the factors enhancing bone production: small periosteal

strips from young animals used as grafts, the presence of fibrin (an active stimulator of osteoblasts) and an abundant recipient blood supply.

Davis and Hunnicutt (1915) believed that periosteum was an osteogenic tissue but with the introduction of MacEwen's monograph in 1912, they decided to re-examine their ideas. They repeated some of MacEwan's experiments and devised their own. Most experimental animals were surgerized under aseptic conditions while experimental results were examined both radiographically and histologically. They found that femoral or tibial periosteal grafts on dogs and young rabbits, autogenously transplanted into soft tissue produced no bone. Femoral osteoperiosteal flaps autogenously transplanted in dogs, however did produce bone. Using dogs of unspecified age, they observed radial periosteal flaps entwined around muscle produced no bone, although the donor cortical surface did become roughened in some cases. Periosteal flaps raised from ribs or femurs and turned around muscle produced new bone. They saw no radiographic evidence of bone necrosis after stripping periosteum from bone surfaces and several times a tightly adherent fibrous membrane regenerated over the denuded cortical bone.

In another series of experiments using dogs, segments of both ribs and radii were subperiosteally resected. Metal caps were placed over the cut ends in some of the specimens. Where metal caps were not placed, bone regenerated almost completely. Where caps were placed, bone grew from the stumps behind the caps rather than the subperiosteal cavity. In one case subperiosteal bone was found in the defect, this being

attributed to bone chips left behind during resection. When ribs in dogs were subperiosteally resected and both autogenous and isologous bone grafts were placed, superiosteal bone formation occurred. The authors did not explain this observation.

Davis and Hunnicutt repeated Duhamel's classic experiment of wrapping a silver wire around the shaft of a femur, humerus or radius. The periosteum was left intact, stripped off, or stripped and the bone surface scraped before placing the wire. Both dogs and rabbits of unspecified age were used. The most abundant new bone growth occurred in two cases where a mild infection was present or where the bone was scraped before applying the wire. The most abundant bone growth occurred where muscle was torn, the authors explaining this to be the area of the greatest bone trauma. Rabbits generally produced more bone than dogs. Little bone formed around a wire placed subperiosteally and resorption of the bone occurred beneath the wire when it was placed over periosteum.

The authors concluded that periosteum was primarily a limiting membrane and would not regenerate bone unless bone chips were included with it. They recommended periosteum be included on all bone transplants as it seemed to favour the production of more callus while periosteum appeared to slow the rate of bone resorption. Several years later, Davis (1917) commented on the behavior of osteoperiosteal flaps turned into soft tissue. While new bone initially formed, it was resorbed with time.

Brooks (1917a) performed a number of experiments with intravital bone

staining. He subperiosteally resected a full thickness portion of cortical plate in an adult dog, subsequently feeding the dog madder root for 17 days. New subperiosteal bone was discovered both within the defect as well as the surrounding margins. In another experiment, he fractured a rabbit's foreleg, later feeding and subcutaneously injecting sodium alazarine sulphate. Only the fracture callus and adjacent subperiosteal bone stained bright red. In a third experiment he subperiosteally resected one centimeter of a young rabbit's ulna, again feeding and injecting (subcutaneously and intravenously) sodium alazarine sulphate for 16 days. The new subperiosteal bone across the gap stained bright red as did the new bone on both sides of the epiphyseal cartilage. Irregular staining was also noted on the periosteal and endosteal cortical surfaces. He concluded madder stains newly forming bone only during the time it is administered while its essential dye, sodium alazarine would stain new bone and recently formed bone for up to one week following administration.

Brooks (1917b) continued his researches with autogenous bone transplants in both young and adult dogs. He resected 20-40 mm of ulnar shaft with its periosteum, transplanting an equal length of autogenous femur into the defect. In one series the periosteum and endosteum were left on the bone fragments while in a second series these tissues were removed. In a third series of experiments, the ulnar defect was repaired with a single block of dry allogeneic bone. All animals were given a postoperative course of sodium alazarine sulphate postoperatively and sacrificed up to 105 days after surgery. Using intravital staining as a guide, Brooks discovered that periosteum (and endosteum) remained vital,

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producing new bone within the graft. No osteogenic activity was seen in the other two series. He concluded that periosteum produced and protected new bone in a graft as well as providing early vascular nutrition. He suggested osteogenesis was a function of the periosteum and cortical bone working together.

Using older dogs and intravital staining techniques, Brooks repeated his experiments of autogenous periosteal-femoral transplants into ulnar defects. He concluded that autogenous bone grafts covered with periosteum succeeded in both young and old dogs although the frequency of success was greater in young, growing animals (Brooks, 1919).

Todyo (1917) experimented with dogs 1 to 2 months of age, a removing radius and replanting it after inversion into the same dog, or a different dog. Periosteum was either included with, or stripped from the graft before implantation.

Four dogs received an autogenous periosteally covered bone graft. By 11 days, the periosteum appeared vital with ingrowth of new blood vessels from the surrounding tissue. No subperiosteal osteogenesis was evident. Osteocytes in the diaphyseal cortex were losing their ability to stain while none of the diaphyseal cells were at all stained. The epiphyseal tissues appeared only slightly shrunken. By 14 days, blood vessels appeared to pass from vital periosteum into the diaphyseal cavity. The cortical cells were now non-staining while a delicate intramembranous bone formation was seen in the diaphyseal cavity. The osteogenic activity decreased towards the epiphyses, while the cells of

the epiphyses had lost most of their staining ability.

By 42 days, the entire bone was undergoing complex resorption and repair with zones of both intramembranous and endochondral osteogenesis. Marrow had regenerated and both epiphyseal plates were present. No mention was made, however, of the presence or absence of a definite periosteal tissue. Results taken at 60 days were similar although increased ossification was present and the dog was bearing weight on the foreleg. Again, no mention was made of periosteum.

Two dogs received autogenous transplants without periosteal cover. By 12 days, the cambial layer, here defined as part of cortical bone, appeared vital, while the remaining tissues appeared similar to the dog sacrificed at 11 days. No new bone formation was evident. By 65 days the tissues appeared to have healed by both intramembranous and endochondral ossification (as in the previous series at 60 days) while only parts of the marrow had regenerated. The periosteum had regenerated to cover the entire transplant.

Two dogs were given homotransplants of periosteally-covered bone grafts. By 19 days sections of the cambial periosteum had degenerated although the cortex was undergoing resorption and replacement with new bone. A dog sacrificed at 20 days demonstrated similar findings.

Todyo defined periosteum as the outer fibrous layer only while the cambial layer belonged to the bone cortex. In this context he felt that transplanted bone could grow with or without periosteal cover although

grafts without periosteum healed more slowly. As periosteal tissues survived and proliferated Todyo argued for retention of periosteum with bone grafts. (Epiphyseal plates were the only other tissues to survive and proliferate.)

Berg and Thalhimer (1918) provided an excellent literature review to 1918. They felt periosteum was a bone producing tissue, as well as giving rise to the endosteal layer. They used full grown cats, autogenously transplanting tibial fragments subcutaneously, into the spleen, and onto costochondral surfaces. They examined their results histologically, finding a lively subperiosteal bone growth originating from the cambial layer. They also found this layer continuous with the Haversian system, the latter showing a lively osteogenesis as well. Berg and Thalhimer felt periosteum slowed the rate of bone graft resorption, while osteocytes on the bone surface from periosteally-stripped cortex began to produce bone.

Davis (1919) presented many of his concepts in his book of Plastic Surgery. He described flaps with periosteum and thus attached bone spicules produced bone while free periosteal grafts did not. He felt reconstruction of the mandible had to be postponed until healing was complete. Reconstruction could include use of the Cole procedure (described later), or implanting bone into soft tissues, delaying several weeks and then transferring the bone pedicle flap. Davisfurther concluded that clavicular flaps did not work well in reconstructing the mandible.

Ely (1919a) described periosteum as a "suitable matrix" for the formation of bone, as was fibrous, granular, or necrotic tissue. He felt that both blood vessels and an external stimulus (physiologic or pathologic) were necessary for bone production.

In a series of poorly described experiments using dogs, he transplanted tibial heads and femoral condyles to muscles of the thigh. Some of the transplants were boiled and some fresh. One dog given a fresh transplant was sacrificed at 17 days and new bone was found growing subperiosteally. He concluded that the bone formed only in periosteum (Ely, 1919b).

Mayer (1919) described 2 cases of osteogenesis following bone grafts. A 39 year old male was given an Albee tibial graft for Pott's diseae. The patient died 2 months later, and the graft site was examined histologically. A vigorous subperiosteal bone formation was noted on the graft at a site away from the host bone. This bone was gradually replacing tibial cortex.

The second case was of an 8 year old boy again given an Albee tibial graft for Pott's disease. The patient died 18 months later and the graft site was also biopsied. The tibial bone had changed to resemble tubular bone with periosteum, cortex and marrow.

Delangeniere and Lewin (1920) describe the clinical usefulness of free osteoperiosteal grafts. They removed grafts from the anterior tibia, claiming the graft survived to produce osseous tissue even if not

contacting host bone. They described the graft as being useful for repair of cranioplasties, non-union repair of long bones or mandibles and in supplementing large grafts to facial bones. They claimed a success rate of over eighty percent in a variety of cases receiving this graft.

Eloesser (1920) used overlay rib grafts for repair of both long bone and facial bone defects. He retained periosteum, and split the rib graft longitudinally using periosteum as a hinge. The graft was laid over the defect with the host periosteum sutured over the graft. He presented a series of 22 cases, using this technique to repair defects in 21 long bones and 1 mandible. Thirteen cases, including the mandibular reconstruction were complete successes.

Eloesser concluded that periosteally-covered bone grafts were clinically useful procedures as they maintained viability for a longer period than larger bone grafts. Even though they were quickly resorbed and tended to fracture through their centre, he observed the graft ends were always united to the host bone.

Ivy (1920a) surveyed treatment methods for mandibular fractures during the First World War, finding tibial periosteal grafts were successful in 71% of patients treated. Ivy felt this procedure was good for repairing loss of osseous tissue of any amount or in any position. Ivy (1920b) reviewed the treatment of 22 patients with mandibular non-union following gunshot wounds of the mandible. He found that osteoperiosteal grafts proved most successful in 11 of 14 patients as a primary or

secondary reconstructive procedure. By contrast, the Cole pedicle technique was successful in 3 of 5 patients while iliac crest grafts were successful in 5 of 6 patients.

McWilliams (1921) reviewed all methods of bone grafting, except Albee's. In the 1390 cases reviewed, he judged 82.3% to be successful. Of 426 osteoperiosteal grafts, 87.3% were successful (the second most successful after the use of bone pegs) while 82.9% of bone grafts with periosteum were considered successes. Bone grafts without periosteum were successful in 82.6% of cases. He concluded the best method of osseous repair involved filling defects with small bone chips overlaid with strip osteoperiosteal grafts, bridging the fragments of host bone.

Albee (1923) reviewed bone transplantation methods, describing both osteoperiosteal grafts and Cole pedicle flaps as poor choices due to their lack of osteogenic stimulation from functional stress.

Henderson (1923) felt non-unions could be treated a number of ways, including placement of semi-inlay bone graft with an autogenous osteoperiosteal overlay graft.

Kolodny (1923) argued that grafted periosteum would produce bone provided a good blood supply was present, the animal was young or if adult, the periosteum was in a "reactive stage" rather than a "latent" stage. The periosteum could be reactively stimulated by fractures of bone, direct trauma or mild infection. Kolodny's work will be more

fully described in a later section.

Thomas (1923) suggested both ends of ununited fractures should be disturbed as little as possible while an osteoperiosteal overlay graft ("wafer graft") could be used to stimulate new bone formation from the graft itself. Thomas harvested osteoperiosteal grafts from the upper tibial surface taking a thin shaving of bone to ensure removal of all osteoblasts. He placed the graft over a conventional cortical bone graft and host bone or over the defect itself. Thomas described one case of a 24 year old male with compound infected fractures in his right leg.

Four and a half years after injury, the fractures were treated with autogenous bone grafts and an osteoperiosteal graft taken from the left tibia. Three months later the fractured leg was reported to be healing well.

Haas (1922, 1924) believed that periosteum, along with endosteum and the osteogenic cells of the Haversian system were able to survive in an autogenous transplant - subsequently producing bone. He described periosteum as containing both an outer fibrous and an inner cellular layer. In one experiment, he removed a metatarsal bone with a strip of periosteum from the foot of an adult dog. The bone was fractured and transplanted autogenously into the animal's back. After 71 days the fragments were seen to be united with minimal callus formation. Haas attributed the repair, in part, to periosteal acitvity. The bone and callus contained empty laumae which Haas attributed to lack of normal

functional stimulus.

In another series of experiments, he removed metacarpal bones from young and full grown dogs. Periosteum was included with the specimens. The bones were fractured and either placed in sterile bottles or incubated in normal saline at 37°C for various periods of time. The specimens were reimplanted in the back muscles of the same dogs. Ten of the 20 animals died in less than two weeks while the remainder were sacrificed up to 51 days after reimplantation. Haas found microscopic evidence of subperiosteal bone formation in 2 animals and 3 cases of subperiosteal cortical bone formation in an otherwise necrotic transplant. The bone had been stored in sterile bottles for up to 19 hours. In 3 specimens nuclei of cortical bone were stainable and there was slight subperiosteal bone formation in 2 animals from bone stored in normal saline up to 19 hours (Haas, 1923).

Haas continued to use the same experimental model, storing bone specimens both at near freezing and at room temperature. He found subperiosteal callus in specimens kept near freezing for up to 5 days and no subperiosteal callus from bones stored at room temperature (Haas, 1925).

In order to finally prove the role of periosteum in osteogenesis, Haas once again repeated his experimental model removing periosteum by sharp dissection from some specimens and removing endosteum by reaming in others. (He admitted that osteogenic cells could remain on the cortical surface giving rise to new bone and altering the experimental results).

The bones were again fractured and either reimplanted or autogenously transplanted into back muscles of the animal. After 14 to 116 days the specimens were examined. He found that fractured bones without periosteum healed in 1/3 of the reimplanted or transplanted specimens. Fractures without endosteum united in one third of reimplanted animals and 2/5 of the transplanted animals. Fractures without periosteum or endosteum failed to unite in any animal.

Haas concluded that while periosteum was not essential for the production of bone, the osteloblasts of this tissue did react and were more important than endosteum in fracture repair (Haas, 1924).

PERIOSTEUM WITHOUT OSTEOGENIC FUNCTION 1839 - 1924

Paget (1839) analyzed contemporary theories of bone growth revealed throughintravital madder staining. He accepted Duhamel's observations that long bones grew in circumference by the addition of bone in layers on its external surface. He felt the new bone was produced by the bone already present, rather than any function of periosteum.

Barth (1893) created defects in the skulls of dogs repairing the defect with either immediate replacement of the autogenous covered skull bone or sterilized, macerated bone. He evaluated the results histologically. Barth's main conclusion was that all parts of living, autogenous periosteum-covered graft died; to be absorbed and replaced by the surrounding bone. While Barth worked only with skull tissue over short periods of time his conclusions contradicted the work of Duhamel and Ollier.

By 1908, however, Barth was so influenced by Axhausen's work (1908) that he changed his views admitting that living periostially-covered bone grafts were superior in osteogenesis.

MacEwan was thoroughly convinced that periosteum was a limiting membrane without osteogenic function. In 1884, he treated a 9 year old boy presenting with a comminuted skull fracture. The defect was extensive with most bone fragments stripped of periosteum, and multiple dural tears. In all, 11 fragments were removed, stripped of periosteum, cleansed and returned to place. After 21 days MacEwan described only 2

fragments being clinically dead, the remainder of the wound healing after one month.

In 1878 MacEwen was presented with a 2 year old boy, suffering from suppurative periostitis of the right humerus (MacEwen, 1878). Except for the proximal and distal epiphysis, the bone was necrotic. MacEwen subperiosteally resected the diseased tissue without finding evidence of subperiosteal bone regeneration. Fifteen months later the upper arm was only 2 inches in length without evidence of clinical bone regeneration. MacEwen grafted tibial cortico-cancellous fragments from a 6 year old boy into the defect, with a further graft placed 2 months after the first. Seven years later the patient's arm was 7 3/4 inches in length, and completely functional (MacEwen, 1887). By 1909 the patient was a labourer in his 30's, using his arm with no ill effects. The humerus was now 11 inches long (MacEwen, 1909, 1912a).

In 1912, MacEwen produced a monograph, based on 30 years of laboratory experiment and clinical experience, arguing that epiphyseal cartilage and not periosteum produced bone. In one experiment he resected 1 1/2 inches of a dog's radius, no attempt being made to strip the periosteum in its deep plane as this would "detach plaques of bone". He found no bone regeneration, but only rounding of the fragment ends. In another experiment he elevated a strip of radial periosteum wrapping it around muscle and finding no bone formation under periosteum except at the angle of reflection.

MacEwen repeated Duhamel's silver ring experiment, first removing the

periosteum before placing the ring. Twelve weeks later bone had completely buried the ring.

In another series of experiments on dogs, MacEwen subperiosteally removed the entire diaphysis from the right radius except for 1/4 inch from each epiphysial plate. He placed metal caps on each fragment and found after 7 weeks, the metal caps had been pushed into the defect by 2 1/4 inches overall while no bone was formed between the caps.

From his experience, MacEwen concluded that new bone was created from the existing bone tissue itself while the periosteum was only a vascular organ and limiting membrane (MacEwen, 1912).

Baschkrzew and Petrow (1912) felt their animal experimental evidence demonstrated that periosteum alone could not form bone tissue. They admitted that periosteum covering a bone graft could live in part and supply nutrition, protection and guidance as a limiting membrane for osteogenesis.

McWilliams (1912) felt that periosteum lived when transplanted but acted only to guide the migration of osteoblasts arising from bone. He described seeing a 12 year old boy, suffering from a mandibular giant cell sarcoma. In 1910 he removed the entire left mandible. Two years later, he reconstructed the mandible with an autogenous subperiosteally resected rib. The rib was perforated several times to allow increased circulation. Two months later McWilliams described the rib as solidly united to the mandible.

In one of several experiments he subperiosteally resected a 3/4 inch length from the diaphysis of a cat's humerus. The fragment was transplanted into a similar defect made in the radius, while the radial fragment was placed into the humeral defect. Both fragments had united solidly after 72 days. From this and other experiments he mentioned that many of his grafts stripped of periosteum regenerated a covering membrane macroscopically identical to normal periosteum. He expressed concern that periosteum might delay healing by preventing blood from reaching the graft.

Two years later, he had changed his views completely. A periosteally stripped rib that he had transplanted into the mandible had resorbed completely after 5 months. From his animal researches, he concluded that periosteum was necessary to preserve the life of a graft as well as being able to produce bone on its own (McWilliams 1914a, 1914b).

Brown and Brown (1913) used mongrel dogs, resecting bone and transplanting periosteum or periosteum and bone intramuscularly or subcutaneously. They were unable to reproduce bone with free autogenous periosteal grafts and concluded periosteum was not essential for preservation of viability of the adult bone.

Geddes (1913) studied endochondral bone formation histologically, concluding periosteum was only a limiting membrane. He felt osteoblasts derived from embryonic ectoderm and only migrated through periosteum to form new bone.

Hey Groves (1913) felt periosteum was only the fibrous outer layer surrounding bone while the deep layer of osteoblasts belonged to bone tissue proper. He created comminuted fractures in the long bones of both cats and rabbits. The fragments were fixed by various methods until sacrifice at 7-71 days. He found bone forming at the angle of reflection between periosteum and unfractured bone but not along the deep surface of periosteum over the fracture site.

Hey Groves concluded that reparative callus developed from the bone and not periosteum although loss of periosteum both delayed recovery and reduced the amount of bone produced. He did not explain this latter observation further. Cartilaginous callus appeared to be formed from pressure and movement between fragments.

He stated that the osteoblastic layer of cells between fibrous periosteum and bone belonged to bone tissue proper, and called this cellular layer "eposteum". Thus periosteum was only osteogenic when the cellular (eposteal) layer could be elevated with it, as with young bones, or adult bones thickened after trauma or inflammation. He felt that periosteum should be included in bone grafts to provide a blood supply to cortical bone, but most importantly to conserve the inner layer of osteoblasts. He recommended cutting the periosteum in several places after bone grafting to release the osteoblasts (Hey Groves, 1917).

Bancroft (1914) stripped periosteum from the humeri of dogs (age not given) and created a full thickness defect in the cortical bone. He

achieved hemostasis by filling the cavity with hot oil, packing with plain gauze, packing with gauze sterilized in oil or filling with bone wax. The gauze was removed after 24 hours before the soft tissues were closed. The animals were sacrificed from 30 to 190 days postoperatively. He found that bone appeared to grow into the cavities from the cut edges of cortex. Some specimens developed a definite fibrous tissue-like covering membrane over the cavity, continuous with the surrounding periosteum and producing connective tissue cells which seemed to be undergoing metaplastic change to osteogenic cells. He concluded that periosteum was primarily a limiting membrane of bone. Gallie and Robertson (1914) reflected periosteum from bones in fox terrier puppies and made defects in the bone. In some cases the defects were filled with wax before replacing periosteum. No subperiosteal bone growth was found in any of the specimens. They felt periosteum was only a limiting membrane, while the subperiosteal osteoblasts belonged to the osseous tissue. They admitted however that occasional islands of bone could be removed with periosteum, creating in essence a small bone graft which was capable of regenerating a limited amount of bone (Gallie and Robertson, 1920).

Lewis (1914) questioned both the osteogenic and the blood carrying activity of the periosteum. He resected 3 to 4 cm of the radial or ulnar mid-diaphysis of young dogs. Periosteum, endosteum and marrow were removed, the cortical bone crushed into 1.0 mm to 3.0 mm fragments and reimplanted autogenously. The recipient stumps were either left open or covered with periosteum, fascia or muscle. The dogs were sacrificed from 21 days to 9 weeks postoperatively. Most of his results

showed that the cortical bone fragments fused both clinically and histologically.

Moore and Corbett (1914) felt periosteum functioned as limiting membrane in adults, a space for bone to grow in children and a collecting area for osteoblasts following irritation or injury. They briefly described a number of their experiments although not giving the species or ages of animals they used. In one series the tibial bone was exposed and the tibia was fractured. After destroying the tibial nutrient artery and reaming out the medulla, a wooden intramedullary peg was placed. Little subperiosteal callus was evident after 2 weeks. When the nutrient artery was not cut, abundant subperiosteal and medullary callus were radiographically evident after 10 days. They concluded that cutting the nutrient artery, even if periosteum was intact prevented formation of subperiosteal callus. They recommended conserving periosteum during fracture treatment but removing it "if not convenient" as it was not necessary in bone healing.

Rhodes (1914) used autogenous tibial bone grafts covered with periosteum to repair depressed skull fractures. He felt periosteum was useful only as an anchor for the bone graft while new bone grew out from the host cranium. Four cases of fracture treatment with this technique were described although no follow-up results were given.

Dobrowolskaja (1916) described a vague series of experiments in which component bone tissues from young animals were cultured in "homogeneous plasma" solutions. He found that cortical bone alone or with

periosteum, endosteum and marrow produced the most abundant growth while periosteum alone produced very little new growth. He concluded that bone with a periosteal covering was necessary.

Cole (1918) drew on his experience in treating mandibular fractures to conclude that periosteum was only a limiting membrane. He described an intact periosteal envelope as being advantageous in fracture healing by keeping soft tissue away from the healing fracture. If periosteum interposed between fragments he described it as hindering rather than aiding in union. He felt periosteum should be included with free bone grafts as it prevented graft absorption.

Ely (1922) studied fracture healing in both young and adult cats. He fractured the humerus by external force, with the animals being examined histologically and radiographically from 4 to 77 days after injury. New subperiosteal bone appeared by 8 days while cartilage appeared in the callus by 16 days. Thereafter the fragments united with fibrous tissue or cartilage callus undergoing later transformation to bone. Bony union occurred in all 4 cats allowed to live 57 days or longer. In a second series of experiments, the periosteum was incised circumferentially and the underlying bone fractured with a saw or bone forceps. Specimens were examined from 11 to 65 days after surgery. Healing occurred as firm union (1 of 6 cats), fibrous union or false joint formation, with cartilaginous callus creating areas of new bone. (One animal developed a suppurating infection by 15 days.) In a final series the periosteum was longitudinally incised before the underlying bone was fractured with bone forceps. One cat died after 4 days while the other animals were

sacrificed 22 to 62 days after surgery. The injuries healed with fibrous union, false joint formation or firm union (2 of 5 cats) with cartilaginous callus proceeding to new bone. All of the specimens presented widely displaced or overlapped fracture ends, and no mention was made of the presence or absence of any type of fixation at the time of surgery.

Ely felt periosteum was valuable during early healing in holding fragments together as well as keeping external callus against the fracture. He interpreted external callus as arising from the external cortical surface and not the periosteum. He concluded also that while endosteal callus may repair small bone defects, it had no function in repair of fractures.

Neuhof (1923) argued that periosteum was not necessary for the healing of an autogenous bone transplant. He postulated that the dying bone graft gave off a substance which induced host tissue to undergo metaplastic change into osteogenic tissue. He felt periosteum underwent partial necrosis but did allow the graft to unite earlier with the host tissue (3 days) as well as preventing early resorption of the bone. After 2 days the periosteum was freely supplied with the host vessels while union with host tissues occured by 3 days. After 6 days Neuhof felt grafts with and without periosteum were equally well vascularized. As the graft cells furthest from the blood supply (periosteum) disappeared first, he felt it was an illusion that periosteum survived and created new bone.

PERIOSTEUM AS AN OSTEOGENIC TISSUE: 1925-1949

Bast and coll, (1925) studied the action of fibrous periosteum. They created longitudinal saw cuts, 10 to 20 mm in length through the tibial cortex in "young adult" rabbits, histologically examining the experimental sites up to 24 days after surgery. By 3 days postoperatively, they noted a marked proliferation in both the fibrous periosteum and cellular periosteum. By the 5th day small cartilage nodules were present in the external callus of one animal. After 7 days 1 specimen presented with cartilage nodules in external callus, appearing to undergo resorption. Both fibrous and cambial periosteum had united across the defect by 12 days and the external callus had largely disappeared by 18 days after surgery. Bast and coll. observed that fibrous periosteum could be stripped easily from the cambial layer and without osteoblasts, could produce cartilage from its cut ends which underwent metaplastic change to osseous tissue while the osteoblasts left on the bone surface were capable of osteogenesis. If periosteum was stripped from bone with an intact cambial layer, the osteoblasts would then produce bone. Bast and coll. regarded the cambial layer as a separate tissue from fibrous periosteum, (which they regarded as periosteum proper). They argued cambial osteoblasts were continuous with the Haversian-endosteal system although they could produce bone if attached to either fibrous periosteum or cortical bone.

Rhode (1925) presented a comprehensive investigation on the osteogenic properties of bone and related tissues. He described the outer layer of

periosteum as fibrous in nature, rich in blood vessels for nourishment of bone and a barrier to the ingrowth of nonspecific connective tissue. Rhode felt the inner periosteal layer was fibro-elastic, rich in osteoblasts but poor in vascularity. The periosteum was bound to the bone surface through blood vessels, Sharpey's fibres and elastic fibres; loosely in young animals (except at the epiphyseal plates) but more tightly in older animals. He believed the cambial layer produced bone through action of osteoblasts, receiving a blood supply from the outer fibrous layer. As this outer layer received its blood supply from the surrounding connective tissues, Rhode emphasized careful stripping of periosteum and with the surrounding connective tissue. As proof of these statements he described an experiment involving subperiosteal resection of an ulnar segment in a young rabbit. The nutrient artery was destroyed and the marrow cavities curetted. After replacing the soft tissues a plaster cast was applied and left for 4 weeks. By 82 days, new subperiosteal bone had completely bridged the gap. The experiment was repeated in a young cat, removing a radial segment, plugging the marrow cavity with autogenous bone pegs and dissecting the periosteum free of the overlying tissue. No new bone was formed after 2 months while the periosteum underwent fibrous degeneration with complete loss of the cambial layer.

Rhode subperiosteally resected a 1.5 cm long segment of radius, and resutured the tissues as a tube over the defect. No mention was made of the nutrient artery. By 39 days only a small amount of new bone was seen on the distal fragment.

The experiment was repeated in an "old" rabbit, this time including cortical slivers with the periosteum. Rhode mentioned that the ulna fractured during placement of a plaster cast. This fracture united with abundant callus formation, which encroached on the healing radius. The periosteum surrounding the radius showed radiologic evidence of independent subperiosteal bone formation by 6 weeks and almost complete healing by 11 weeks. Rhode concluded that periosteum was tightly adherent to cortical bone in older animals and must be removed with underlying bone slivers to assure inclusion of the cambial layer. He felt periosteal bone regeneration was slower in older animals but otherwise identical in its healing mechanisms. To further confirm his findings Rhode described the transplantation of periosteum from a fracture site to a subcutaneous recipient bed in a 60 year old man. New bone was seen arising from the cambial periosteum after 14 days. It is noteworthy that the periosteum was removed in a metabolically reactive state; that is thickened and activated by the stimulus of the underlying fracture.

Rhode felt periosteum was the most powerful osteogenic tissue, capable of reproducing itself providing adequate vascularity and stability of fragments were present. When a cylinder of long bone and half the periosteum was removed in a young rabbit (marrow cavities also curetted) new subperiosteal bone bridged the defect by 3 months forming an inner medullary bone, an outer cortical bone and a new periosteal layer covering the region of periosteal resection. Soft tissues of Haversian canals were also capable of regenerating periosteum on denuded bone. He felt that mature connective tissue of any type (including the fibrous

layer of periosteum) could not produce bone through metaplasia; rather osteoblasts in their correct tissue relationships with a proper blood supply were the only producers of osseous tissue.

Rhode argued that mild functional stress ("early static stimulus") aided bone regeneration while excessive stress or movement such as improper fixation only delayed healing.

Cowan (1928) examined the periosteal response in fracture healing by performing numerous experiments on young kittens as well as observing his own patients. He felt periosteum was important in binding fracture fragments together as well as limiting the spread of the fracture hematoma. Cowan observed a hematoma to arise from both the bone ends and any areas of torn periosteum. The hematoma induced periosteum to send out vascular buds into the fracture area developing granulation tissue. Periosteal osteoblasts ("osteal fibroblasts") were subsequently seen to form external callus. Cowan felt transitory cartilage nodules produced in subperiosteal callus arose from pressure on the external surface of bone although he didn't explain the origin of these forces.

Allison (1928) used tibial osteoperiosteal grafts for spinal fusions. He recommended the graft be placed into the prepared bed immediately after its harvest. No experimental evidence case studies were used to supplement his paper.

Dorrance (1930) harvested tibial osteoperiosteal grafts in dogs of unspecified age, autogenously grafting them to bridge the mandible

across its lower border. The grafts were laid in place and left for up to 226 days. In 3 dogs, one had resorbed while 2 grafts formed an osseous union at one side only. He varied the experimental technique, laying the graft subperiosteally from the angle to the premolar region of the same side. One graft formed a strong bridge after 100 days. In a final series, Dorrance bridged the mandible with a periosteal graft, wiring it in place. Two grafts formed secure bone bridges, one formed a bridge joined by fibrous union while another formed bone attached to one side only. Dorrance concluded periosteum alone would not form bone but osteoperiosteal grafts would produce satisfactory results if securely fixed to the recipient bed.

Mock (1928) reviewed the literature, concluding that all authors agreed periosteum played a role in bone healing and regeneration even if they disagreed on its osteogenic potential. He experimented with adult dogs, finding non-union in fracture sites where periosteum was stripped from the cortical surface. Mock felt periosteum was invaluable for bone repair. He removed periosteal grafts by blunt dissection, and recommended placing them over bone defects of delayed union, non-union or loss of bone substance with as little disturbance to the already damaged host periosteum as possible.

Mock described 8 cases of free tibial periosteal graft or flap procedures to repair non-union or delayed union in fractures of the radius, femur and tibia. The grafts were supplemented with external splints, new bone arising in all cases to heal the defects from 2 months to 3 years after surgery. Mock recommended the use of osteoperiosteal

grafts for the repair of larger bone defects where mass and rigidity were needed. He felt, however, that osteoperiosteal grafts were less effective than "pure" periosteal grafts or flaps in producing bone.

Burman and Umansky (1930) reviewed the literature on periosteal transplantation. They began their experiments admitting their belief in the osteogenic ability of periosteum. In one series of experiments they used young rabbits 2 to 6 months old, autogenously wrapping anterior tibial periosteum around the tibialis anticus tendon. In 21 experiments, the cambial layer was placed next to the tendon and in 15 experiments it was placed facing the surrounding connective tissues. All experiments lasted from 14-100 days.

In all cases where periosteum was seen, bone was present without cartilage formation. The bone was inconsistently presented as nodules, large masses, or tubes while evidence of bone resorption was seen in several specimens. Unfortunately, no detailed account relating the placement of periosteum and its subsequent bone formation was given. The tendon always underwent change to a fibrous tube while the reflected edges of the tibial donor periosteum produced bony nodules. Burman and Umansky admitted periosteum did produce bone but felt that its unpredictable osteogenesis precluded its use.

Ham (1930) created fractures in both ribs and fibulae of adult rabbits examining the repair mechanisms up to 28 days after operation. He felt that periosteum in both growing and adult animals had a deep layer of osteoblasts or osteogenic cells, continuous with and identical to cells

of both the Haversian canals and endosteum. He described a dramatic thickening of this layer for a distance on either side of the fracture by 4 days. He found the cells nearest the fracture in a relatively avascular field, differentiating into chondrogenic cells and producing an initial carilaginous matrix. The cells farther from the fracture appeared to be in a highly vascular environment, differentiating into bone producing cells and beginning to lay down osseous tissue. Ham felt bone was produced by osteogenic cells of periosteum rather than metaplastic transformation due to the lack of granulation tissue prominent in metaplastic bone formation, but not at all present in fracture healing.

Ham briefly described endochondral bone formation, and replacement of cartilage by bone in mature subperiosteal callus. He noted that osteogenic cells produced bone providing they had adequate blood supply and local availability of calcium. He felt absence of these two factors led to cartilage formation by periosteum.

Furstenburg (1931) argued that periosteum with adherent bone chips on its inner surface was an osteogenic tissue, as was the dura. He described calvarial periosteum as returning to an embryonic connective tissue morphology after injury, with some nonspecific connective tissue cells differentiating into osteoblasts. Although he did not have experimental evidence to back up this concept he very briefly presented a patient series to prove the regenerative role of calvarial periosteum. Fourteen patients from 6 to 56 years of age were treated for calvarial osteomyelitis. The defects ranged from small frontal bone

openings to almost complete loss of the cranial vault. Eight patients survived the disease with complete regeneration of cranial defects.

Leadbetter (1931) viewed autogenous periosteum as ideal internal suture material for repair of long bone fractures, when combined with external fixation and early mobilization. He felt periosteum had adequate tensile strength for use as siture, while the osteogenic properties arose from cortical shavings included with the graft. Leadbetter felt periosteum grew best when contacting cortical bone, being able to form an early external callus which could supply strength and vascularity to the later appearing (but stronger) endosteal callus.

He performed a number of experiments on rabbits of unspecified age, fracturing the tibia and fibula. In one case without splinting, periosteal callus alone was seen to bridge the defect after 7 days. In another rabbit, the periosteum was elevated after fracture to allow wax plugging of the medullary cavities. The leg was not splinted. After 21 days the fragment ends were displaced from each other while a mass of well vascularized periosteal callus united these fragments. In a third experiment, Leadbetter created a long bone fracture, reflected the periosteum and replaced it as a free suture graft through holes drilled in each fragment. The medullary canals were not plugged. Fixation was placed for 26 days. At sacrifice, firm union had occured with abundant, vascular subperiosteal callus. The bone was more mature than the non-splinted cases while endosteal callus had begun to form.

From these and other experiments, Leadbetter concluded that periosteal

grafts, containing small chips of cortical bone formed the first callus in fracture healing while this function was markedly impeded when no splint was applied. Leadbetter recommended using autogenous tibial periosteal strips as suture material by passing them through holes drilled in the bone fragments and tying the ends securely. The recipient periosteum in all cases was reflected before placing the suture and subsequently fixed over the graft. He presented 6 cases treated by this method: 4 fractured olecranon processes, 1 fractured humerus, and 1 case of fractured radius and ulna. One olecranon process fixation failed due to infection while the fractured forearm bones were evaluated after only a week's fixation. Leadbetter described a further case successfully treated with local periosteal flaps to repair a fractured tibial shaft.

Phemister (1931) felt all layers of bone should be included with a graft, although the layers did not have to perfectly match those of the host. He felt onlay splint and osteoperiosteal grafts were less traumatic to use than other types of grafts although he felt the osteoperiosteal graft was weaker and provided slower union.

Haldeman (1932) studied the action of periosteum during fracture healing, using adult rabbits and dogs as experimental models. He created saw-cut fractures in the radii of a rabbit. On one side, the periosteum was disturbed as little as possible with subsequent bone union after 4 weeks. On the other side periosteum was stripped 1 cm on both sides of the saw cut, and no union was seen after 6 weeks. Subperiosteal bone was present, however, at the angle between bone and

reflected periosteum. The experiment was repeated, removing 1/2 cm of bone. When periosteum was disturbed as little as possible new subperiosteal bone filled the gap. Nonunion with fibrous replacement resulted from stripping periosteum from the defect. When a defect was created in the radius and periosteum tied over the cut ends, healing occured with non-union or partial union from endosteal callus formation. The periosteum on the opposite side was pushed back to 1 mm from the fracture ends. The gap healed by new bone bridging. Haldeman repeated this last experiment, removing fibular defects in dogs, he found similar results, with a slower healing rate.

He concluded periosteum would produce bone if the cambial layer was present as well as haematoma between separated periosteum and cortical bone surface. Haldeman felt the cambial layer of periosteum was most important in healing fractures while endosteum played only a minor part. He argued that separation of periosteum and cortex by haematoma was necessary to induce fracture repair.

Haldeman used rabbits 4 to 8 months old in studying the influence of periosteum on bone graft healing. Full thickness radial defects were created and repaired with autogenous tibial or fibular grafts. He found that full thickness bone grafts, covered with periosteum healed completely in most aminals as did free periosteal and osteoperiosteal grafts. Osteoperiosteal grafts appeared to heal through the action of the periosteal component and not the bone. He found cortical bone alone or with endosteum healed very slowly. Haldeman concluded periosteum was the most important tissue in a graft both in aiding survival and

securing early union (Haldeman, 1933).

Bisgard (1933) investigated the healing potential of different grafts in spinal fusion. He autogenously transplanted rib and tibial bone grafts as well as periosteally-covered tibial grafts into prepared spinal beds of half-grown goats. His results with the different tissues were somewhat ambiguous, although he did notice subperiosteal (and endosteal) osteoblastic proliferation after 7 days. Rib grafts were undergoing resorption with new bone replacement by this time while the same process was not apparent in the periosteally covered tibial transplants until 14 days. All grafts had been replaced by new bone after 155 days.

Bisgard felt the cellular tissue on the surface and in Haversian canals survived in a graft, beginning to proliferate by 7 days. He felt osteoperiosteal grafts were acceptable although rib grafts were superior.

Fang and Miltner (1933) took strips of periosteum from the middle ribs and tibiae of adult dogs, autogenously transplanting the grafts into tibialis anticus muscle fascia. The grafts were left in place for 7 weeks to 8 months. They found rib periosteum produced intramembranous bone islands at 7 weeks and all layers of bone tissue (cortex, marrow) at 8 months, while tibial grafts produced no bone. In a second group of dogs, they created rib and tibial periosteal flaps 3 cm long, implanting them into muscle. Rib periosteum produced 25% more bone than free periosteal grafts while the tibial flap produced only slight calcification.

Fang and coll. (1934) studied the osteogenic potential of autogenous periosteal grafts taken from different areas of the tibia. Young adult rabbits and adult dogs were used, with material being examined up to 10 weeks after operation. They found free grafts taken from the upper anterior tibial third and transplanted into tibialis anticus fascia had the most potential for osteogenesis. Periosteum from the lower third of tibia showed less potential while the middle tibial third had the least osteogenic capacity. They felt their results corresponded to fracture healing rates in similar locations on tibial bones in man.

Ghormley and Stuck (1934) attempted to produce spinal fusion in adult dogs. Autogenously transplanted tibial periosteum, tibial cortex, cancellous tibia, cancellous ilium and chips of bone elevated in situ were placed between spinous processes. No fixation was used postoperatively and specimens were taken 2 to 3 months after surgery. Periosteal grafts produced no bone although the authors did admit the grafts were thin and ischemic after harvesting. The densely trabecular bone of iliac crest produced the most frequent and abundant new bone formation. They concluded, with respect to periosteal grafts, that resting periosteum in younger persons may produce bone while that from older persons, especially on long bones free of muscular attachments, had no osteogenic function.

Keith (1934) argued that osteogenic cells within a bone graft would survive and produce new bone if given an adequate blood supply. He removed segments of radii in dogs of varying ages, stripping the periosteum and endosteum and reducing the bone to fine shavings.

Reimplanting the bone shavings, he found almost complete regeneration after 20 days. When bone was subperiosteally removed new subperiosteal bone regenerated in the defect, moreso in younger animals. From these and other experiments, Keith concluded that osteogenic cells could survive and produce new bone, especially in younger animals if an adequate blood supply was present. This was achieved by keeping periosteum adherent to its overlying connective tissue, and reducing bone into small chips.

Orell (1937) described the production and use of a periosteal derivative "os novum" in humans. Beef bone, freed of fat and protein from both the bone surfaces and Haversian systems ("os purum") was subperiosteally implanted on the anterior tibial surface. Up to 2 months later, the induced, highly vascular subperiosteal osseous tissue ("os novum") was removed for autogenous transplantation. Orell claimed success in using os purum for spinal fusion as well as aiding replacement of bones in hands and feet. He advised placing "os purum" under the host periosteum in the above situations.

Kazanjian and Converse (1940) described a treatment plan for managing osteomyelitis of the frontal bone. Wide full thickness forehead flaps were created to expose the diseased bone and left open until the acute phase had passed. The flaps were subsequently primarily closed from a series of 16 patients, complete bone regeneration occurred in only 2 boys, eight and nine years old, 18 months after treatment. They argued that while periosteum could be an osteogenic tissue, it was too unreliable to be depended on. They preferred reconstruction with tibial

osteoperiosteal grafts.

Urist and McLean (1941) produced tibial fractures in 7 week old rats. They found strong evidence of subperiosteal trabecular bone forming from the fracture site distally to the limit of traumatically stripped periosteum. The subperiosteal callus (along with endosteal callus) was actively engaged in replacing the first formed fibrocartilage callus.

McBride (1943) argued that osteoperiosteal grafts were excellent for producing new bone but had no strength. He added a plate over the graft to add strength and compress the graft on to the bed. McBride used plated osteoperiosteal grafts in beds of chronically inflamed tissue where the inflammation provided a mild stimulus for bone formation. He briefly presented a series of seventeen cases, with only 2 failures from his procedure.

Urist and Johnson (1943) described fracture healing, using biopsies from 48 of their clinical cases. They felt several different processes could occur simultaneously making the overall mechanism confusing. By the end of the first week, the inflamed periosteum was seen to produce osteoblasts from the spindle-shaped cells of the inner layer of periosteum. The osteoblasts were actively involved in producing bony callus. Both hyaline cartilage and fibrocartilage masses were abundant in the first formed callus under the subperiosteal callus, possibly through the increased vascularity of the intitial granulation tissue. They had no explanation for cartilage formed in subperiosteal callus proper.

Bourne (1944) argued that many stages of bone healing occured at the same time and histological study of serial sections was inaccurate unless the same area was viewed in every section. He drilled 1.0 mm holes through the periosteum as well as the outer cortical plate of th femur and parietal bone in rats and guinea pigs. All animals were sacrified at intervals up to 2 weeks after surgery.

He observed bone healing to be essentially similar in all cases. By 3 days he noted a generalized periosteal reaction with fibrous subperiosteal callus being formed. He observed several small bone chips in the defects; those without periosteum were undergoing resorption while chips covered with periosteum were undergoing subperiosteal osteogenic activity. Periosteum did not cross the defect (5-7 days) until the endosteal callus had reacted first to close the defect with osteoid trabeculae (3 days). Healing appeared to be species-related as rat femora healed more rapidly (7 days) than guinea pig femora (10-14 days). Healing was also location specific as guinea pig calvaria were not healed in many cases by 2 weeks. Cartilage was occasionally seen to arise from the femoral periosteum in both animals.

He repeated his experiments, placing rats and guinea pigs on a scorbutic diet. Small groups were given subcutaneous injections of vitamin C pre and postoperatively. Minimal periosteal reaction was seen with a scorbutic diet while trabeculae were not seen unless 1.0 mgm daily supplements were given. By contrast a definite endosteal reaction was not seen unless 1.0 mgm of vitamin C was given daily (Bourne 1942, 1944).

Byars (1946) advised extraoral resection of benign mandibular tumors so as to leave as much periosteum bridging the defect as possible. Following resection he advised securing the fragments with an internal bar, finally closing the oral and skin defects as carefully as possible. An 8 year old girl was given this procedure to remove a right body ossifying fibroma, leaving only the lingual periosteum intact. Firm clinical union with radiographic evidence of new subperiosteum was evident by 8 weeks. The patient was re-evaluated at 23 years of age, with no change in occlusion or loss of full mandibular movements (Byars and Schatten, 1960).

Byars and Schatten (1960) described another case of left mandibular body resection for fibrous dysplasia. The lingual periosteum and only part of the buccal periosteum was left intact. New subperiosteal bone with firm clinical union became evident by 5 weeks after surgery. The subperiosteal bone mass increased until the patient was lost to follow-up one year later. By this time the occlusion was normal, mandibular movements full and external appearance excellent.

Hoyer (1946) subperiosteally removed entire fibulae in rabbits of unspecified age. One cavity was filled with bone paste and the wound closed. The other cavity was either filled with course bone chips in or left empty. The rabbits were sacrificed after 3 weeks with the most new bone produced from the bone paste-filled cavity and the least bone produced from periosteum alone. Hoyer concluded the results were due to the relative ease of vascularization of the bone paste.

Kazajian (1946) removed (intraorally) a giant cell tumor from the left mandibular body and ramus, in a 10 year old male, leaving periosteum intact. Eight months later bone regeneration had occurred in the mandibular body. By 7 1/2 months the mass, now diagnosed as an ameloblastoma, had recurred and was resected from the mandibular body to the sigmoid notch. Five months after surgery subperiosteal bone regeneration had occurred sufficient to remove the fixation appliance.

Pritchard (1946) argued that defects of the cranial vault heal primarily by fibrous union and smaller amounts of bone were formed than in healing of long bones. He created saw cut linear defects (from .15 mm x 1.0 mm) in parietal bones of 12 to 18 month old rats. The cut passed through periosteum, bone and dura. By 4 days the first subperiosteal callus was laid down on either side of the defect (as it was on the dural surface). While the callus extended 1 mm from the margin on sound bone into the gap, healing occurred by fibrous tissue infiltration. No bone bridges were seen. Healing was complete between 16 to 24 days.

A second series of rats were subject to knife cut fractures (15 mm x 0.1-0.5 mm), with healing occuring by the same mechanisms. One rat produced a subperiosteal cartilage nodule by 10 days although no explanation was given for this. The defect healed by fibrous union although a number of bone bridges uniting the fragments were observed. Pritchard concluded local factors contributing to calvarial healing included tight contact of fragments, intact dural and periosteal bridges and lack of interposing fibrous connective tissue.

DeBruyn (1947) devitalized bone, marrow and periosteum by freezing with either dry ice or liquid nitrogen. The grafts were transplanted autogenously or allogenically into the thighs of rabbits while a similar series was carried out with fresh tissues. All rabbits were sacrificed from 6 to 24 days. None of the allogenic grafts formed bone and there was no real difference in graft activity between the two freezing agents. None of the 12 frozen periosteal grafts produced bone while 3 of 12 fresh periosteal grafts produced osseous tissue. Both fresh and frozen cortical bone and marrow produced bone more frequently than periosteum. DeBruyn concluded that freezing kills cells and may have destroyed a bone inducing substance if one was present. He suggested bone may have been produced from the frozen grafts by an unknown mechanism.

Weinman and Sicher (1947) described periosteum as having two layers similar to the observations of previous authors. They added a description of an abundant periosteal nerve supply in the outer fibrous layer; mostly sensory fibres with the occasional small Vater-Pacini corpuscle. Weinman and Sicher argued that periosteal blood vessels entered cortical bone through Volkmann's canals and the Haversian system to anastamose with vessels of the medullary artery. They felt periosteum had a definite osteogenic function, especially in correcting bone loss from disease.

Macomber (1948) used tibial autogenous osteoperiosteal bone grafts as the primary graft to repair bone defects of less than 1/2 cm in 36 cases. Clinical union by 10.3 weeks (average) was achieved. Macomber

felt the osteoperiosteal graft could also be used as a "feeder" graft where a secondary graft was necessary.

PERIOSTEUM WITHOUT OSTEOGENIC FUNCTION 1925-1949

Stump (1925) reviewed the contemporary literature, concluding that periosteum was only a limiting membrane of bone, and an end stage of periosteal connective tissue development. He argued that primitive connective tissue independently formed osteoblasts as it could form osteoblasts, chondroblasts, fibroblasts, perichondrium or periosteum depending on local needs.

Leriche and Policard (1926, 1928) described periosteum as a fibrous membrane covering bone, except where muscles inserted, and separating bone from the surrounding connective tissue. They felt periosteum developed as condensation of soft tissues surrounding bone, being condensed and forced back as the bone grew. They made no mention, however, of subperiosteal bone formation in endochondral osteogenesis. The adult periosteum developed an outer layer of loose connective tissue plus an inner layer of fibrous and elastic tissue, interspersed with fusiform connective tissue cells. They denied the presence of a cambial layer.

Leriche and Policard did not believe periosteum could produce bone. They argued that periosteum irritated by trauma or infection would revert to its embryonic state ("congested periosteum") becoming an area for osteogenesis to occur ("an ossifiable medium"). Periosteum would seem to form bone provided small slivers of bone were adherent to its deep surface. As the bone slivers died, they produced "calcareous

materials" which induced new bone formation. As an example, they described these bone slivers being resorbed in the presence of new formed bone. (J. Key, their translator took exception to this point, stating the adherent bone slivers lived and acted as nuclei for new bone formation). They rejected Ollier's belief that periosteum created bone, stating he removed bone chips with his grafts through sharp dissection. MacEwen, on the other hand, was careful not to remove bone chips (blunt dissection) with no bone arising from the graft.

Bancroft (1929) described the events of fracture healing, but felt the origin and function of cells contributing to these events were as yet unknown. Although he admitted periosteum did play a role in fracture healing and regeneration when transferred as a graft, he questioned whether the periosteum was directly responsible for osteogenesis or whether the changed environment induced connective tissue cells to undergo metaplasia.

Murray (1930) described fracture healing in the adult. He observed when intact periosteum bridged the fracture site, periosteum, endosteum, marrow and soft tissues of Haversian canals all provided soft tissues for healing. Torn periosteum appeared to undergo repair from overlying connective tissue. The repair ("pseudoperiosteum") resembled its normal counterpart except for the absence of a vascular inner layer. Both periosteal tissues appeared to function normally, providing the first formed granulation tissue at the fracture site. He felt other soft tissues within bone or surrounding it could supply the granulation tissue. He presented the view that osteoblasts originally present or

derived through metaplastic change from the surrounding fibroblasts may have played a part in bone production, but more important were local disturbances of blood flow.

Pollock and Henderson (1940) transplanted bone segments from one femur to the other on the same dog (ages of dogs not given). One fragment was transplanted with periosteum and one without. Five dogs were sacrificed from 99-113 days postoperatively. Where periosteum was removed, a fibrous periosteal tissue regenerated over the graft, while 4 of 5 transplants showed new bone growth. Only 2 of 5 periosteally covered grafts demonstrated osseous formation. Pollock and Henderson concluded that periosteum was not an important tissue for bone grafting success.

Schram and Fosdick (1943) believed periosteum had an osteogenic activity, based in part on a series of briefly summarized experiments. They removed 2 cm segments of radial bone and periosteum from dogs of unspecified age. The defect consistently healed by fibrous union. When bone was resected and synthetic bone paste implanted, partial bone regeneration was observed. Shram and Fosdick did not give the one paste composition. When the previous experiment was repeated and the defect wrapped with Tantalum (Tm) foil, essentially complete bone union was observed. No bone union occured when the defect was wrapped with tantalum foil, excluding bone paste fill. They concluded periosteum was only a limiting membrane, preventing embryonic bone cells from reaching the injured area.

Hutchison (1949) transplanted iliac crest bone both autogenously and

allogeneically into the anterior chamber of rabbits' eyes. The bone was fragmented with chips 1-2 mm in diameter. By 180 days the grafts had developed a new periosteum, supposedly from both graft and host cells. None of the allogeneic grafts survived. He concluded that small autogenous bone grafts would survive in this environment regardless of the presence or absence of periosteum.

PERIOSTEUM AS AN OSTEOGENIC TISSUE 1950-1969

Vainio (1950) argued that previous researchers performed similar experiments but arrived at different conclusions since it was impossible to tell if osteogenic cells arose from the grafted osseous tissue or the surrounding connective tissues.

He created full thickness ulnar defects in rabbits aged 7 months to over 2 years old. The bone segment was split and autogenously replaced with or without periosteum. In some animals the graft was replaced with the marrow facing outward, again with some and some without periosteum. The younger rabbits were kept up to 90 days while the older rabbits were kept up to 360 days.

In all cases where periosteum was maintained, a proliferation of periosteal tissue was seen as early as 55 days while new bone formed after 2 weeks. Subperiosteal callus arising from both normally-placed grafts and host bone united by 3 weeks.

In some cases of inverted periosteum covered grafts, the subperiosteal host callus passed deeply to meet the subperiosteal graft callus. Most of the periosteum covered grafts preserved their shape and produced new bone thicker than the original graft. The periosteally covered transplants, correctly placed, regenerated in young animals by 4 months, while the inverted graft regenerated more slowly. Grafts without periosteum developed a thin osteoid layer 15 to 30 days after surgery,

blending with both the overlying connective tissue and underlying cortical bone. No callus developed, and the subperiosteal host callus united with fibrous tissue on the cortical surface. When the graft was inverted, host subperiosteal callus joined with the medullary callus. As healing progressed, a new membrane, similar in appearance to resting periosteum developed on the cortical bone surface.

The periosteal free grafts tended to resorb early, healing with thinner bone than the original graft. Both types of grafts healed more slowly than those covered with periosteum. In all cases healing was produced more slowly in the older animals. Vainio noted transitory cartilage forming in early callus, in regions of pressure or shearing.

He concluded that subperiosteal osteoblasts were primarily responsible for union and healing of the graft. He postulated that trauma, such as taking a graft released a substance from the bone cortex which could dissolve the superficial layers of bone, freeing osteocytes to the subperiosteal cambial layer. He also postulated this same substance could induce a minor degree of osteogenic metaplasia from the surrounding connective tissue. As periosteally covered grafts maintained a proper anatomical shape, Vainio concluded periosteum also carried an organizing function.

Marrangoni (1951) transplanted cortical bone grafts from an unnamed source to the radii of dogs (age not given). All grafts were stored at $-15^{o}C$ to $-25^{o}C$ for an unspecified period of time before transfer. Autogenous grafts showed a marked dilatation and necrosis of the

Haversian systems during the first two weeks, thereafter 25% of the laumae near periosteal or endosteal surfaces contained viable osteocytes. Similar necrosis was seen with homogeneous grafts, with no lacunar filling during the same time period. Marrangoni concluded that frozen grafts contributed nothing to osteogenic activity, this arose instead presumably from host periosteum and endosteum. The healing process appeared to take 50% longer in allografts than autografts.

Peer (1951) briefly described a number of experiments on human subjects. He found that autogenous rib, tibia and iliac (endochondral) grafts with or without periosteum were replaced by fibrous tissue after 6-8 months after transplantation into soft tissues. Iliac and rib grafts autogenously transplanted over mastoid and malar periosteum suffered a similar fate. When the grafts were stripped of periosteum, placed against bone and subject to functional stress, they tended to survive. Nasal, vomer (intramembranous) and perpendicular plate of ethmoid (endochondral) grafts autogenously transplanted into soft tissues survived for up to 5 years.

Peer subperiosteally resected a rib segment, finding the defect completely regenerated by 18 months. A similar result was seen when a periosteally stripped rib segment was returned to its bed. While he admitted periosteum, or host bone healed the defect, he suggested that bones unable to regenerate after resection (nasal bone, vomer, perpendicular plate of ethmoid) survived the longest whether in contact with host bone or not. Conversely, bones able to regenerate new osseous tissue (rib, tibia, ilium) were soon resorbed whether transplanted with

periosteum or not.

Urist and McLean (1952) transplanted autogenous tibial periosteum into the anterior eye chamber of three week old rats. Half the grafts produced intramembranous bone after 15 days, transforming into cortical bone without marrow by 5 weeks. Tibia of adult rats were fractured and the thickened periosteum transplanted to the eye after 1 to 4 days. New bone formed after 24 hours while unstimulated adult tibial periosteum transplanted as a control produced none. All periosteal grafts transplanted 96 hours after fracture demonstrated cancellous bone although the authors admit the bone may have come from the healing tibial fracture.

Fibrocartilaginous callus grafts from 10 to 12 day old closed tibial fractures were autogenously transplanted into the eye. Only those specimens without bone were evaluated. The graft invariably attached to the iris, a subsequent acute inflammatory reaction following during the first week. The transplant developed a fibrous capsule during the first week with the capsular tissues appearing to arise from the host. The capsule developed an inner vascular and cellular layer with the formation of new subcapsular bone. By 5 weeks the graft was replaced by an ossicle of cortical bone containing active red marrow. No description of the surrounding capsule was given. When fibrocartilaginous callus was devitalized by boiling or freezing, three specimens produced similar ossicles after 4 to 5 weeks. Other tissues including bone marrow, blood clot, muscle cortical bone and cartilage were implanted with variable results.

The authors concluded that autogenously grafted periosteum was capable of surviving and producing intramembranous-type bone. They termed periosteum "histogenic" as it produced only a single tissue. The fibrocartilaginous callus produced new bone earlier and in greater amount than the other transplants. Urist and McLean argued that the tissue was both osteogenic and osteoinductive as the host tissue appeared to supply the cells causing new bone formation in both thefresh and devitalized transplants. They called the fibrocartilage "organogenic" as it appeared to form a complex structure of many tissues.

Urist and McLean used their theory of osteoinduction to describe fracture repair. They felt periosteum had "osteogenic potency" as it was capable of directly producing new bone after injury or transplant. Adult resting periosteum could not produce bone but was "modulated" by injury into a temporary osteogenic function. Fibrocartilaginous callus, however, behaved as an embryonic inductor, influencing the adjacent tissues to begin osteogenesis. They felt both factors, osteogenesis and osteoinduction were locally operational in a heaing fracture (Urist and McLean, 1953). They further argued that periosteum could regenerate both itself or new bone, this property decreasing with the age of the individual (McLean and Urist, 1955).

Cohen and Lacroix (1955) argued that many factors influenced the success of bone grafts, including source of graft, size of graft, tissues taken, age of host or donor, and abundant blood supply. They felt experimental investigation of bone grafts could be evaluated if only one variable was

investigated. In their experiments all factors except the host bed were standardized.

Anterior tibial periosteal grafts were autogenously transplanted into the anterior eye chamber, the kidney surface under its capsule, and onto the cortical surface of tibia. All animals used were 5 week old rabbits, sacrificed at 10-14 days. The least amount of woven bone was found in 6 of 8 intraocular grafts and no cartilage was present.

All 8 subcapsular grafts produced an intermediate amount of bone resembling callus, while 2 grafts produced hyaline-like cartilage. Two adult rabbits produced a lesser amount of subcapsular periosteal bone after 30 days. No difference was seen whether the cambial layer faced kidney or capsular tissue. All 8 tibial grafts (taken from middle tibia and placed on upper tibia) produced the greatest amount of mature callus-like bone as well as hyaline-like cartilage. The largest trabeculae were found near the free edge of host periosteum while a generalized periosteal reaction was present over half the shaft circumference in the graft site. Variable amounts of bone were found between the periosteum and cortical bone interface. Most cartilage formed with maximum bone production although no explanation was given for this. Although none of the grafts exhibited necrosis, vascularity did not appear to be a controlling factor as the most vascular bed, the kidney, did not produce the most bone.

Cohen and Lacroix concluded that unspecified chemical factors influenced bone production. They recommended including periosteum with bone

grafts, however as it was a proven bone producer.

Axhausen (1956) transplanted autogenous rib cortical bone grafts into muscle tissue of adult dogs. When periosteum or endosteum was included, new bone was produced by 4 days. New bone formed much later and in smaller amounts when cortical bone alone was grafted. The bone appeared to arise from the graft surfaces.

Free autogenous periosteal grafts produced bone when stretched and placed in contact with recipient cortex. Periosteally covered grafts were frozen for 24 hours and transplanted. After 10 to 35 days the grafted tissues were necrotic and undergoing resorption. No new bone was present. After 30 days new bone was seen at the sites of bone resorption, rather than the graft surface. Bone grafts denatured with alcohol began resorbing by 4 weeks while new bone was seen after 12 weeks.

Axhausen argued that bone formation occurred in two phases. The first bone was produced by osteogenic tissue (including periosteum) of the graft while necrotic organic bone products induced surrounding connective tissues to develop a second osteogenic function. As proof he cited frozen bone as producing osseous tissue much earlier than organically denatured bone. He felt necrotic bone had to be resorbed to expose the organic components while necrotic periosteum impeded this process. As stripping periosteum damaged the cambial osteogenic layer, Axhausen recommended using osteoperiosteal grafts for maximum new bone formation.

Tonna (1958) studied the periosteum in both infant and young rats. He found the inner layer to be highly cellular, with osteoblast-like cells arranged along the bone and an outer fibrous layer containing spindle-shaped fibroblast-like cells. With age, the fibrous layer became thicker while the osteoblastic layer became more fibrous and less cellular. The cells came to resemble fibroblasts lying in a random pattern. Alkaline and acid phosphatase activity was localized in the osteogenic layer, the activity increasing during osteogenesis and decreasing but not disappearing with age. The respiratory enzymes cytochrome oxidase and succinic dehydrogenase were most active in the osteogenic layer of infant rats. Activity was greatest during osteogenesis, sharply decreasing by 5 weeks of age. Tonna postulated their activity may have been related to mitochondral aging.

Tonna (1959) drilled holes through periosteum and the full cortical thickness of femurs in rats 1 to 104 weeks old. Animals were sacrificed 1 to 2 weeks after surgery, and tissues were treated specifically to evaluate enzyme activity. Acid and alkaline phosphatase activity was elevated in all age groups studied and found to be especially strong in cells and fibres of both periosteal layers growing into the defect. The respiratory enzymes cytochrome oxidase and succinic dehydrogenase showed levels elevated beyond normal, especially adjacent to the area of trauma. The enzyme elevation became less with age after 5 weeks but was always present in some degree.

Tonna felt the periosteal cells were stimulated to repair injured bone at all ages, except during the time of maximum bone growth (in rats 5

weeks of age) when trauma could produce no further stimulation of periosteal activity.

Tonna (1960a) created mid diaphyseal fractures in femurs of female mice 1 to 52 weeks old, the animals being sacrificed up to 2 weeks after injury. Each animal was given tritiaded thymidine one hour before sacrifice. Periosteal cells near the fracture became labelled by 24 hours after injury, the osteogenic cells being labelled more than others. The degree of labelling decreased with the age of the animal. The chondrogenic cells about cartilagenous callus were densely labelled although chondrocytes proper were not. As bone callus was being formed both osteogenic and osteoprogenitor (osteoblastic) cells were labelled. No diagrams or photos were given with the abstract. Tonna concluded that fracture repair was accomplished primarily by periosteal cells although their potential for healing decreased with age. He did not mention which layers of periosteal cells produced bone although he inferred the cambial osteoblasts were responsible for this function.

Tonna (1960b) repeated his experiments as before, labelling mice with tritiated thymidine 1 hour before sacrifice. He again described increasing density of periosteal labelling around the fracture site (less so in older animals) although osteoclasts (and osteocytes) did not take up the label. Tonna postulated from his serially static series of slides that periosteal osteoclasts were formed by fusion of osteocytes and dissolved to form osteoprogenitor cells. Tonna did not explain why a labelled osteoblast could not incorporate with an osteoclast at least partially labelling the latter cell.

Tonna (1961) took female Swiss Albino mice, 1 to 52 weeks of age giving them tritiated thymidine 1 hour before sacrifice. Femurs were removed and examined histologically. Osteogenic cells in the periosteum were most frequently labelled, osteoblasts less so and fibroblasts least of all. The most densely labelled areas were around perichondrial regions (and frequently the endosteum). Overall labelling decreased in mice up to 8 weeks of age then remained constant in mice beyond this age. In young animals, labelled osteogenic cells were seen extending into the epiphyseal disk while chondroblasts were labelled only in the hypertrophic and proliferative zones. In a second group of 5 week old mice, the animals were labelled and sacrificed 1 hour to 25 days after labelling. Labelled cells appeared to migrate from perichondrial periosteum to the distal articulating surface and epiphyseal disk. Tonna concluded that periosteal osteoblastic precursors and osteoblasts in young mice could divide while periosteum was directly responsible for appositional bone growth and indirectly for endochondral growth.

Tonna and Cronkite (1961a) fractured the femurs of 5 week old Swiss Albino mice, examining the fracture healing from 1 to 32 hours and 2 to 14 days after fracture. Mice were given tritiated thymidine one hour before sacrifice. The preosteoblasts of normal diaphyseal periosteum labelled to no more than 2% of the total preosteoblast population throughout the experiment. The preosteoblasts more frequently took up the label than either fibroblasts or osteoblasts. In the fractured leg an increased labelling of preosteoblasts and surrounding mesenchymal cells occurred along the length of the diaphysis by 16 hours. Later, osteoblasts and fibroblasts became labelled, indicating cell

proliferation. By 32 hours after injury, a maximum of 25.4% of periosteal cells were labelled. By contrast only 0.01% of endosteal cells were labelled by 24 hours after injury. Cells distant from the fracture decreased their labelling frequency beginning at 32 hours after injury to reach a normal control value by 5 days. Periosteal cells in the fracture area remained more densely labelled for up to 14 days. Dense labelling of all cells in the zone of callus formation were present until the fracture was healed. Tonna and Cronkite suggested viable osteogenic cells such as preosteoblasts were necessary for new bone formation as was the possibility of a substance released at the time of injury causing a relatively widespread periosteal response.

Tonna and Cronkite (1961b) continued their researches using female Swiss Albino mice aged 1 to 52 weeks, fracturing the right femur and administering tritiated thymidine one hour before sacrifice. Animals were sacrified 1 hour to 2 weeks after fracture. The authors found a normal femoral periosteum decreased in overall thickness (136 microns to 48 microns) with increased age as did the thickness of the osteogenic layer (77 microns to 14 microns). The 52 week old fibrous layer decreased 41% of its thickness from 1 week while the osteogenic layer decreased 81%. Tonna and Cronkite found rats of all ages increased the thickness of their periosteum after injury by cellular proliferation although this happened much more slowly in older animals, because of less precursor cells present at the time of fracture. Fibroblast production appeared to be autonomous from osteoblast production while the fibrous periosteal layer contributed little, if any, to the osteogenic proliferation. Preosteoblasts appeared to produce

chondrogenic cells and osteoblasts which in turn produced fracture callus. Osteocytes and osteoblasts, being non labelled were formed from transformation or fusion of these preosteoblastic parent cells.

Tonna and Cronkite (1962) studied 4 week old Brookhaven National Laboratory female mice, administering tritiated thymidine and sacrificing the animals 5 minutes to 25 days after injection. The femurs were removed and examined for labelled periosteal and bone cells. Their findings led them to believe the osteogenic periosteum was self-generating, increasing in size to produce osteoblasts which produced new bone as well as accommodation of larger cortical diameter during growth. They felt periosteum decreased in thickness from stretching during growth, loss of cells to the osteocyte pool and reduction of regenerative ability. They estimated a cell cycle of 7 days to 19.4 days for a cell in the osteogenic layer of periosteum.

Tonna and Cronkite (1963) reported a series of mice given tritiated thymidine 24 hours after birth. The mice were allowed to live normally for 9 months, and no alterations in growth were seen. After 9 months labelled cells were occasionally found of fibrous periosteum, cartilage cells near the epiphysis, epiphyseal osteocytes (derived from periosteum?) and fibrocartilaginous cells. The series demonstrated at least some cells of periosteum and bone possessed a long life span. Tritiated thymidine was given by daily continuous infusion to rats (species and age not given) over 30 days demonstrating areas of dense labelling in both periosteum and endosteum. Labelling was also seen in osteocytes of both cortical and trabecular bone. Tonna and Cronkite

felt the results suggested periosteal cells in the osteogenic layer were of one population, dividing regularly and increasing their proliferation when stimulated by trauma. In a further experiment "young" mice were subjected to 836 rads of total body radiation with one femur fractured in half the mice immediately after irradiation and half 6 days later. All rats were given tritiated thymidine 29 hours after fracture, and sacrificed 1 hour later. The irradiated fractures began healing but with less labelling than nonirradiated fractures. More cells were labelled in fractures sacrificed 6 days after irradiation than those sacrificed 30 hours after irradiation suggesting some healing from radiation injury had occurred. Cronkite again suggested periosteum produced new bone purely from the osteogenic layer.

Tonna and Cronkite (1964) stimulated periosteal response in the femurs of 5 to 6 week old Brookhaven Swiss Albino mice by manually fracturing the bones or injecting normal saline, pooled fresh mouse whole blood with EDTA or fresh mouse serum over the periosteum at mid-diaphyseal level. Twenty-nine hours after treatment the mice were administered tritiated thymidine and sacrificed one hour later. In a second series, mice were administered 876 rads of total body radiation in an effort to destroy their white blood cells. Whole blood from this series was pooled 30 hours or 6 days after irradiation. Non-irradiated mice were injected supraperiosteally with the 30 hour post-irradiation whole blood, or 6 hour post-irradiation whole blood. The mice were subjected to both femoral fracture and injections of tritiated thymidine as in the previous experiment. The results showed a periosteal response was not initiated solely by its manipulation in a fracture. Response was

similar after injection of saline and blood products suggesting factors in blood alone did not stimulate a periosteal response. They felt the only common factor of periosteal stimulation was local hypoxia following rupture of vascularity, producing a specific tissue factor capable of stimulating periosteum.

Sirola (1960) created calvarial defects in rabbits from 4 days to 3 years of age and 8 cats, aged 1 week to adulthood. Periosteum and full thickness skull bone was removed from the fronto-parietal region of rabbits. After 4 months, complete regeneration of calvarium, including sutures, occurred in very young rabbits while substantial regeneration occurred in adults. Similar results were seen when cats of different ages were similarly treated. When periosteum was retained as a lateral flap, in 6-8 week old rabbits, and calvarium removed, healing was similar to the first group.

When polyethylene film was placed between dura and subcutaneous tissue after removal of periosteum, most bone regeneration occurred between dura and the film. Little bone was found arising from bone edges and none from the subcutaneous tissue. When polyethylene was placed to isolate the bone margins, bone islands were seen to arise from the dura. When dura was removed the defect remained present 3 months later. When polyethylene film was placed between arachnoid and periosteum, bone islands formed on the inner surface of the film.

Sirola concluded that calvarial regeneration occurred from periosteum, dura, bone margins, and connective tissue within the defect. Both dura

and periosteum supplied vascularity to calvarial bone, although dura had the more important role. In none of the cases did periosteum produce bone without dural influence. Other factors, such as animal species, age, and location of the defect were thought important in the amount of regeneration.

Dineen and Gresham (1962) described their experiences with osteoperiosteal rib grafts from the 7th and 8th rib, taken at the midaxillary line. They used the grafts in over 50 patients finding the tissues easy to harvest and capable of being placed almost anywhere a fracture or nonunion had occurred except for size-limited procedures such as spinal fusion. Supplementary internal fixation was found necessary for success.

Frankel and Derian (1962) subperiosteally dissected out the femoral head and neck in adult mongrel dogs, such that no blood supply remained attached. The femoral head was disarticulated, cut through the neck and replaced as a free graft with fixation to the femoral neck by a stainless steel screw. After 60 days all 10 dogs not infected, had developed a vascular necrosis of the femoral head, 50% of these showing a vascular necrosis with nonunion. The operation was repeated except either gluteus medis or vastus lateralis muscle was pedicled to the femoral neck before closure. Eighteen dogs were used, 2 showing vascular necrosis, 2 showing nonunion and 14 showing viable femoral heads after 40 days.

Frankel and Derian concluded that femoral subcapital circulation and

bone regeneration could be achieved with a muscle pedicle implanted onto the free graft at least in dogs.

Young (1962) injected tritiated thymidine intraperitoneally into 32 six day old Long-Evans rats, sacrificing the animals from one hour to two weeks after injection. One proximal tibia and three ribs were taken for evaluation. Osteoprogenitor cells took up the radioactive label in the majority of cases. Periosteal cells initially took up the least label, endosteal cells took up more label and metaphyseal tissues took up the greatest amount. Periosteal cells became labelled later (2 hrs) than metaphyseal and endosteal cells (1.5 hours) of both ribs and tibia. Labelled osteoblasts were first seen in the endosteum and metaphysis of both bones by one hour but not in tibial periosteum until 20 hours or rib periosteum until 36 hours. The numbers labelled osteoblasts only occasionally exceeded the number of labelled osteoprogenitor cells in each tissue. Osteoclasts took up the label by 9 hours in all tissues of both ribs and tibiae. Rib periosteum presented labelled osteoclasts before labelled osteoblasts.

Young felt that osteoprogenitor cells were capable of temporarily becoming osteoblasts, osteocytes or contributing to osteoclast formation depending on local immediate needs. He argued these changes were reversible when the original stimulus was removed. The cells were thus "modulated" rather than being "transformed". Periosteal osteoblasts appeared later than endosteal or metaphyseal osteoblasts, presumably through a much longer osteoprogenitor division time.

Wilderman (1963) created split thickness mucoperiosteal flaps in the maxillary and mandibular buccal mucoperiosteum of young dogs. The subsequent soft tissue defects were 5 mm x 33 mm and extended from the alveolar crest to the mucobuccal fold. Dogs were sacrificed up to 180 days after surgery. Wilderman noted that osteoclastic activity began at the alveolar crest, reaching a maximum by 6 days. Defects with connective tissue overlying periosteum greater than 0.45 mm showed osteoclastic activity primarily from the periosteal surface while defects with thinner overlying tissue demonstrated osteoclastic activity primarily on medullary and periodontal surfaces. Areas without soft tissue cover on periosteum showed periosteal necrosis and healing by secondary intention.

Wilderman created a horizontal notch in alveolar cortex at the base of each defect before replacing the flap. Periosteal resorption began at 10 days after surgery, reaching maximum activity on all surfaces by 28 days. By this time medullary bone repair had begun. No conclusions beyond the experimental results were drawn.

Branemark and Breine (1964) created 15-20 mm full thickness defects in the 10th, 11th or 12th rib of 6-10 month old albino rabbits. The periosteum and surrounding blood supply was left intact. Trabecular bone was first seen in the cavity by 21 days, cortical bone by 49 days and a normal rib by 90 days. They repeated the experiment, resecting 30-35 mm rib segments in 3-4 year old dogs. The first trabecular bone was found after 35 days while the defect had regenerated by 150 days. Both animals retained a normal appearing periosteal cover. They

concluded that an adequate blood supply to normal periosteum would completely regenerate new rib segments with cortical bone, cancellous bone and marrow in both adult rabbits and dogs. Rabbit periosteum had a greater ability to form new bone than canine periosteum.

Skoog (1965) argued that growing periosteum had a tremendous osteogenic power and would continue to function when transferred as a flap and sutured over clefts of the primary palate. Skoog developed periosteal flaps from the antero-lateral maxillary surface rotating them approximately 180 degrees to cover the defect. The flaps contained periosteum with only a thin layer of underlying connective tissue and Skoog admitted the blood supply may have been impaired sufficiently to make the flap essentially a free graft. He described four cases of primary palatal clefts in three month old infants repaired by this procedure. (The cleft was at first exposed while palatal microperiosteal flaps were sutured together.) Three and six month radiographic follow-ups showed new subperiosteal bone bridging the clefts with no retardation of facial growth. If the opposite cleft was later surgically treated, Skoog explored the originally operated side. He found a narrow strip of new subperiosteal bone bridging the cleft, the transferred periosteum thinner than normal while a new periosteum, more adherent to bone covered the donor site.

In a series of 52 patients, 3 to 6 months old, nasal periosteal flaps were developed to cover the inner surface of the cleft while a buccal periosteal flap was rotated over the buccal surface. Cortical-cancellous bone bridges were found in 83 clefts of this series. Skoog

placed Surgicel (Tm) in a number of the palatal clefts before covering with periosteum, claiming more abundant bone formation from both periosteum and cleft margins. The new trabecular bone replaced Surgicel (Tm) by 3 months (Skoog, 1967).

Fonkalsrud and coll. (1966) created 3.5 cm x 2.0 cm defects in the anterior cervical trachea of adult mongrel dogs. Free autogenous rib periosteal grafts were placed to cover the defect. When the grafts were loosely placed, minimal calcification occurred; when the grafts were stretched similar results were found. Pedicled periosteal flaps freshly transferred were found to calcify. Pedicled periosteal flaps, delayed for 2 1/2 weeks calcified sufficiently to provide immediate rigid support. Fonkalsrud and coll. concluded that pedicled costal periosteal flaps produced new bone by 2-3 weeks providing a satisfactory tracheal repair if the vascular pedicle remained intact.

Murakami and Emery (1966) studied the wings of chick embryos, and described elastic fibres among periosteal cells at the outer edge of osteoblasts, beingincorporated into the newly formed osseous matrix. Murakami and Emery (1967) continued their researches, studying humeral fracture healing in 500-600 gm guinea pigs. They found an elastic fibrous layer between the outer fibrous and inner cambial layers of periosteum with elastic fibres running parallel to the long axis of the bone. The elastic layer increased to a maximum thickness 5 days after fracture, returning to normal 2 weeks later. In contrast the osteogenic layer reached maximal thickness by 8 days, returning to normal 2 weeks after injury. Elastic fibres were seen to pass from this layer

perpendicularly either into bone, or into the centres of endochondral bone formation. The elastic layer appeared to act as a limiting membrane to bone growth. Murakami and Emery felt an increased thickness of the inner elastic layer corresponded with an increased number of osteogenic cells. They concluded the fibrous layer of periosteum was composed largely of elastic fibres while the cells between these fibres (fibroblasts) could form osteogenic cells, elastoblasts or other cells.

Lindholm and coll. (1967) fractured the tibia and fibula of adult white rats, finding mast cells in the mesenchymal regions of periosteal callus. The cells appeared on day 3 after injury increasing to day 13 and finally returning to normal levels, 19 days after injury (corresponding to clinical union). Lindholm postulated mast cells arrived by invasion and correlated with the periosteal callus activity.

Miller (1967) dissected the midshaft of mice femora, burnishing the underlying periosteum with a spatula. Six to 8 days after the procedure, 64% of mice produced a reactive response, while 44% showed histologic evidence of subperiosteal cartilage production. Twenty percent of the surgical mice demonstrated normal periosteal response with osteophyte growth. Labelling of mice with tritiated thymidine showed the heaviest uptake in the osteogenic layer of periosteum. Guinea pigs and rabbits treated similarly demonstrated bone production without cartilage formation. Miller concluded that cartilage was formed directly from subperiosteal osteoblasts and may have been a species specific reaction.

Westin (1967) described a procedure for lengthening of the femur, first creating a periosteal sleeve 5 inches above and below the proposed transverse osteotomy site. A variable amount of subperiosteal dissection was carried out prior to creating the sleeve. The fragments were stabilized with an intramedullary nail and external fixation splint. In some cases autogenous fibular bone grafts were placed subperiosteally between the fragments of femur. Subperiosteal bone began forming 12-24 days with columnar bone appearing by 6-10 weeks. Complete bone healing was seen 8-18 months after operation. Westin argued the periosteal sleeve contributed to new bone formation as well as limiting osteogenesis to the gap. He felt the technique carried a high degree of complication even if an interpositional bone graft was placed and the lengthening was carried out in stages.

Choucas et al (1968) reflected the inferior two thirds of masseter muscle from the infero-lateral mandibular border of albino rats (age not given). Periosteum was removed from muscle except at the inferior mandibular border where muscle insertion was more tendinous. The muscles were laid back in position. Periosteum remaining on bone laid down new bone for at least 10 days after surgery creating a new tendinous insertion for masseter muscle at the inferior mandibular border.

Costich and Ramfjord (1968) removed full thickness mucoperiosteal blocks 4 mm x 6 mm from the anterior labial alveolar crest of human subjects. Biopsies were taken from the surgical site as healing progressed. (All patients were to have teeth in this region removed for dental and

periodontal disease at a later date.) By one week after surgery the denuded buccal cortex showed signs of sequestration while granulation tissue from the mucosa and periodontal surface began covering the bone. The labial periosteum was thickened for "some distance" from the defect, and showed a chronic inflammatory reaction which extended into the periodontal membrane. Osteoclasts from the medulla and periodontal membrane began resorbing the denuded bone at this stage, later spreading to the periosteal surface. After 3 1/2 weeks the mucosa had regenerated although no mention was made of a regenerated periosteum. The chronic inflammatory response with bone resorption and little new bone formation continued until the denuded labial bone was almost completely resorbed by 6 weeks. Net bone loss was 1.02 mm (range +0.5 mm to -3.8 mm) in 10 patients. Costich and Ramfjord advised retaining epithelium or as much connective tissue as possible over periosteum during surgical manipulation.

The experiment was repeated on 12 patients, leaving a thin layer of connective tissue over the periosteum. Healing with bone resorption on periodontal, medullary and periosteal surfaces continued as before although less bone was lost (0.4 mm average, +.05 to -3.9 mm range) (Ramfjord and Costich, 1968).

Nichols, Toto and Choucas (1968) fractured the right fibula in young rats, subsequently labelling the rats with tritiated thymidine almost immediately before sacrifice. Twelve mice were normal in development while 12 were hypophysectomized before creating the fracture. Normal animals sacrificed 6 and 12 hours after fracture showed a thickening of

the periosteal osteogenic layer with a sharp increase in the number of osteogenic layer labelled cells by 24 hours. Hypophysectomized animals showed an increase in labelling after 18 hours, peaking at 24 hours. Nichols, Toto and Choukas concluded that the periosteal osteogenic response of hypophysectomized rats was less than the periosteal response of unoperated rats.

Saunders (1968) described the use of a pericranial flap in repairing forehead, medial canthal and auricular defects. He argued the flap gave an excellent blood supply for placement of a skin graft.

Melcher (1969) created isolated periosteal flaps over parietal bones in adult Wistar rats. The skin flaps were replaced with and without the underlying separate periosteal flap. He found that subperiosteal bone formed at the periosteal wound edges although none formed under the periosteal flap. In some experiments, millipore filters were placed between periosteum and bone. Osteogenesis was found between the periosteum and the filter, but none between filter and bone. Melcher concluded new bone was produced only by "undisturbed" periosteal cells at the flap edge while an inhibitor substance might be present to prevent division of osteogenic precursor cells in the periosteum.

PERIOSTEUM AS AN OSTEOGENIC TISSUE 1970-1984

Lindholm and Lindholm (1970) fractured the brachium in rabbits, investigating healing from 2-17 days after injury. Periosteal callus appeared after 6-8 days while endosteal callus appeared by 4-5 days. Mast cell appearance however, was twice as great in periosteal as in endostial callus on days 5-7. Lindholm and Lindholm argued that since mast cells carried calcium ions to calcifying areas more cells migrated to periosteum due to mechanical stimulation of fracture fragments. They postulated this migration correlated with increased periosteal cartilaginous callus as well.

O'Brien (1970) used Skoog's periosteal flap, based at the lateral piriform margin, to repair 10 unilateral and 2 bilateral clefts. Follow up extended from 2 months to 4 years. In all cases the first bone formed in the primary cleft by 6 months, continuing to increase with time. At second operation to repair the secondary palate, the maxillary periosteum was seen to have regenerated.

Thompson and Casson (1970) also investigated the fate of onlay bone grafts applied to the mandible of adult mongrel dogs. In one series, cortico-cancellous grafts from iliac crest were stripped of periosteum. The cancellous surface was applied to maxillary and mandibular cortex as autografts or delayed allogenic grafts. In a second series, grafts with periosteum were placed on the maxilla (autogenously or as allografts) while those applied to the mandible were stripped of periosteum with

cortical surfaces placed next to host bone. The recipient bed was prepared by removal of the cortical plate. Specimens were examined up to 52 weeks after surgery. Inclusion of periosteum, on maxillary autogenous grafts only, appeared to produce new subperiosteal bone. This tissue united with host bone growing into and replacing grafted bone tissue. After two weeks, periosteally covered grafts contained at least 10% of lacunae containing living osteocytes while those without periosteum retained only 10%. The graft with periosteum retained 60% of its initial volume after 32 weeks and 50% by one year. In contrast, bone without periosteum retained only 5% of its volume after one year. All homografts elicited an inflammatory host reaction, with delayed union and increased resorption of the graft. Periosteum on the graft surface decreased bone resorption and increased graft survival. Thompson and Casson concluded that periosteum extended viability of bone grafts, allowed early vascular union with subsequent appearance of osteoblasts and decreased the rate of graft resorption. Fresh autogenous bone grafts were superior to all others in these experiments.

Budal (1970) described a case of subsequent healing following removal of an osteofibroma involving almost the entire mandibular body in a 31 year old Cameroun female. The tumor was removed intraorally. By 2 weeks new bone was evident radiographically while a full new subperiosteal bone bridge had formed 3 weeks after resection. Budal followed the case (Budal, 1979) describing a complete functional regeneration of the mandibular body 5 years after surgery.

Beasley (1971) described a pilot study using 28 adult male albino rats

200-250 grams in size. The inferior border of the mandible was dissected to the level of periosteum.

In one group the periosteum on both sides was reflected and the bone on the right side burnished.

In a second group bone was burnished without reflecting periosteum soft tissues were replaced and all animals were sacrificed at 1-21 days after surgery. By the first day, the group with reflected periosteum showed a greater inflammatory reaction, mild resorption by 3 days and new lamellar bone (remodelling) by 21 days. The group without reflected periosteum demonstrated new bone formation by 10 days, but with poor bone production by 21 days.

Beasley concluded that stripping periosteum stimulated bone growth after 5 days with remodelling of new bone after 10 days. Burnishing cortical bone resulted in a greater osteoblastic response. Injury to intact periosteum, such as burnishing, delayed osseous proliferation until 14 days with minimal new bone formation.

Ham and Harris (1971) reviewed the events of fracture healing, describing the first formal external callus as arising from periosteum, specifically from the deep cellular layer. Tearing or excessive manipulation of periosteum destroyed the compensating periosteal blood supply, allowing fibrous tissue to interpose between the fragments. Both factors caused non-union.

Melcher (1971) continued his studies on the behavior of periosteum with monkeys (Macaca Irus) 2.2-2.9 kg. Through an extraoral approach, the mandibular periosteum with some overlying connective tissue was exposed. On each side bur cuts were made through buccal cortex into medulla. A periosteal flap between 2 cuts was developed on one side, elevated and replaced. Animals were sacrificed 10 days after surgery. New periosteal bone was found on both sides of each bone cut where periosteum was not raised. Where a periosteal flap was raised, new subperiosteal bone was found only where slivers of cortex were elevated with the flap. Subperiosteal bone was routinely seen beside the bone cuts where a flap had not been created. New endosteal bone was routinely seen on both types of treatment.

Melcher concluded that new subperiosteal bone was created by osteoblasts of the cambial layer, stimulated by trauma but destroyed by creation of a mucoperiosteal flap. He felt mucoperiosteal flaps would produce more bone if raised as osteoperiosteum, thus protecting the osteoblasts. Melcher felt endosteum being undisturbed played a greater role in healing wounds of the mandible.

Melcher and Accursi (1971) created osteoperiosteal-periosteal flaps in the calvaria of 12 middle aged rats. The flaps were raised posteriorly to the trapezius muscle, a bone chisel being used to raise the osteoperiosteal component from the anterior parietal bone. The left flap was removed with a thin layer of bone while the right flap contained most of the cortical outer table. The remainder (posterior portion) of the flap was composed of periosteum alone. Each flap was

placed into a pocket of trapezius muscle on the ipsilateral side.

All flaps formed new bone between osteoperiosteal bone and periosteum, the bone itself appearing necrotic. New bone appeared to be similar in amount whether the osseous position of the flap was thin or thick. New subperiosteal callus was also found at the base of all flaps. No new bone was found arising from the purely periosteal flap.

Melcher and Accursi argued that the cambial layer of growing animals was thick enough to allow elevation of a periosteal flap without damaging all of the osteogenic cells. The adult periosteum with only a thin layer of osteoprogenitor cells would, however, be damaged by flap elevation unless a thin layer of bone was included.

Melcher (1976) summarized his findings on the response of periosteum to trauma. He described the cambial layer of growing periosteum as having several layers of cells, the deeper layers being osteoprogenitor cells dividing and supplying osteoblasts to the layers nearest bone. Mature periosteum, however, had only a single layer of osteoprogenitor cells next to bone that would first have to divide and produce osteoblasts before new subperiosteal bone could be formed. He argued that elevation of a mature periosteal flap did not produce new bone as the osteo-progenitor cells were destroyed. The periosteum surrounding the flap however would be stimulated to divide, their osteocytes producing new bone. If a mature osteoperiosteal flap were raised the osteoprogenitor cells would not be destroyed but produce osteoblasts and further new bone. Young actively growing periosteum, being multilayered, would not

have osteoprogenitor cells destroyed after creation of a flap. The osteoprogenitic cells actively producing osteoblasts would readily produce new bone after trauma.

Piatier-Piketty and Zucman (1971) experimented with adult rabbits 17-21 weeks old, scraping the tibial diaphyseal periosteum (presumably completely around its circumference) at a height of 4.0 cm or circumferentially stripping periosteum from the diaphysis. Periosteal samples were taken up to 48 hours after surgery and placed in a tissue culture medium containing tritiated thymidine for 4 hours. First evidence of cambial cell proliferation was seen by 18 hours with a dramatic increase in labelled cells by 24 hours. By 48 hours no difference was seen in the number of labelled cells although the cambial layer had thickened.

Periosteum was also removed 24 hours after trauma (type not given), placed in tissue culture as before and autogenously grafted to tibial diaphysis along with fresh autogenous marrow from the opposite tibia. The graft survived, with labelled cells from the preosteoblastic region seen after 6 days.

Piatier-Piketty and Zucman concluded that traumatic separation of periosteum initiated division of cells in the deep layers by 18-24 hours, while cells deep in free periosteal grafts could maintain vitality and the ability to divide for at least four days. The authors felt that cells of the deep periosteal layers had the ability to become chondrocytes but did not explain or prove this concept.

Rokkanen and coll. (1971) created fractures in the left distal radius and ulna of full grown dogs, leaving the fracture unfixated for 3 weeks. A dorsal tibial cortical flap pedicled to periosteum, was raised from the fracture site and autogenous iliac cancellous bone placed into the defect. A cast was applied for 3 days to 12 weeks after surgery. By 3 days the periosteum surrounding the injury was producing abundant new bone callus while clinical union was evident by 6 weeks. Rokkanen and coll. postulated the bone marrow also produced new bone as well as forming a scaffold for new bone replacement.

Engdahl (1972) removed part of the premaxillary-maxillary suture in 2-5 week old rabbits. In one group inner and outer periosteum was retained with bone and the cavity filled with blood clot, Surgicel (Tm), autogenous trochanter marrow or autogenous split rib graft. In a second group periosteum was removed and the cavity allowed to fill with blood alone. In a third group only inner periosteum was retained while the cavity was filled as in the first group. A fourth group was treated similarly to the third except inner periosteum was cauterized. Most rabbits were sacrificed at 6 1/2 to 7 months.

Engdahl found periosteum was the most important factor in bone regeneration. Both periosteal layers produced a quicker and earlier repair than the inner layer alone, while total absence of periosteum allowed minimal bone production.

Blood within the cavity provided the minimum amount of bone in the shortest time. Regeneration was seen after the first week while normal

anatomy was restored by 2 to 3 weeks. Occasionally, normally functioning sutures were regenerated. Surgicel (Tm) caused slow and incomplete bone filling. Bone marrow acted like blood but seemed to produce bone independently. Bone grafts produced rapid, massive bone formation, locking the premaxilla and maxilla together. The grafted bone was gradually replaced by host bone even if periosteum was not present.

Hellquist (1972) studied the effects of intramembranous periosteal excision in young rabbits and guinea pigs. He resected periosteum from the sutures of various facial bones, with animals being sacrificed up to 25 weeks after surgery. Periosteal resection across the suture always caused the snout to deviate towards the surgerized site. Hellquist admitted there was a difference in anatomy and vascularity between rabbits and guinea pigs although both showed growth disturbances. Periosteum completely regenerated over denuded bone although remained more adherent to the bone surface. Bone under regenerated periosteum had a rougher surface than unoperated cortex. Hellquist concluded periosteal stripping caused hypervascularity of regenerated periosteum, subsequently causing appositional bone formation.

Histologic examination showed hematomas present in the surgical site up to 7 days after surgery with periosteal remnants including osteoblasts on the bone surface. By 10 days the periosteum had completely regenerated into an abundantly cellular inner layer and outer fibrous layer. Periosteum was 2-4times thicker than normal on the surgerized side, this thickness being due to the outer fibrous layer from 2 months

onward. By 6 months the tissues assumed their normal thickness. Surface cortical osteocyte necrosis was evident up to 22 days after surgery. The necrosis had disappeared, however by 32 days. Seven days after surgery, subperiosteal cancellous bone was seen on the cortical surface, with periosteal osteoblasts becoming osteocytes. By 10 days new periosteal woven bone was seen developing from the inner cambial layer of periosteum, this activity spreading beyond the surgical site. Periosteal activity was present up to 6 months after surgery in the surgical site.

Hellquist concluded that periosteal behaviour may have been species or location specific in its reaction to injury or irritation. He suggested newly formed periosteum may have arisen from an inner osteogenic layer as well as locally available undifferentiated mesenchymal cells.

Ritsila and coll. (1972a) autogenously transplanted either tibial (with muscle fibres) or calvarial periosteum across the submandibular gap between hemi-mandibles in rabbits aged 11-149 days old. By one week fibroblasts of both types of periosteum appeared to becoming cartilage cells, with osteoid forming at the cartilaginous periphery by 2 weeks. The first clinical periosteal callus appeared at 2-3 weeks while true bone with Haversian systems, marrow and overlying periosteum was present by 4 weeks. Calvarial periosteum produced structurally different bone of inferior quality than tibial periosteum. Better quality bone was formed when periosteum met muscle, rather than fascia or subcutanous tissue. Bone bridges formed between hemi-mandibles did not resorb by 9 weeks provided the transplant was adjoining bone. Allogenic periosteal

grafts also produced bone although differences to autogenous bone were not described.

Ritsila and coll. (1972b) used autogenous tibial periosteal grafts to repair anterior or posterior clefts in humans 8-10 weeks old. Radiographic examination showed callus by 2 weeks with definitive bone bridges by 6 weeks.

Alhopuro and coll. (1973a) autogenosly transplanted tibial periosteum in 14 day old rabbits, placing the graft over extirpated frontonasal sutures. The defect filled with bone, while the snout grew, deviating to the opposite side. When a periosteal graft was placed between suture and periosteum, the suture fused with definite growth arrest.

Alphopuro and coll. (1973b) carried out similar experiments using 14 day old guinea pigs, autogenously transplanting tibial periosteum to the intact premaxillary-maxillary suture. By 4 weeks a bone bridge had fused across the suture with deviation of the snout to the affected side during growth.

Alhopuro (1978) exhaustively reviewed his previous experiments on rabbits 13-15 days old. In summary: When tibial, scapular or parietal free periosteal grafts (stripped from bone with a blunt elevator) were placed into tibialis anticus muscle, all produced bone with marrow by an endochondral mechanism by at least 300 days. Tibial periosteum, however produced bone more quickly than the other tissues. As all types of periosteum (both tubular and membranous) formed similar types of bone,

Alhopuro concluded host environment influenced bone formation.

When periosteum was placed between premaxillary-maxillary suture and underlying periosteum, first cartilage and then trabecular bone was produced under the graft with sutural fusion occurring by 10 days. Again, tibial periosteum was superior in bone production. When the frontonasal suture was overlaid with periosteal grafts, tibial periosteum again proved superior in its ability to fuse the suture.

Alhopuro concluded that various free periosteal grafts from bones of different embryonic origin produced bone at differing amounts and rates while host environment influenced the types of bone architecture.

Ritsila and Alhopuro (1973a) removed part of the ulnar shaft in growing rabbits, placing an interpositional fat graft for 2 weeks. Free periosteal grafts subsequently transplanted aross the gap (after removal of fat) produced strong bony union. Free periosteal grafts were also used to cause early closure of the normal maxillary palatal defect in growing rabbits. Details of the technique and results were not given.

Ritsila and Alhopuro (1973b) created anterior tracheal defects in rabbits 2-10 weeks old, repairing the defects with autogenous tibial periosteum. Radiographic evidence of callus was present by 2 weeks while bone was evident a week later. Most transplants produced bone, histologically similar to membranous rather than tubular bone. Rigid bone developed over the site 3 weeks later. Ritsilia and coll. suggested tibial periosteum produced membranous-like bone due to local

recipient environmental influences.

Ritsila and Alhopuro (1975) grafted free tibial periosteum (from the middle 1/3 of tibia) between the spinous and articular processes of the vertebrae in rabbits, 4-77 days old. The grafts were placed across 2-5 vertebrae from T8-L7. Of 49 spaces grafted unilaterally, fusion occurred in 39. Of 8 interspaces grafted bilaterally fusion occurred in all 8. Nineteen rabbits with unilateral grafting developed a lateral curvature while 11 of 12 rabbits with posterior fusion developed a lordosis. Radiographs showed bone forming by the 2nd-3rd week. Histologic examination showed cartilage forming by 2 weeks, developing into a callus-like mass. This had turned with a well vascularized bone tissue by 2 months with Haversian systems and marrow.

Ritsila and Alhopuro concluded that their high percentage of ossified specimens may have been due to their preparation of periosteum. The periosteal grafts produced bone through a short cartilaginous stage but did not have a resorptive stage as with bone grafts. They admitted their findings may not be strictly applicable to humans. Ritsila and coll. (1977) did suggest the ability of periosteal grafts to change spinal growth may have application in treatment of scoliosis.

Ritsila and coll. (1975) used free tibial periosteum to close both unilateral and bilateral maxillary clefts in humans. The surgery was carried out at 8-10 weeks after birth and follow-up studies were done by radiograph. By 2 weeks callus was seen in the graft site while evidence of new bone was present by 6 weeks. Teeth were seen to erupt through

the newly formed bone bridge.

Kernek (1973) fractured the right tibia in rats of unspecified age, administering tritiated thymidine at various intervals. The cambial layer of periosteum showed the most intense labelling 2-3 days after fracture, suggesting the cambial layer of periosteum was the site of new cell formation. Cellular proliferation was most intense at the fracture site becoming less along the diaphyseal shaft. Minimal cellular labelling was seen in the surrounding soft tissue. Early callus developed cartilaginous tissue with labelled chondroblasts suggesting periosteal cells, chondrogenic and osteogenic cells derived from the same cell line.

Atherton and coll. (1974) created soft tissue flaps in a growing pig, placing palatal periosteum on both nasal and palatal surfaces and treating nasal periosteum similarly. The surgical site was histologically examined after 7 weeks. The oral mucoperiosteum, folded on itself, retained normal morphology. Bone deposition appeared to take place on the superior palatal surface with resorption on the inferior surface. The nasal epithelium changed to a pseudostratified squamous type in transfer to the oral cavity while bone beneath the temporal nasal periosteum was resorptive in character. Atherton and coll. concluded periosteum retains its resorptive or depository character when transposed as a flap.

Locke and Norton (1974) described the care of a 16 year old male suffering a comminuted tibial fracture. After debridement and removal

of a 6 inch segment of bone the limb was immobilized. Five months after injury radiographic evidence of subperiosteal bone was apparent while the bone had regenerated across the gap allowing some weight bearing 2 months later. Locke and Norton felt retained fragments of cambial layer regenerated the bone.

Craft and coll. (1974) used young New Zealand White rabbits, creating subperiosteal bone defects in the lower border of mandible as well as the zygomatic arch. Surgical sites were examined 1-3 weeks after fracture. They found periosteum was more active than endosteum in producing callus by the first week. Cartilage was occasionally present but disappeared by the fifth week.

Hyldebrandt and coll. (1974) created mid-shaft femoral fractures in one tibia of 5 week old mice, following cellular response with tritiated thymidine. The cambial layer proliferated 8-16 hours reaching a maximum 20-28 hours after fracture. Response was greatest at the fracture site but extended on both sides along the diaphysis. Hyldebrandt and coll. also found a small transitory rise in subperiosteal activity on the control side that did not correspond with activity on the fracture side.

Knize (1974) used female New Zealand White rabbits 2.3-3.3 kg removing iliac crest bone blocks and autogenously grafted them into a pocket between nasal bone and nasal periosteum. Surgical sites were examined radiographically while specimens were evaluated up to 9 weeks after surgery. Fifty-five percent of grafts placed with the graft cortical surface next to host cortex showed complete resorption, while 30% of

grafts with cortex next to host periosteum were completely resorbed. Twenty-two percent of periosteally covered grafts, with periosteum placed next to host cortex were completely resorbed while none of the grafts with periosteum placed next to host periosteum were completely resorbed.

A second group of animals was given intramuscular injections of calcitonin, but otherwise treated similarly to the first series. The grafts with periosteum placed next to host periosteum showed the least resorption of any graft while those grafts with periosteum next to bone showed slightly more resorption. On the average all animals treated with calcitonin showed more graft rentention than untreated animals. Pelvis donor sites in the groups treated with calcitonin showed remodelling and calcification as opposed to the resorption of the untreated groups. In rabbits given calcitonin, periosteum placed next to host cortex showed activation with subperiosteal bone formation.

Knize concluded that preosteoblasts of periosteum probably produced osteoblasts and hence new bone in the autogenous periosteally covered grafts, while the periosteum revascularized quickly, probably sustaining life in the outer cortex. Knize further argued that iliac crest would be the best donor site because of its highly vascular periosteum. Calcitonin seemed to stimulate osteoblasts to produce new bone, stimulate blood vessel formation and inhibit osteoclastic resorption, especially when periosteum was placed next to soft tissue.

Prydso and coll. (1974) took biopsies from 9 children 22-24 months old

who received a palatal-vomereplasty at an unspecified earlier age. The biopsies were taken after closure of a complete unilateral cleft, from both the hard palate and the nasal septum. Both the cleft and non-cleft sides demonstrated periosteal resorption on the nasal surface and periosteal apposition on the palatal surface. The normal side showed normal sutural architecture while the cleft side showed no sign of a developing palatal suture.

Rintala and coll. (1974) reviewed a series of 63 children treated for repair of primary palatal clefts by Skoog's technique of rotated periosteal flaps. The Surgicel (Tm) modification was carried out either at 3 months of age or, in some cases after presurgical orthodonties. Overall, well formed bone bridges were found in 54% of clefts. No statistical trials were carried out between variables such as unilateral or bilateral clefts, width of cleft, application of presurgical orthodonties, quality of bone formed or time after surgery. They mentioned, however, that in unilateral clefts, the width of the cleft made no significant difference in the amount of bone produced. Surgicel (Tm) was placed in 40 clefts, allowing clear radiographic evidence of bone bridging in 53% of clefts. Twenty-three cases were operated without placing Surgicel (Tm) allowing radiographically distinct bone bridges to form in 56% of cases.

Rintala and coll. felt their series was not followed long enough to fully evaluate the clinical efficiency of Skoog's treatment.

Tam and coll. (1974a) administered intravenous tetracycline to New Zealand White Rabbits (ages not given) at various times, sacrificing the animals 15 minutes after administration. They found three zones of mineralization in the organic bone matrix; a rapidly mineralized zone, a slow mineralizing zone and a fully mineralized zone. The tetracycline was taken up primarily by the rapidly mineralizing zone.

Tam and coll. (1974b) continued their research, administering intraperitoneal tetracycline to rats 300-400 grams in weight. Humerus, femur and iliac crest were evaluated for evidence of tetracycline staining. Both cortical and cancellous bone picked up the tetracycline, suggesting appositional bone growth had a central as well as local control. Non linear growth suggested periodic variation of periosteal osteogenic activity.

Tam and coll. (1974c) gave intraperitoneal injections of oxytetracycline to New Zealand White rabbits. Humerus, femoral, iliac crest, vertebral body and mandible were examined. They deduced bone growth in rabbits occurred on a regular cycle of roughly 2 days and was similar in all bones studied. Tam and coll. did not know however if active and inactive phases of bone growth were of equal duration. They suggested each bone formation site was a group of cells working together to create a field of growth, perhaps arising from a single osteogenic precursor cell. They cautioned the growth field was of unknown size and the cyclic activity was probably species-specific.

Jenkins and coll. (1975) described a technique for lengthening lower

limb long bones. Forty-five patients, 6 to 15 years old, suffering from polio were treated by stripping periosteum as for as the metaphysis at each end. No further treatment after stripping the periosteum was considered necessary. All patients showed increased long bone growth over 3 years. Jenkins and coll. thought the bone grew through vascular activity as well as increased blood the circuli vasculosus and hence the epiphyseal plates.

Swinson and coll. (1975) administered tetracycline to humans aged 31 to 373, all scheduled to undergo orthopedic surgery for any number or regions. Statistical analysis of their results suggested that human periosteum formed bone with a three rhythm of 3 days.

Golan and coll. (1976) grafted autogenous tibial periosteum as free grafts in young rabbits weighing 750 grams. One graft was placed in the muscles lateral to tibia while the graft from the opposite leg was placed in a tunnel between the lower borders of the mandible. Four of the 9 rabbits received no further treatment while 5 were given calcitonin daily for 8 weeks. Radiographic evidence of bone formation was seen in all animals, and first evidenced in muscles by 2 weeks. The bone increased rapidly in size and density during the first 10 weeks, forming a solid bridge of compact bone across the mandible. In one rabbit the periosteum separated from mandible forming a slender bone tube as opposed to the denser bone on the opposite side where periosteum was firmly anchored. Calcitonin had no effect on bone formation between the 2 groups.

Golan and coll. concluded that free tibial periosteal grafts formed bone of good quality in young rabbits, with the graft necessarily contiguous to bone for best results.

Lozano and coll. (1976) autogenously transplanted iliac crest bone both with and without periosteum into the skull of rats 150-200 grams in weight. The experiment was vaguely described. All animals were sacrificed at 1-21 days after operation. Vessels were seen entering the graft from all surfaces after 3 days, while an abundant vascularization to all surfaces occurred by two weeks. There appeared to be no difference in vascularization in any of the experimental groups while vascularization appeared to take place through vessel in growth rather than host graft link up.

Narang and Laskin (1976) removed 6 mm of fibular bone with its periosteum in 40 three month old rats. No new bone regeneration was radiographically or histologically evident by 12 weeks. When 12 mm of bone and periosteum were removed in a second group, no new bone had formed by 12 weeks. When 6 mm of fibular bone were subperiosteally resected the gap gradually closed over a 12 week period with most specimens showing complete bony union. Similar results were found when 12 mm of bone were subperiosteally resected. When fibular periosteum was removed but multiple fibular fractures left in place, there was gradually increasing evidence of bone union. Similar results occurred when periosteum was left intact.

Narang and Laskin concluded small and large fibular gaps healed more

completely when periosteum was present. When periosteum was not present bone contact was most important for clinical union. The authors felt periosteum, besides being directly osteogenic, acted as a barrier to prevent soft tissue from growing into the bone gap.

Thompson and Lewis (1976) took iliac bone; one segment covered with periosteum and one segment without in New Zealand White rabbits of unspecified age. The periosteally covered graft was autogenously transplanted between nasal bone and nasal periosteum. The periosteal free graft was sutured between nasal bone and a normal periosteal graft. All animals were followed radiographically and sacrificed one year after surgery. Animals with periosteum placed as a free graft showed an average 25% increased bone graft survival after 9 months, and 8% after one year over animals with periosteal-bone grafts. The bone grafts with overlying free periosteal graft demonstrated earlier vascularization with greater osteoblastic activity and earlier replacement of graft by the host bone.

The authors concluded that periosteum initially removed for contouring of the graft stimulated the periosteum to produce a superior bone graft than grafts with periosteum left in place.

Adekeye (1977) described the removal of an ameloblastoma in a 15 year old Nigerian boy. Using an extraoral approach, the tumour was subperiosteally removed from second molar to second molar. Seven months later there was radiographic and clinical evidence of functional mandibular regeneration from the periosteum.

Altonen and coll. (1977) exposed a 4 x 7 mm area around the mandibular incisor roots of adult rabbits, subsequently covering one half of the defect with an autogenous periosteal graft. The opposite side was covered with a local mandibular periosteal flap. By 8 months the bone formed under the graft was thicker, more cellular (more osteoblasts), with less compact bone and more fibrotic marrow than bone formed from the local flap. Altonen and coll. concluded that a free tibial graft possessed better osteogenic potential while neither type of periosteum interfered with tooth eruption.

Lee and Wright (1977) used mongrel dogs of unspecified age, subperiostally and subperichondrally resecting portions of the nasal bones, subsequently replacing the periosteal and perichondral flaps. Six cases were reported on 2 to 8 months after surgery. No significant regenerated bone was found in the animals irrespective of whether the gap was filled with blood clot, crushed bone or crushed cartilage. Most defects healed with fibrous connective tissue.

Samuel and coll. (1977) drilled holes of unspecified size in the lower of rats' mandibles (species, age not given). One side of the mandible was covered with calvarial periosteum and all animals were histologically examined) from 4 to 30 days after surgery. The uncovered defect filled with granulation tissue while the side covered with periosteum completely filled with bone.

Snellman and coll. (1977) grafted free tibial periosteum to lumbar vertebrae in human subjects for the treatment of scoliosis. Fifty-five

patients averaging 14.5 years of age were followed in the study. By 24 weeks after surgery, compact bone had developed in the grafted sites, effectively fusing the spine.

Tornberg (1977) injected 0.6 mgm of calf freeze dried bone and cartilage extract juxtaperiosteally in the radial midshaft of mature Sprague-Dewley rats, sacrificing all animals up to 7 days after operation. Tritiated thymidine was injected intraperitoneally before sacrifice and samples were examined by light microscopy, electron microscopy and autoradiography. By 48 hours, approximately 50% of all periosteal cambial cells were labelled while cells in fibrous periosteum were only moderately labelled. The cambial layer had increased in thickness by four fold. From 60 to 84 hours osteoid was seen developing between cortical bone surface and cambial layer while mineralization occurred from 60 hours to 7 days after surgery.

Tornberg felt periosteum was "activated" (rather than induced to osteogenesis), the mechanism perhaps being partly or wholly electrical due to the strong negative charge of polyglycans in the extract.

Goldman and Smukler (1978) created multiple periosteal wounds in an "old" dog by penetrating the buccal mucoperiosteum (maxillary and mandibular) with a 25 gauge hypodermic needle. The operation was repeated at weekly intervals for 4 weeks. Results demonstrated an acute inflammatory response after the first week, with resorption areas of the buccal cortex. By the second week, the traumatized periosteum demonstrated hypertrophy of both the fibrous and cambial layers. New

subperiosteal osteoid was evident as was an increase of local vascularity. By three weeks new subperiosteal bone was apparent with similar activity in the adjacent endosteum. A similar experiment on a human subject produced similar results.

The authors argued that adult periosteum having few osteoprogenitor cells in a single layer would not produce new bone if a periosteal flap was lifted, as the cells would be left behind. If, however, an osteoperiosteal flap was raised the stimulated periosteal osteogenic cells would be stimulated to produce new bone. They felt their experimental results confirmed this hypothesis. New bone, however, was produced when periosteal (not osteoperiosteal) segments were elevated from bone. Goldman and Smukler also concluded that adult periosteal cells could be stimulated to produce new bone.

Plezia and coll. (1978) studied the behavior of frozen bone and periosteal grafts. They first removed the teeth in young adult mongrel dogs. After 10 weeks of healing, the anterior mandibular bodies and symphysis were removed through an extraoral approach, half of the resections with periosteum and half without. The mandibles were then split at the symphysis. Multiple bur holes were placed in the mandible and the bone was frozen in liquid nitrogen (-196.6^{o}C for 10 minutes) thawed, refrozen and rethawed before replantation. Specimens were taken from 3.9 to 24.6 months after replantation. All animals demonstrated clinical union within 3 weeks, with subsequent mandibular regeneration of varying degrees. The specimens frozen with their periosteal cover allowed regeneration of less bone than specimens with periosteum left

behind. Histologic examination was not described well. but showed generalized bone regeneration and remodelling about the graft.

Pleiza and coll. briefly mentioned a series of 9 patients treated by mandibular removal, freezing and replacement. No mention was made of the treatment of periosteum. The grafts were successful in 4 of the patients for at least 2 years.

Uddstromer (1978) created full cortical defects in the anterior tibia of 3 month old rabbits, filling the defect with a hollow teflon cup, the cup opening facing periosteum. The cup was either allowed to fill with blood, or packed with autogenous bone marrow or Surgicel (TM). The periosteal flap reflected to create the cortical defect was replaced over the cup. A similar procedure was carried out on the parietal bone. All rabbits were sacrificed from 2-15 months after surgery. By 2 weeks, bone covered the cup implanted in the tibia, the new bone being the same thickness as surrounding normal cortical bone. This bone appeared to be coming entirely from the deep surface of overlying periosteum. Bone marrow was first seen in the cup by 4 weeks with complete normal bone and marrow architecture developing by 6-7 weeks. Small islands of subperiosteal bone were seen in the calvarial cup by 3 weeks healing (without passing through a woven bone stage) to roughly one third normal calvarial thickness by 8 weeks. Red bone marrow and blood inside the cup produced no difference in quality or quantity of new bone. Surgicel (TM) was found to decrease the amount of marrow formed by approxiately 1/3.

Uddstromer concluded that tibial periosteal flaps were 7 times as potent

as calvarial periosteum in producing new bone, while isolated local periosteal flaps were capable of producing new bone similar to that removed surgically. The fine structure of newly formed bone appeared to depend somewhat on local factors. As calvarial periosteum produced only 1/3 that cortical bone of tibial periosteum Udderstromer concluded dura may also have been necessary in calvarial bone production.

Udderstromer and Ritsila (1978) repeated the experiments with three month old rabbits, covering the tibial cup with a free parietal periosteal graft and the reverse. All cups were allowed to fill with peripheral blood. The rabbits were sacrificed from 2-15 weeks after surgery. Tibial free grafts applied to the skull produced uneven bone by 2 weeks with complete repair of compact bone and marrow by 8-10 weeks. The parietal periosteal grafts applied to tibia formed the first woven bone by 4 weeks. The final healed bone was tubular with compact cortex and internal marrow (6-12 weeks postoperatively).

Udderstromer and Ritsila concluded that tibial periosteum transplanted to calvarium halved its osteogenic power but was still 3 times greater than parietal periosteum in situ. Conversely, calvarial periosteum transplanted onto a tibial defect appeared to increase its osteogenic power five fold. The authors concluded both the nature of host tissue and recipient environment influenced subperiosteal bone production.

Udderstromer and Ritsila (1979) modified their research to isolate endosteal and periosteal osteogenic influences again using a teflon cup. When the cup orifice faced periosteum, tibial periosteum produced

bone and marrow while parietal periosteum incompletely regenerated calvarial-like bone. When the cup orifice was turned internally, the tibial periosteum regenerated compact bone on the cup's base while parietal periosteum produced varying amounts of cortical bone. When both periosteal and endosteal surfaces were exposed to the cup's interior, the tibiae healed almost completely while calvarial defects did not heal more than with dural influence alone. When periosteal and endosteal influences were excluded by teflon sheets, more subperiosteal bone (sometimes with marrow) was found in both tibia and parietal bone.

Uddstromer and Ritsila concluded that tibial periosteum was a more potent osteogenic organ than parietal periosteum. In the tibia periosteum appeared to be the major osteogenic organ while calvarial periosteum functioned with dura to accomplish this. The authors also admitted their experiments had limitations in the pressure from teflon cups on the underlying tissue membranes as well as a possible leakage of tissues under the teflon.

Carroll and Keates (1979) used a tibial periosteal graft to repair a scleral bed secondary to chemical burns, the periosteum subsequently producing new bone. The tissue was removed to be replaced with a second periosteal graft and no bone was formed on this occasion.

Friedlander and coll. (1979) used rats 90-100 gm removing femoral periosteum and transplanting it autogenously between vertebrae T8-L2. Cartilage formation was seen by 10 weeks while subperiosteal lamellar bone formation was seen by 15 weeks. They felt periosteum was a highly

osteoinductive tissue but free periosteal grafts were not recommended for use on humans because of their unpredictable results.

Freng (1979a) treated 12 patients suffering from choanal atresia by elevating the palatal mucoperiosteum, and resecting some amount of hard palate to correct the atresia. Five patients (6-13 years old) underwent a first operation while seven (10-26 years) were given a second operation. Specimens of resected bone were sent for histologic examination. In the 7 reoperated patients, new bone had formed on the posterior surface of the hard palate, this bone being a greater amount with increased time between primary and secondary operations. None of the defects contained islands of bone or cartilage. Freng surmised little bone regeneration in the posterior hard palate may have resulted from a difference in periosteal activity of the posterior hard palate.

Freng (1979b) reflected palatal microperiosteum in the hard palate of fifteen 2 month old cats creating a full thickness midpalatal defect 5 mm wide extending the full length of the hard palate. Nasal periosteum was not disturbed. A similar soft tissue palatal reflection was carried out on fifteen 2 month old cats without resecting bone. Animals were sacrificed at 13 months of age or less. Suture morphology was completely re-established as early as 4 1/2 months after surgery in only 5 animals, the rest developing a synostosis. Of 15 animals given only a mucoperiosteal flap, 3 developed a synostosis. Cartilage was not found in any of the results.

The author felt new bone arose by incremental apposition to the existing

palatal margins, presumably from the periosteum or bone itself.

Freng (1979c) repeated his previous study on 2 month old cats, this time completely destroying the nasal periosteum. The animals were sacrificed at 13-5 months or 2 1/2 years after surgery. In all operated animals abundant new bone production occurred, and bone bridges interlocking palatal halves were never seen. The cleft was generally larger posteriorly than anteriorly with the newly-formed bone thinner and more compact than the 2 layered periosteal experiment. In general, one layer palatal periosteum produced less bone than an environment with both palatal and nasal periosteum. The new bone appeared to be laid down appositionally from the palatal edges, most likely arising from the palatal periosteum.

Poussa and Ritsila (1979) demonstrated a folded border at the periosteum-bone interface of 6 week old rabbits. They felt that it was possible to leave osteogenic buds on the bone surface after stripping periosteum, as osteogenic cells. Using 6 week old rabbits, they removed tibial periosteal and 200 micron osteoperiosteal grafts autogenously grafting them across vertebral spinous and mammary processes. All animals were sacrificed 3-84 days after surgery. By one week, both periosteal and osteoperiosteal tissues were vital, producing woven bone from cartilage in an endochondral pattern. The cortical component of the osteoperiosteal graft was dying. By 2 weeks the periosteal graft had formed a bone bridge, while the osteoperiosteal graft was continuing to form cartilge. After 3 weeks the cortical bone of the osteoperiosteal graft was resorbing. By 4 weeks bone bridges

were formed on both sides while the periosteal graft had formed red marrow. Remodelling into vertebral-like compact bone continued to the end of the series.

The results showed that a periosteal graft, in young rabbits, was a more vigorous bone former than 200 micron-thick osteoperiosteal grafts. Poussa and Ritsilia presumed bone had to be resorbed to allow the insulation necessary for periosteal bone formation.

Poussa and coll. (1980) repeated the experiments on 6 week old rabbits, using both periosteal grafts 100 microns thick and full thickness osteoperiosteal grafts. All grafts were taken from the proximal third of tibia, and placed between the spinous and mammary processes of autogenous lumbar vertebrae. Results of the periosteal and 100 micron osteoperiosteal grafts were similar to the previous series. Full thickness osteoperiosteal grafts underwent necrosis and resorption up to 10 days after surgery with some new bone formation subperiosteally as well as resorption. From 28-84 days bone continued to be resorbed and replaced with bone bridges as before.

Poussa and coll. surmised the cartilage seen in free periosteal and 100 micron osteoperiosteal grafts produced cartilage up to 14 days after surgery due to low oxygen tension. They found full thickness osteoperiosteal grafts were inferior to free periosteal grafts while thin osteoperiosteal grafts were only slightly less inferior perhaps due to inhibition of vascularity.

Puckett and coll. (1979) compared the quality of bone formation in revascularized periosteal grafts of minimally stressed bone; specifically the fibulae of mongrel dogs (age not given). A segment 2.0 cm. long was removed and transferred to a similar defect in the opposite leg. One graft retained its periosteum and one did not. All dogs were sacrificed at 120 days. Both types of grafts proved successful, with bony union in 80% of cases retaining periosteum and 70% of cases with periosteum removed. Stress intolerance reached 78% of normal with periosteum and 75% of normal without.

Rib periosteum with attached intercostal vessels was removed and placed over the fibular defect. On one side vessels were anastomosed while on the other side, they were not. The graft form was maintained with a Surgicel (Tm) core. Thirty-eight percent of revascularized grafts demonstrated gross subperiosteal bone formation ("lacunar bone") while the free grafts were replaced by scar tissue, without bone formation.

When rib with periosteum and intercostal vessels was placed into the fibular defect, subperiosteal callus appeared earlier on the revascularized side although healthy bone was seen on both free and revascularized sides. Stress tolerance was 90.5% of normal on the revascularized side and only 68% normal on the free graft side.

The authors felt the grafts failed to produce substantial new bone due to lack of adequate functional stimulus. They felt this might be a factor in the failure of facial onlay grafts to persist. The authors admitted a factor in stimulating periosteum to produce bone may have

been lacking. Since both types of grafts (free and revascularized) behaved similarly they concluded free periosteally covered bone grafts were suitable for most clinical situations. In contrast, revascularized grafts should be saved for those donor sites poor in vascularity, such as scar or irradiated tissue.

Kisner (1980) described treatment of a 12 year old male, injured in a shotgun blast, with destruction of the mandible from tooth #34 to #46. A Kirshner wire was placed as the initial reconstruction. Ten months after reconstruction radiographic follow-up showed new bone bridging the gap with a regenerating symphysis. Kisner felt several factors contributed to the successful regeneration, including multiple bone fragments, multiple fragments of retained periosteum, young age of the patient and possible metaplastic change.

Kuylenstierna and coll. (1980) exposed one hemi-mandible in young rabbits, leaving the periosteum intact. The exposed periosteum and bone was frozen twice with a cryoprobe to -160^{o}C. By 2 weeks the undamaged periosteum apparently produced new bone (although most new bone was endosteal in origin). The authors concluded adequate vascularization always preceded new bone formation.

Nappi and coll. (1980) placed Surgicel (Tm) into clefts of 5-8 year old human patients according to Skoog's method of the cleft palate repair, but found no new bone formation after 12 months.

They subsequently described their experiments on New Zealand White

rabbits of unspecified age, subperiosteally resecting ribs to create periosteal tubes with cut bone at each end. One rib was resected only, one resected and filled with Surgical (Tm) while one rib was resected and filled with Avitene (Tm). Periosteum and overlying tissues were resutured into place. Animals were evaluated up to 8 weeks after surgery. The resected rib showed good bone regeneration, complete bridging with mature bone and some regeneration of bone in the Avitene (Tm) filled cavity, similar to the first but less extensive. Fibrous tissue only filled the cavity originally containing Surgicel (Tm).

The Avitene (Tm) was completely gone by 8 weeks although Surgicel (Tm) particles were still present.

Nappi and coll. concluded periosteum does produce abundant new bone in this experimental model bone although bone forms in spite of the presence of Surgicel (Tm), not because of it.

Nwoku (1980) described two cases of mandibular resections, where as much periosteum as possible was retained. In one case, removal of a recurrent ameloblastic fibroma in a 15 year old Negro male resulted in complete functional mandibular regeneration by 6 months. The second case involved removal of an ossifying fibroma in a 12 year old Negroid female. By 6 months the mandible had functionally regenerated.

Barro and Latham (1981) investigated palatal periosteal response in mongrel dogs of unspecified age. They elevated palatal mucoperiosteum on one side, immediately replacing it. The periosteum had proliferated

by 15 days, producing new subperiosteal cancellous-like bone.

The experiment was repeated by reflecting both sides of the palate, through a midline incision and removing an 8 mm x 10 mm bone segment from one side. New bone appeared to arise from the edges of the resected cavity by 3 days, with new bone bridging the cavity 23-40 days after surgery. The traumatized palatal and nasal mucoperiosteum appeared reactive but did not seem to produce significant new bone.

Barro and Latham concluded that stimulated periosteum interrupted bone production, subsequently causing an osteogenic cellular multiplication without forming bone on its own.

Habal and Maniscalco (1981) felt pericranium was a successful osteogenic tissue because of its very rich blood supply. They argued pericranium provided vascularity to underlying bone, cells for bone formation and became a stimulus for the production of new bone. Calvarial bone being membranous, would heal by calcification of a fibrous union rather than callus formation.

Kufass (1981) evaluated the response of cranial and tibial periosteum used as autogenous patch or circumferential free grafts to repair tracheal defects in 6 week old (growing) adult rabbits. In general, both cranial and tibial periosteum produced cartilage and endochondral-type bone, the cartilage and bone appearing to arise from the grafts rather than the recipient bed. In young animals tibial periosteal grafts were superior to calvarial grafts. In adult animals window

tracheal defects were better repaired when the cambial layer was placed next to the defect. Grafts placed on cartilaginous defects produced more rapid healing when the cambial layer was placed next to the host cartilage. Results were similar when cranial periosteum was used to repair defects in young or adult rabbits.

Kufaas concluded that autogenous free tibial periosteum had potential as a repair tissue for tracheal defects in both young and adult animals.

Marciani and coll. (1981) subperiosteally removed hemimaxillas (anterior to tuberosity and inferior to infraorbital foramen) in young monkeys, twice freezing the bone in liquid nitrogen before subsequent reimplantation. A second series of hemimaxillas were similarly surgerized without freezing. All animals were sacrificed from 3 weeks to 1 year after operation. All grafts were clinically firm 3 weeks after surgery and all grafts appeared smaller than the untreated side at the end of the experiment. Periosteum overlying both types of grafts showed both new subperiosteal deposition and resorption although no detailed description was given regarding periosteal activity.

Ranta and coll. (1981) studied the effect of adult periosteum in healing bone defects of adult rabbits. They exposed the lower root surfaces of both mandibular incisor teeth in rabbits 5-23 months of age. After allowing blood to fill the cavities one defect was covered with the overlying mandibular flap while the opposite side was repaired with a free autogenous tibial graft. Animals were sacrificed at 3.5-8 months after surgery. Local periosteal flaps allowed bone healing to restore

normal architecture while in all but one case grafted tibial periosteum allowed a 2-3 fold increase in normal bone thickness. The new bone in all cases was compact and lamellated.

Ranta and coll. suggested that autogenous tibial periosteal transplants survived while the cambial cells of the transplant as well as the adjacent host periosteal cells may have produced new bone. Bone chips included with the graft presumably aided in osteogenesis. They also suggested free periosteal grafts acted as semipermeable membranes and may have contained bone inducing compounds. They offered no explanation for the increased bone mass under the free graft although they suggested it may be permanent, as it persisted over 8 months.

Poussa and coll. (1981) tested the effect of chondrotrophic environments on periosteal cells. They used 6-8 week old rabbits, taking free tibial periosteum, autogenously transplanting the tissue into various sites. Grafts transplanted across costal cartilages (after removal of perichondrium and placement of periosteal cambial layer next to cartilage) produced osteoid-like tissue by one week. Cancellous bone with marrow was produced over bone and muscle from periosteal grafts by 3-8 weeks, although new cartilage produced by the recipient bed cartilage separated the new bone from costal cartilage. Periosteum placed between ear cartilage and perichondrium allowed a lively cartilage production and scanty bone formation by 8 weeks. Periosteum placed into an ear defect between skin layers created by removal of perichondrium and cartilage produced an osseous nodule at 1 week, evolving to mature cortical bone by 8 weeks. Periosteum transplanted

into knee joint synovial fluid as a loose body developed cells resembling chondrocytes by 2 weeks with gradual resorption of the periosteum after 4 weeks.

Poussa and coll. argued that sufficient vascularization was necessary for periosteum to produce bone. Periosteum placed next to vascular intercostal muscle rapidly produced cortical bone. Periosteal grafts placed in a relatively avascular bed such as the rabbit ear or synovial fluid produced cartilage. Periosteum from endochondrally ossified tubular bone tended to produce bone in a richly vascular bed and cartilage in an oxygen poor, relatively avascular bed.

Reid and coll. (1981) studied the role of periosteum and dura in healing parietal defects of New Zealand White rabbits. A laterally based periosteal flap separated from the overlying skin was elevated from parietal bone in rabbits 23-28 days old. A smaller full thickness rectangle of parietal bone was then removed and the flap replaced. Results were analyzed at 8 weeks after surgery. Complete regeneration between the periosteum and dura was found in 6 of 7 animals. The last showed 75% regeneration.

A second group of young rabbits underwent a similar procedure, except the periosteal flap was removed. Four of 7 animals demonstrated 50-70% regeneration, while 3 demonstrated less than 50% repair. When periosteum, bone and dura were removed only 3 animals showed 25-38% osseous regeneration. The remaining rabbits demonstrated less repair of the defect. When the first experiment was repeated on adult rabbits, 5

rabbits evidenced 50-75% regeneration, the remaining two demonstrating 25-50% regeneration. Statistical testing confirmed young rabbits regenerated more bone than adults while removal of dura and periosteum inhibited bone formation more than removal of periosteum alone.

Stanley and Rice (1981) removed all teeth from the right mandibular quadrant in adult mongrel dogs. Three weeks later a 1.0 x 3.0 cm segment of buccal and lingual mandibular periosteum was removed from the lower border of right mandible via the extraoral route. The block of bone thus exposed was removed and autoclaved after extraction of its marrow. The bone was subsequently replaced and covered with local periosteum. Samples were evaluated up to 12 weeks after surgery. Fibrous union of bone was evident by 4 weeks with firm clinical union by 8 weeks. Periosteum was firmly attached by 4 weeks. New bone was observed bridging the host graft interface by 6 weeks while new subperiosteal bone appeared at 8 weeks. Fluorescence at the graft host interface was first seen by 6 weeks while first subperiosteal banding was evident at 8 weeks.

Stanley and Rice concluded that reimplanted heat killed bone covered with periosteum provided a suitable environment for the stimulation of subperiosteal bone formation. The authors felt this technique had value in treatment and reconstruction of some oral malignancies.

Stauffer and coll. (1981) investigated the biologic properties of transposed periosteal flaps, using 3 to 5 month old mongrel dogs. A standardized 10 mm x 7 mm defect was made in the maxillary alveolus and

lined with a local buccal mucosal flap, the submucosa facing the cavity. An autogenous rib bone graft was placed tightly across the cavity and the defect covered with a flap of palatal microperiosteum, nasal mucoperiosteum or buccal mucosa. Animals were sacrificed at varying times. No mention was made of periosteal removal but presumably this was done.

Ribs covered with buccal mucosa demonstrated extensive cortical resorption and replacement of most medulla with fibrous tissue. Rib grafts covered with palatal mucoperiosteum demonstrated increasing degrees of osteogenesis with time (17, 26, 27 days) on both the cortex and in the medulla. The deep surface of bone next to buccal submucosa showed resorption similar to that described before. Ribs covered by nasal periosteal flaps demonstrated almost complete resorption by 26 days. Stauffer and coll. concluded that local periosteal flaps retained their growth activities similar to their behavior during normal growth, even when transferred to other anatomic regions.

Satol and coll. (1983) repaired comminuted fractures of the right lower leg in a 17 year old male using a periosteal flap 8 x 10 cm long, taken from the patient's ilium along with iliacus muscle and deep circumflex iliac artery and vein. The vessels were anastomosed locally while the entire transplant was covered with a split thickness skin graft. Radiographic evidence of calcification appeared after 2 weeks while a definite callus was present 6 weeks after surgery. Clinical union occurred by 5 weeks and the patient was bearing full weight 6 weeks after the repair. Satol and coll. stressed the importance of retaining

as much periosteum as possible in the injured site or adding to it by using local flaps.

Wildenberg and coll. (1983, 1984) agreed with previous authors on the validity of periosteum as an autogenous graft, and investigated the possibility of an increased osteogenic potential using a revascularized periosteal graft. They experimented with adult female African Pygmy goats creating a mid-shaft 2 cm diaphyseal defect, removing both bone and periosteum. After closure of the defect, a 2 pin external fixation apparatus was secured in place. The defect closed without periosteal cover showed only fibrous tissue repair after 8 weeks.

The defect was also treated with a revascularized autogenous rib periosteal graft placed as a spiral over the defect. The posterior intercostal arteries and veins were re-anastomosed to anterior tibial artery and vein with an ischemia time of approximately 100 minutes. By 4 weeks, 1 of 13 goats demonstrated fibrous healing with woven bone trabeculae in the defect. By 8 weeks, 1 of this group presented a small amount of woven bone. Evidence of subperiosteal bone was seen in 2 of 8 cases. A third group was similarly treated without re-anastomosing the vessels. Small amounts of woven bone were evident by 2 weeks in 2 of 12 goats with no reported increase beyond this point. Bone of subperiosteal origin was seen in 10 of 11 specimens examined.

A fourth group was treated by creating a local tibial periosteal collar and replacing it with microvascular re-anastomoses after creating the defect. The ischemia time was approximately 97 minutes. Two goats of a

possible 16 showed evidence of peripheral woven bone in the defect with 2 goats showing complete osseous healing of the defect by 4 weeks. After 8 weeks 2 goats demonstrated complete osseous healing with both cancellous and compact bone as well as islands of cartilage. The last group was treated with a similar tibial periosteal graft, without microvascular anastomoses. Again woven bone was present in the defect by 2 weeks (in 2 goats) incomplete filling of the defect with woven bone and cartilage was seen in 1 of 2 specimens by 8 weeks. Bone of subperiosteal origin was seen in 4 of 11 specimens examined.

Vascular patency was present throughout the experiment for both types of revascularized grafts. Mineralized bone was detected in a small amount in the revascularized rib periosteal transplant and free tibial periosteal graft while large amounts were found in the revascularized tibial periosteal graft.

Three specimens of the revascularized tibial periosteal graft were tested for fracture strength after 8 weeks of healing. The bones fractured through the new bone of the diaphysis and at lower loads as opposed to fractures occurring through the metaphysis of unoperated tibias subjected to similar strain tests.

The authors concluded that factors influencing the osteogenic potential in periosteal grafts included the sites for donor periosteum as well as the nature of the recipient bed. Immediate revascularization of the graft also seemed to increase the quality and quantity of bone healing.

Schultz (1984) described a technique for repairing clefts of the primary palate using local microperiosteal flaps to close the mucosa and an underlying sheet of free autogenous tibial periosteum placed over the labial surface of the cleft for bone regeneration. Schultz's series of 14 patients ranged from 9-30 years of age and all were followed up to 3 months after surgery. The results, briefly described, included clinical stability through the cleft, radiographic or computerized tomographic evidence of subperiosteal osteogenesis from the graft and eruption of teeth through the newly formed radio-opaque areas. No complications during follow-up appeared to be related to the periosteal grafting.

PERIOSTEUM WITHOUT OSTEOGENIC FUNCTION 1972-1975

Cestero and Salyer (1974) implanted porous carbon, porous polypropylene or porous polyethylene discs above or below the pericranium of rats (ages not given). Calvarial bone in contact with discs produced new bone growth while periosteum in contact with the discs produced no new bone. The experimental method and results were presented in a summarized and vague manner. Cestero and Salyer felt the role of periosteum in producing new bone was uncertain while bone grafts were predictably successful. The authors performed a similar series of experiments (1975) implanting porous carbon discs between various pericranial, periosteal and bone graft tissues or in the soft tissues of young guinea pigs or rats of unspecified age. Both the experimental method and the results were vaguely described. The authors found that periosteum in contact with the carbon discs did not produce bone while calvarial bone or bone grafts in contact with carbon discs produced new bone in every case. Cestero and Salyer felt bone grafts were imperative to new bone formation while "further research" was necessary to evaluate the osteogenic potential of periosteal tissues.

PEDICLED PERIOSTEAL FLAPS

Phelps (1891) described one of the first osteoperiosteal flap transfers. One of his patients was a young male suffering from persistent nonunion of his right tibia, despite four attempts at repair. As a last resort, Phelps removed part of a dog's right radius, leaving it pedicled to both soft tissues and the brachial artery. The bone was placed into the one inch gap between the tibial fragments, stabilizing all parts with an intramedullary rod. The man and dog were kept together for 11 days, when the brachial artery was finally severed. Blood was seen oozing from the radial graft at this point. Phelps removed the graft, noting new bone had formed on both its periosteal and endosteal surfaces, however no change had occurred to the host fragments throughout the time of observation.

Muller (1890) used osteocutaneous flaps to repair cranial defects; specifically a segment of outer table pedicled to a large cutaneous scalp flap.

Curtis (1892, 1893) presented the case of a 33 year old male suffering from a compound fracture of the left tibia and simple fracture of the fibula. Five months after injury the tibia healed with a 1 1/2 inch loss of the diaphysis. Curtis created a 2 inch fibular fragment, pushing it with its surrounding soft tissue into the tibial gap. Five months after surgery the fragments had healed solidly, the patient being employed as a camping guide.

Reichel (1905) described using a skin flap to transplant vital bone and periosteum from one leg to the other in treatment of congenital pseudoarthrosis. He postulated that 10-12 cm pieces of rib could be similarly transposed.

Huntington(1905) described a two stage pedicled osteoperiosteal flap procedure. A seven year old boy, suffering from osteomyelitis of his left tibia underwent a complete diaphyseal sequestrectomy in 1902. The periosteum was retained as much as possible with the cut edges being sutured together. Six months later the tibia had regenerated except for 5 inches of the diaphysis. In 1903 Huntington created an upper fibular fragment, moving it medially as a composite flap to unite the tibia. Although clinical union was complete after 6 months, he transposed an inferior fibular fragment as a composite flap to stabilize the ankle. He claimed the transposed fibular segment increased to 3/4 inches in diameter and was able to function normally.

Stone (1907) described a modification of Huntington's treatment for transfer of a fibular segment to repair a tibial defect in a 5 year old male patient of his. Stone split the fibular shaft to retain lateral malleolar continuity. Periosteum was preserved except at the points of union, as was the surrounding fibular soft tissue. Nine months after the final operation the transposed fibula was vital and continuing to increase in both diameter and length.

Hibbs (1911) ankylosed the knee joint by grafting the patella directly

to cortical bone of the femur and tibia. He originally maintained the upper patellar ligament and periosteum intact as a vascular pedicle but later felt this step unessential.

In a review article, Roberts (1912) surveyed a number of contemporary "osteoplastic flaps". He described the empirical construction of pedicled osseous flaps in gaining access to surgical sites, extending this concept to purposefully developing pedicled flaps for reconstruction procedures. Included in his survey was a mandibular coronoid process pedicled to temporal muscle and rotated to replace an orbital floor.

Hey Groves (1917) felt that while pedicled osteocutaneous flaps would remain viable, their disadvantages of further surgical procedures and increased rash of infection rendered pedicled flap repair "almost obsolete".

Blair (1918) described a number of composite pedicled bone and soft tissue flaps for reconstructing mandibular defects. Among his procedures were transplantation of a rib fragment into the subcutaneous tissues of the neck. Three or four weeks later the rib fragment with surrounding soft tissues and platysma muscle as pedicle was rotated into the defect. Blair also described a flap using a saggitally split clavicle pedicled to neck skin. Blair felt pedicled bone grafts were superior to free grafts as the pedicle maintained osseous viability. He felt the attached osseous component could be treated as an open fracture, and even moved into an aseptic area. Blair suggested using a

cutaneous forehead flap, with underlying periosteum and pedicled on the temporal artery for use in repairing middle face defects. He recommended including calvarial periosteum but only as a surface for accepting a skin graft.

Cole (1918) firmly believed pedicled bone grafts were superior in reconstructive surgery, as they received an uninterrupted blood supply from their independent muscle pedicle. He felt mandibular defects could be best repaired using a segment of the lower mandibular body pedicled to both platysma and anterior digastric muscles. Once mobilized, the pedicled graft could be moved to bridge the mandibular defect. Cole fashioned pedicles with bone fragments to up to 3 1/2 centimeters in length. He was enthusiastic about the procedure, claiming 70% success in a series of 30 patients. In later papers, he described success of three and a half years (Cole, 1940) and seventeen years (Cole, 1938) with this procedure.

Tainter (1919) used Cole's technique of pedicled bone flaps for mandibular repair in his own series of 30 patients. He felt the procedure was superior to free bone grafts as the osseous fragment would not resorb. Tainter cautioned against performing the repair until the intraoral mucosa was intact.

Ivy (1920) carried out a limited survey of mandibular fracture treatments during the first World War. He found Cole's pedicle graft gave the best results of any method (87%) and was best used for repairing small losses of bone.

Limberg (1926) described a number of mandibular osteotomies for correction of facial deformities. He commented on the high failure rate of bone grafts, especially when secretions from the oral cavity contaminated the surgical site. To overcome this problem, Limberg recommended transplanting a 3-4 cm segment of periosteally-covered rib under the mandibular periosteum of the future surgical site. Two or 3 months later the mandibular osteotomy was done, pedicling the transplanted bone to adjacent soft tissue and placing it between the osseous fragments. Limberg noted that by the second operation the graft was being resorbed, making it easier for blood vessels to enter. He presented 6 cases using this flap, one procedure being carried out in an infected site. The successful result in this case caused him to question whether the pedicle supplied any vascularity to the bone. After placement of the graft, Limberg emphasized replacing the mandibular periosteum as much as possible to aid in bone production.

Hibbs (1926) achieved extra-articular hip fusion by detaching the trochanter and turning it with its periosteal muscle attachments under the ilium. He described a series of 20 patients from 4-31 years old with successful joint fusion in 90% of cases. Follow-up was 6 months to 2 1/2 years after surgery.

Pilcher (1926) described a pedicled bone flap for mandibular repair identical to the technique outlined by Cole. Pilcher felt the anterior inferior border of the mandible was well supplied with vascularity by anterior digastric muscle, as evidence by bleeding from the cut bone

surface. The composite flap could be moved widely with tension put on the pedicle if necessary. Up to 12 cm of bone could be successfully transplanted with this technique. One patient died of unknown causes 3 years after being given this procedure. At autopsy, the bone pedicle was firmly united to the host bed, with extensive bone callus and remodelling. He used the procedure in 80 cases, admitting only 3 failures.

Watson-Jones (1933) described repair of calvarial defects using a flap of outer table of calvaria, pedicled to pericranium at the temporal region. He felt the pericranium would provide adequate vascularity for the bone.

Watson-Jones presented one case of a parietal bone flap being used to close a frontal bone defect six months after surgery the flap had healed in its new position.

Hellstadius (1942) commented on the frequent complications of bone grafts, including resorption, graft fracture, pseudoarthrosis and long healing periods. He felt more reliable results could be achieved by retaining a periosteal blood supply through a pedicle of soft tissue. Hellstadius carried out a series of experiments on mature or nearly mature rabbits to investigate his hypothesis. Segments of radius were freed and replaced, maintaining skin or muscle pedicles. After 6 days one skin pedicle and one muscle pedicle bone flap showed vital osteocytes in the outer cortex. After 3 weeks, the skin pedicled bone contained viable osteocytes in the outer quarter of cortex with a lively periosteal bone formation. In another series of experiments a muscle

pedicle trochanteric flap was created and replaced. By 6 days most bone cells were vital. By 3 weeks, most osteocytes were nonstainable although a vigorous subperiosteal osteogensis was present. When radial bone segments were created leaving only the nutrient artery and periosteal blood supplies intact, the majority of osteocytes became non-stainable after 2-3 weeks. New bone formation was present in all cases.

Os novum, as introduced by Orell (1937) was created by rasping the surface of a rabbit's radius. Three weeks later a radial defect was made and replanted, keeping the os novum between cortical bone and an overlying skin pedicle. The tissues were examined after 2-3 weeks. While the tibial bone appeared as in previous experiments, the os novum was more abundant in production than in similar free grafts while non-staining osteocytes were more abundant. Hellstadius felt his os novum grafts and flaps maintained a higher degree of vitality than Orell's due to his shorter observation period (2-3 weeks as opposed to 42 days for Orell). He concluded older osteoid or bone tissue was more sensitive to decreases of vascularity, as the cells were further from the blood supply.

Hellstadius believed dying cortical bone produced a bone-inducing substance which stimulated the surrounding soft tissues including the muscle pedicle to resorb the graft and form new bone. He felt the greater vascularity of the pedicle allowed this process to occur more quickly.

Penton and Brantigan (1951) created 3 x 3 cm or 3 x 5 cm defects in the trachea of adult dogs. The defect was repaired with a pedicled flap of rib periosteum, intercostal muscle and parietal pleura pedicled to associated intercostal vessels. After 2 weeks the periosteum had produced an osseous skeleton with complete repair of the defect.

The authors argued that a pedicled flap was superior for repair of large tracheal defects as the continued blood supply increased healing and decreased the amount of stricture. They used a composite chest wall flap to repair surgical defects of the trachea and both bronchi. By 8 weeks the flap was healing well although no mention was made of subperiosteal bone formation (Penton and Brantigan, 1952).

Blair (1958) found similar results when tracheal defects were repaired in mongrel dogs using a composite intercostal pedicled flap. In some cases a mesh was placed over the defect before flap closure. Bone was formed in 8 of 34 dogs after 1 1/2 months while hyaline masses were found in the flap after 7 months. Ross (1956) reported using this technique on a 28 year old male with good healing 3 years after surgery. Narodick and coll. (1964) reported similar results after repair of a tracheal defect in a 29 year old male.

Davis and Taylor (1952) compared pedicled and free bone grafts in dogs 6-9 months of age. They pedicled a 1.5 x 3.0 cm block of anterior iliac crest on adjacent muscle, placing the bone against denuded femoral head and acetabulum. A similar procedure was carried out on the opposite hip, using a free bone graft of anterior iliac crest. The dogs

were sacrificed 1 1/2-10 weeks after surgery. By 3 weeks the pedicled bone graft was partially vital with some osteoblastic activity while the free graft appeared to be completely nonvital with some osteoclastic function. By 10 weeks the pedicled graft was united to the acetabulum with active trabecular bone formation on the recipient lamellar bone. The free graft appeared to be united with the acetabulum, while most original lamellar bone was replaced by a looser trabecular structure. Davis and Taylor concluded that a cancellous bone flap with an intact periosteal blood supply and muscle pedicle could remain vital during healing.

Brown and Fryer (1953) argued that large deficits of mandible could be repaired by using pedicled bone from a distant region, the pedicle providing a permanent blood supply.

Davis (1954) used his technique on humans, using the anterior iliac crest (6.4 cm x 2.5-4 cm) pedicled to tensor fascia lata, gluteus medius (primary blood supply) and gluteus minimus for fusion of the ipsilateral hip joint. In a series of 9 patients aged 23-70 years only one case did not form a union after 33 months.

Hartley and Silver (1954) experimented with mature and nearly mature rabbits removing a 1.0 x 2.0 cm of outer ilium or greater trochanter and pedicling it to gluteus muscle. The flap was enclosed in Tantalum (Tm) foil and replaced on its bed. In 9 experiments the iliac graft was fractured, and in 3 experiments it was left intact. Specimens were examined from 29 to 38 days after surgery. In 11 cases the graft

remained vital in 1/7 to 9/10 of its mass. In all cases bone next to periosteum remained vital while bone near the cut edge was nonvital. The remaining 4 grafts were completely nonvital and related to a nonvital muscle pedicle. The fractured bones showed evidence of repair in 6 of 9 experiments, the bone appearing to arise from endosteum.

Free grafts were used as controls and treated similarly to pedicle grafts. In all cases the grafts became nonvital. Where free grafts were placed into the host bed, new bone formation was seen between host and nonvital graft. Hartley and Silver concluded that bone with a healthy muscle pedicle would maintain most of its viability and undergo new bone formation.

Davis (1959) applied his researches to humans, describing the use of a pedicled iliac crest fragment to attempt repair of an ununited ulna. Although the arm was subsequently amputated, histologic examination confirmed the bone flap viability.

Woodhouse and coll. (1962) argued that a bone graft would survive if transplanted with a pedicled blood supply. Using adult mongrel dogs, they transplanted a 7.5 cm segment of the sixth rib with the costochondral junction into adjacent skin tubes. The graft was pedicled to inferior intercostal vessels and remained viable for up to 54 days. After that period of time, collateral circulation had developed sufficiently to allow the graft to be moved into a humeral defect. The flaps retained a viable blood supply after 6 weeks while free bone grafts immediately transplanted into similar defects underwent

resorption.

Baadsgaard and Medgyesi (1965) studied the different properties of pedicled and free bone grafts on mature or nearly mature rabbits. Cancellous and cortical bone flaps pedicled to one cm. of muscle were isolated with polyethylene film. Free cortical and cancellous bone grafts were taken, freed of periosteum and placed against adjacent muscle "in exactly the same way". The authors do not mention if the free grafts were also isolated. Specimens were examined up to 13 days after surgery. The periosteum in both cortical and cancellous pedicled flaps survived, producing subperiosteal bone after 6 days in both cases. Most of the cancellous bone survived while up to half of the cortical bone remained vital.

Osteocytes in the periphery of cancellous free grafts survived, with a new periosteum forming by 6 days. Subperiosteal callus appeared by day 13. All osteocytes disappeared from the free cortical grafts, with periosteum appearing by 10 days.

Angiography and autoradiographic findings confirmed these observations. A second group of rabbits were given pedicled cortical and cancellous flaps and observed for up to 7 weeks. The cortical bone resorbed from the periphery towards periosteum, the latter creating a subperiosteal bone nodule, with both cortex and medulla. The cancellous bone necrosed only slightly while new subperiosteal and endosteal bone formation retained the original architecture. Baadsgaard and Medgyesi confirmed that periosteal-endosteal vascular anastomoses existed sufficiently to

allow a muscle pedicle to adequately nourish a bone flap. As cortical bone flaps produced more bone, they further concluded that the cortical bone largely devitalizes, providing stimulation for new subperiosteal bone formation.

Medgyesi (1965) developed an experimental model to isolate the host bed influence on healing myo-osseous flaps. Mature or nearly mature white rabbits were used. A 10 x 5 x 5 mm graft from the greater trochanter was pedicled to 1.0 cm of gluteus medius and minimus muscles. A free iliac crest graft was butted to the pedicled trochanteric bone (medullae adjacent) and the bones were isolated by a polyethylene film. The procedure was performed on both sides oof each of 13 rabbits. Two rabbits were treated with a pedicled graft of ilium butted to a free graft of the trochanter. Specimens were examined from 1-12 weeks after surgery. In the series of 26 grafts, the pedicled bone remained alive in 15, except for a zone of empty laumae adjacent to the cut margin. The remaining flaps showed varying degrees of more extreme aseptic necrosis. Periosteal (and endosteal) callus began forming after one week, the amount of bone increasing with time. Bone union was recorded for 23 cases. In all successful unions, new bone arose from the pedicle to invade and begin replacing the free graft tissue. Medgyesi concluded that bone and periosteum from a pedicled flap could provide the entire mechanism for healing. The cancellous bone flaps appeared to survive more frequently than cortical grafts.

Medgyesi (1968) repeated his experiments, placing the muscle pedicles under tension or 180^{o} torsion. All specimens were examined after 2

weeks. Three of the 5 bone fragments with a pedicle under tension were completely dead while 2 specimens showed substantial necrosis with good subperiosteal osteogenesis. Five composite flaps were put under extension and torsion, subsequently 2 of the bone fragments underwent complete necrosis. The control flaps with no strain on the pedicle survived the best. Medgyesi concluded that muscle pedicles under the least strain provided the best periosteal blood supply. He felt some of the subperiosteal bone formation may have been stimulated by necrotic bone.

Bell (1969) performed anterior maxillary osteotomies on 10-14 year old Rhesus monkeys retaining only a labial mucosal pedicle or a full palatal and incomplete labial mucosal pedicle. Surgical sites were examined by angiography and conventional histology from 1-6 weeks after surgery. After 1 week both groups showed good vascularization from soft tissue through periosteum to bone. Bone necrosis was present, however, adjacent to the osteotomy margins. By 6 weeks the osteotomies were well healed with no evidence of necrosis. Similar types of results were obtained when Bell performed posterior maxillary osteotomies with buccal mucosal pedicles (Bell, 1971), Le Forte I osteotomies with palatal mucosal pedicles (Bell and coll., 1975), vertical ramus mandibular osteotomies (Bell and Kennedy, 1976) saggitally split mandibular osteotomies, (Bell and Schendel, 1977) and one tooth osteotomized segments (Bell and coll., 1978).

Santoni-Rugiu (1969) recommended the use of outer table of calvaria, pedicled to periosteum as a method of repairing cranial defects. He

found no complications in a follow-up of 12 patients, although hyperostosis of the donor area was seen, persisting from 4 to 12 months after surgery.

Snyder and coll. (1970) presented a series of eight patients with mandibular defects, not suitable for reconstruction by conventional grafting. They created tubed flaps around the entire clavicle or saggitally split clavicular fragments, subsequently moving the flaps by stages into the defect. Periosteum was left intact. Seven of the 8 cases were successful with the clavicular bone undergoing active remodelling.

Baadsgaard (1970) investigated the ability of a pedicled bone graft to repair a long bone defect. He used rabbits, 6 to 12 months old creating a 3/4 cm long full thickness defect each proximal ulna. A cortical ulnar bone graft pedicled to flexor carpi ulnaris muscle was developed and used as an onlay graft to repair the defect. A similar repair was carried out on the other side using a free ulnarbone graft. Rabbits were sacrificed up to 41 days after surgery. The pedicled grafts showed periosteal hypertrophy with subperiosteal osteogenesis adjacent to muscle pedicle as well as viable osteocytes within the lacunae. Endosteal callus arose early from the donor bed, spreading to meet the pedicled cortical bone. By contrast, free bone grafts showed poor subperiosteal bone formation with most lacunae empty of osteocytes.

In a second series, pseudoarthrosis 3/4 cm long were created in the proximal ulnae and repaired in a manner similar to the first series. In

both pedicled and free grafts, periosteal bone formation was poor. The pedicled grafts produced more subperiosteal bone during the first 2 weeks. Endosteal callus arose more abundantly from the pedicled flaps especially after the 4th week.

Baadsgaard concluded that pedicled bone grafts revasularized earlier, producing more subperiosteal callus and faster healing during the first 6 weeks after surgery. He admitted that the muscle pedicle was longer than their previous experiments (2.0 cm) and distally based, implying a poorer blood supply. He felt these factors decreased the difference in quantity of bone productionbetween muscle pedicle flaps and free grafts.

Medgyesi (1970) investigated the contribution of a living bone graft towards replacement of bone in a free nonvital graft. He joined the femoral head of mature or nearly mature rabbits to the greater trochanter, the latter having a 1.0 cm muscle pedicle. The entire flap was isolated with polyethylene film and all animals were sacrificed from 4 days to 14 months after surgery.

Medgyesi admitted the flap's vascularity may have been compromised through manipulation. The pedicled bone demonstrated normal cells in half the specimens except in the region of the saw cut where the cells were necrotic. The other specimens demonstrated varying degrees of bone damage, the osteocytes being most sensitive to the cutting procedure. Complete necrosis occurred in 2 grafts. In the grafts, new bone trabeculae were both subperiosteally and endosteally. The abutted bone

graft was invaded by connective tissue both from its periosteum and the interlong connective tissue, followed by trabecular bone formation. Callus united both pieces of bone after 2 weeks.

In a second series, the muscle pedicle was directly joined to cancellous bone of the femoral head. The flap was isolated and examined 4 days to 6 months after surgery. No primary callus developed although invading connective tissue with some new trabecular bone formed within the graft.

Medgyesi felt that isolation with polyethylene demonstrated that only the periosteum and endosteum provided vascularity for connective tissue invasion. He felt the invading connective tissue induced new bone formation.

Strauch and coll. (1971) resected the anterior 6 inches of the 7th rib in mongrel dogs, 50 to 75 pounds in weight, maintaining their intercostal and internal thoracic vascular pedicles. The grafts, containing skin, subcutaneous tissue, periosteum and bone were transferred subcutaneously to the ipsilateral submandibular soft tissues, adjacent to the mandibular border. The flap skin was brought to the body surface and sutured in place. Three of 7 dogs demonstrated good healing after an unspecified period of time.

A second group was treated similarly to the first, except the sternum was not split for surgical access as in the first group. By 10 weeks the skin paddle died in all 5 animals although bone and muscle survived.

A third group of animals underwent a similar procedure to the first group except the osseous portion included half the sternum and overlying skin. All elements of the flap in the 5 dogs survived 3 months after surgery.

Stauch and coll. felt this procedure was of great value in reconstructive surgery as long as all tissues of the flap, including periosteum and bone, remained viable.

Conley (1972a) felt there was a risk in moving a pedicled flap with attached bone into heavily irradiated or infected areas since the osseous component was usually attached to its pedicle only by areolar connective tissue. He felt muscle pedicles delivered the best blood supply. Conley (1972b) described using temporalis muscle with attached skin and temporal bone for repair of the orbit, maxilla or upper mandibular ramus. He warned that even though the bone maintained its own blood supply contamination with saliva could compromise its success. Conley and coll. (1973) summarized their past experiences with composite flaps. They felt creation of a pedicled bone flap severely compromised all osseous vitality except for the area supplied by periosteum.

Jurkiewicz (1973) commented on the report by Conley and coll. He felt the reported results were invalid as bone samples were taken from infected wounds and further, taken several months after flap reconstruction.

Judet and Patel (1972) argued that composite muscle pedicle bone flaps needed to be developed without supraperiosteal dissection while some bone should be removed with a chisel allowing bone chips to cover both periosteum and muscle. They described repair of both aseptic and septic pseudoarthroses by this method. They felt the pedicled osteoperiosteal flap could survive in a septic environment regenerating new reparative bone. Of 1,068 patients treated with this procedure between 1962 and 1971, 92% had clinical successes.

Medgyesi (1973) created composite flaps of scapular margins pedicled to 3 cm supraspinatus muscle pedicles, scapular spines pedicled to skin and trapezius muscle, ribs pedicled to skin tubes, femoral diaphyses pedicled to 3 cm of gluteus maximus muscle and radial or tibial diaphyses pedicled to skin. Procedures were carried out on goats of unspecified age. After isolating the pedicles, India Ink was injected into the local vessels. Bone vessels perfused well through muscle pedicles but poorly through skin pedicles. One femur perfused poorly probably because the muscle pedicle also perfused poorly. Medgyesi suggested muscle pedicle bone flaps were superior to skin pedicle bone flaps although the latter were superior to free grafts.

Meyers and coll. (1973) reviewed 150 cases of femoral neck fractures treated with a quadratus femoris muscle pedicle bone flap and internal pin fixation. Residual bone defects, primarily in the femoral neck were repaired with autogenous cancellous iliac bone chips. One hundred and forty-six patients were followed up to 40 months after surgery. After exclusion of 10 patients for various reasons, 15 fractures failed to

unite yielding an 89% rate of clinical union. Radiographically, the bone of the pedicle appeared to remain vital, loosing none of its mass and occasionally increasing in size. While many factors such as time of operation after injury, degree of injury, age or compliance of the patients influenced the results, the authors felt the technique gave a high success rate even after several days of delay between injury and surgery. The authors believed the pedicle gave stable fixation and increased both the speed and amount of femoral head revascularization.

Adelaar and coll. (1974) took rib grafts with the associated intercostal artery, periosteum and muscle pedicle, autogenously transplanting them into radial defects of unspecified length in adult mongrel dogs. The proximal and distal intercostal arteries were anastomosed to branches of the radial artery, although no mention was made of venous drainage. The opposite limb was treated by a similar graft without anastomosis. Both limbs were fixated with plaster up to 90 days after surgery. Nine of 16 anastomoses were patent and there was no significant difference in clinical union between both types of grafts although the grafts without anastomoses showed less periosteal callus formation.

Ketchum and coll. (1974) attempted to repair the mandible of a 37 year old male lost to a gunshot injury with free rib grafts. When free grafts failed, the mandible was reconstructed with a segment of rib pedicled to local vessels. The graft was greenstick fractured in 2 places to provide a more acceptable contour with success reported 4 months after surgery.

Adelaar and coll. (1974) created 3 cm full thickness defects in the radii of adult mongrel dogs. The defect was immediately repaired with a periosteal rib graft and immediate vascular anastomoses while the opposite radial defect was repaired with a rib graft without vascular anastomoses. Animals were evaluated up to 90 days after surgery. The results were presented in summary form, there being no significant difference in the healing of either graft or anastomosed flap.

Ostrup (1974) removed the posterior 10 cm of a rib in adult mongrel dogs, including overlying soft tissues, muscles, the posterior intercostal artery and vein. The graft was autogenously transferred to a defect in the inferior border of mandible via an extraoral approach. Ischemia time averaged 90 minutes. Periosteum was removed from both the host and recipient contact areas while the graft was fixated with stainless steel wires. Ten dogs were treated with ring pin anastomoses while 6 dogs were given free composite grafts. All animals were sacrificed from 4 weeks to 6 months after surgery. Radiographic studies demonstrated callus in the anastomosed grafts at 4 to 8 weeks while callus was present in only one free graft after 8 weeks. Healing was clinically solid in 8 of 10 dogs after 7 weeks while nonunion was always seen in the free grafts. Histologically, 6 of 8 pedicled grafts demonstrated good marrow architecture while two specimens demonstrated fibrous tissue replacement of marrow. The free grafts showed empty lacmae in all cortical bone, degeneration of marrow, loss of vascularity and loss of periosteum.

Ostrup and coll. (1975a) repeated their earlier experiments with adult

mongrel dogs, first irradiating the mandibular body with 5,000 rads of CO 60 in twenty fractions. A 9th rib graft was transferred 4-48 weeks after radiotherapy. Five of 10 dogs retained successful grafts with clinical union after 16 days. Vessels were seen growing from the irradiated mandible to the graft. New subperiosteal (and endosteal) bone formation was present with remodelling of the grafted cortical and cancellous bone. Four failures were present with histologic evidence of blocked vessels and total tissue necrosis. All recipient vessels, however, appeared healthy.

Ostrup and coll. concluded that a composite bone graft transferred with its own blood supply into the hostile environment of irradiated tissues had a greater chance of survival. Vitality of pedicled composite grafts seemed to depend on good arterial supply, good venous drainage and good bone tissue perfusion. Under these conditions, periosteum had the ability to survive and produce new bone after transplantation (Ostrup and coll., 1975b)

Taylor and coll. (1975) repaired a tibial defect in a 28 year old male by transferring a 22 cm fibular segment from the opposite leg into the defect. Iliac bone chips were placed around both graft ends. The anoxic time was 1 1/4 hours. Callus was evident by 10 weeks although angiography showed no patent anastomoses by 14 weeks.

A 19 year old male was treated for gunshot wound to the leg by fibular transposition. The attempt was unsuccessful and a free vasularized fibular graft, 22 cm in length, was transferred into the defect with

vascular anastomoses. Forty-four days after surgery the vascular anastomoses were patent although no comment was made on new bone formation or the use of the leg. The authors felt the technique should only be used where amputation was the only alternative because of the sacrifice of major vessels for graft anastomoses.

Canalis and coll. (1977) created osteocutaneous deltopectoral flaps in 18 adult Rhesus monkeys, using the attached distal third of clavicle to repair a defect made in the inferior border of mandible. The opposite side was given a free clavicular graft as a control. All animals were sacrificed from 2-24 weeks after surgery. Both free and pedicle grafts were firm by 4 weeks while later specimens demonstrated progressive bone resorption. Pedicled flaps always resorbed more slowly than free grafts, however two of the later pedicle flaps demonstrated seemingly thicker bone than in the original flap. Both flaps and graft demonstrated loss of osteocytes up to 6 weeks after surgery while the pedicled graft demonstrated active subperiosteal remodelling with incorporation into the graft site after this time.

In a second series forehead flaps containing the anterior wall of frontal sinus were created. The flap was tubed, with pedicled bone being wired to the zygomatic arch. Bone from the upper anterior wall of frontal sinus was fixed as a free graft to the opposite zygomatic arch. Dogs were sacrificed up to 16 weeks after surgery. All free grafts were resorbed while pedicled bone flaps were thicker and solidly fused to the zygoma. The pedicle demonstrated a rich vascularity. The free grafts showed some new bone formation with resorption predominant while pedicle

grafts showed persistence of vital osteocytes as well as early subperiosteal bone formation.

Canalis and coll. concluded that free autogenous bone grafts demonstrated greater bone resorption than production. Pedicled flaps were superior as periosteum remained viable, providing osteoblasts for new bone production as well as continued viability of the bone graft, allowing rapid union of the bone to the defect. They emphasized fixation and immobilization were imperative for success.

Canalis and coll. (1979a) and Canalis (1979b) continued their researches, with adult mongrel dogs. They created forehead flaps of skin containing anterior wall of frontal sinus and sinus mucosa, suturing the flap to the ipsilateral fascia of parietal bone. A flap was created on the opposite side without bone, the periosteum being sutured directly to muscle fascia. Free bone was implanted subcutaneously in the calvarium as a control. Specimens were taken at 8 to 40 weeks. All pedicled periosteal flaps showed change to a fibrous tissue without evidence of new bone production.

All free bone grafts were completely resorbed as the experiment progressed. The osteoperiosteal flaps generally retained their size while one specimen of 40 weeks showed a small increase in size. The osteocytes remained vital while subperiosteal bone formation continued throughout the duration of the experiment. This new subperiosteal bone gradually replaced the entire bone flap. Only the richly vascular periosteum appeared to be producing new bone.

Canalis and coll. felt the success of pedicled bone flaps depended on a continuous abundant blood supply, continued normal vascularity of the transplanted bone somehow impeding host resorption. The bone was produced in a completely stress free environment, in young animals. The authors suggested an "inductor substance" released from devitalized or damaged bone could have a part in bone production.

Serafin and coll. (1977) described using an osteomyocutaneous rib graft, 25 cm x 10 cm, delayed for a week and then transferred with microvascular anastomoses to repair the anterior mandible and floor of the mouth. Serafin and coll. reviewed the various free complete grafts with microvascular anastomoses in 1980, emphasizing their success depended on an intact blood supply to bone.

Finley and coll. (1978) used 9 mongrel dogs of varying ages, autogenously transplanting a rib with its intercostal vessels into the groin. One half of the rib was placed as a free graft while half was implanted as a revascularized flap. After 6 weeks, the free graft was almost completely resorbed while the revascularized grafts demonstrated new bone in 3 of the 9 dogs showing vascular patency.

In a second series, 5 cm of tibia and periosteum were removed while in the opposite limb a similar defect was made, followed by repair with rib periosteum anastomosed to local vessels. By 6 weeks no new bone had formed in the resected limbs while all of the 5 revascularized limbs exhibited new bone formation. New bone bridged the defect by 6 weeks in every case. The new bone after 2 months healing appeared thicker than

the original bone with the dogs able to bear weight freely on the limb. Finley and coll. concluded that periosteum with an adequate blood supply produced new bone in dogs.

Acland (1978b) in a letter subsequent to his article (PRS [Finley & coll.] 61:1, 1978) felt the procedure should be applied with caution to humans as experiments with dogs showed no significant bone formation in regenerating cranial defects even though 60% of the vascular anastomoses remained intact. When the technique was tested on ulnar defects, grafts with both patent (5 of 7) and occluded (2 of 5) periosteal vessels produced new subperiosteal bone while most (7 of 8) free periosteal grafts produced new bone. No indication as to the amount or quality of new bone was given. Factors influencing the results were not mentioned but rather were "being studied".

Harashina and coll. (1978) described two cases of mandibular reconstruction using ribs with posterior intercostal vessels as the intact periosteal blood supply. Good bone remained after 8 months in one case with no clinical resorption. A biopsy on one case at 3 1/2 months showed normal cortical and medullary bone in the grafted site. Daniel (1978) commented on this paper, arguing no substantial proof was given for the continued uninterrupted viability of the revascularized grafts. Daniel felt that facial reconstruction could be better managed with free osteocutaneous groin flaps, osteocutaneous rib flaps or dorsalis pedis flaps.

Ariyan and Finseth (1979) argued that taking a posterior rib graft would

risk damage to a spinal nerve. Removing an anterior graft allowed harvest of intercostal vessels supplying only periosteum although they felt this was sufficient to supply blood to the entire rib cortex. They described one case of a 54 year old male who underwent mandibular resection plus radiotherapy (7000 rads). A skin paddle (10 x 30 cm) with muscle and associated segment of 5th rib were taken and anastomosed into position. By 4 months, angiography showed increased diameter periosteal vessels after 6 months. The vessels travelled from periosteum in straight lines through the cortex.

Krepsi and coll. (1979) created free pleuroperiosteal grafts from the eighth rib in mongrel dogs of different ages, preserving the intercostal vessels. The graft was used to repair a tracheal defect spanning two cartilaginous rings. One graft was not anastomosed while 4 grafts had their vascular pedicles anastomosed to local vessels. Ischemia time was not given. All dogs were sacrificed at 8 and 12 weeks. The flap taken at 5 weeks and free graft taken at 12 weeks showed satisfactory fibrous repair of the defect without osseous or osteoid formation. The remaining 3 pedicled grafts showed satisfactory tracheal repair with submucosal "plates of supporting bone".

Kufaas (1979) created tracheal defects in mongrel dogs of different ages, repairing the windows with 10 cm x 4 cm free pleuroperiosteal flaps anastomosed to local vessels. The pleural surface was placed next to the tracheal lumen. The animals were evaluated at 12 weeks, all showing successful repair with mature subperiosteal bone formation.

O'Brien and coll. (1979) described several cases of composite osseous grafts including free composite dorsalis pedis flap with microvascular anastomoses used to repair both limb bones and mandible. They felt large nutrient osseous vessels were not imperative as periosteum itself properly vascularized would supply adequate vascularity to underlying osseous tissue.

Ariyan (1980) continued to argue anterior rib grafts with internal mammary vessels provided better blood flow both to overlying soft tissues as well as adequate periosteal blood supply to bone in composite free grafts. He described the case of a 60 year old male with mandible resected from angle to angle reconstructed successfully with rib osteocutaneous flaps. They described another case involving a 58 year old woman with mandibular reconstruction utilizing autogenous rib with pedicled periosteal blood supply. Tetracycline markers showed new bone laid down below periosteum as well as within the deeper ostems.

Ariyan concluded periosteum supplied the only blood for continued viability of the graft as well as new bone production within it.

Cuono and Ariyan (1980) transferred a rib osteomyocutaneous flap to repair the mandible in a 53 year old male 1 year after the initial resection. Tetracycline was administered after reconstruction with a subsequent biopsy showing new bone formation in the osseous component of the flap.

Kowalik (1980) described using composite bone flaps on muscle or skin

pedicles to repair mandibular defects. The flaps maintained a constant blood supply and were thus able to be transferred to irradiated recipient sites.

Green and coll. (1981) described using sternal cortex and overlying skin pedicled to pectoralis major muscle. They briefly mentioned its use on 6 patients over one year although no detailed follow-up studies were shown.

PERIOSTEAL BLOOD SUPPLY

Langer (1876) first identified periosteal arteries and veins entering cortical bone, anastomosing with medullary vessels from the nutrient artery.

Many early authors recognized the importance of a rich blood supply in producing new bone (Albee 1913; Bancroft 1914; McWilliams 1914a; McWilliams 1914b; Phemister 1914a; Davis and Hunnicutt 1915; Gill 1915; Hey Groves 1917; Gallie and Robertson 1920), but little detailed investigation on the influence of periosteal vascularity to osteogenesis was carried out.

Kolodny (1923) questioned this relationship with his experiments on adult dogs. In one series, a 6 cm. segment of radius was supraperiosteally dissected in the middle or distal diaphysis and a Gigli saw fracture was created. Both periosteal and part of the medullary blood supplies were thus destroyed. Radiographic examination was made of the surgical site from 14 to 42 days after surgery. None of the fractures united, while subperiosteal callus did not form until the periosteal circulation was re-established at approximately 28 days. By this point fibrous tissue had bridged the fracture gap, creating a non-union. Endosteal callus did not form until much later when intraosseous circulation had re-established (specific time not given). As a further observation, increased vascularity about the fracture site was present after 42 days, was compared to simple forearm fractures in

dogs where the hyperemia disappeared by 25 days.

In another series of experiments, a similar dissection was performed. The nutrient artery was cut and the fracture made through the entrance of the nutrient artery at the proximal portion of the radius. No unions occurred and little callus of any type was produced.

Kolodny concluded that periosteum was given a rich blood supply primarily from the overlying soft tissues as well as the nutrient artery in the area of the nutrient foramen. The periosteum was felt to produce a more important healing callus than endosteum, while a proper subperiosteal callus could not develop unless a sufficient blood supply was present.

Neuhof (1923) argued that a periosteally covered bone graft would unite with the host soft tissues, establishing a periosteal blood supply by 3 days. He felt that by 6 days there was no difference between vascularization of transplants with or without periosteum, although periosteum did slow down resorption of the bone graft by postponing the eventual death of the bone.

Johnson (1927) studied the blood supply to the tibial diaphysis of dogs by isolating each component (periosteal, nutrient artery, and metaphyseal vascular system) as the only blood supply. Dogs were sacrificed immediately after surgery up to 4 weeks postoperatively. Injections were made through the aorta or iliac vessels with India Ink, prior to histologic preparation. Throughout the period of examination,

the periosteal blood supply was seen to vascularize the outer cortex from just below the cortical surface to half its outer thickness. In no case were vessels seen passing through cortex from periosteum to the medullary cavity. The periosteal vessels were always finer than medullary vessels. In isolating the nutrient and metaphyseal arteries, the periosteum was sharply dissected from the diaphyseal bone surface. Bone wax was rubbed over the cortical surface and three full thickness cortical holes of 6 mm. diameter were made before replacement of periosteum. A small area of periosteum was also elevated to block nutrient and metaphyseal vessels when testing the periosteal blood supply. Johnson does not mention if a supraperiosteal dissection was carried out during initial exposure.

By 4 days, the outer cortex had become necrotic, while the periosteum was now thickened. The periosteum had reattached by 2 weeks with a beginning subperiosteal osteogenesis. By 4 weeks the now abundant new subperiosteal bone had begun to invade and replace the underlying necrotic bone. Superiosteal callus was found at the margins of the defect while its central zone was repaired with fibrous connective tissue. The periosteum appeared to vascularize new bone and the callus beneath it. By 2 weeks, vessels were seen passing through the fibrous plug into the medulla. In contrast the re-established medullary supply created almost complete bridging bone callus across the inner surface of the drill holes by 2 weeks. Cartilage was found only in the subperiosteal callus.

Johnson concluded that periosteum supplied the outer half of cortical

bone and was the only system to do so during the period of observation. As well, the periosteum was less important than endosteum in repairing the cortical defects.

Cowan (1928) described the periosteal blood vessels of young animals dividing many times before entering cortical bone. The vascularity decreased with age. He followed healing of humeral fractures in young kittens, arguing the first formed subperiosteal hematoma induced the periosteum to send out vascular buds. The granulation tissue thus formed periosteal osteoblasts to lay down the external callus.

Leriche and Policard (1928) observed periosteal vessels entering Volkmann's canals, providing a vascular supply to the outer cortical layers of bone.

Gallie (1931) felt subperiosteal osteoblasts were part of a bone graft rather than the periosteum. He felt however that periosteum should not be stripped extensively from host bone as it provided a major blood supply for healing.

Kistler (1934) created infarcts in femurs of young rabbits by injecting powdered charcoal and gum acacia into the nutrient artery. The animals were sacrificed from 20 hours to 150 days after operation. He found charcoal initially produced necrosis of the medulla and inner 1/4-1/2 of cortex. In a few areas the entire cortical bone thickness was infarcted. Subsequently new subperiosteal bone of varying thickness developed over the necrotic bone. The new bone was more vascular than

the original cortical tissue, as well as being more abundant where muscles or tendons made attachment to the necrotic bone. New endosteal bone was also found near the ends of the infarcted areas. Kistler found the most striking changes in very young rabbits. He concluded that extracortical vascular interference did not alter either periosteal bone formation or the periosteal blood supply to outer cortex.

May (1937) investigated the relationships between vascularity and healing of bone transplants, using dogs of unspecified age. In each case, a single radius was subperiosteally removed and immediately replanted. In no case was the periosteum separated from overlying connective tissues. The dogs were sacrificed from 5 weeks to 10 months and the axillary arteries were injected with a mercury-turpentine solution. By 5 weeks a greatly increased number and size of periosteal vessels had begun penetrating dead cortex through the Haversian system, most densely beneath joint cartilages and around epiphyses (especially in growing animals). The vessels penetrating into marrow began to revascularize this tissue early while the osteoblasts and vessels derived from periosteum replaced bone cortex from 2 1/2-4 months after surgery. External subperiosteal bone formation occurred during the healing phase, as well. The larger vessels became "the intra-osseous system".

In areas of the original incision, periosteum did not cover the bone. Here, osteoclastic bone resorption with fibrous tissue infiltration was dominant while osteogenesis was a less dominant process. In all, 25 experiments were performed with 21 sites being contaminated. No mention

was made of inflammation or infection in describing the results. May concluded that periosteum alone supplied new vascularity to a bone graft, priority being given to areas of greatest need, such as epiphyseal plates. He further concluded that periosteum supplied osteoblasts to replace the entire cortical bone tissue.

Urist and Johnson (1943) followed the course of fracture healing in humans, from biopsies taken at operation. They found periosteum supplied the most vessels to fracture callus during the first week, until the medullary system reformed.

Weinman and Sicher (1947) described periosteum as supplying vessels to the cortical bone, entering through Haversian and Volkmann's canals to anastomose with branches of the medullary artery. No mention was made of the cortical area supplied exclusively by periosteal vessels.

Foster and coll. (1951) destroyed the nutrient vessels in one femur of growing rabbits, subsequently almost completely stripping periosteum from the same bone. The opposite femur was left intact as a control. All animals were sacrificed up to 5 months after surgery. The experiment was repeated on mature rabbits, some showing endochondral bone growth. By day one most diaphyseal cortical osteocytes in young animals had ceased staining while marrow showed extensive necrosis. These findings persisted for 2 months after operation. By 3 days, new bone was forming on the cortical surface, the authors presuming it arose both from the reattached periosteum and associated fascia. By 3 weeks, reorganization of the dead cortex was well marked and almost complete

after 3 months.

Repair of infarcted bone and marrow was similar in older animals except that it proceeded more slowly and both periosteal and endosteal reparative layers were seen. As the infarct involved the entire thickness of cortex in the middle femur the authors concluded both endosteal and periosteal blood supply contributed to the vitality of cortical bone.

Trueta and Cavadias (1955) reviewed previous work on blood flow through bone. They observed that interruption of blood flow through metaphyseal and nutrient vessels would cause an increase in periosteal blood flow, while increased vascularity of periosteum always preceded new bone formation. Severing the nutrient artery produced necrosis of the inner 2/3 of cortex while the outer third, supplied only by periosteum remained vital. When the periosteum was stripped and separated from outer cortex, only the outer 1/3 of cortical bone developed necrosis. They felt Kuntscher nailing produced ischemic changes of the nutrient artery with subsequent periosteal bone formation, and evaluated the hypothesis experimentally using "young" rabbits of unspecified age. Gigli saw fractures were made in rabbits' radii and the ulna was used as a splint. Periosteum was incised and the nutrient artery divided in every case. On one side a medullary nail was placed while the opposite side was left undisturbed. Polyethylene plugs were inserted to prevent metaphyseal blood flow to the diaphyseal cortex. After 7 to 10 days periosteal bridging callus had formed; more abundantly on the nailed than the undisturbed side. Periosteal vasculature into cortex increased

on the nailed side as soon as 21 days after surgery with trabeculae following the straight direction of the vessels. In nailed bones, the diaphyseal bone diameter became greater due to subperiosteal deposition. Angiograms showed radiopaque material in the medullary cavity after interruption of the medullary supply, the material presumably arising from periosteal vessels. The inner half of cortex appeared empty of osteocytes, this zone gradually blending into viable periosteal cortex. Vessels were seen passing from the periosteal surface to the medulla. In adults, the cortical thickness increased 2 months later and to a lesser extent than in young animals. Periosteal callus occurred earlier than the undisturbed side. In 4 cases periosteal callus of greater volume than in young animals occurred but was quickly absorbed. One third to two thirds of inner cortex became necrotic after destruction of the nutrient artery. Union occurred at approximately the same time in both groups; (6 1/2 months) although earlier on the nailed than the cortical side.

Trueta (1963) summarized his findings of 15 years in 1963. He felt periosteal vessels did not perfuse well with radiopaque solutions misleading researchers to believe no arteries perforated cortex from periosteum. Veins, however, penetrated the cortex in great abundance. He found that suppressing gastrocnemius muscle contraction in experimental animals caused cortical sinus and venous engorgement with bone rarefaction. Restoration of muscle activity caused return of normal bone architecture. Trueta felt dying osteocytes, chondrocytes or endothelial cells released a vascular stimulating factor which stimulated angioblasts to proliferate forming osteoblasts, osteoclasts

or their precursors. The cells all maintained cytoplasmic connections, the future canalicular cores. His conclusions were indirect inferences as no definite cytoplasmic connections were demonstrated.

Lewis (1956) described his findings of studies on long bones of embryonic and young rabbits. He found endochondrally formed bone arose from the nutrient artery while periosteally derived bone was supplied by periosteal vessels. In later development periosteum supplied the metaphysis and epiphyseal plate.

Cohen and Harris (1958) studied femoral Haversian systems in adult mongrel dogs. They found uniformly thin walls in all Haversian and Volkmann canals, suggesting blood could flow in either direction through cortical bone depending on pressure differences at periosteal and endosteal surfaces.

Herfert (1958) presented a summary of his research into correction of cleft palates. He reflected a full thickness mucoperiosteal hemi-palatal flap in 6 week old terrier pups, ligating or tearing the posterior palatal artery before replacing the flap. Eight weeks after operation the palate had narrowed 16% to 23% on the reflected side. The periosteum had become a fibrous, scar tissue-like structure. A second group of dogs underwent a similar procedure without arterial ligation. Palatal narrowing was decreased 10.8% to 13% of the normal side and osteogenic cells were seen in the periosteum. Herfert concluded that damaged palatal periosteum hindered palatal growth although palatal periosteum would retain most of its osteogenic power provided the

palatal artery was not damaged. He also recommended delaying palatal surgery on humans until 6 years of age when the maxillary arch reached its maximum width.

Morgan (1959) studied the periosteal blood supply in growing rabbit tibia. He found the vessels passing close to cortical bone were parallel to the bone surface, and interconnected by vessels passing at 90^{o} to them. The major vessels also gave off smaller vessels, primarily arteries, which passed perpendicularly through the cortex to medulla. The number of vessels in the shaft was smaller, becoming greater at the epiphyses. There s-shaped branches passed to the epiphyseal plate with assistance from metaphyseal and epiphyseal arteries. Other branches formed the primary supply to the circumferential artery of the epiphyseal plate. (The "Circulus Articuli Vasculosus" described by Hunter, 1743.) The metaphysis was found to be rich in anastomoses of veins between medulla, bone cortex, marrow and periosteum. A venous plexus was found passing from the metaphyseal cortex to join the periosteal plexus of veins.

Brookes and Harrison (1957) injected different concentrations of Micropaque (Tm) or Thorotrast (Tm) into the abdominal aorta or external iliac veins of fully grown rabbits to study osseous blood flow. Periosteum was stripped from all bones before histologic examination. They found endosteal arteries freely penetrated the endosteal cortex, traversing the inner half with only the occasional artery penetrating the entire cortical surface to anastomose with periosteum. This, however may have been due to the size of radiopaque particles. Veins

injected with Thorotrast (Tm) revealed abundant periosteal capillaries penetrating cortex to link with medullary sinusoids as well as an intermediate cortical plexus. Brookes and Harrison concluded this plexus formed a link between arteries and capillaries in cortex while arteries crossing the entire cortex were periosteal in origin. They postulated blood entered the medullary arterioles, passing to a thin walled vascular lattice in the Haversian canals, and thence to the endosteal, or more commonly, the periosteal surface, at least in the rabbit.

Brookes (1960) injected 50% Barium Sulfate (Micropaque Tm) intravenously in humans aged 59-80 years; all 2-24 hours before amputation of a lower limb. (The limbs were amputated for senile atherosclerosis causing peripheral gangrene.) The solution was injected into the anterior tibial artery, nutrient tibial artery or draining tibial vein. He found ischemia from the nutrient vessel resulted in increased vascularity to cortical bone from periosteum, the blood flow changing from medullary centrifugal to periosteal centripedal in nature.

Brookes and coll. (1961) injected salicylic acid, urea and hypaque into the cortex of the acromion process in human subjects. Radiographs showed a "spray-like effect" in the surrounding muscles. A similar result was obtained in a 6 hour old cadaver while histologic examination showed the material (India Ink) passing into medulla, on into periosteum and hence muscle. Brookes and coll. used these results as further proof for blood flow passing though a "cortical capillary mesh" outwards to periosteum. They felt blood flow was aided by the pumping action of

attached muscle.

Brookes and coll. (1970) created upper humeral osteotomies just below the surgical neck in mature rabbits, re-uniting the fragments with an orthopedic plate. Animals were sacrificed one week to one year after surgery. Blood flow through the osteotomized segment was recorded as a measure of perfusion, pH and histologic presentation. They found abundant spongy bone in the injured area, with increased arterial supply directed towards cut cortical bone cartilaginous callus and screws used in fixation. The mixed blood pH was elevated up to 6 weeks after surgery. From 4-8 weeks, the bone changed to compact cortex with a newly formed marrow cavity, with decreased vascularity and gradual decrease in pH to that of the unoperated side. By 8 weeks compact bone united the fracture, burying the fixation plate, and persisting at least 1 year after osteotomy. The blood pH had dropped below that of the unoperated side by 24 weeks on the osteotomized side, reaching a maximum by 7 weeks after surgery and remaining elevated for at least one year (50% greater than the unoperated side). By contrast, some venous dilatation was seen on the unoperated side during the first 4 weeks after surgery.

Brookes and coll. concluded that bone injury directed vessels towards the site of injury and newly formed callus, with increased blood flow, elevation of pH and formation of spongy bone. Concurrent with increased blood flow to the site of injury, was increased blood flow to the opposite limb. As vascular pH fell over 8-12 weeks, corresponding to a fall in blood flow as more cortical bone was produced.

Nelson and coll. (1960) studied the blood supply of 14 lower limbs amputated for tumors by gross dissection, injection of India Ink into the arterial tree, and microangiography. They described the three classic vascular systems to the tibia epiphyseal-metaphyseal, main nutrient artery and periosteal. An abundant periosteal vascular network on the tibial periosteum was supplied primarily by the anterior tibial artery. The vascular branches, consisting of an arteriole with two venules passed along the anterior surface of tibia giving off regularly spaced collateral vessels "like rungs of a ladder". while this pattern presented itself in the posterior and lateral tibial surfaces, the medial surface presented only a random anastomotic configuration.

All vessels entering or leaving the cortex had different diameters and thin walls lined by endothelium. The authors felt larger vessels were veins, while smaller vessels were capillaries entering the cortex from periosteal arterioles. Capillaries were infrequently seen, perhaps representing a collateral source of circulation. Occasional cell nests were seen lying in cortical depressions, with arterioles originating from these nests and entering long cortex. The authors postulated that Haversian systems developed around these nests while the periosteal circulation supplied vessels for new Haversian systems that formed on the external bony cortex.

Zucman (1960) studied the vascular connections between muscle, periosteum and bone using rabbits of unspecified age, species or sex. In one group the nutrient artery of extensor hallicus longus muscle was

cut and all fascia divided. A ligature was placed around the tendon and only the periosteal blood supply was left intact. Nerves were preserved where possible. Animals were sacrified 4 hours to 30 days after surgery. Injections of Berlin Blue and Micropaque (Tm) through the abdominal aorta showed muscle revascularization from periosteum beginning at 12 hours, reaching a hypervascular stage by 48 hours and returning to a preoperative pattern by one month. Both arteries and veins showed similar filling patterns. The muscles themselves atrophied after 15 days.

When the series was repeated, this time also severing the surrounding periosteal blood supply, the muscles atrophied with no evidence of vascular perfusion. In a third series, periosteal attachments were stripped while nutrient vessels were left intact. The muscular vessels became markedly dilated up to 30 days after surgery. Zucman carried out a final series, separating nutrient vessels on one leg as in the first series, and separating both nutrient vessels as well as stripping periosteum on the opposite limb. By 24 hours the muscles with best periosteal attachments demonstrated marked vascular dilatation while the muscle itself was less atrophic after 5-6 weeks. Zucman concluded that division of nutrient arteries caused muscular ischemia with revascularization occurring after 12 hours, the mechanism being development of anastomoses through periosteum. Vascular connections through bone to periosteum proved inadequate to revascularize muscle. Periosteal stripping, however, seemed to enhance the rate of revascularization.

Rhinelander and Baragry (1962) digitally fractured either long bone of the forelimb of dogs of unspecified age or sex, allowing all animals to walk on their fractures immediately after injury. The animals were sacrificed up to 8 weeks after surgery, their vascular trees being perfused with Micropaque (Tm) in normal saline solution. They found a highly vascular periosteum producing callus of bone and fibrocartilage, earlier (3-4 days) and more abundantly than endosteum, especially if the fracture was more displaced and less stable. Subperiosteal cartilage was found most frequently under sites of torn and displaced periosteum. By 2-3 weeks the medullary blood supply had re-established itself, becoming the primary bone producing tissue at the fracture site.

In another series of experiments, midshaft displaced radial and ulnar fractures were made in mongrel dogs of unspecified age. One week after injury, there was a marked increase in periosteal and endosteal blood supply with extensive branching of vessels along the fracture line into external callus as well as the first anastomosis from periosteum to endosteum. By 12 weeks, however, the medulla appeared to supply the endosteum and most of the cortex with vessels, while the periosteum appeared to supply the outer cortical third after 20 weeks. Periosteum appeared to be the primary blood supply during the first stages of healing as the initial hematoma formed. Periosteal callus rapidly bridged the gap in displaced features. After 3 weeks endosteal vessels bridged the gap with endosteal callus uniting the fragments after 6 weeks.

Rhinelander and coll. concluded that with more complex fractures, the

medulla became more disrupted and the cortex took over as the former of rapid, abundant first callus until endosteal circulation re-established. In some cases an endosteal callus bridge became evident after 3 weeks while incomplete periosteal callus bridges were also seen. This was attributed to periosteal cortical surfaces always being more widely separated in fractures. The authors felt muscles were crucial in supplying vessels to periosteum while torn muscles became the immediate blood supply to periosteum in long bone fractures. Results also suggested anastomoses between periosteum and endosteum varied in size and number in different cortical regions, as disruption of periosteal blood supply occasionally suppressed the adjacent medullary blood supply. (Rhinelander and coll., 1968a, Rhinelander 1968b).

Hulth and Olerud (1962) amputated the lower limb between upper and middle tibia on rabbits of unspecified age. Healing was observed for up to 12 weeks after surgery. By one week, new vessels were seen radiating outwards in a fan shaped appearance with trabeculae paralleling the course of the vessels. By two weeks, both periosteal and endosteal callus appeared, the periosteal callus also oriented parallel to periosteal vessels (Hulth and Olerud, 1962).

Trueta and Caladias (1964) stripped periosteum from the radial diaphysis in rabbits, separating it from the cortex with a polythene sheet. Alternatively, the nutrient artery was severed and prevented from re-attaching to the metaphyseal-epiphyseal blood supply. Animals were studied from 1 day to 8 months after surgery, both radiographically and angiographically using Maropaque (Tm) and Berlin Blue. Trueta and

Caladias found periosteal vessels of the unoperated animal were small, penetrating bone from muscle and interseptal attachments. Large vessels were present running along the bone, originating from the metaphysis. The latter vessels sent off multiple smaller vessels to cortex. The periosteal blood supply, especially venules, appeared especially abundant. The vessels maintained the same diameter even after branching many times. In the normal cortex, they concluded cortical vessels connected primarily to the marrow and to a lesser degree, the periosteum.

Results of blood flow interruption showed the marrow and most diaphyseal cortex necrosed. Total necrosis of cortex did not occur due to abundant anastomoses of periosteal or metaphyseal endosteal vessels. Adult rabbits demonstrated sufficient anastomoses between all systems of vessels to prevent necrosis although this system was not present in adults. Trueta and Caladias concluded that periosteal vessels supplied the outer third of cortex while periosteal vessels alone supplying cortex produced new bone if the inner two-thirds of cortex became ischemic.

Yabsley and Harris (1965) stripped periosteum to within 2-3 mm of the epiphyseal plates in long bones of young rabbits, sacrificing the nutrient artery. They found endochondrial ossification was decreased, causing temporary thickening of the central epiphyseal plate and stimulation of bone growth.

Zucman and coll. (1968) created three fractures in the tibia of rabbits

15 to 18 weeks old after widely excising the periosteum. All fractures were stabilized with medullary pins and plaster casts. During healing, callus formation was preceded by increased vascularization in the grafted periosteum, while fractures denuded of periosteum did not unite. Zucman and coll. concluded that periosteum increased bone union when used as an autogenous free graft (Zucman and coll., 1968).

Holden (1969) studied revascularization in healing tibial fractures of rabbits. He demonstrated circulations of periosteum, bone and muscle communicated at the capillary level. Loss of blood in bone vascular supply allowed the other system to supply complete vascularity provided the periosteal blood supply was intact. When the medullary blood supply was destroyed, blood flow changed from centrifugal to centripedal, thus maintaining osseous vascularity.

Danckwardt-Lillistrom and coll. (1970) created tibial midshaft osteotomies in female rabbits, 7 to 8 months of age. The medulla was reamed and a compression nail subsequently placed. When the medullary blood supply was lost but periosteal blood supply remained intact, there appeared to be a rapid re-establishment of medullary circulation primarily through periosteum. When cortical vessels were plugged, revascularization was incomplete with formation of large cystic bone cavities. Because stability was present from the intramedullary pin, the authors felt loss of marrow circulation and cortical bone necrosis was the largest factor in the periosteal reaction. Blood flow was thus changed from its normal centrifugal pattern to centripedal flow.

Brookes (1964) described periosteal vessels radiating outwards from the primary ossification centre of the diaphyseal shaft corresponding to the outward growth seen in periosteal osteogenesis. In areas of fleshy muscle attachment, the fibrous periosteum was extremely tenuous, allowing close association between muscular and periosteal vessels. He described the cortical surface as displaying tertiary foramina of Testuti, having uniform diameter and orderly orientation, corresponding to the radiation pattern of vessels in the underlying periosteal bone. Brookes felt vascular continuity between bone and periosteum occurred almost entirely at the capillary level.

Expanding on his ideas in a later publication (1971), Brookes argued that vessels in compact bone were thin walled endothelial tubes, allowing blood to flow in either direction between periosteum and bone. He felt the cortical arterial supply arose from marrow and not periosteum. Bone formed from periosteal apposition displayed a radiating pattern of vessels and bone trabeculae extending outwards from the primary centre of ossification. This pattern arose from both longitudinal endochondral and expanding periosteal growth.

Branches of vessels supplying various muscles entered fibrous periosteum to form vascular circles around the bone shaft and sending longitudinal anastomoses along the osseous borders. Smaller branches extended deeply into the cambial layer of periosteum as capillaries. In areas of muscle attachment, both muscle venules and periosteal capillaries were directly linked. In either case, periosteal capillaries were directly linked to efferent cortical capillaries, while no arteries were seen entering cortical bone from periosteum. (In opposition to Morgan's [1959]

finding.)

Brookes argued that blood travels only from cortex into periosteum as evidenced by oozing blood after periosteal stripping. Brookes did not mention the possibility of backflow from these cortical vessels which may have been arterioles, or the nature of blood flow from severed periosteum. Brookes explained cortical necrosis after periosteal stripping on the basis of venous obstruction.

Brookes felt periosteal arterioles supplied only the surface osteons of diaphyseal cortical bone. Flat bones however, received a large part of their medullary and cortical blood supply from periosteum entering the bone at one or two nutrient foramina. When the nutrient artery was ligated close to the nutrient foramen, periosteal and metaphyseal arteries became larger to assume the blood supply to medulla. The bone itself changed to accommodate the centripedal flow, becoming more cellular and vascular; these changes being permanent. Brookes postulated the changes may be due to different pressure gradients in the cortex, changes in gas tension or changes in pH. Brookes felt periosteal stripping produced cortical death through thrombic venous obstruction.

Cavanaugh and coll. (1974) used radioactive isotopes to determine blood flow after subapical osteotomies, using dogs as the experimental model. Immediately after surgery, blood flow to the osteotomized segment was 25% less than intact mandible. From 1 to 3 weeks the blood flow to the osteotomized segment increased beyond that to normal bone, decreasing to normal by 6 weeks after surgery.

McNab and de Haas (1974) described periosteal vessels as running transversely to the long axis of bone, supplying blood to the outer cortex. They felt this arrangement to be most important since in a tibial fracture at least, an intact periosteum would maintain vitality to each fragment and actively re-establish a blood supply for the inner cortex and medulla. The integrity of intact periosteum also prevented fibrous tissue from interposing between fragments.

Trueta (1974) noted different blood supplies of tibial periosteum, the upper tibial periosteum having a rich blood supply and the lower tibia having one of the sparsest. He described periosteal vessels entering cortex as being small in size and few in number near the diaphyseal centre, becoming larger towards the distal of the diaphysis. Vessels were greater in children than adults, forming a vascular network surrounding the cortex. Age tended to decrease periosteal vascularity while rupture of vessels through injury tended to stimulate vascular proliferation.

The periosteal vessels gave off perpendicular branches extending into cortex. Trueta (in conflict with Brookes, 1971) felt both arteries and veins entered cortex, some reaching the marrow. At each diaphyseal end, the periosteal vessels entered the epiphyseal plate as "Circulus Articuli Vasculoses".

Trueta felt that elevated periosteum caused an increased number of vessels to appear, especially from the attached muscles. The vascular

proliferation increased even more if the principal nutrient artery was torn. Bone richly supplied with vessels from periosteum healed more quickly (upper tibial fractures healed sooner than lower tibial fractures). The very close association of vessels to new trabeculae suggested an active osteogenic activity of the vascular wall while the pumping activity of muscle action circulated blood and sped up bone regeneration. Trueta felt plates and screws interfered with bone union through compression of periosteal vascularity.

Rhinelander (1974) felt periosteal arteries supplied the outer third of diaphyseal cortex in certain bones although their exact distribution was controversial. He argued that periosteum contained many more efferent vessels (venules and capillaries) than afferent vessels (arterioles entering bone at fascial attachments). While overall blood flow seemed to pass outwards from cortex to periosteum, Rhinelander felt the periosteal blood supply could persist in supplying vascularity to bone if the medullary blood supply was interrupted. An efferent blood supply was as vital as an afferent since plates compressing periosteum tended to block efferent circulation and thus necrose bone. Periosteum gave the first blood supply to external callus until medullary blood supply became established although periosteum could continue to give vascularity to the callus. He concluded that periosteal blood supply supplied the first vascularity to external callus and outer cortex until the medullary blood supply took over.

Forman (1976) felt presenile mandibular atrophy was due, in part, to a shift in blood flow from the nutrient artery centrifugally to the

periosteum flowing centripedally. He described using an extra oral tunneling procedure to reinforce the lower mandibular border with autogenous bone; care being taken to preserve as much periosteum and thus blood supply, as possible.

King (1976) used 6 week to 6 month old mongrel puppies exposing the hind limb to the level of periosteal arteries. In one experiment periosteal flaps bared on the middle third of tibia were sharply dissected and turned back onto each other as tubes with deep periosteal surface now the tube lumen. The animals were sacrificed from 3 weeks to 6 months. Some bone formation was found in every tube, with mature woven bone in the last cases done (as the surgical technique improved). In a second series, 0.5 cm of bone and periosteum was removed from the middle tibial third and tube grafts placed to bridge the gap. After 2-6 months, 9 of 10 dogs showed bone replacement, one with pseudoarthrosis. In a final series, a pseudoarthrosis was created in the middle tibial third with subsequent closure by periosteal tube flaps. After 4-6 months repair through new subperiosteal bone occurred in all 24 dogs. King concluded that periosteal flaps based on a vascular pedicle provided a predictable capacity for producing new bone, this potential being far greater than a random periosteal flap.

Nathanson (1978) felt revascularization was imperative for bone growth. He removed a 15 mm x 5 mm bone and periosteal segment from the inferior border of mandible in 6 month old rabbits. The defect was restored with either a periosteally covered autogenous iliac crest or humeral diaphysis. All animals were examined 9-30 days after surgery.

Microangiography showed progressive revascularization of both grafts although the humeral graft vascularized earlier than the graft from iliac crest.

The new capillaries seemed to be invading the graft primarily through the interfragmentary region. From the second week periosteal vessels supplied the outer humeral cortex, but hardly perforating the iliac graft. After 9 days subperiosteal bone was formed in both grafts. While the humeral graft revascularized earlier the iliac graft seemed to revascularize more completely.

Siemssel and coll. (1978) argued that maintenance of nerve supply to muscle was imperative for maintaining adequate blood supply through periosteum to bone. They described a series of 18 patients 16-32 years old, given clavicular flaps pedicled to sternocleidomastoid muscle for reconstruction of their mandibles. One patient 16 years old had donor clavicular periosteum remaining in situ apparently regenerating the clavicle 2 years after surgery.

Whiteside and coll. (1978a) used hydrogen washout techniques on New Zealand White rabbits (immature and mature) and mongrel dogs of unspecified age to study periosteal blood flow. Stripping periosteum from 50% of the circumference of the tibial epiphysis did not significantly slow blood flow nor did reaming the epiphyseal centre. Both stripping and reaming significantly reduced blood flow in all immature animals and most mature animals. Similar stripping or reaming of the epiphyseal cortex did not significantly reduce blood flow in the

middle cortical layers but combined procedures prevented any blood flow in both animals.

Their findings supported the arguement that periosteum supplied a major portion of the vascularity to the epiphysis in growing rabbits and somewhat less in adult animals. Their findings in procedures on the diaphysis suggested an arterial and venous supply were present on both the periosteal and endosteal surfaces. Both seemed able to communicate through cortex as well as maintaining independent circulation to the bone.

Whiteside and coll. (1978b) exposed the flexor digitorum muscle in New Zealand White rabbits, measuring muscle blood flow rates before and after subperiosteal dissection in one tibia and extraperiosteal dissection on the opposite tibia. Blood flow rates did not change in either type of dissection. When the muscle was crushed several times in its centre, perfusion seemed to increase after subperiosteal dissection while extraperiosteal dissection eliminated or substantially reduced blood flow. When the main artery to muscle was cut no significant change in blood flow occurred. A subsequent subperiosteal dissection produced no change but extraperiosteal dissection drastically reduced or eliminated blood flow.

Whiteside and coll. concluded that transecting the major artery to muscle or crushing that muscle did not stop blood flow due to the presence of collateral circulation from periosteum. Extraperiosteal dissection destroyed the collateral periosteal circulation while subperiosteal circulation did not. Subperiosteal dissection thus

appeared superior to extraperiosteal dissection in a surgical approach to bone. Maintenance of this blood flow from shared vessels of muscles and periosteum could aid fracture healing and bone transplantation.

Whiteside and coll. (1978c) studied the effects of compromised periosteal blood supply on fracture callus formation, using New Zealand White rabbits weighing 2.5-4.0 kg. In one group, the distal tibia was exposed subperiosteally and a complete diaphyseal saw cut fracture was created. The opposite leg was fractured after an extraperiosteal exposure. All fractures were stabilized with intramedullary wires. A second group of rabbits was treated by first cutting all soft tissues to the level of bone, creating muscle trauma alone, then making the cut after spontaneous retraction of soft tissues. The opposite leg was treated by exposing the bone subperiosteally (muscle trauma plus subperiosteal dissection) before making the cut. The third group was treated similarly to the second, except one tibial diaphysis was exposed to the level of periosteum (extraperiosteal dissection) before osteotomy-fracture.

All specimens were evaluated at 3 weeks after surgery. Tibiae exposed to muscle trauma alone or extraperiosteal dissection developed a soft cartilaginous callus with clinical instability while those given a subperiosteal dissection without muscle trauma allowed the formation of bone callus and good clinical stability in 71% of fractures. All specimens with muscle trauma failed to heal irrespective of the type of dissection. Subperiosteal dissection without muscle trauma produced the most dry weight callus, extraperiosteal dissection produced less callus

and muscle trauma regardless of the type of dissection produced the least callus. Tensile strength showed similar trends. Calcium levels in callus were lowest in all cases of muscle trauma.

Whiteside and coll. argued that since stripped periosteum due to injury initiated a massive generative response and bone repair; the processes demanded a large amount of blood borne oxygen and nutrients. It would follow subperiosteal dissection and muscle trauma, compromised blood supply to periosteum and thus decreased the amount of callus formation. Their experimental results lent support to their arguments.

Trias and Fery (1979) injected India Ink into the femoral nutrient artery or common trunk supplying the femoral nutrient artery of adult mongrel dogs. Specimens showed longitudinal thin walled vessels of uniform diameter in parallel rows within cortex. A second radial branching pattern of vessels demonstrated thick and thin walled vessels of varying diameters freely anastomosing with periosteum, the longitudinal vessels of cortex and membrane. While the authors conclusively demonstrated vascular anastomoses from between periosteum and bone, they were not able to show patterns of blood flow.

Poussa (1980) autogenously grafted free tibial periosteal and 108 micron thick osteoperiosteal grafts to the spinous and mammary processes of lumbar vertebrae in 6 week old rabbits. The animals were sacrificed up to 21 days after surgery, the tissues being perfused with Chromopaque for microangiography. By day one no vessels were seen in either graft although the cambial layer of free periosteum was proliferating. By the

second day capillaries were growing into the free graft, primarily the thickened cambial layer. After 3 days the entire periosteal graft was revascularized through both cambial and fibrous layers while the cortical component of the osteoperiosteal graft was showing evidence of revascularization. Circulation to both grafts continued to increase from 4 days on. Bone bridges were seen on both grafts by 21 days with a tremendous increase of circulation to the areas of new bone formation.

Poussa could not tell for certain if vascularization arose from anastomosis of host-graft tissues or from new capillary sprouting. The muscles above the graft had been damaged and this may have affected the course of revascularization.

Berggren and coll. (1982a) studied the behavior of revascularized bone grafts with periosteal and medullary blood supplied as well as a periosteal blood supply alone. In one series of "large" (presumably adult) mongrel dogs they removed a posterior segment of the ninth rib (both periosteal and medullary blood supply). The 6-8 cm segment of rib was autogenously buried in the subcutaneous ipsilateral groin tissues, (very poorly vascularized), anastomosing vessels of the graft to the deep circumflex iliac artery and vein. Ischemia time averaged 140 minutes.

In a second group a 6-8 cm anterior rib segment (periosteal blood supply alone) was harvested and transplanted in a similar manner. The ischemia time averaged 200 minutes.

A 4 cm segment of anterior ninth rib with covering periosteum was

autogenously transplanted as a free graft into subcutaneous abdominal tissues of both groups. All animals were sacrificed up to 180 days after surgery. The free bone grafts were gradually resorbed to less than 50% of their original size by 180 days. Only 1 of 9 animals demonstrated cortical bone vitality while three grafts showed evidence of subperiosteal bone formation. No viable marrow or endosteal bone formation was evident. The pedicled grafts both anterior and posterior resorbed to some degree. All posterior grafts retained cortical vitality although marrow showed loss of haemopoetic cells and partial bone resorption. Vessels demonstrated a full periosteal and medullary blood supply. At 4 months subperiosteal and endosteal new bone was evident although resorption had removed new bone by 180 days.

Only 3 of 4 anterior rib grafts showed cortical and marrow viability. Animals sacrificed earlier than 6 months showed evidence of periosteal and endosteal bone formation although after 6 months most bone was resorbed. Five tortuous medullary vessels were evident after 2 weeks, 4 weeks and 6 months.

Berggren and coll. concluded that autogenous rib grafts with only a periosteal blood supply could survive in a recipient site with little vascularity. The mechanisms for this process however remained unknown.

Berggren and coll. (1982b) continued their studies on adult mongrel dogs, autogenously grafting a segment of ninth rib with medullary and cortical blood supplies to a defect in the inferior border of mandible. Vessels were anastomosed locally with an ischemia time averaging 3.45

hours. A similar anterior rib graft with only periosteal vascularity was similarly grafted. The ischemia time averaged 4.15 hours. Dogs were sacrificed up to 14 weeks after surgery. All dogs but 2, 1 from each group, showed clinical union after 2 weeks.

Seven of 11 dogs with dual blood supplies were evaluated. In general, all graft tissues were viable with new subperiosteal bone in 5 grafts and endosteal bone in one graft. Seven of 10 dogs with a periosteal blood supply alone were evaluated, with 4 grafts demonstrating varying degrees of viable marrow. Here all cortical and endosteal osteocytes appeared viable. The remaining 3 dogs demonstrated necrotic marrow with few or no viable osteocytes. Five dogs showed new periosteal bone with some endosteal bone uniting the graft to the recipient bone.

Berggren and coll. discovered cortical bone resorption (25-60%) in grafts with a dual blood supply with less (15-20%) or no resorption in periosteally supplied grafts. They felt the excess bone resorption might have been caused by a higher oxygen concentration from a more abundant blood supply. The authors felt periosteally supplied rib bone grafts would survive in a recipient bed even if all of the marrow did not unite, and resorb less than a graft with a dual blood supply.

Berggren and Ostrup (1982) carried out a similar series of experiments on adult female Beagle dogs. In one group a posterior segment of the ninth rib was isolated in situ with saw cuts leaving intercostal soft tissues and thus periosteal blood supply intact. The fragments were subsequently fixated with a bone plate overlying periosteum. The

nutrient intercostal vessels were cross clamped for 90 minutes before closure of the wounds. A second group was similarly treated except both periosteal and medullary blood supplies were left intact. Animals were sacrificed up to 4 weeks after operation.

When only periosteal vascularity was present, 2 of 4 dogs showed complete vitality of cortex and marrow. The remaining 2 dogs developed nonvital bone marrow at the distal part of the segment. Periosteal vessels were seen extending into cortex and marrow in 3 of 4 dogs with bridging from the intact periosteum and endosteum into the distal osteotomy segment. Both blood supplies showed viable bone and marrow in 3 of 4 dogs except at the distal region of the fragment. Vascular patterns were similar to the first group with cortical and marrow vessels. Evidence of new bone formation (sites and distribution not given) was seen in both groups.

Berggren and Ostrup concluded that periosteum probably gave an adequate blood supply for vitality to the isolated segment while an overlying plate compromised blood supply, probably through venous obstruction.

Berggren and coll. (1982c) studied the effects of prolonged ischemia time on the cell survival of anastomosed bone grafts. Adult mongrel dogs were used. In one group, the posterior segment of ninth rib was removed and kept at +5^{o}C for 1.5-4.8 hours before being autogenously transplanted into subcutaneous groin tissue. Vessels were re-anastomosed on transfer. In a second series, the grafts were transplanted into the neck. Specimens were examined after 2 weeks.

None of the grafts made ischemic for 46 hours or longer could receive successful microvascular anastomoses.

Up to 4 hours of ischemia did not compromise complete vitality of cortex and marrow. Longer periods of ischemia compromised vitality in increasing amounts and after 26 hours of ischemia, marrow was necrotic while cortex showed less than half cortical vitality. No graft made ischemic for more than 46 hours showed new bone formation.

Berggren and coll. concluded that periosteum, cortical bone endosteum and marrow could survive ischemia of up to 25 hours, provided the graft was properly stored and full vascularity was re-established. The periosteum and in particular the osteoblasts survived to produce varying degrees of new long bone.

TEMPORALIS MUSCLE FLAP SURGERY

Gillies (1920) described using the anterior two-thirds of temporalis muscle to reconstruct loss of maxillary bone as well as to provide an orbital floor. He recommended incising the temporalis fascia from zygomatic arch, allowing temporalis muscle to be brought to the nasal septum. One of his patients retained temporalis contraction ability for 18 months after surgery.

Halle (1938) described using temporalis muscle freed from the coronoid process for secondary repair of the frontalis muscle, eye or mouth admitting some degree of success after 3 or 4 months.

Milton Adams (1946) described using temporalis muscle flaps cut longitudinally to support and activate frontalis muscle, the eyelid or oral canthus. Four to 6 weeks were allowed for active contraction of the temporalis flaps. Milton Adams observed that using temporalis to support the lateral canthus would sacrifice its neuromuscular supply.

Campbell (1948) used a temporal muscle flap to reconstruct a resected maxilla lost 10 years previously. He used the anterior one-third of temporal muscle swung into the midface to create an orbital floor and maxilla. A palatal flap was used to close the new oral surface of temporal muscle. In a subsequent operation, iliac bone was used to create a new maxilla and alveolar process.

Campbell (1954) described the use of a temporal muscle flap in

reconstructing the resection defect following left maxillectomy in a 38 year old male. The reconstruction was followed (in a second operation) with placement of a polyethylene sheet to support the orbit and free osteoperiosteal graft to rebuild the alveolus. Forty-seven days later a mucobuccal fold was created. The graft had laid down enough new bone to be "quite firm" on palpation.

Webster (1955), Naquin (1956), Reese and Jones (1961), Cramer (1962), Deitch and Callahan (1964) all described using temporalis muscle, or parts of it, to reconstruct the maxilla and orbit following resection for tumor. While the esthetic and functional results were satisfactory, no mention was made of possible bone production by temporalis periosteum.

Bakmajian (1963) advocated using a temporalis muscle flap to cover exposed dura, bone orbital floor or to provide bulk after maxillary loss.

Anderson (1961), Rubin, Bromberg and Walden (1969) described using temporalis muscle for animation of the face in Moebus syndrome, using temporal fascia as a tendon.

Clodius (1972) described reconstruction of the nasolabial fold after resection for malignancy or loss of facial nerve activity by several methods including the use of temporalis muscle flap transfer. No reference was made to dynamic results obtained with this technique.

Rubin (1974) expanded on this reconstructive technique advising the dynamic aspects of temporalis muscle in control of facial movements.

Holmes and Marshall (1979) described using a temporalis muscle flap for repair of orbital defects. They split the muscle either saggitally or longitudinally noting any surface could freely accept a skin graft. They cautioned leaving the temporalis origin to coronoid process intact as a protection of the neurovascular supply.

More recent presentations using temporalis muscle for orbital-facial reconstruction (Bradley and Brockbank, 1981; Wolfe, 1981) have reviewed the use of this muscle for orbital reconstruction without mention of bone formation.

Hauben and van der Meulen (1983) created periosteal tubes with temporalis muscle pedicles in young pigs; the tubes being wrapped around a 4 mm diameter silicone rod. Arteries to the opposite temporalis muscle were ligated. Animals were followed radiographically from 6 weeks to 12 months after surgery while samples were evaluated histologically 12 months after operation. Radiologic evidence of bone formation was present by 3 weeks in the tubed specimens while none was seen on the ligated specimens. By one year lamellar woven bone was present in 86.6% of cases. No cartilage was found and no mention was made of the control specimens. Hauben and van der Meulen felt periosteum produced bone in a stress free environment nourished only by its musclar pedicle.

McCarthy and Zide (1984) briefly described the use of a composite osseous-myoperiosteal flap based on the superficial temporal artery in the repair of calvarial defects in 12 patients. Other than briefly describing the creation of the flap, little mention was made of the detailed techniques or the follow-up. McCarthy and Zide suggested using this method where vascularity of the recipient bed was in question.

SUMMARY OF THE LITERATURE REVIEW AND OVERVIEW OF THE EXPERIMENTAL PROCEDURES

Periosteum is now recognized as an osteogenic tissue and is used in reconstructive procedures either as a free graft or a component of predicled composite flaps. The outer fibrous layer of periosteum provides an abundant blood supply to the underlying loosely adherent osteoblastic layer. This inner layer is tightly adherent in turn to the underlying cortical bone. Normally the osteoblastic periosteum can receive a blood supply from either fibrous periosteum or cortical bone and is capable of osteogenesis when attached to either tissue.

By the turn of this century, two philosophies had developed regarding the osteogenic ability of periosteum, undoubtedly because none of the researchers defined their concept of periosteum before discussing either their experiments or case studies. Those aligned with Ollier were correct in assuming periosteum would generate new bone provided the osteoblastic layer was included with the graft. Researchers aligned with MacEwan were also correct in believing that periosteum possessd no osteogenic potential, if the osteoblasts were left adherent to the underlying cortical bone. Considering the friable attachment of fibrous to cambial periosteum, rapid periosteal harvest would almost certainly leave the osteoblasts adherent to bone. With this experimental model osteoblasts adherent to the surface of a bone graft would produce new bone while the overyling fibrous periosteum would be incapable of producing osseous tissue.

Most of the early researchers after Ollier used a variety of experimental animals and with a wide range of age. Whatever the evidence, the results seemed to be interpreted towards proving one of the two predetermined conclusions. Further, the early clinical reports were anecdotal in nature. While interesting from a historical perspective, they also seemed tailored to a predetermined philosophy of the author.

By the middle of this century, periosteum was routinely being defined as possessing both fibrous and osteoblastic components. Although some resistance to periosteum as an osteogenic tissue persisted (Cestero and Salyer, 1974, 1975), repeated experiments on a wide variety of experimental animals (all of them in a growing, immature stage) demonstrated the ability of free periosteal grafts to produce new bone.

An adequate blood supply was found to be necessary for success of periosteal grafts. Situations such as chronic fibrosis from infection or radiation, potential infection from adjoining hollow viscera or a graft of large size all worked against the early graft revascularization so necessary for successful osteogenesis. The early investigators discovered through trial and error that pedicled periosteal flaps were superior to free periosteal grafts in reconstruction of osseous defects especially in the clincial situations listed above. Later research confirmed that an uninterrupted blood supply through a soft tissue pedicle would maintain periosteum in the optimum condition for osteogenesis. Muscle and vascular pedicles were subsequently found to be superior to skin pedicles for this function. If a pedicled

periosteal flap was placed in an infected recipient bed (such as a tracheal defect) healing progressed satisfactorily. If a pedicled osteoperiosteal flap was transferred for repair of a bone defect the osseous component died while the fully vascularized periosteum produced new bone which subsequently replaced the necrotic osseous tissue.

Pedicled periosteal flaps, then, were found to be superior in their ability to produce new bone especially when transferred to recipient beds of either poor vascularity or potential infection. These same problems are present when facial bone is lost through trauma or ablative surgery. Temporalis muscle alone has been used to provide some degree of structural or functional reconstruction of the face with no attempt, however, to reconstruct the skeletal base. The most current research has demonstrated that temporalis myoperiosteal flaps can produce bone when wrapped around inert material and placed in subcutaneous sites of low functional stress. (The animals used in these experiments were actively growing). A need exists, therefore, to evaluate the ability of a pedicled myoperiosteal falp to produce new bone in a totally functional recipeint bed, such as a functioning mandible.

Most of the research leading to the current knowledge of periosteal behaviour was carried out in young, growing animals. Immature experimental animals seem to produce more bone of better quality than older animals following osseous injury. Further, this principle is commonly accepted in clinical practice from anecdotal experience while no research has defined the actual potential of periosteal osteogenesis

in older animals. Using a very real clinical application, facial bone is lost through trauma or ablative surgery more frequently in adults, and a need exists to develop a method of osseous reconstruction for these patients.

In view of the almost complete lack of knowledge regarding periosteal behaviour in the adult animal following transfer by various techniques, this thesis was designed to investigate the following questions:

EXPERIMENT 1

Does the temporalis muscle provide a blood supply to the underlying periosteum?

This initial experiment was carried out to confirm that a temporalis myoperiosteal flap was indeed a true composite flap rather than a free periosteal graft with attached overlying muscle tissue.

CONTROL EXPERIMENT

Does a mandibular defect devoid of periosteum heal in the same manner as a defect covered with autogenous periosteum?

In most of the experiments, a standardized mandibular defect was created and subsequently allowed to heal with various periosteal flaps or grafts

(to be described) secured closely over the defect area. The osseous injury sustained in creating the defect would stimulate both the endosteal tissue and surrounding periosteum to produce a variable amount of new bone as is commonly seen in fracture repair. The contribution of endosteal tissue alone in repair was evaluated by first removing periosteum from each of the defect borders and subsequently allowing the mandible to heal without the influence of periosteal cover.

EXPERIMENT 2

Does a temporalis myoperiosteal flap include osteogenesis? If so, does this differ qualitatively or quantitatively from a free tibial periosteal graft?

This experiment was performed to evaluate the ability of a temporalis myoperiosteal flap to produce new bone in a standardized mandibular defect, with the mandible remaining in function throughout the duration of the experiment. The opposite mandible of the same rabbit was prepaired and treated with a free tibial periosteal graft. Autogenous tibial periosteal grafts are superior osteogenic tissues to most other free periosteal grafts, including parietal periosteum. This experiment, then, allowed comparison between a autogenous free tibial periosteal graft and a temporalis myoperiosteal flap with its continuous blood supply (although the parietal periosteum in the free graft form is inferior to tibial periosteium as an osteogenic tissue).

Specimans were taken at 2 weeks to evaluate initial healing; 5 and 9 weeks to evaluate both further and final healing, and 12 weeks to evaluate the long term osseous behaviour of the treated defects.

EXPERIMENT 3

Does a free tibial periosteal graft transplanted to temporalis muscle receive a blood supply from this muscle?

Tibial periosteum is a superior osteogenic tissue in the form of a free graft. Would its performance be superior if it could be grafted to a muscle pedicle, receive a blood supply from that pedicle and subsequently be transferred as a myoperiosteal flap to repair a mandibular defect? This experiment was carried out to evaluate the ability of an autogenous free tibial periosteal graft for successful transplantation onto the belly of a temporalis muscle. After 2 weeks of healing this artifically created myoperiosteal flap was evaluated for vascular connections between periosteum and underlying muscle.

EXPERIMENT 4

Does a free tibial periosteal graft applied to temporalis muscle and subsequjently transferred as a vascularized flap induce osteogenesis? If so does this differ either qualitatively or quantitatively from a free tibial periosteal graft?

The ability of a presumably superior and artifically created tibial myoperiosteal flap in repairing a standardized mandibular defect was compared to a repair of the opposite mandible using a free autogeneous tibial periosteal graft. The tibial graft was chosen to provide a standard of comparison in producing new bone for the previous and following experiments. Specimens were harvested at similar times to Experiment 2.

EXPERIMENT 5

Does a Vitallium (Tm) mesh placed over a mandibular defect influence the amount or quality of bone produced by a surrounding myoperiosteal flap?

Mandibular defects are frequently repaired with a Vitallium (Tm) mesh framework, the cavity being filled with autogenous bone chips. Results are for the most part good, but the bone chips are still free grafts and interrupted from their blood supply. This experiment was carried out to evaluate the ability of a temporalis myoperiosteal flap (delivering an uninterrupted blood supply to its underlying periosteum) wrapped over a Vitallium (Tm) mesh in producing new bone within the defect. The opposite mandibular defect was repaired with a free autogenous tibial periosteal graft, again as a standard of comparison. Specimens were evaluted at 2,5,9 and 12 weeks as in previous experiments.

Adult New Zealand White rabbits were chosen as the experiment models - (1) because of the similarity in osteogenesis between rabbits and humans, and (2) because of the relative speed with which new bone

develops, allowing a practical time frame for evaluation of experimental results.

The main thrust of this research has been to qualitatively evaluate new bone formation. However, some attempt has been made at quantitating the experimental data, with the small population sizes used in each series.

EXPERIMENTAL PROCEDURES
OBSERVATIONS
DISCUSSION
CONCLUSIONS

EXPERIMENTAL PROCEDURES AND OBSERVATIONS

a. Peri-Operative Animal Care

Mature (as confirmed by radiographic examination of randomly selected epiphyses) (Fig. 1) New Zealand White male rabbits were used in the experiments. Weights varied overall from 2.83 kg-4.1 kg. The animals were kept under appropriate conditions of hygiene and care and fed either Wayne Guinea Pig Diet (Tm) or Regal Rabbit Pelletts (Tm) with water available ad libitum.

All rabbits were given the following anesthesia: Acepromazine (Tm) 15 mg/kg intramuscularly as premedication, followed 10 minutes later by an intramuscular combination of Ketaset (Tm) 50 mgm/kg and Rompun (Tm) 3.0 mgm/kg. Supplementary intramuscular injections of 75 mgm Ketaset (Tm) were administered as required. Once anesthetized, the rabbits in experiments 2, 4, 5 and the control experiment were given procaine penicillin 300,000 IU IM and Benzathine penicillin 300,000 IU IM. All intramuscular injections were given in the posterior thigh muscles. Postoperatively, each rabbit from experiments 2, 4, 5 and the control experiment was administered a 60 cc subcutaneous bolus of Ringer's lactate solution and allowed to recover spontaneously in a quiet, warm environment. Each rabbit was given a further 60 cc subcutaneous bolus of Ringer's lactate the following day.

b. Preparation of Specimens

Rabbits were sacrificed as indicated in Table 1. Sacrifice was achieved through intravenous injection of 100 mgm Sodium Amytal (Tm) or 150 mgm intramuscular injection of Ketaset (Tm) followed after onset of unconsciousness (non-reaction to a painful stimulus) by a 2.0 cc intravenous air embolus (for experiments 2, 4, 5 and the control experiment). Each hemi-mandible with attached soft tissue was immediately removed, trimmed and stored in 10% buffered formalin solution.

Each specimen was subsequently laid flat on a piece of dental x-ray film (Kodak Ultra (Tm) group 8, emulsion #5259427356 9) and images created with a Ritter (Tm) model J x-ray machine, using 12" FFD, 75 KVP, 5 ma and 1/16 second exposure. The film was processed with the SS White Auveloper (Tm) automatic processor at 85° F.

The radiographs were enlarged to 210% of actual size and the radiographic patterns of healing were carefully copied on tracing paper from the magnified image. Paper blocks including the surgical site were cut from the tracing and weighed with a Mettler M3 (Tm) balance. The areas of bone were then cut from the blocks and weighed in the same balance to give an indication of the quantity of new bone formation.

Quantitative estimates of healing were calculated from the following formulae:

(1) Weight of remaining defect (mgm) = Weight of surgical site (mgm) (including existing bone, new bone and remaining defect) - Weight of estimated bone (mgm)(both existing and new bone formation)

(2) Percent of remaining defect (mgm)

$$= \frac{\text{weight of remaining defect (mgm) (calculated in (1))}}{\text{weight of a standardized block (mgm) 4.0 mm x 4.0 mm (7.86 mgm by direct measurement)}} \times 100\%$$

(3) Percent healing = 100% - % defect

Specimens were dehydrated through graded alcohols and imbedded in glyco methacrylate. The undecalcified blocks were mounted and two 3 micron thick slides cut from each section. Clear nail polish was painted over the block to provide support during cutting. After mounting the tissue sections, the nail polish was removed by washing in acetone. One section of the pair was stained with haematoxylin and eosin for transmitted light microscopic evaluation. The second section was examined unstained with ultraviolet microscopy using a 100 angstrom halogen bulb and Leitz E2 (Tm) filter.

TABLE 1

SACRIFICE OF SPECIMENS

EXPERIMENT	WEEKS SACRIFICED AFTER SURGERY	NUMBER OF RABBITS SACRIFICED
1	Immediately	2
2	2	2
	5	2
	9	2
	12	2
3	2	2
4	2	2
	5	2
	9	2
	12	2
5	2	2
	5	2
	9	2
	12	2
Control	2	1
	5	1

EXPERIMENT 1

Question: Does the temporalis muscle provide a blood supply to the underlying periosteum?

Material: Two rabbits were used for this experiment.

Under anesthesia, hair was shaved from the scalp, posterior mandible and lateral neck. The skin was prepared with Povidone (Tm) aqueous solution and the operative areas isolated with sterile towels. A midline incision was made from the vertex to the roof of the nose and flaps were reflected laterally to expose the temporalis muscle. The muscle together with periosteum was elevated from the calvarial cortex using sharp dissection. A muscle paddle approximately 2.0 cm long and 1.0 cm wide was created, leaving only the tendon and neurovascular pedicle intact. The periosteum appeared as a milky white slightly dull membrane on the under-surface of the muscle. The exposed calvarial bone contained an abundance of irregularly sized Volkmann's canals.

Ipsilateral skin anterior to the sternomastoid muscle was incised and the tissues sharply dissected to the carotid artery. The external carotid artery was isolated and cannulated with a #20 polyethylene catheter. One hundred and fifty mg of Sodium Amytal (Tm) were given via the intravenous catheter followed one minute later by a slow intravenous infusion of 3.5 cc of India Ink. After 1.4 cc of India Ink were administered, the dense black pigment was seen returning through the adjacent internal jugular vein. After sacrifice, the left temporalis muscle, now noticeably blacker, was removed and placed in formalin.

Observations:

Histologic examination showed both muscle tissue and attached periosteum in numerous vessels containing India Ink. (Fig. 2 & 3)

CONTROL EXPERIMENT

Question: Does a mandibular defect devoid of periosteum heal in the same manner as defect covered with autogenous periosteum?

Material: Two rabbits were used.

After preparing the posterior border of the mandible (size chosen at random) a 1.0 cm skin incision was made. Soft tissues were dissected to the level of posterior jugal process of zygoma. This structure was removed with rongeurs exposing Masseter muscle. The muscle was reflected from the lateral and posterior mandible above the angle and a 4.0 mm x 4.0 mm square of bone removed from the posterior border using dental burs and abundant normal saline as coolant. (Fig. 4-6) Periosteum was carefully curetted from all mandibular surfaces to a distance 2 mm from the bone defect borders. After creating the defect in the posterior border of the mandible, the surgical site was irrigated with normal saline and the wound closed.

One rabbit died of a respiratory infection 14 days after surgery while the second rabbit was sacrificed at 5 weeks. Unfortunately the first rabbit was not available for histologic or radiographic evaluation. Specimens were radiographed in a manner similar to Experiment 2. The tissue was subsequently dehydrated in graded alcohols and mounted in paraffin blocks. Sections were stained with haematoxylin and eosin for examination by light microscopy.

Observations

a. Radiographic:

The defect observed at 5 weeks showed extensive internal remodelling as well as resorption at the superior angle and inferior angle. There was no radiographic evidence of new bone formation. (Fig. 7 & 8)

Table 2 lists the quantitative estimate of osseous regeneration.

TABLE 2

ESTIMATION OF QUANTITY OF BONE

REPAIR IN THE CONTROL EXPERIMENT

Total Weight of Block (mgm)	Weight of Bone (mgm)	Weight of Defect (mgm)	% Defect Remaining	% Healing
20.531	11.507	9.024	>100	0

b. Histologic:

The specimen was striking in its appearance of minimal osseous activity, with the bulk of defect still present. The bone surface presented either a smooth cortex or mature trabeculae. The spaces between trabeculae were filled with accumulations of what appeared to be newly deposited bone and a central core of loose connective tissue with few fibres and few cells. The bone/periosteum interface

demonstrated the almost total lack of periosteal architecture. A few small round or thin fusiform cells were seen at the bone border, with zones of active osteoclastic activity. Little osteoblastic activity was present.

The soft tissue adjacent to this cellular zone demonstrated dense fibrous bundles with relatively few cells, and few blood vessels. (Fig. 9 & 10)

EXPERIMENT 2

Question: Does a temporalis myoperiosteal flap induce osteogenesis? If so, does this differ qualitatively or quantitatively from a free tibial periosteal graft?

Material: Eight rabbits were used in this experiment.

The temporalis myoperiosteal flap (from a side chosen at random) was elevated as in experiment 1. The skin incision was extended along the posterior border of the mandible and a defect created in the posterior border in a manner similar to the control experiment. (Fig. 5) The surrounding periosteum was then removed to at least 2.0 mm from all defect margins and the temporalis muscle and folded so that its periosteum covered the defect in all planes. (Fig. 11) Tissues were then closed in layers, with 4/0 plain gut for deeper layers and 4/0 silk for skin.

The opposite mandible was prepared by exposing the posterior border and creating a 4.0 mm by 4.0 mm defect in a similar manner to the first side. Soft tissue flaps were passively replaced until harvesting of tibial periosteum.

The anteromedial tibial surface (side chosen at random) was prepared, draped and a 2.0 cm longitudinal skin incision was made along this surface. Tissues were dissected to the level of periosteum, exposing a broad flat area for harvest. At this point, large numbers of vessels

were seen coursing randomly through the periosteal tissues. A 1.0 x 1.5 cm rectangle of periosteum was outlined by incision and a fresh scalpel was used to carefully elevate the tibial periosteal tissue from the tibial cortex (Fig. 12). After removal, the graft immediately contracted by approximately 1/3 of both its length and width. Few Volkmann's canals were visible on the tibial cortex as compared to the parietal bone. The recipient mandibular defect was exposed and irrigated to wash away clotted blood and the free tibial periosteal graft was draped around the defect, placing its cambial surface against bone. The graft was secured with 4/0 plain cat gut sutures. Total time for removal and transplantation of the graft averaged 12 minutes.

Soft tissues were closed in layers on both the donor and recipient sites. All rabbits were placed on their side in a quiet, warm environment as described previously.

Rabbits were given intramuscular tetracycline or oxytetracycline at 50 mg/kg and sacrified at intervals according to Table 3. Specimens were taken as previously described.

OBSERVATIONS

a. Radiographic and Clinical

Specimens 2-1-L and 2-1-R disintegrated while in formalin storage; specimen 2-2-R was partially disintegrated. None of these specimens were suitable for histologic examination although specimen 2-2-R (flap) was evaluated radiographically. All specimens were visually evaluated for the presence of new bone. Bone regeneration occurred in combinations of 4 basic patterns:

i. Regeneration along the posterior border of the defect upwards or downwards as a spur extending into the cavity for variable distances. This pattern suggested periosteal osteogenesis occurring where periosteum folded around the open border of the defect. (Fig. 13)

ii. Osteogenesis from the inner corners and surfaces of the originally square defect giving it a more rounded appearance. This pattern suggested bone regeneration from both the periosteum and the mandible itself as would be expected. (Fig. 14)

iii. Varying degrees of radiopacity within the defect itself, suggesting new bone formation from both transplanted periosteum and endosteal defect margins. (Fig. 15)

TABLE 3

Experimental Protocol for Experiment 2

RABBIT	FLAP	GRAFT		TETRACYCLINE (T) OR OXYTETRACYCLINE (OX) WEEKS POSTOP.		SACRIFICE WEEKS POSTOP.
		TIBIAL DONOR	RECIPIENT MANDIBLE			
2 - 1	L	L	R	1	T	2
2 - 2	R	L	L	1	T	2
2 - 3	L	R	R	1, 4	T, T	5
2 - 4	R	R	L	1, 4	OX, OX	5
2 - 5	R	L	L	1, 4, 8	T, T, T	9
2 - 6	L	L	R	1, 4, 8	T, T, OX	9
2 - 7	R	L	L	1, 4, 8	T, T, T	12
2 - 8	L	L	R	1, 4, 8	T, T, T	12

iv. Bone resorption from walls or external corners of margins.

Terms used to describe the surfaces and angles related to the defect are given in Fig. 16.

Specimens 2-4-R (flap)(Fig. 22) and tibial 2-4-L (graft) harvested at 5 weeks demonstrated dense radiopacities filling the defect and extending beyond its boundaries in all directions. Clinically, the radiopacities presented as hard nodules on the lateral and posterior surfaces of the mandible, completely obliterating the former defect. Each nodule measured approximately 1.0 cm superoinferiorly x 1.2 cm anteroposteriorly x 1.0 cm mediolaterally. The nodules appeared to be composed of osseous tissue with interspersed islands of cartilage and fibrous tissue. Specimens 2-5-R (flap)(Fig. 23) and 2-6-L (flap)(Fig. 26) presented the radiographic appearance of complete healing,

In general, healing progressed rapidly with an amount of new bone formed equal to or surpassing that removed from the original defect by 5 weeks after surgery in both specimens of rabbit 2-4. The second rabbit sacrificed at 5 weeks demonstrated a 46.883% repair on the defect treated by myoperiosteal flap cover and 69.504% repair on the defect treated by free tibial graft. By 9 weeks, the specimens treated by myoperiosteal flap cover retained 87.137% and 90.229% osseous healing compared to 60.751% and 54.402% healing from free graft repair. By 12 weeks only 7.659% and 32.354% healing remained from the myoperiosteal flap repair while 73.414% and 40.433% healing

TABLE 4

RADIOGRAPHIC EVALUATION OF EXPERIMENT 2

SPECIMEN	TIME OF SACRIFICE	MAJOR PATTERNS OF BONE REMODELLING
2-1-R(G)	2 weeks	disintegrated
2-1-L(F)	2 weeks	disintegrated
2-2-R(F)	2 weeks	(2) internal remodelling (3) central nodular opacity
2-2-L(G)	2 weeks	disintegrated
2-3-R(G)	5 weeks	(2) internal remodelling (1) posterior bone bridge creating new post. border
2-3-L(F)	5 weeks	(2) internal remodelling (1) bone spur from inferior angle (4) superior angle resorption
2-4-R(F)#	5 weeks	obliteration of defect extending beyond borders evidence of previous fracture
2-4-L(G)#	5 weeks	obliteration of defect extending beyond borders evidence of previous fracture
2-5-R(F)	9 weeks	complete obliteration of defect
2-5-L(G)	9 weeks	(1,3) diffuse bone spur from superior angle extending down and into defect (2) internal remodelling
2-6-R(G)	9 weeks	(1) bone spur extending superiorly from inferior angle (2) internal remodelling (4) superior angle resorption
2-6-L(F)	9 weeks	regeneration of defect (3) nodule within former defect boundary at post. border
2-7-R(F)	12 weeks	(1) bone spur extending downward from superior angle (2) remodelling internal (4) resorption at superior margin and inferior border angle
2-7-L(G)	12 weeks	(1) fine bone bridge along entire posterior border of defect (2) internal remodelling
2-8-R(G)	12 weeks	(1) bone spur from inferior angle extending superiorly across defect (2,3) bone mass from superior & anterior walls extending into cavity (4) superior corner resorption
2-8-L(F)	12 weeks	(1) spur extending from superior angle inferiorly along margin (2) internal remodelling (4) inferior angle resorption

TABLE 5

ESTIMATION OF QUANTITY OF BONE REPAIR IN EXPERIMENT 2

Specimen	Total Weight of Block (mgm)	Weight of Bone (mgm)	Weight of Defect (mgm)	% Defect Remaining	% Healing
2-1-R(G)	disintegrated				
2-1-L(F)	disintegrated				
2-2-R(F)	16.447	10.280	6.167	78.461	21.539
2-2-L(G)	disintegrated				
2-3-R(G)	14.565	12.168	2.397	30.496	69.504
2-3-L(F)	12.782	8.607	4.175	53.117	46.883
2-4-R(F)	23.193	23.401	0.0	0.0	100
2-4-L(G)	22.083	22.253	0.0	0.0	100
2-5-R(F)	12.377	11.366	1.0	12.863	87.137
2-5-L(G)	17.631	14.546	3.085	39.249	60.751
2-6-R(G)	23.059	19.475	3.584	45.598	54.402
2-6-L(F)	24.588	23.820	0.768	9.771	90.229
2-7-R(F)	20.595	13.337	7.258	92.341	7.659
2-7-L(G)	20.312	18.227	2.085	26.586	73.414
2-8-R(G)	20.183	16.148	4.682	59.567	40.433
2-8-L(F)	19.242	13.925	5.317	67.646	32.354

remained from the free graft repair.

Radiographic analyses of bone healing are given in Table 4, patterns of bone healing, and Table 5, estimation of the quantity of bone repair.

Experiment #2 provided an opportunity for statistical analysis of flap versus graft performance. Using a matched pair analysis flap versus graft performance was evaluated for the specimens from 5 weeks, 9 weeks and 12 weeks. The one specimen at 2 weeks was not included in the analysis.

The null hypothesis was formulated; that is, no overall difference existed between defects covered with temporalis myoperiosteal flaps or free tibial periosteal grafts.

Calculations were carried out as follows:

	Flap	Graft	d (Flap-Graft)		$\bar{d}$
T2	46.883	69.504	d_{21}	-22.621	-11.311
(5 weeks)	100	100	d_{22}	0	
T3	87.137	60.751		26.386	31.107
(9 weeks)	90.299	54.402		35.827	

and so on.

(1) Using the t-test $t = \dfrac{\bar{\bar{d}} - 0}{\sqrt{S^2_p / 6}}$

(2) where: $\bar{\bar{d}} = \dfrac{\bar{d}_2 + \bar{d}_3 + \bar{d}_4}{3}$

(3) $S^2_p = \dfrac{Sd_1^2 + Sd_2^2 + Sd_3^2 + Sd_4^2}{4}$

(4) $Sd_2^2 = (d_{21} - \bar{d})^2 + (d_{22} - \bar{d})^2$

similarly for Sd_3^2 and Sd_4^2

With 3 degrees of freedom, $t = -0.1998$

$< t_{(.975)}\ 3.182$

The difference seen is insufficient evidence to conclude, from the very small sample size, that there is a significant difference in these experiments between defects covered by temporalis myoperiosteal flaps and free tibial periosteal grafts. This result must be interpreted by the extremely small sample size and the actual observation that bone defects covered by temporalis myoperiosteal flaps did produce more bone than the corresponding grafts at 9 weeks (Table 5).

A second analysis was performed to evaluate the interaction of time (2 weeks versus 5, 9 or 12 weeks) on the bone produced between myoperiosteal flap versus the free graft.

Null hypothesis: Time has no significant effect on the potential of myoperiosteal flaps versus free periosteal grafts in the production of new bone.

$$F_{(2,3)} = \frac{\sum 2(\bar{d}_i - \bar{d})^2}{3} \Big/ s^2_p = 0.928 < F_{(.975)} 9.5521$$

There is insufficient evidence to conclude that time has an influence on differences seen in the two treatments. That is, the absolute differences between bone produced by myoperiosteal flaps or free grafts harvested at similar times after surgery is not influenced by the passage of time.

b. Histologic:

This study defines periosteum as a three layered tissue; an outer fibrous vascular layer, an inner vascularized cortical bone layer and a middle osteogenic (or cambial) layer.

Specimens taken at 2 weeks demonstrated similar features. The bone demonstrated early lamellar architecture for both types of periosteal transfer with simultaneous osteoblastic and osteoclastic

activity at their surfaces. (Fig. 29, 30, & 31)

The zone of periosteum closest to growing bone varied from one to several cell layers in thickness. The cells were round or plump ovals in shape, suggesting cellular activity related to the new bone formation. Osteoclasts were fewer in number and presented as typical multinucleated cells within Howship's lacunae. Blood vessels resembling capillaries were abundant in this zone. The region of periosteum adjacent to the cellular zone but furthest from bone demonstrated bundles of fine-textured fibrils generally arranged parallel to the bone surface. The cells in this layer were numerous although less abundant than the inner zone. Their shape varied from plump oval to spindle shaped, suggesting various degrees of metabolic activity. Blood vessels were also numerous in this region. The outer layer blended into either loose irregular connective tissue or muscle tissue undergoing atrophy and fibrous replacement.

Specimens taken at 5 weeks demonstrated increasing maturity of the new bone adjacent to periosteum. (Fig. 32, 33, 34) The osseous tissue in this region showed few trabeculations resembling immature newer bone and a greater degree of adjacent osteoclastic and osteoblastic sites along the healing bone-periosteum interface. The periosteum itself demonstrated a similar appearance as at 2 weeks. Specimen 2-4-L was destroyed during processing. Specimen 2-4-R (Fig. 34) however presented features similar to other tissues taken at 5 weeks, except for a mass of cartilage developing adjacent to new bone and periosteum.

Specimens at 9 weeks demonstrated mature or maturing bone with surface remodelling activity. (Fig. 35-39) The inner cellular zone of periosteum was generally thinner with only 3 or 4 cell layers at its thickest points. The cells ranged in shape from round or plump ovals near the bone surface to more spindle in shape in their outer regions. Blood vessels were less apparent. The outer fibrous zone was thinner while fibres were arranged in bundles both perpendicular and parallel to the bone surface. There did not seem to be a relation between the bone-periosteum interface and fibre bundle orientation. The fibre bundles were fine or coarse in appearance. Cells were fewer in number, mostly fusiform in shape although a few round cells were present. Blood vessels were less abundant. The periosteum generally reflected decreasing response to the original surgical injury as evidenced by increasing thinness, decreasing cellularity and more quiescent appearance of the cells. The periosteum blended into irregular connective tissues or muscle tissue generally undergoing atrophy and fibrous replacement. Specimen 2-6-L (flap)(Fig. 37, 38, 39) demonstrated masses of cartilage blending into periosteum and bone.

Slides of specimens 12 weeks old demonstrated some intramembranous bone formation, mature cortical bone and osteoclastic activity. (Fig. 40, 41, 42) Developing marrow was seen in speciments 2-7-L (graft) and 2-8-L (flap). Although the bone surface was remodelling in many areas, the periosteum had now shrunk to almost the thinness of resting periosteum. The inner cellular zone was only 1 or 2 cells thick except for specimen 2-8-L (flap) which showed an active

periosteal bone formation. The outer fine to coarse fibre bundles were arranged generally parallel to the bone surface. Cells were fewer in number and predominantly spindle shaped suggesting a decrease of metabolic function. Blood vessels were seen more frequently in this region. Specimen 2-7-L (graft) demonstrated areas of hyaline-like connective tissue just beyond the fibrous layer of periosteum.

A tetracycline fluorescence was seen throughout the osseous tissues in this experiment as well as experiments 4 and 5. Fluorescence was evident within the mass of newly formed bone along osteon seams as well as the subperiosteal osteoid border. No comparison could be made, however, between newly formed bone on slides stained with haematoxylin and eosin and those evaluated by fluorescence.

EXPERIMENT 3

Question: Does a free tibial periosteal graft transplanted to temporalis muscle receive a blood supply from this muscle?

Material: Two rabbits were used.

After achieving general anesthesia, the temporalis muscle (on a side chosen at random) was exposed as in experiment 1. An anterior autogenous tibial periosteal graft, 1.0 cm x 1.6 cm was harvested (from a side chosen at random) and sutured to the outer surface of the temporalis muscle, cambial surface of the graft facing outwards. (Fig. 43, 44) The skin flaps were closed. Two weeks later the temporalis muscle with the grafted periosteum was isolated and the India Ink perfusion repeated as in experiment 1.

Observations

The tibial grafts had the gross appearance of white, thick fibrous squares overlying healthy temporalis muscle. Both the graft and the muscle darkened, as in experiment 1, after infusion with 3.5 cc of India Ink. Microscopically, the periosteum appeared thicker, more fibrous and less cellular than resting periosteum while the muscle tissue appeared normal in structure. India Ink was seen in vessels coursing through both the muscle and the grafted periosteum (Fig. 47). One graft demonstrated nodules of maturing bone trabeculae within its substance. (Fig. 45 & 46)

EXPERIMENT 4

Question: Does a free tibial periosteal graft applied to temporalis muscle and subsequently transferred as a vascularized flap induce osteoneogenesis? If so, does this differ qualitatively or quantitatively from a free tibial periosteal graft?

Material: 8 rabbits were used in this experiment.

Tibial periosteum (from a side chosen at random) was harvested and grafted to a temporalis muscle (side chosen at random) in a manner similar to experiment 3. Two weeks later a defect was made in the mandible using a technique similar to experiment 1. The grafted periosteal-temporal muscle flap was transferred so that the tibial periosteum could be wrapped around the defect. A free tibial periosteal graft from the unoperated leg was harvested and placed over a defect made in the opposite mandible. All rabbits were given tetracycline and sacrificed according to Table 6. (Rabbits 4-2 and 4-6 appeared to be going into shock during surgery and were given 0.1 mgm of Depo-Medrol (Tm) subcutaneously during the operative procedure). The rabbits showed no abnormal behavior during their recoveries from surgery.

Observations

a. Radiographic

The maximum amount of healing occurred by 9 weeks (4-5-R) when a myoperiosteal flap was the only tissue to produce complete healing. Multiple small radiopacities posterior to the former defect suggested focal osteogenesis or fibrosis related to host tissue surgical disruption.

Bone formation occurred very rapidly in one specimen covered by a myoperiosteal flap harvested at 2 weeks (4-1-R; 80.56% healing) as opposed to the opposite free graft (4-1-L; 53.521%). The amounts of bone formed at 5 weeks and 12 weeks were less than the amounts formed at 9 weeks, following the results of experiment 2.

Rabbits 4-2 and 4-6 were given Depo-Medrol (Tm) intraoperatively. Both produced less bone with myoperiosteal flap repair (4-2-R, 68.24%; 4-6-R, 63.079%) as well as free graft repair (4-2-L, 10.903%; 4-6-L, 58.028%) as compared with the second rabbits sacrificed at the same time (4-1-R flap 80.56%, 4-5-R flap, 100%; 4-1-L graft, 53.321%, 4-6-L graft, 58.028%).

A matched pair analysis similar to experiment 2 was performed. All specimens from 2 weeks were available, thus expanding the sample sizes.

The calculated $t = 2.152 < t_{(.975)} 2.776$

$DF = 4$

The evidence is insufficient to conclude that there is a statistical difference between the amount of bone produced by defects covered with myoperiosteal flaps as contrasted with defects covered by free tibial periosteal grafts. The closeness of the calculated t value to the listed t value indicates, however, a strong statistical trend (in this small sample size) towards a statistically significant difference. As well, the amounts of bone formed by tibial graft-myoperiosteum flaps was observed in most defects of this experiment (Table 8).

The interaction with time analysis:

$F_{(3,4)} 2.83 < F_{(.975)} 6.5914$

left the evidence insufficient for time being a significant factor of the absolute difference in bone production between flaps and grafts at each period of sacrifice.

Patterns of bone production are listed in Table 7 while estimated quantities of bone production are listed in Table 8.

TABLE 6

Experimental Protocol for Experiment 4

RABBIT	DONOR GRAFT	RECIPIENT FLAP	GRAFT		TETRACYCLINE (T) OR OXYTETRACYCLINE WEEKS POSTOP.	SACRIFICE WEEKS POSTOP.
			TIBIAL DONOR	RECIPIENT MANDIBLE		
4 - 1	R	R	L	L	1 T	2
4 - 2	L	R	R	L	1 OX	2
4 - 3	L	L	R	R	1T 4T	5
4 - 4	R	R	L	L	10X, 40X,	5
4 - 5	R	R	L	L	10X, 40X, 80X	9
4 - 6	L	R	R	L	10X, 40X, 80X	9
4 - 7	R	R	L	L	1T, 40X, 80X	12
4 - 8	R	R	L	L	1T, 4T, 8T	12

TABLE 7

RADIOGRAPHIC EVALUATION OF EXPERIMENT 4

SPECIMEN	TIME OF SACRIFICE	MAJOR PATTERNS OF BONE REMODELLING
4-1-R(F)	2 weeks	(1) bone spur extending superiorly from inferior angle (2) internal remodelling
4-1-L(G)	2 weeks	(1) bone spur extending downward from superior angle (2) internal remodelling (3) central radiopacity (4) superior and internal margin resorption
4-2-R(F)	2 weeks	(1) spur extending inferiorly from superior angle (2) internal remodelling
4-2-L(G)	2 weeks	(1) spur extending inferiorly from superior angle (2) interior remodelling (3) circular opacity in defect by inferior angle
4-3-R(G)	5 weeks	(1) spur extending inferiorly from superior angle (2) internal remodelling
4-3-L(F)	5 weeks	(2) internal remodelling
4-4-R(F)	5 weeks	complete bone fill except at inferior border of defect (2) bone spur extending superiorly
4-4-L(G)	5 weeks	(1) bone spur extending superiorly from inferior angle (2) internal remodelling (4) superior angle resorption
4-5-R(F)	9 weeks	almost complete healing of defect mult radiopacities posterior to former defects
4-5-L(G)	9 weeks	(2) internal remodelling
4-6-R(F)	9 weeks	(1) superior and inferior spurs along posterior border (2) internal remodelling
4-6-L(G)	9 weeks	(1) superior and inferior spurs along posterior border (2) internal remodelling

TABLE 7 (Cont'd)

RADIOGRAPHIC EVALUATION OF EXPERIMENT 4

SPECIMEN	SACRIFICE	MAJOR PATTERNS OF BONE REMODELLING
4-7-R(F)	12 weeks	(3) diffuse radiopacity within defects extending from superior and anterior margin
4-7-L(G)	12 weeks	(1) superior and inferior bone spurs along posterior border (3) radiopacity within defect
4-8-R(F)	12 weeks	(2,3) bone spur extending posteriorly from anterior border
4-8-L(G)	12 weeks	(2) internal remodelling inferior angle (4) inferior angle resorption

TABLE 8

ESTIMATION OF QUANTITY OF BONE REPAIR EXPERIMENT 4

Specimen	Total Weight of Block (mgm)	Weight of Bone (mgm)	Weight of Defect (mgm)	% Defect Remaining	% Healing
4-1-R(F)	16.480	14.952	1.528	19.44	80.56
4-1-L(G)	15.266	11.597	3.669	46.679	53.321
4-2-R(F)	16.426	13.914	2.512	31.960	68.04
4-2-L(G)	25.426	18.423	7.003	89.097	10.903
4-3-R(G)	24.344	20.836	3.508	44.631	55.369
4-3-L(F)	25.555	19.416	6.139	78.104	21.896
4-4-R(F)	23.819	21.185	2.634	33.511	66.489
4-4-L(G)	28.928	25.879	3.049	38.791	61.209
4-5-R(F)	10.812	14.125	0.0	0.0	100
4-5-L(G)	16.631	14.349	2.282	29.033	70.967
4-6-R(F)	17.551	14.649	2.902	36.921	63.079
4-6-L(G)	22.294	18.995	3.299	41.972	58.028
4-7-R(F)	20.756	17.554	3.202	40.738	59.262
4-7-L(G)	19.648	15.154	4.494	57.176	42.824
4-8-R(F)	20.009	14.508	5.501	69.987	30.013
4-8-L(G)	16.417	11.791	4.626	58.855	41.145

b. Clinical

All specimens demonstrated normal tissue contour during their removal and exostoses were not clinically evident as in experiment 2.

c. Histologic

Histologic patterns generally mirrored those of experiment 2. Both grafted myoperiosteal flaps and free tibial periosteal grafts presented similar histologic patterns as healing progressed. (Fig. 60-74)

Tissues harvested at 2 weeks demonstrated young, active periosteum with a highly cellular cambial layer similar to experiment 2. (Fig. 60-64) This zone was intimately associated with intramembranous patterns of new bone formation. The periosteal cells were round to plump ovoid in shape, suggesting active bone formation. Two sections (4-1-L graft Fig. 62 and 4-2-L graft Fig. 64) prepared with free periosteal grafts presented areas of hyaline cartilage within the substance of the active periosteal tissue. The fibrous layer was thick with fine fibre bundles either parallel to the bone surface or randomly arranged. One section (4-2-L graft) demonstrated an active, abeit thinner periosteum, presumably associated with the plane of section.

Specimens taken at 5 weeks reflected a continuing lively periosteal osteogenic activity. The cambial layer remained multilayered with

round to plump ovoid cells, and a fibrous layer with fine to coarse fibre bundles. The bundles were oriented as in the earlier specimens. The trabecular bone showed a continued maturation. Elements of embryonic marrow associated with new bone were seen in all tissue sections taken at 5 weeks. (Fig. 65-68)

Sections taken at 9 weeks reflected continuing healing of the osseous defects. The periosteum remained multi-layered in its cambial layer with round or oval shaped cells. Both features suggested a metabolically active periosteum. The outer fibrous zone remained thick with fibre bundles oriented either parallel or in random arrangement to the bone surface. The cells of this zone were spindle shaped, suggesting less metabolic activity. The bone surface demonstrated active osteoblastic and osteoclastic areas, suggesting active surface remodelling. (Fig. 69-72)

Three defects (4-3-R, graft, Fig. 65; 4-4-R, flap, Fig. 68; 4-5-R, flap) demonstrated embryonic marrow. One specimen covered by a free tibial graft demonstrated cartilage islands and sheets blending with regenerating bone and periosteum (4-6-L, Fig. 72).

Specimens at 12 weeks showed continuing maturation of bone with areas of osteoclastic and osteoblastic activity. (Fig. 73 & 74) The bone itself appeared more cortical and less trabecular in architecture. Marrow was seen in one of the regenerated defects covered by a myoperiosteal flap (4-7-R) and one regenerated defect covered by a free graft (4-8-R). The cambial zone of periosteum

varied from several to one layer in thickness while the outer fibrous layer demonstrated a similar architecture as in the 9 week specimens.

Blood vessels remained abundant in the fibrous periosteum from 2-12 weeks although fewer vessels were seen in the cambial layer.

Both animals 4-2 (Fig. 63, 64) and 4-6 (Fig. 71, 72) were given a 1.0 mgm subcutaneous dose of Depo-Medrol (Tm) during the operative procedure. Tissue sections from these animals showed an increased deposition of cartilage adjoining bone and the active periosteum, for both myoperiosteal flaps and grafts. Bone formation otherwise did not appear to be altered by the drug.

Cartilage islands were seen throughout the series, primarily in the grafted defects (4-1-L, 4-2-L, 4-6-L, and 4-7-L) although one specimen repaired with a myoperiosteal flap taken at 12 weeks (4-7-R) demonstrated cartilage-like tissue within the periosteum and against remodelling bone.

EXPERIMENT 5

Question: Does a Vitallium (Tm) mesh placed over a mandibular defect influence the amount or quality of bone produced by a surrounding myoperiosteal flap?

Material: Eight rabbits were used in this experiment

The temporalis muscle and the posterior mandible were exposed in a manner similar to experiment 1. After creating the defect and removing local periosteum, a Vitallium (Tm) mesh crib was wrapped around the bone defect and secured by turning its edges slightly inwards. (Fig. 75) The temporalis myoperiosteal flap was subsequently wrapped over the mesh with periosteum facing the metal. An equivalent defect was made in the opposite mandible and this was covered with autogenous free tibial periosteal graft in a manner similar to experiment 2. All animals were given tetracycline injections and sacrificed in a manner similar to experiment 1. (Table 9)

Observations

a. Radiographic and Clinical

Details of radiographic evaluation are given in Table 10 while representative radiographs are presented in Fig. 76-82. The specimens with Vitallium (Tm) mesh covering the defects were impossible to evaluate radiographically. (Fig. 76)

No radiopacities were seen to extend beyond the margins of the mesh, nor was excessive resorption seen to extend in a similar manner.

The specimens covered with a free tibial periosteal graft produced less radiographic evidence of osteogenesis than in experiments 2 and 4. One specimen harvested at 9 weeks (5-5-L) (Fig. 68) demonstrated complete clinical healing with new bone extending as a lateral cortical exostosis (1.0 cm x 1.0 cm) composed of bone and cartilage.

Radiographically, the specimen appeared as an ovoid cortical radiopaque outline extending posteriorly from the posterior mandibular border and occupying the greater portion of the former defect. (Fig. 80)

In contrast to experiments 2 and 4 maximum bone production occurred after 9 weeks, persisting to at least 12 weeks.

Patterns of bone regeneration are given in Table 10 while estimates of the quantity of new bone are listed in Table 11.

TABLE 9

Experimental Protocol for Experiment 5

RABBIT	FLAP	DONOR GRAFT	RECIPIENT MANDIBLE	TETRACYCLINE (T) OR OXYTETRACYCLINE WEEKS POSTOP.	SACRIFICE WEEKS POSTOP.
5 - 1	L	L	R	1T	2
5 - 2	L	R	R	1T	2
5 - 3	L	R	R	1T, 4T	5
5 - 4	R	R	L	1T, 4T	5
5 - 5	R	R	L	1T, 4T, 8T	9
5 - 6	R	L	L	10X, 40X, 80X	9
5 - 7	L	R	R	1T, 4T, 80X	12
5 - 8	L	L	R	1T, 4T, 8T	12

TABLE 10

RADIOGRAPHIC EVALUATION OF EXPERIMENT 5

SPECIMEN	TIME OF SACRIFICE	MAJOR PATTERNS OF BONE REMODELLING
5-1-R(G)	2 weeks	(2) slight internal modelling
5-1-L(F)	2 weeks	mesh over defect, unable to evaluate
5-2-R(G)	2 weeks	specimen fractured slight internal remodelling
5-2-L(F)	2 weeks	mesh over defect, unable to evaluate
5-3-R(F)	5 weeks	mesh over defect, unable to evaluate
5-3-L(G)	5 weeks	(1) bone spurs from superior and inferior angles (2) internal remodelling (3) round radiopacity adjacent to anterior border
5-4-R(F)	5 weeks	mesh over defect, unable to evaluate
5-4-L(G)	5 weeks	(1) bone spur extending downward from superior angle into centre of defect bone spur extending upward from inferior angle (2) internal remodelling
5-5-R(F)	9 weeks	mesh over defect, unable to evaluate
5-5-L(G)	9 weeks	(1) defect almost obliterated with bone spur extending beyond posterior border of mandible (4) cortical radiopacity over defect
5-6-R(F)	9 weeks	mesh over defect, unable to evaluate
5-6-L(G)	9 weeks	(1) spur extending posteriorly from anterior margin of defect (2) internal remodelling
5-7-R(F)	12 weeks	mesh over defect, unable to evaluate
5-7-L(G)	12 weeks	thick intact posterior border (4) small nodule in centre of residual defect
5-8-R(F)	12 weeks	mesh over defect, unable to evaluate
5-8-L(G)	12 weeks	(1) bone spur extending upwards from inferior angle (2) internal remodelling

TABLE 11

ESTIMATION OF QUANTITY OF BONE REPAIR EXPERIMENT 5

Specimen	Total Weight of Block (mgm)	Weight of Bone (mgm)	Weight of Defect (mgm)	% Defect Remaining	% Healing
5-1-R(G)	22.228	15.278	6.95	88.422	11.578
5-2-R(G)	23.536	16.866	6.67	84.860	15.140
5-3-L(G)	26.508	24.025	2.483	31.590	68.41
5-4-L(G)	26.309	23.410	2.899	36.883	63.117
5-5-L(G)	20.494	18.814	1.68	21.374	78.626
5-6-L(G)	33.379	28.512	4.867	61.921	38.079
5-7-L(G)	11.912	10.337	1.575	20.038	79.962
5-8-L(G)	16.883	15.154	1.729	21.997	78.003

b. Histologic

Specimens from 2 weeks appeared similar in tissue architecture. There was a strong periosteal response with lively new trabecular bone formation against older cortical bone. The multilayered cambial zone presented plump to ovoid cells suggestive of active metabolic response. The outer zone again presented as a fibrous tissue with fine fibre bundles oriented parallel or randomly to the bone surface. (Fig. 83-86)

The 5 week old defects covered by mesh and myoperiosteal flap retained the same appearance as the 2 week old specimens. (Fig. 87-88) Cartilage was seen in the healing tissues of both myoperiosteal flap and free graft of one specimen (5-2-L; 5-2-R). One specimen (5-3-R) demonstrated a lively osteogenic response as well as cartilage under the periosteum.

The defects covered by grafts at 9 weeks showed continued evidence of bone formation and remodelling with zones of periosteal, osteoclastic and osteoblastic activity. (Fig. 89-93) The cellular zone was multilayered, and thinner in many areas. The cells, although round and ovoid in shape were smaller than earlier specimens. These features suggest active although diminishing, periosteal activity. The defects covered by mesh and myoperiosteal flaps appeared similar to the 2 and 5 week specimens. One specimen demonstrated cartilaginous tissue within the periosteum of both myoperiosteal flap (5-5-R) and free periosteal graft (5-5-L). One

defect originally covered with a periosteal graft (5-6-L), Fig. 92) and one covered with mesh and myoperiosteal flap (5-5-R) . demonstrated embryonic marrow within the newly formed bone.

Specimens at 12 weeks demonstrated a thinner periosteum although osteogenesis and bone remodelling was still proceeding. (Fig. 94-96) Cells were smaller and more fusiform in outline. These findings suggest a maturing although still active periosteum. Newly formed and now maturing marrow was seen in these specimens. One section covered by mesh and a myoperiosteal flap demonstrated cartilage-like tissue within the periosteum.

Atrophying muscle was associated with most periosteal tissues in this experiment while most sections demonstrated a high degree of vascularity throughout the series.

DISCUSSION

This study defines periosteum as a three layered tissue; an outer fibrous vascular layer, an inner vascularized cortical bone layer and a middle osteogenic (cambial) layer. The osteogenic cells are given a vascular supply by both superficial and deep layers, capable of osteogenesis if attached to either (Fig. 97). Periosteum of immature animals has potential for producing new bone in normal growth, or following injury such as bone fracture or periosteal transplantation. Although many authors mention that periosteal osteogenic potential decreases with age, the actual potential for periosteal osteogenesis has not yet been demonstrated in animals beyond the post-epiphyseal fusion stage. This study has been directed towards investigation of the osteogenic potential of periosteum in post-epiphyseal fusion stage in male New Zealand White rabbits.

The experiments were standardized for peri-operative care, surgical approach and creation of defect. The only variable in the experimental series was the treatment of the defect sites before closure; without periosteal cover, with a parietal myoperiosteal flap, a free tibial periosteal graft, an autogenous tibial periosteal graft transferred as a flap or a myoperiosteal flap covering a Vitallium mesh (Tm). The research was approached under the null hypothesis, assuming there would be no difference in the different surgical treatments of the defects.

EXPERIMENT 2

Question: Does a temporalis myoperiosteal flap induce osteogenesis? If so does this differ qualitatively or quantitatively from a free tibial periosteal graft?

a. Radiographic Evaluation

Specimens were radiographed to a standardized technique, making comparisons between all of the films possible. Although as much tissue as possible was trimmed from the defect, different thicknesses of tissue were reflected on the films. With this limitation in mind, the films were evaluated visually noting the trends of bone formation and estimating the amount of defect remaining at each period of evaluation by comparing weighted areas of new bone formation. The radiographs were enlarged before tracing to give a more easily measured mass of paper.

Four basic patterns of bone regeneration and remodelling were seen, with one or more patterns present in any specimen. Osteogenic cells of periosteum were in greatest numbers at the posterior margin of the defect where the periosteum folded on itself. In this region spurs of bone were a frequent finding and were seen either descending from the superior angle of the defect or ascending from its inferior angle. The spurs were present either singly or together, occasionally fusing to form a bone bridge and creating a new posterior osseous margin. They followed a course corresponding to the potential space left by the folded periosteum at the posterior margin of the defect.

A second pattern of remodelling occured at the margins and inner corners of the defects representing bone deposition and resorption, giving the defect a more or less C-shaped outline. This almost

constant finding suggests both the cut surfaces of the mandible and the adjacent periosteal wrapping participated in the remodelling activity.

Resorption of the overall bone mass was a finding first present in a flap specimen taken at 5 weeks. Resorption is well known to occur as a late phenomenon in the placement of periosteal grafts. Its presence here in both myoperiosteal flap and grafted preparations reflects an undesirable consequence of the experimental procedure.

The last pattern of osseous change presented as round nodules within the defect cavity, occasionally contacting an osseous margin. As periosteum covered the defect on both sides, leaving a blood-filled space within, radiopacities suggested new subperiosteal bone formation within the defect, arising from the transplanted periosteum.

In general terms, the defects appeared to heal as time progressed with the maximum amount of new bone formation (complete healing) seen as early as 5 weeks after surgery. This amount decreased to approximately 30-40% healing of the defect by at least 12 weeks following operation.

There were notable exceptions. One rabbit (2-4) demonstrated an exuberant callus formation on both myoperiosteal flap and graft treated mandibles. The defects were completely obliterated and a callus-like nodule extending outward from the lateral mandibular

surface. The radiographs demonstrated possible fractures through both defects at some point between surgery and sacrifice. If the mandibles did fracture each would have occurred spontaneously and separately as the rabbit used its mandible throughout the 9 week period of healing. The clinical appearance of the specimens suggests fracture callus, with an exaggerated response due to the constant stress caused by movement of the mandible. The myoperiosteal flaps appeared to deliver the best environment for healing as evidenced by the greater amount of bone filling the defect at 9 weeks in both experiments 2 and 4.

By 12 weeks both myoperiosteal flaps and grafts were associated with increasing evidence of bone resorption. Both defects covered by myoperiosteal flaps in experiment 2 and one defect of experiment 4 produced less new bone than their counterpart defects covered with free grafts. The results suggest that myoperiosteal flaps initially produce an environment condusive to better healing at least to 9 weeks after which they are no better and potentially less advantageous than free periosteal grafts in producing new bone.

Statistical analysis suggests that free tibial grafts applied to temporalis muscle and transferred as myoperiosteal flaps are superior tissues for producing new bone while parietal temporalis myoperiosteal flaps are only slightly (if at all) better than free tibial periosteal grafts in producing new bone. As noted before, the sample size of experiment 2 was very small so that statistical results can only be interpreted relative to the healing trends seen

in Table 5.

The myoperiosteal flap was turned through approximately 110 degrees in its placement. This would almost certainly cause some degree of ischemia to the periosteum and muscle in the initial stages of healing. They were, however, placed in a highly vascular environment of surrounding muscle tissue. The free grafts, were placed into this highly vascular environment of surrounding muscle tissue, allowing rapid revascularization of the graft. This hypothesis would be consistent with other researcher's findings on the revascularization potential of muscle tissue. (Beck, 1935; German and Taffel, 1939; Stuck and Hinchley, 1944, Zucman, 1960).

b. Histologic Evaluation

In general terms, the periosteum transferred as either a myoperiosteal flap or free autogenous periosteal graft demonstrated similar histologic features throughout the healing period.

Specimens taken at 2 weeks and 5 weeks demonstrated the classic features of a stimulated periosteum rapidly producing new bone. The abundant new bone formation at 2 weeks suggests an early revascularization of the free periosteal graft. Two of the specimens demonstrated complete osseous healing at 5 weeks, both specimens being taken from the same animal. Specimen 2-4-L (free graft) was destroyed during the acrylic embedding phase. Specimen 2-4-R (myoperiosteal flap) demonstrated the features as described

previously for an active periosteal tissue with a lively osteogenic reaction. Masses of callus were seen adjacent to both periosteum and newly formed bone. No other evidence of a previous and now healing fracture was seen in the section. The specimens taken at 9 and 12 weeks demonstrated a maturing of bone in the healing defect with the associated redirection of periosteum towards its resting phase. Specific factors included thinning of the periosteum, primarily at the expense of the cambial layer, fewer smaller cells within the cambial layer and remodelling of the existing bone at the periosteal and endosteal surfaces.

Overall, both types of periosteum appeared to produce a similar type of bone at the same time after healing even though one periosteum arose from a membranous bone (parietal periosteum in the myoperiosteal flap and the second from an endochondrally formed bone free tibial graft). The mandible, also intramembranous in origin, may have influenced both types of periosteum to produce bone in a similar manner. The nature of the grafted tissue itself: that is, a periosteal sheet, would seem more likely to produce an intramembranous rather than an endochondral osseous tissue.

Several specimens demonstrated cartilage adjacent to both periosteum and newly formed bone. It is hard to draw conclusions on this finding as other specimens may have shown cartilage at different levels of the section the cartilage may have arisen, however, from ischemia of the transplanted tissues in the recipient bed. As well, sections 2-1-R, 2-1-L and 2-2-R were lost to histologic

examination. Examination of all specimens by fluorescence for evidence of new bone added little information. Tetraycycline fluorescence was seen throughout the osseous tissue in this experiment as well as experiments 4 and 5. Fluorescence was evident within the mass of newly formed bone along osteon seams as well as at the osteoid border between periosteum and newly formed bone. No comparison could be made, however, between the newly formed bone on slides stained with haematoxylin and eosin, and those evaluated by fluorescence. The tetracycline staining did reinforce that new bone was formed in the mandibular defect partly from the transposed or transplanted periosteum and partly from the recipient bed endosteal tissue.

EXPERIMENT 3

Question: Does a free tibial periosteal graft transplanted to temporalis muscle receive a blood supply from this muscle?

Grossly, the periosteum underwent fibrous change during its 2 weeks as a transplanted tissue. Microscopically, the tissues showed loss of the layered periosteal architecture, and increased fibrous tissue formation within its substance. One section demonstrated 2 nodules of maturing trabecular bone surrounded by its own periosteum. These features suggest that the grafted periosteum was undergoing a normal reorganizational response secondary to the trauma of the grafting procedure.

Traumatized periosteum produces new bone in young animals provided vascularity is sufficient. The bone nodules seen in this experiment suggest traumatized periosteum in New Zealand White rabbits beyond the epiphyseal growth stage can produce new bone while the presence of India Ink in the periosteal vessels suggests adequate vascular connections by at least 2 weeks after grafting. The rabbit began to use its jaw, thus moving temporalis muscles on the day after surgery. The constant gentle function of the contracting muscle belly may have provided part of the stimulus for vascular anastomosis and bone formation.

EXPERIMENT 4

Question: Does a free tibial periosteal graft applied to muscle and subsequently transferred as a vascularized flap induce osteogenesis? If so, does this differ qualitatively or quantitatively from a free tibial periosteal graft?

Both the transplanted tibial myoperiosteal flap and free tibial periosteal graft were similar in arising from a similar donor site although on opposite legs. The periosteum on the myoperiosteal flap was stimulated two weeks before the free graft through its harvest for grafting to temporalis muscle. Radiographic evidence demonstrated that myoperiosteal flaps produced an increased amount of bone throughout the healing period. The maximum healing was seen at 9 weeks as evidenced by complete repair of the defect by the myoperiosteal flap. The free graft, however, had regenerated 71% of the defect by this point.

Overall the tibial grafted myoperiosteal flap produced more bone throughout the experiment while in contrast the parietal myoperiosteal flaps produced quantitatively slightly less bone by comparison through each stage of evaluation (except for evaluations done at 2 weeks as specimens 2-1 and 2-2 were destroyed before histologic preparation). The overall results suggest that tibial periosteum grafted to a muscle and transferred as a myoperiosteal flap produced a rapid and sustained osteogenic response up to 9 weeks with slightly less absorption of bone than the parietal myoperiosteal flap. The tibial graft was stimulated in its harvest and transplantation. The rapid vascularization by temporalis muscle could possibly have advanced the activation of the tibial graft such that it had a 2 week "head start" when transferred as a myoperiosteal flap (as opposed to a resting periosteum freshly transferred as a free graft or a flap).

Animals 4-2 and 4-6 given the subcutaneous Depo-Medrol (Tm) produced slightly less bone from both the myoperiosteal flap and free periosteal graft covering the defect as compared with the second set of rabbits sacrificed at the same point after surgery. Depo-Medrol (Tm) does delay healing and this property may be reflected in the smaller amount of bone produced by these 2 rabbits. The histologic picture was similar in both types of defect repair, paralleling the histologic changes seen in experiment 2. The early periosteum was thicker than its resting state with large metabolically active cells, and a lively subperiosteal osteogenic response. With time the periosteum thinned, primarily at the expense of the cambial layer while the newly formed bone was undergoing remodelling and maturation. The animals treated with Depo-Medrol (Tm)

demonstrated a similar tissue architecture to the other specimens sacrificed at similar times.

The observations of these few specimens treated with Depo-Medrol (Tm) suggest this compound reduces the quantity although not the quality of new subperiosteal bone.

EXPERIMENT 5

Question: Does a Vitallium (Tm) mesh placed over a mandibular defect influence the amount or quality of bone produced by a surrounding myoperiosteal flap?

Radiographic observations demonstrate free tibial periosteal grafts produced similar amounts and patterns of new bone as in experiment 2 and 4. These observations suggest that bone newly formed by free tibial grafts, and to a lesser extent calvarial myoperiosteal flaps reaches a maximum in this donor site by 5-9 weeks, subsequently undergoing some degree of resorption and remodelling.

Histologic examination of the defects covered by a Vitalium (Tm) mesh demonstrated a metabolically active periosteum as described previously with a lively subperiosteal bone formation. This appearance persisted at least until 9 weeks. The specimens examined at 12 weeks demonstrated a periosteum returning to its resting state. These observations suggest that a Vitallium (Tm) mesh placed between a myoperiosteal flap and a coagulum-filled mandibular defect retains the metabolically active state

of the periosteum for a prolonged period of time. The free periosteal graft presented a histologic appearance throughout the healing period similar to experiments 2 and 4. The quality of bone formed by both myoperiosteal flaps and free grafts was similar to experiments 2 and 4 again suggesting the influence of the recipient bed in producing new bone.

Defects covered with mesh and a myoperiosteal flap showed radiographic evidence of comparatively less bone formation than their free periosteal graft counterparts although the mesh made more accurate comparisons impossible.

Conclusions

Several conclusions beyond those answering the questions of the study can now be stated:

1. An isolated temporalis muscle with intact vascular pedicle provides a blood supply to its underlying periosteum.

2. A mandibular defect, devoid of periosteum heals poorly and primarily by endosteal reorganization. No new periosteum was regenerated by adjacent mandibular periosteum, endosteum or local connective tissue cover for at least 5 weeks after surgery.

3. A calvarial periosteum, stimulated by surgical removal with an intact connection to the uninterrupted blood supply at the overlying temporalis muscle pedicle (temporalis myoperiosteal flap) does induce osteogenesis in a surgically created defect of a mature male New Zealand White rabbit. The potential for complete regeneration of the defect is evident. The bone produced is membranous in nature.

4. A free tibial periosteal graft will receive a blood supply from a temporalis muscle when transplanted to that muscle at least by 2 weeks after the transplant. The periosteum is further capable of stimulating new bone formation.

5. A free tibial periosteal graft applied to temporalis muscle and subsequently transferred as a vascularized flap does induce osteogenesis as early as 2 weeks after surgery. The bone formed is similar in nature to the first experiment. The amount formed was slightly more than that produced by a free periosteal graft.

6. A Vitallium (Tm) mesh interposed between the mandibular defect and temporalis myoperiosteal flap appeared to delay healing throughout the experiment as evidenced by the smaller amounts of bone produced. The periosteum underlying temporalis muscle retained a histologic appearance of metabolic activity longer than the other myoperiosteal systems. The bone produced was again similar to that produced by the previous myoperiosteal flaps.

7. Free periosteal grafts produced new bone similar in architecture to the myoperiosteal flaps, at slightly less amounts as the tibial graft-myoperiosteal flap and at very slightly less amounts than the parietal myoperiosteal flap.

8. Evidence from performance of the different systems in producing new bone in these experiments suggest from greatest to least potential: tibial graft-temporalis myoperiosteal flap, parietal temporalis myoperiosteal flap, free autogenous tibial graft, defect filled with autogenous blood coagulum and covered by both Vitallium (Tm) mesh and temporalis myoperiosteal flap.

9. Maximum healing for myoperiosteal flaps and free periosteal grafts was seen at 5 weeks. Regression in healing bone volume after this point suggests an impermanence to new bone formed by this system.

10. Factors influencing quality and quantity of new bone include: a highly vascular recipient bed, a recipient bed with bone of intramembranous origin and a recipient bed allowed as normal activity as possible (moving mandible and temporalis muscle) throughout the course of the experiment.

Any research project must discover new questions to be answered as well as answering those which prompted the study. From this research, several questions emerge:

1. Does a temporalis myoperiosteal flap produce bone of a permanent nature beyond 3 months? If so, how much remains after the initial healing phase of 5 weeks?

2. Does a temporalis myoperiosteal flap induce osteogenesis in a mandibular defect treated by pre or post surgical irradiation as would be found in a postirradiated human patient?

3. Does revascularization of a free periosteal graft influence the quality or quantity of new bone produced in a postirradiation mandibular defect?

4. Does the inclusion of Vitallium (Tm) mesh filled with autogenous bone chips or alloplastic bone substitute and wrapped with a temporalis myoperiosteal flap influence the quality or quantity of new bone produced in an unirradiated or postirradiated mandibular defect?

5. Does a cortical bone graft placed in the defect influence the osteogenic activity of a surrounding myoperiosteal flap?

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Figure 1: Knee joint showing distal femur, proximal tibia and proximal fibula.

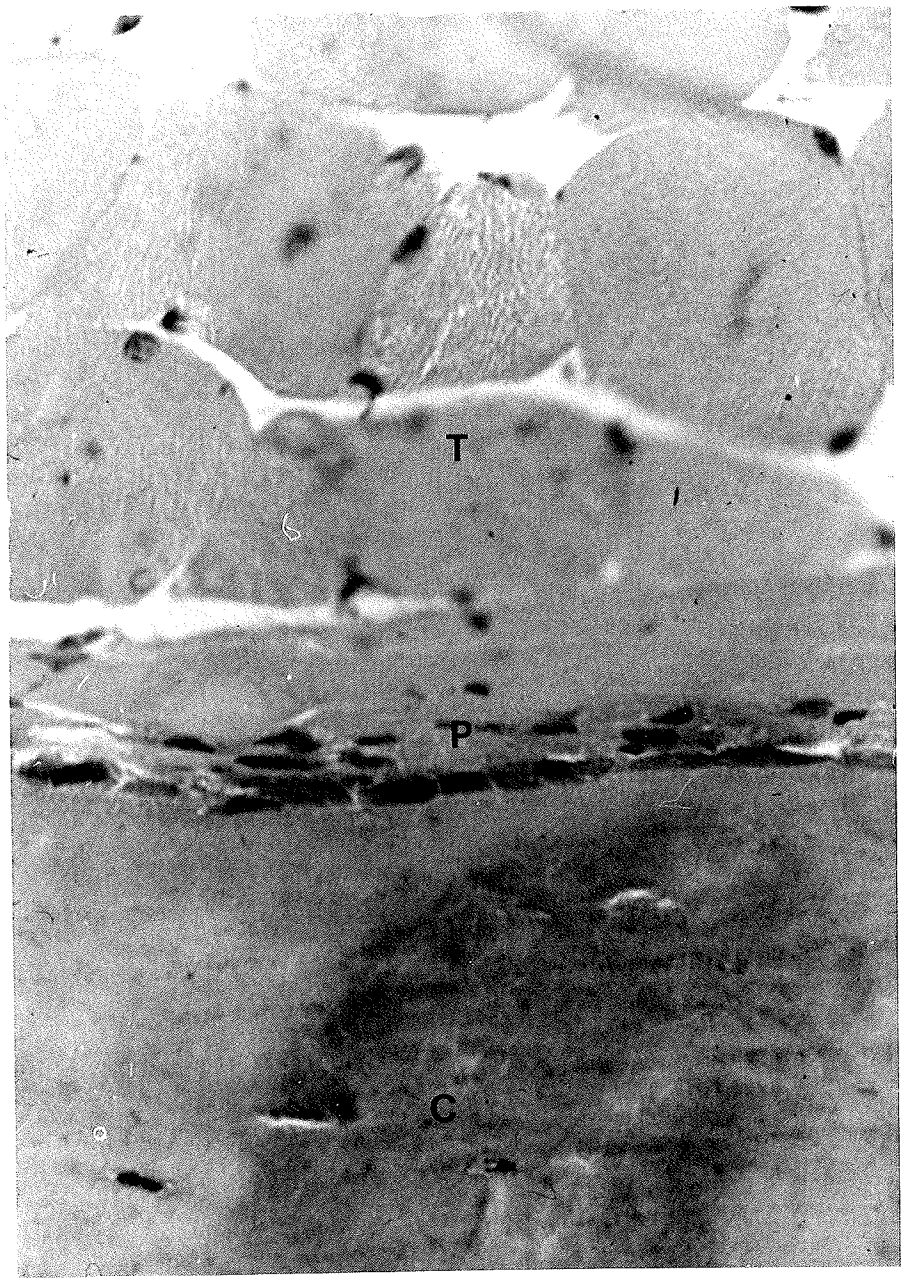


Figure 2: Calvarium (C) with overlying periosteum (P) and temporalis muscle (T) (x100).

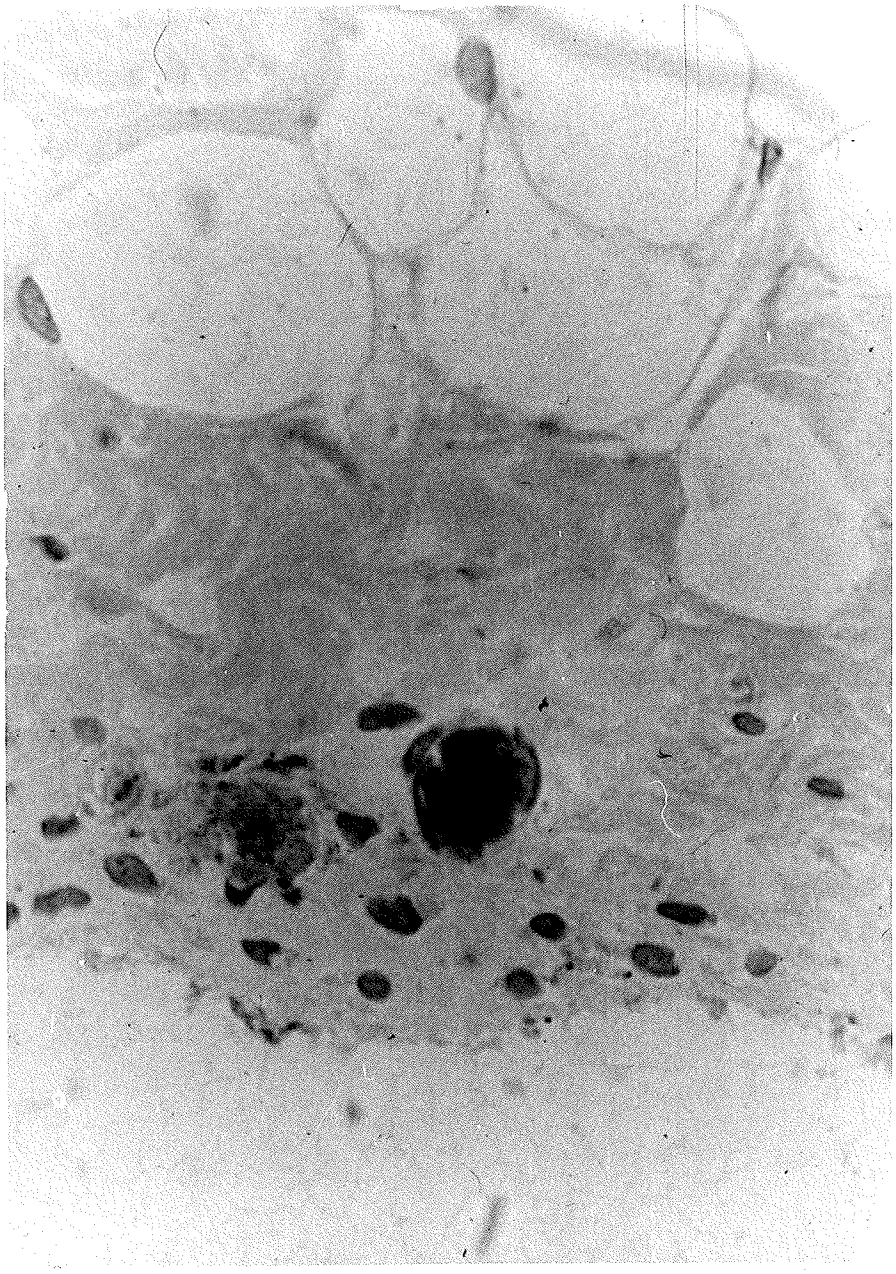


Figure 3: India Ink in temporalis periosteum (x100).

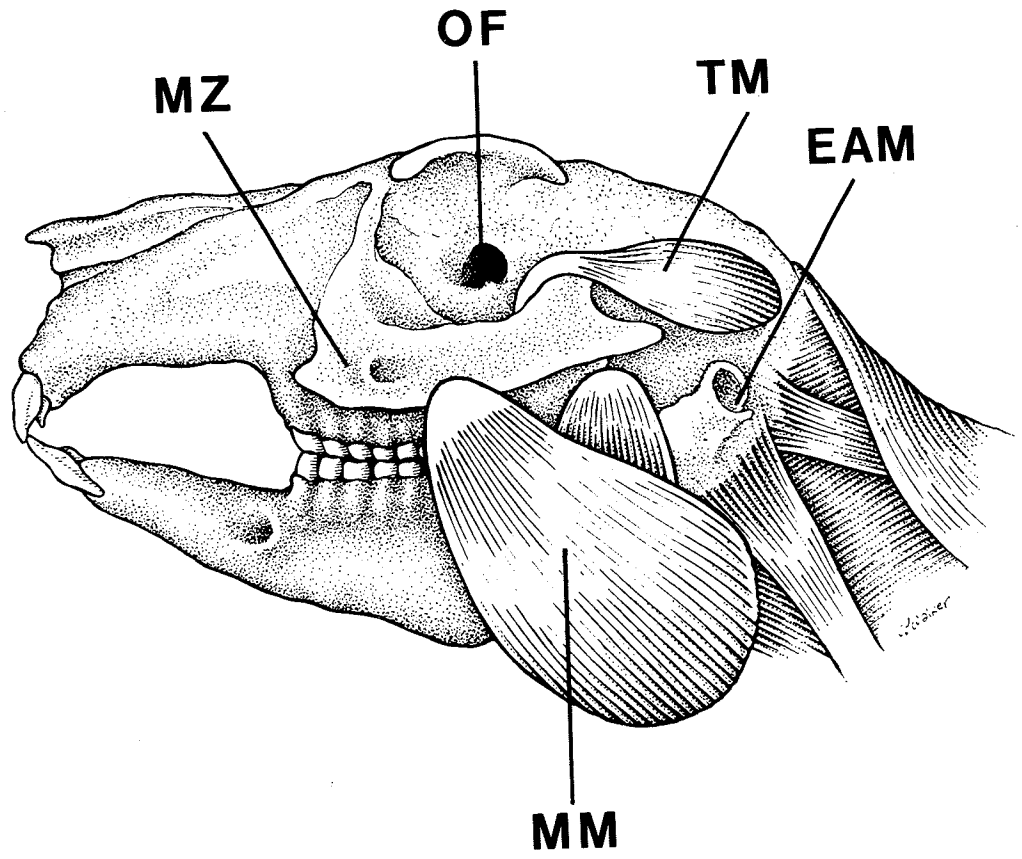


Figure 4: Rabbit skull with attached temporalis muscle (T) and masseter muscles (MM). Maxilla-zygomatic arch (MZ), optic foramen (OF), external auditory meatus (EAM).

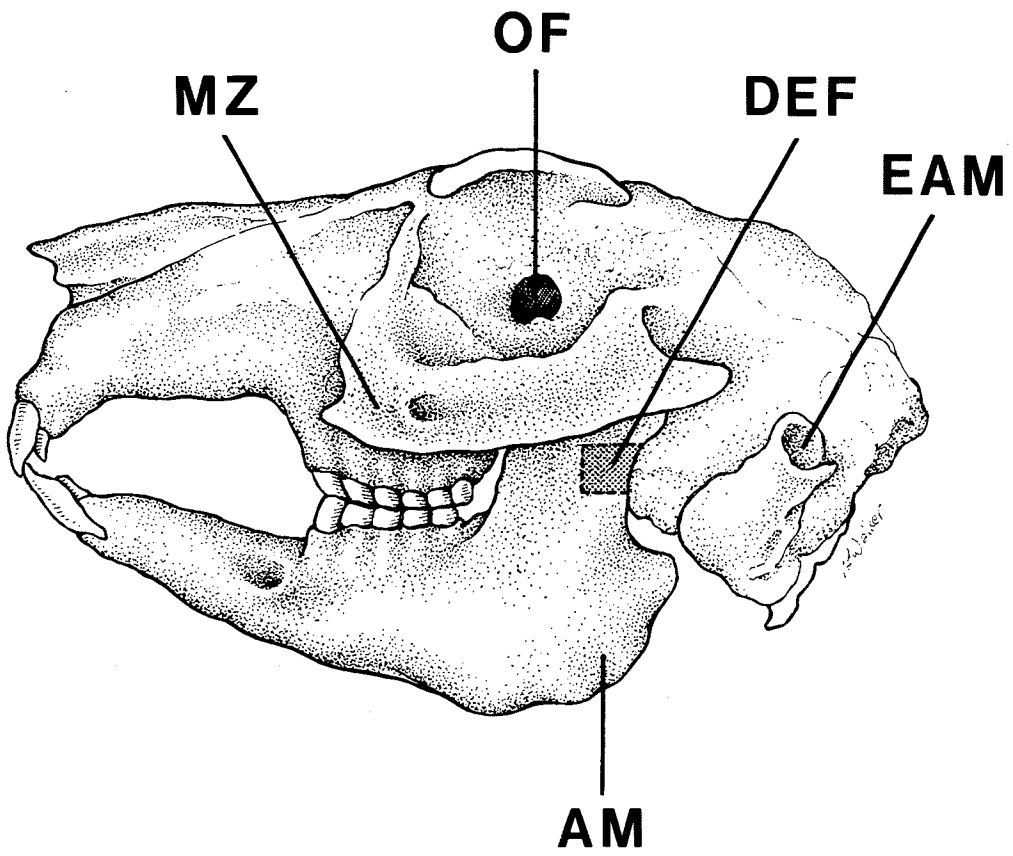


Figure 5: Rabbit skull showing location of mandibular defect (DEF). Angle of mandible (AM).



Figure 6: Defect with temporalis muscle (arrow) ready to be wrapped around defect.

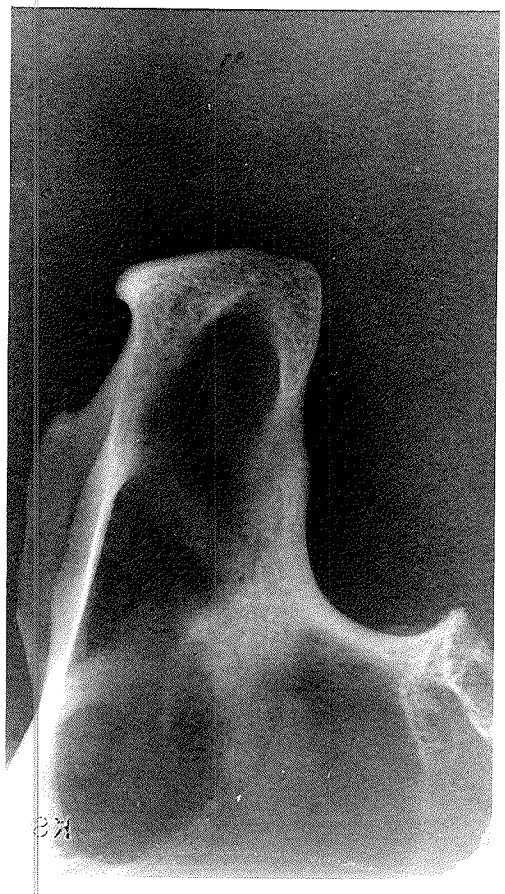


Figure 7: Radiograph of intact mandible

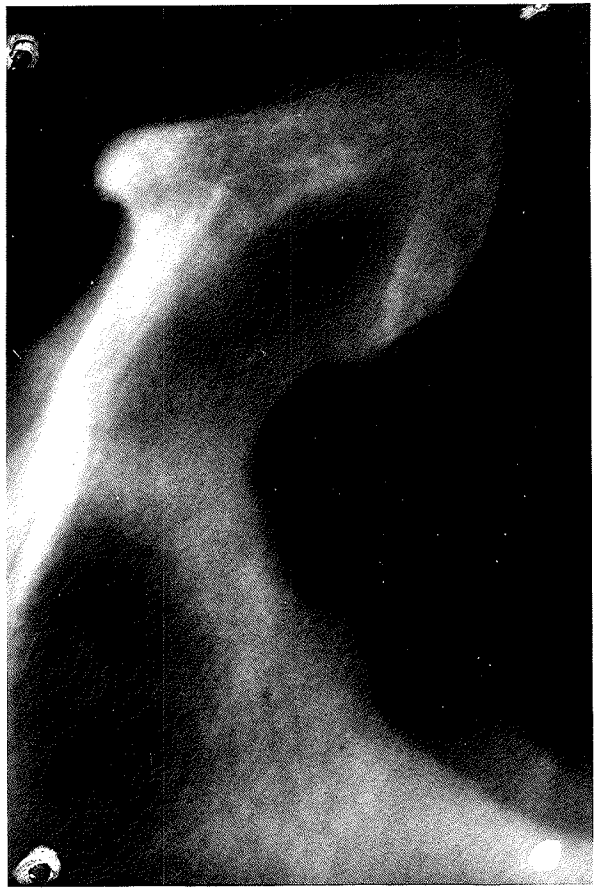


Figure 8: Control experiment, with residual defect five weeks after surgery



Figure 9: Posterior border of unoperated mandibles. Cortical bone (B), periosteum (P), connective tissue (CT) (x100)

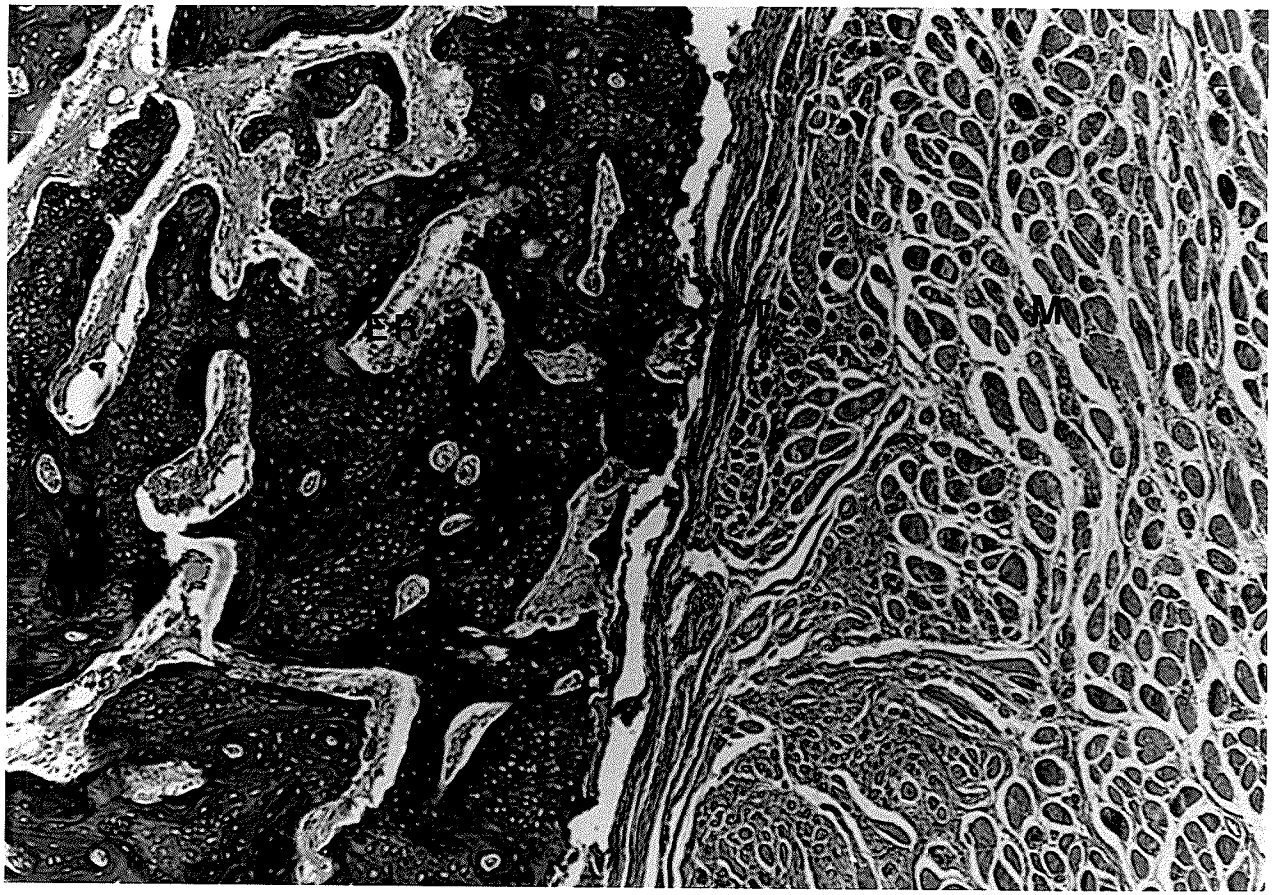


Figure 10: Control experiment. Endosteal remodelling (ER) surrounding connective tissue (CT) and muscle (M) (x100)

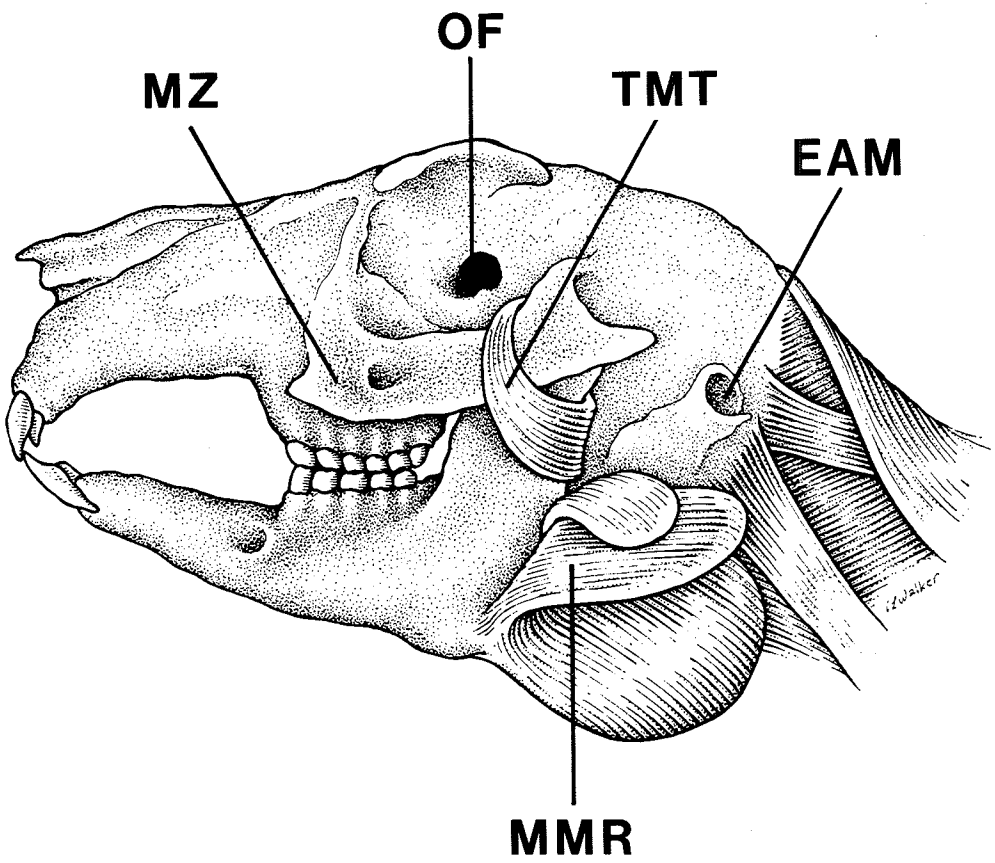


Figure 11: Temporalis muscle folded over defect. Temporalis muscle transfer (TMT), reflected masseter muscle (MMR)



Figure 12: Tibial cortex (T) with resting periosteum (P) and overlying connective tissue (CT)



Figure 13: Bone regeneration at posterior border



Figure 14: Bone regeneration on inner corners of defect

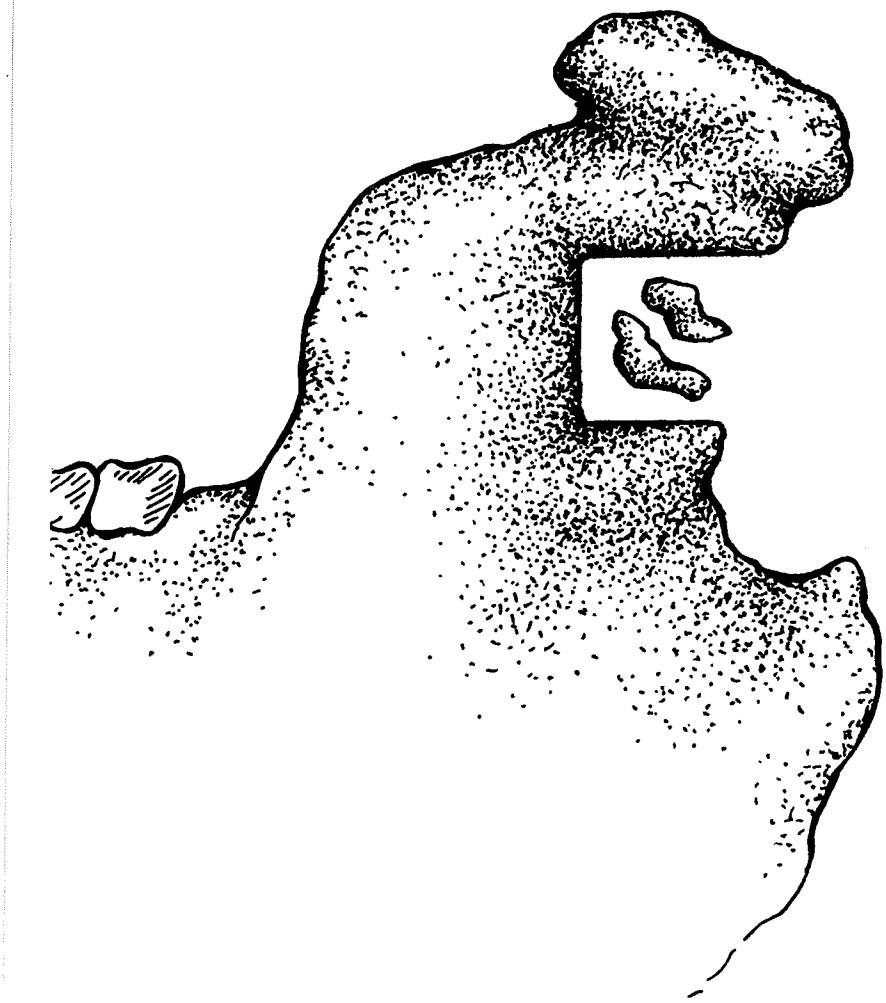


Figure 15: Bone regeneration within defect

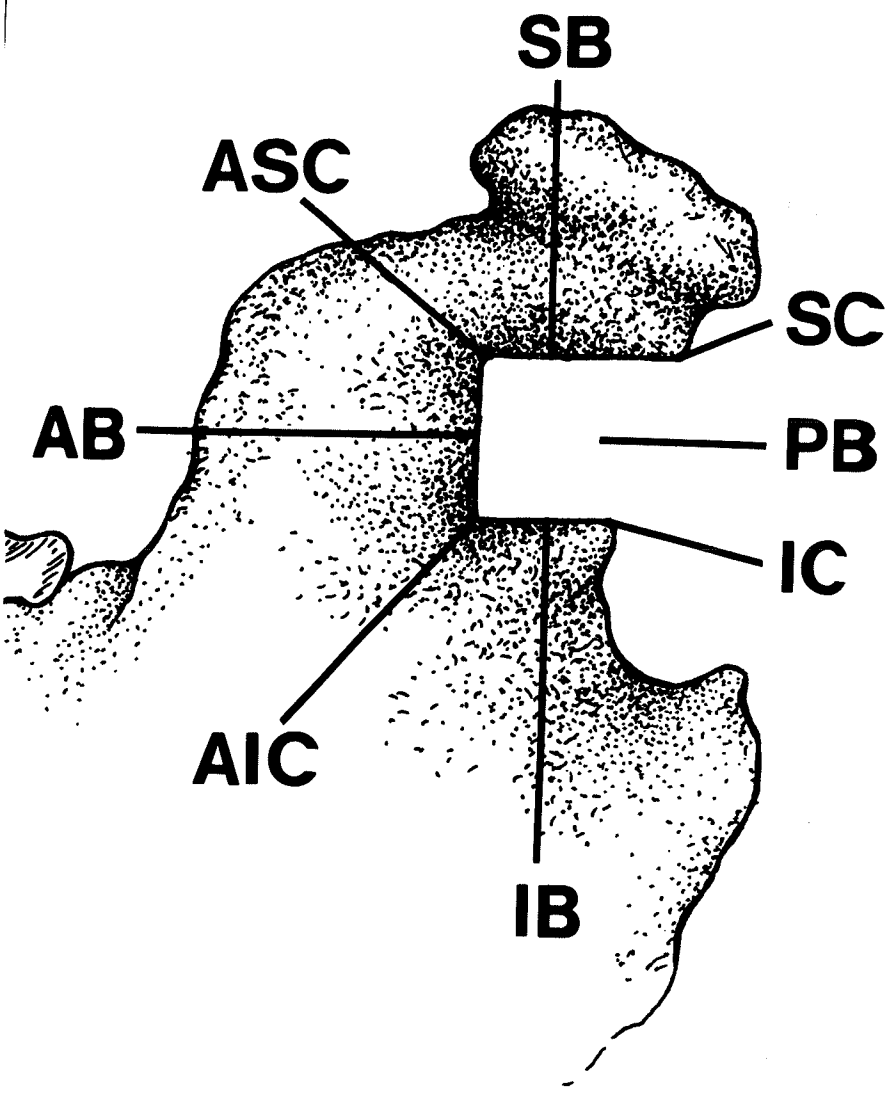


Figure 16: Description of defect angles and borders. Superior border (SB), posterior border (PB), inferior border (IB), anterior border (AB), superior corner (SC), inferior corner (IC), anterior inferior corner (AIC), anterior superior corner (ASC)



Figure 17: Bone regeneration after 12 weeks (2-8-R-Graft)

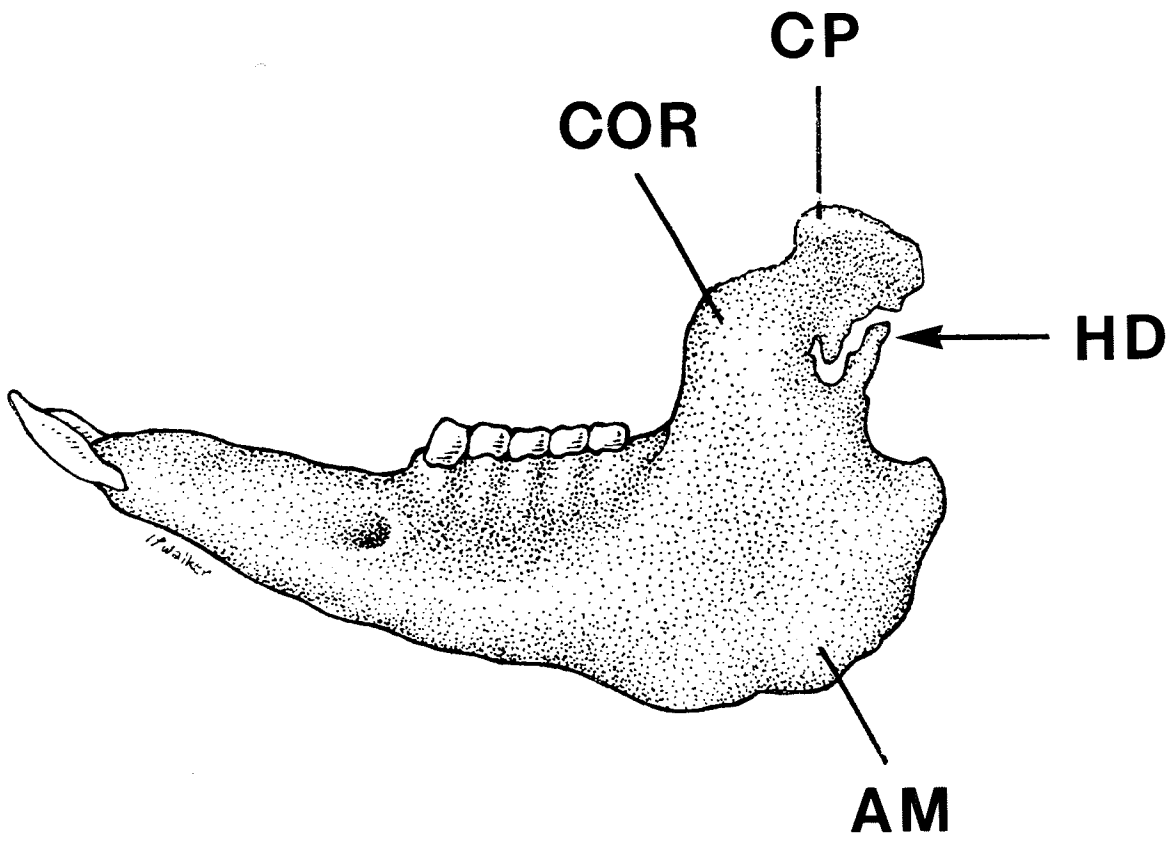


Figure 18: Diagram of Figure 17. Coronoid process (COR), condylar process (CP), healing defect (HD), angles of mandible (AM)



Figure 19: 2-2-Right Flap



Figure 20: 2-3-Right Graft



Figure 21: 2-3-Left Flap

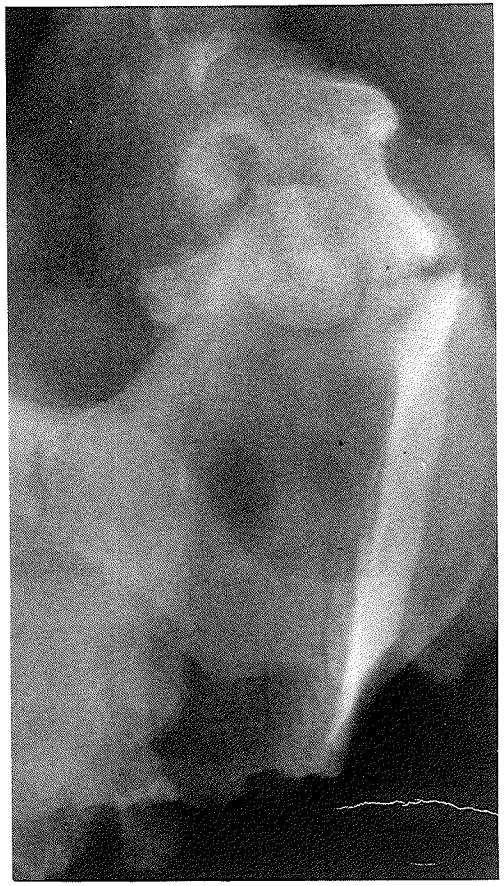


Figure 22: 2-4-Right Flap

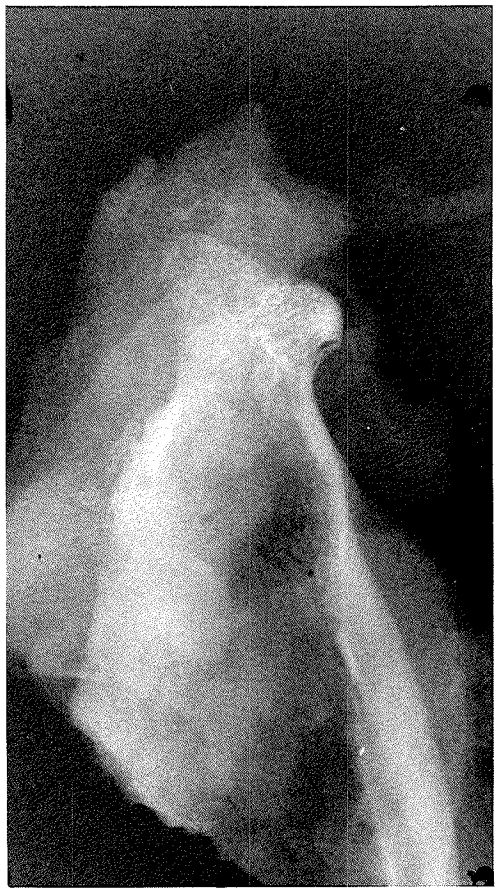


Figure 23: 2-5-Right Flap



Figure 24: 2-5-Left Graft



Figure 25: 2-6-Right Graft

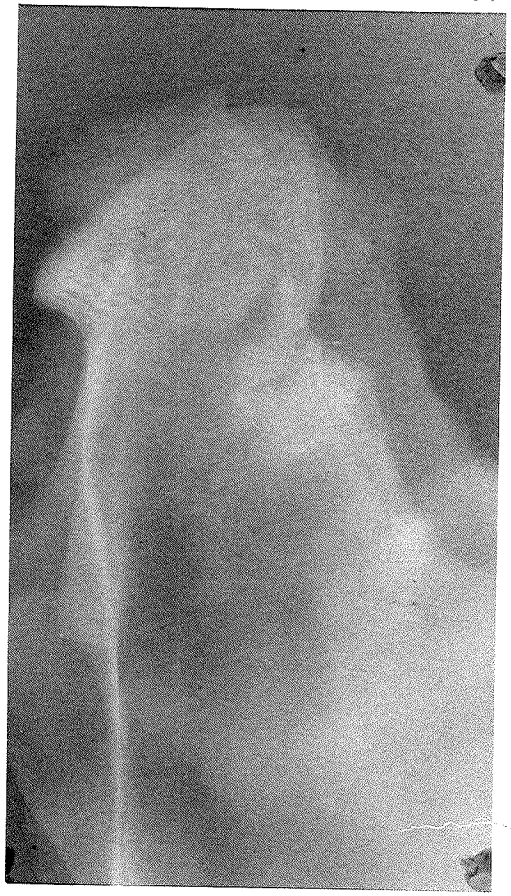


Figure 26: 2-6-Left Flap



Figure 27: 2-7-Right Flap



Figure 28: 2-7-Left Graft



Figure 29: 2-2-Right Flap (x40)

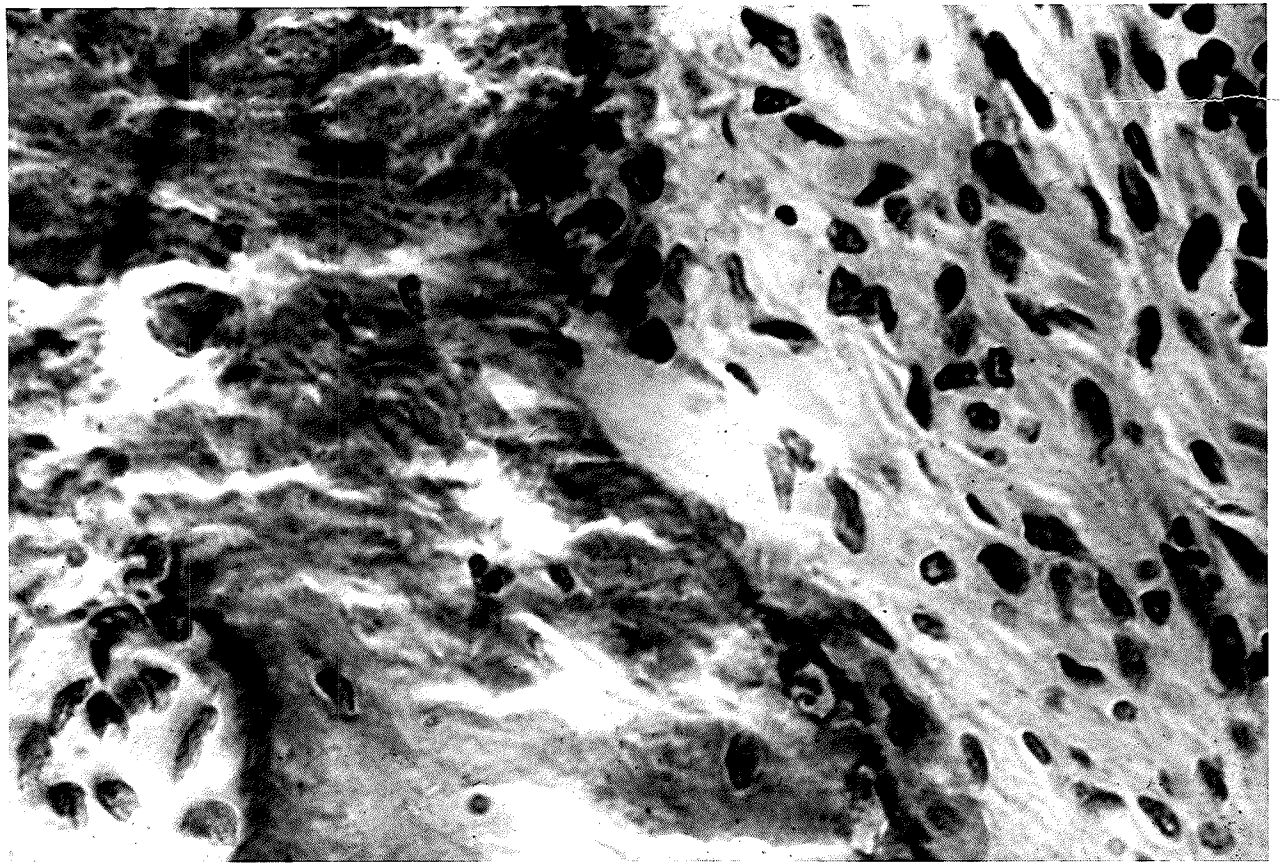


Figure 30: 2-2-Right Flap (x400)

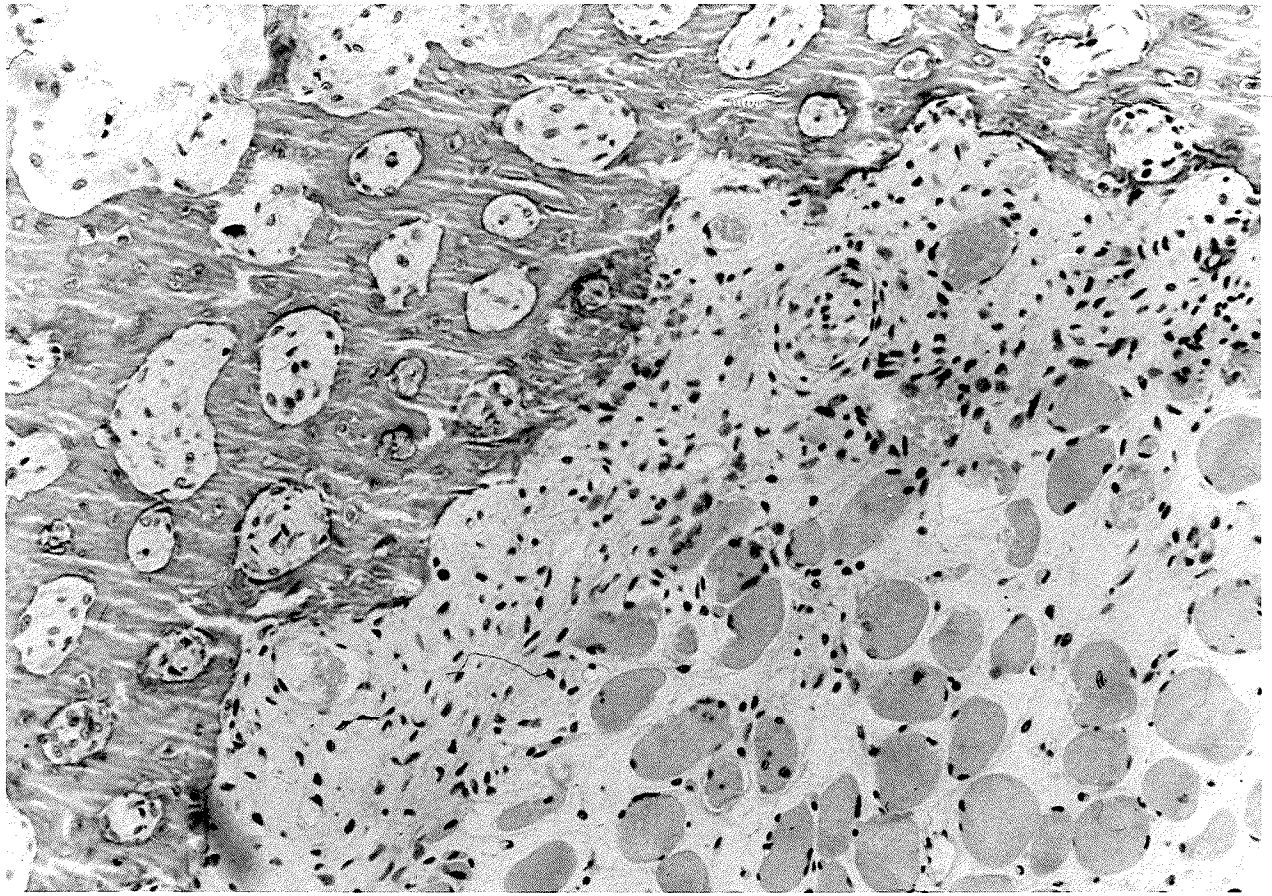


Figure 31: 2-2-Left Graft (x100)

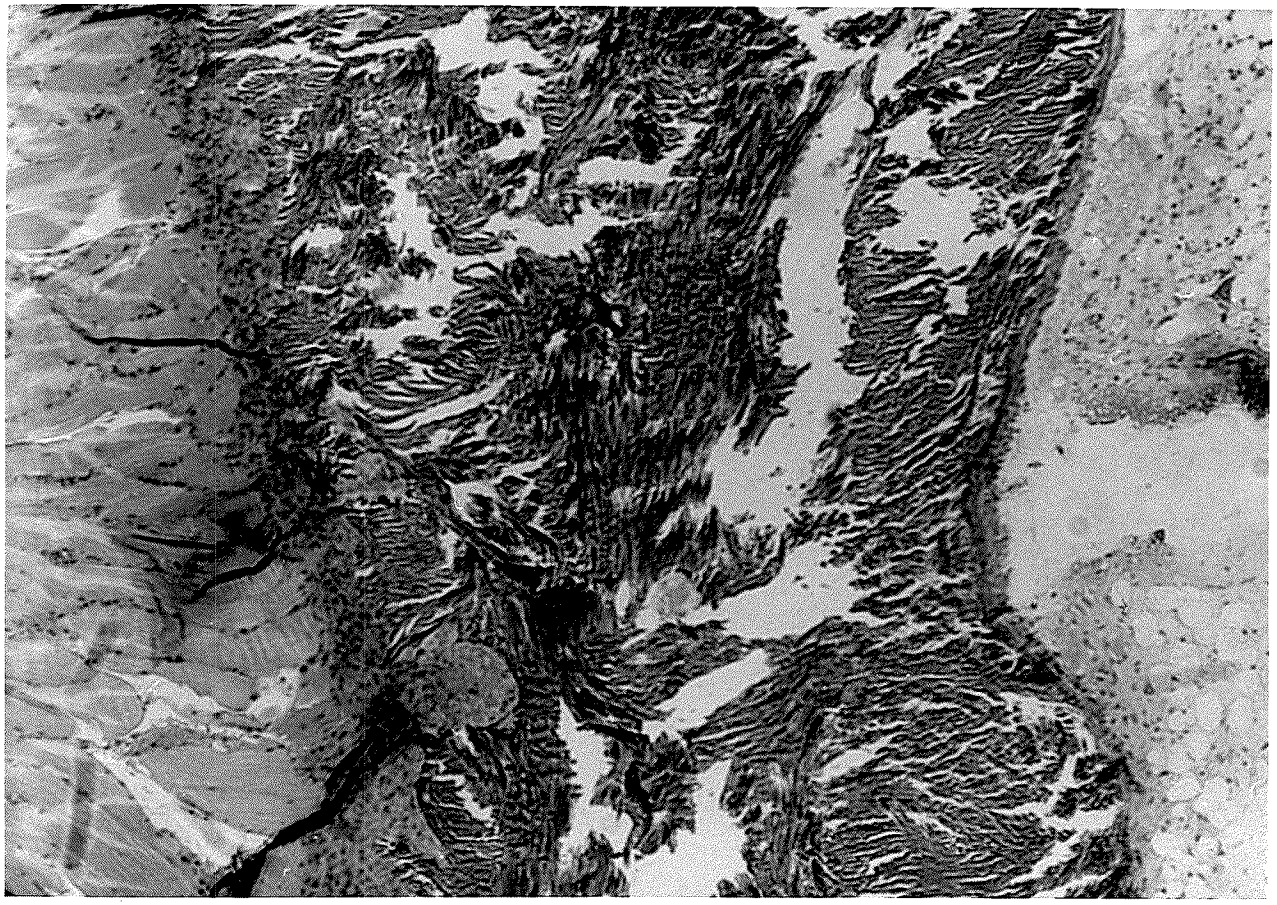


Figure 32: 2-3-Right Graft (x40)

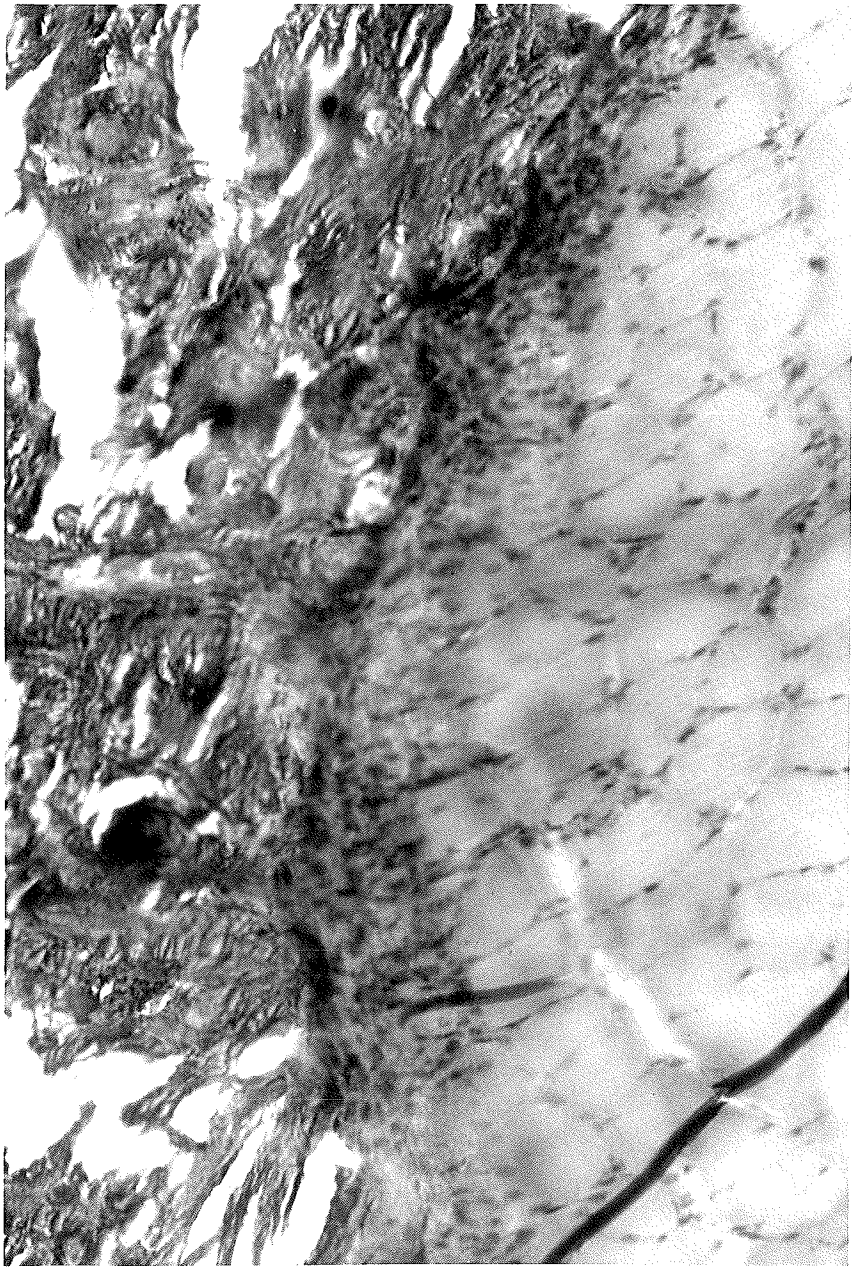


Figure 33: 2-3-Left Flap (x 100)

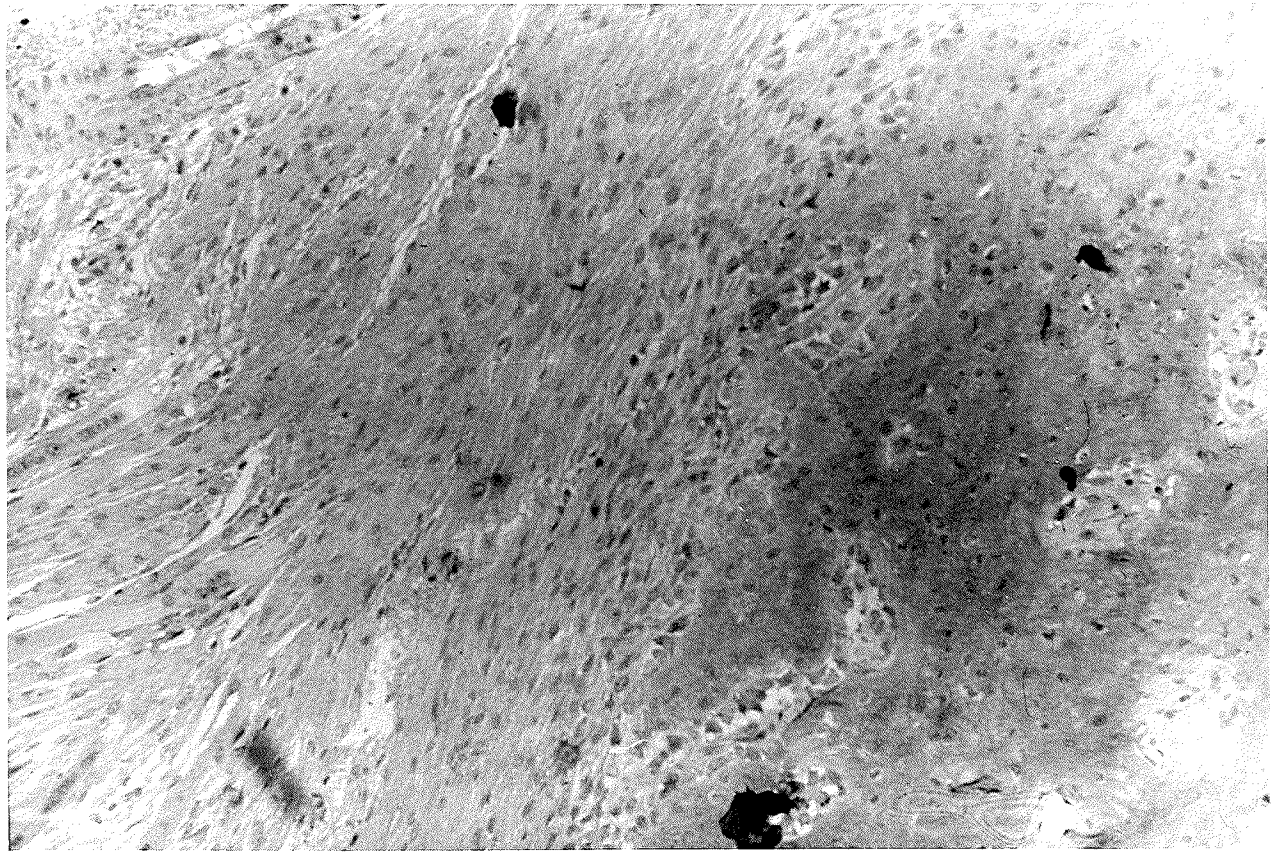


Figure 34: 2-4-Right Flap (x100)

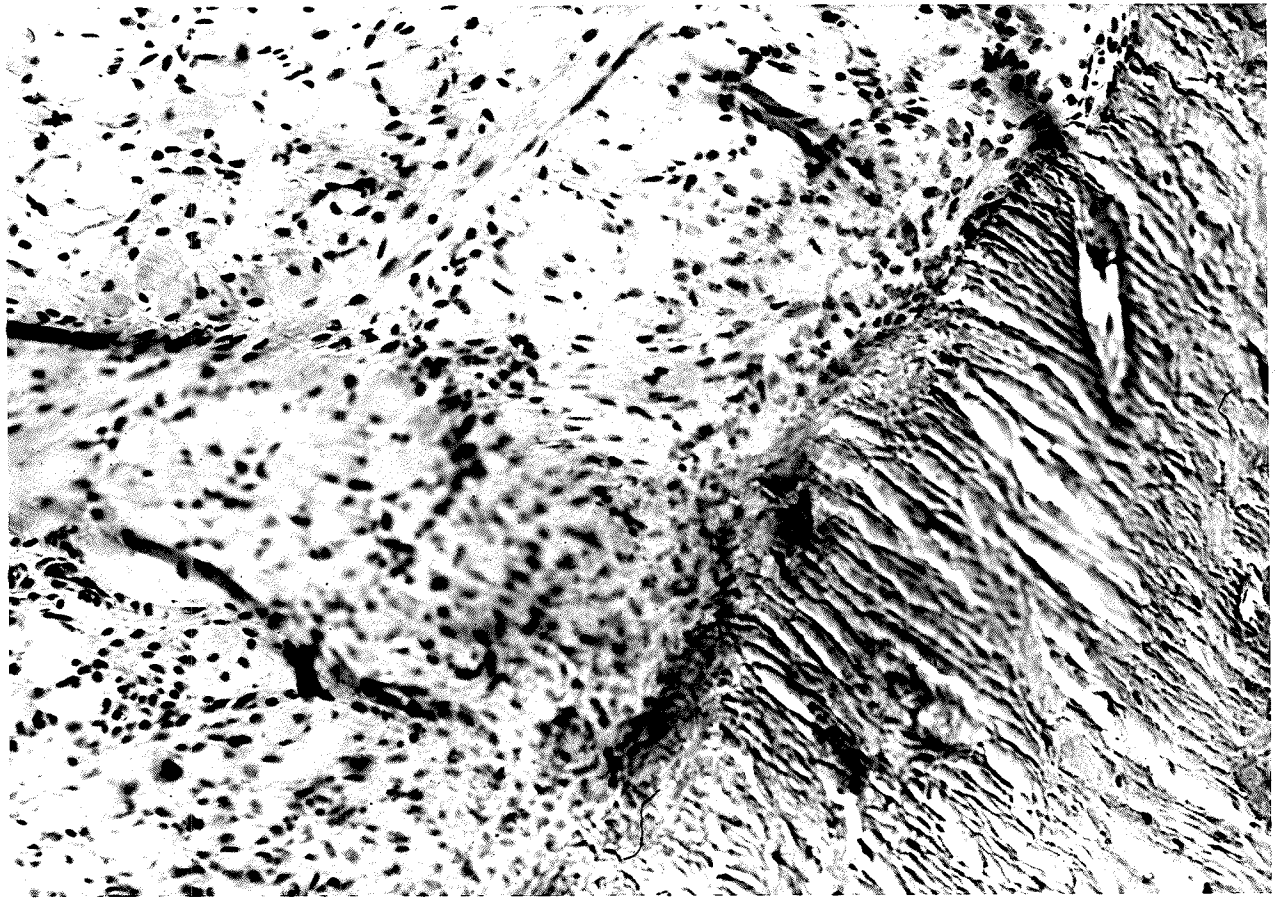


Figure 35: 2-5-Right Flap (x100)

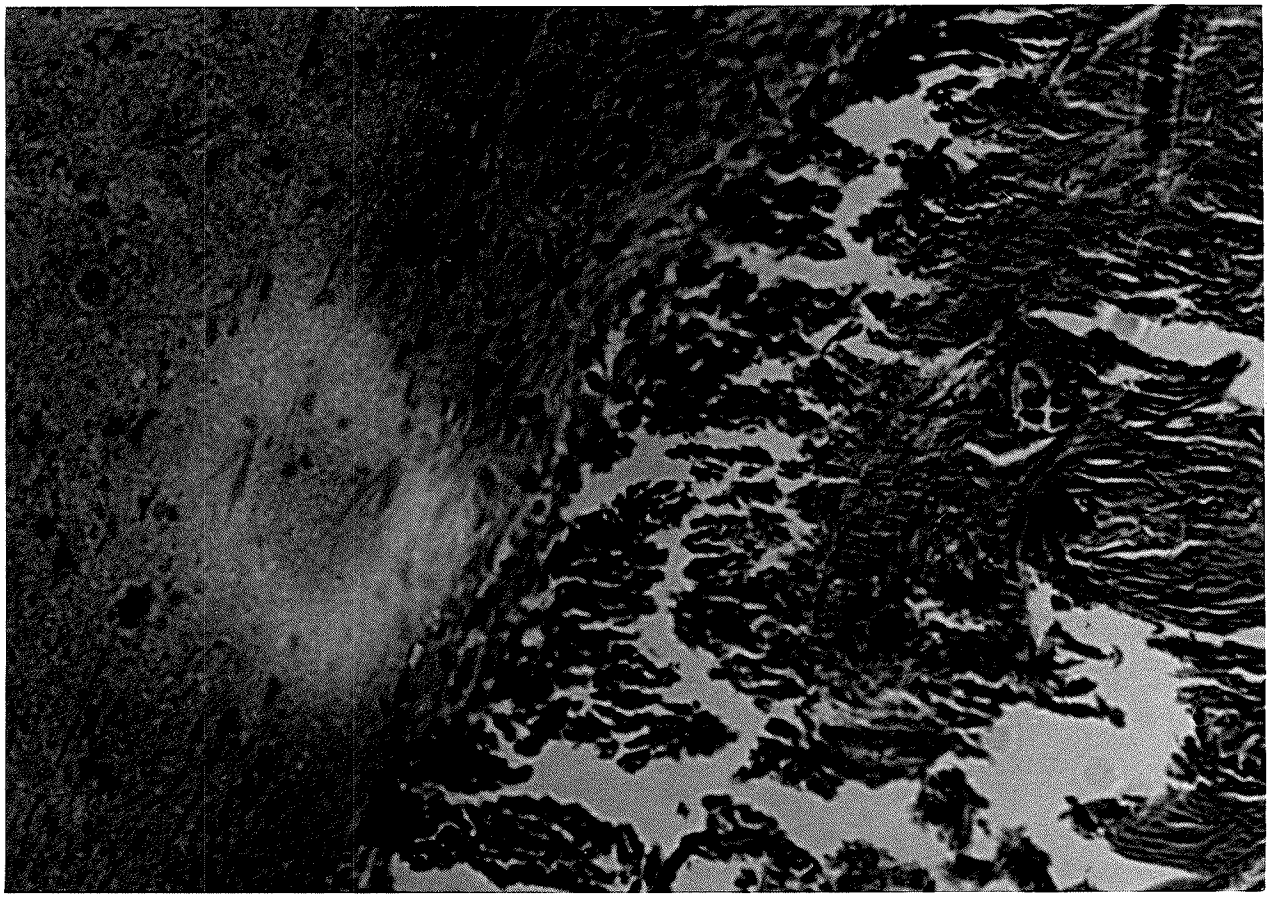


Figure 36: 2-6-Right Graft

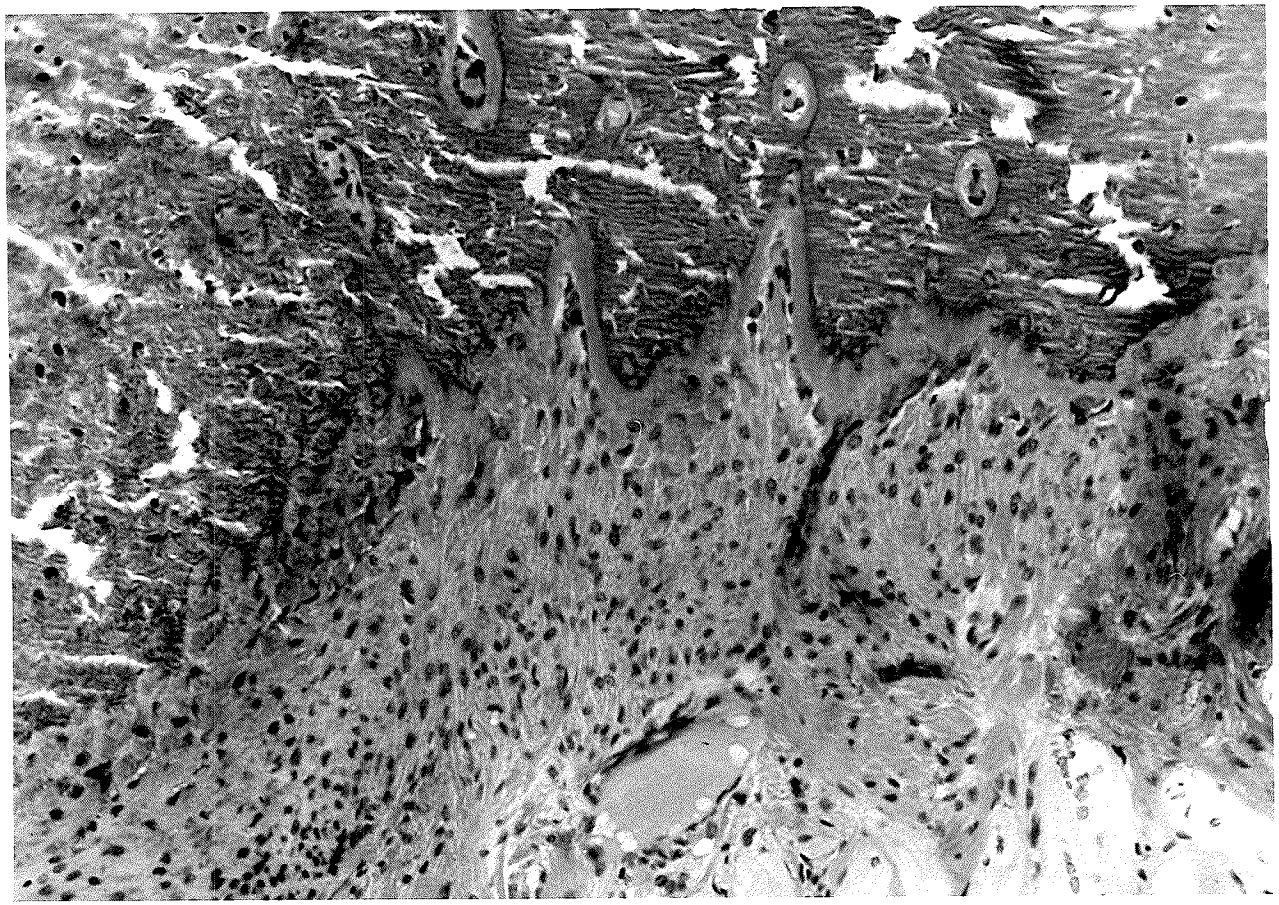


Figure 37: 2-6-Left Flap (x100)

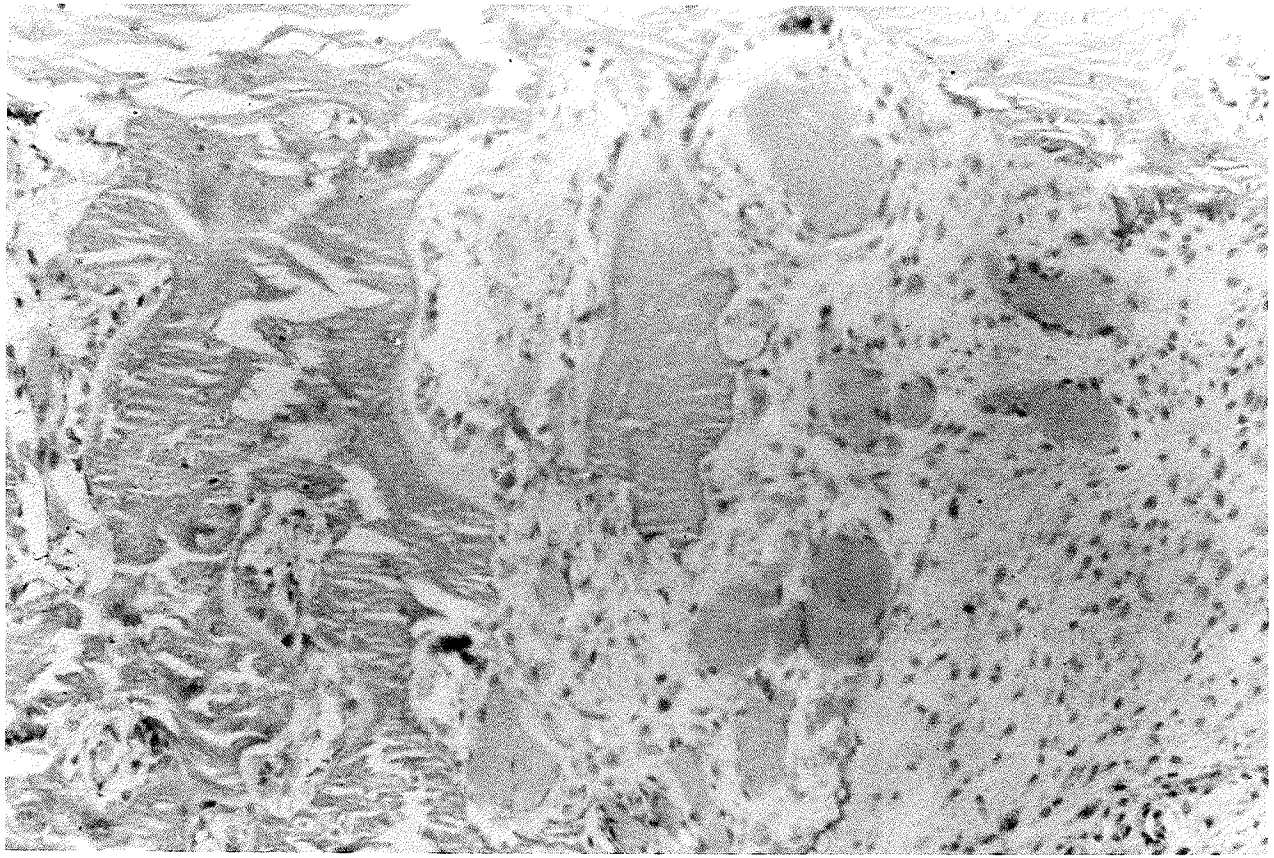


Figure 38: 2-6-Left Flap (x100)

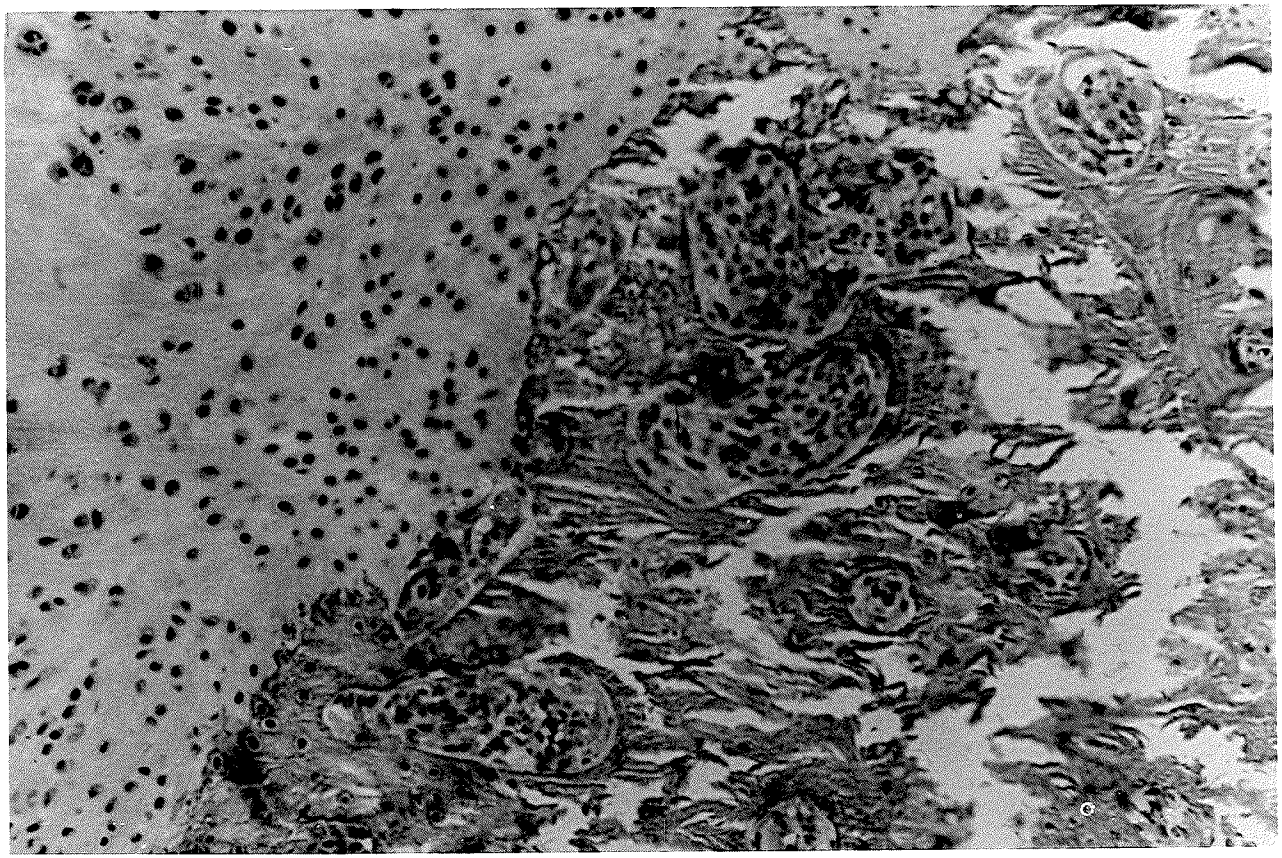


Figure 39: 2-6-Left Flap (x100)

Cartilage and intramembranous bone formation

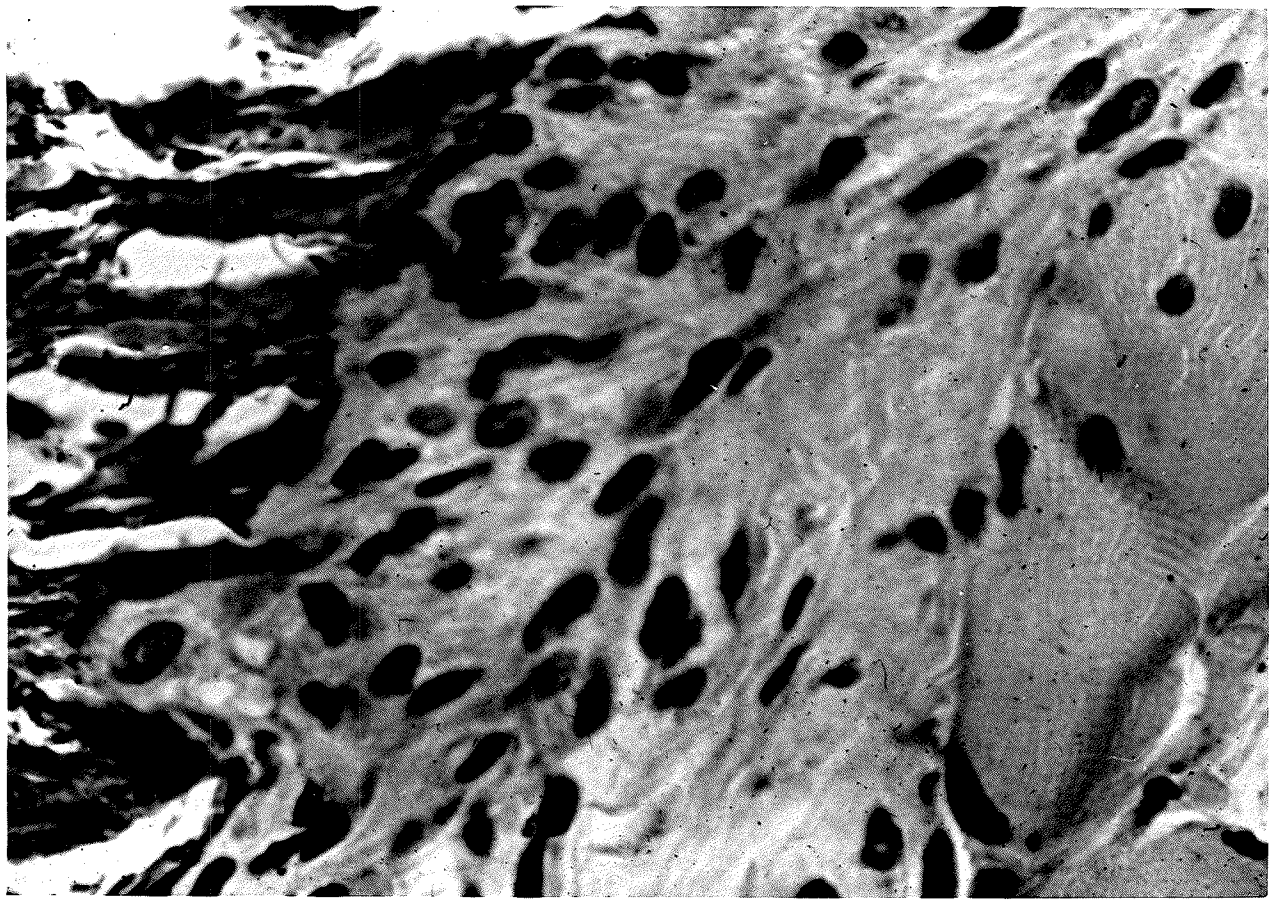


Figure 40: 2-7-Right Flap (x400)

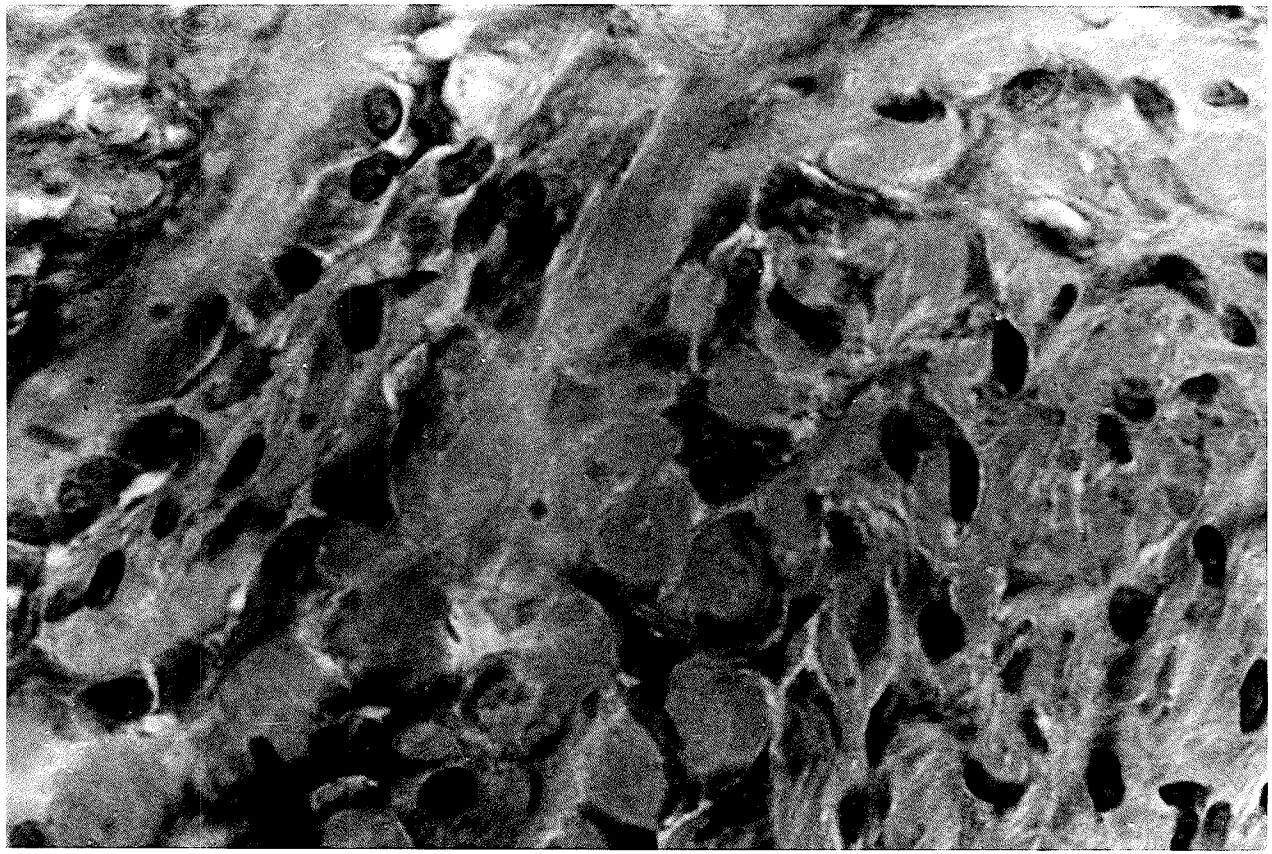


Figure 41: 2-7-Right Flap (x400)

Continuing intramembranous bone formation

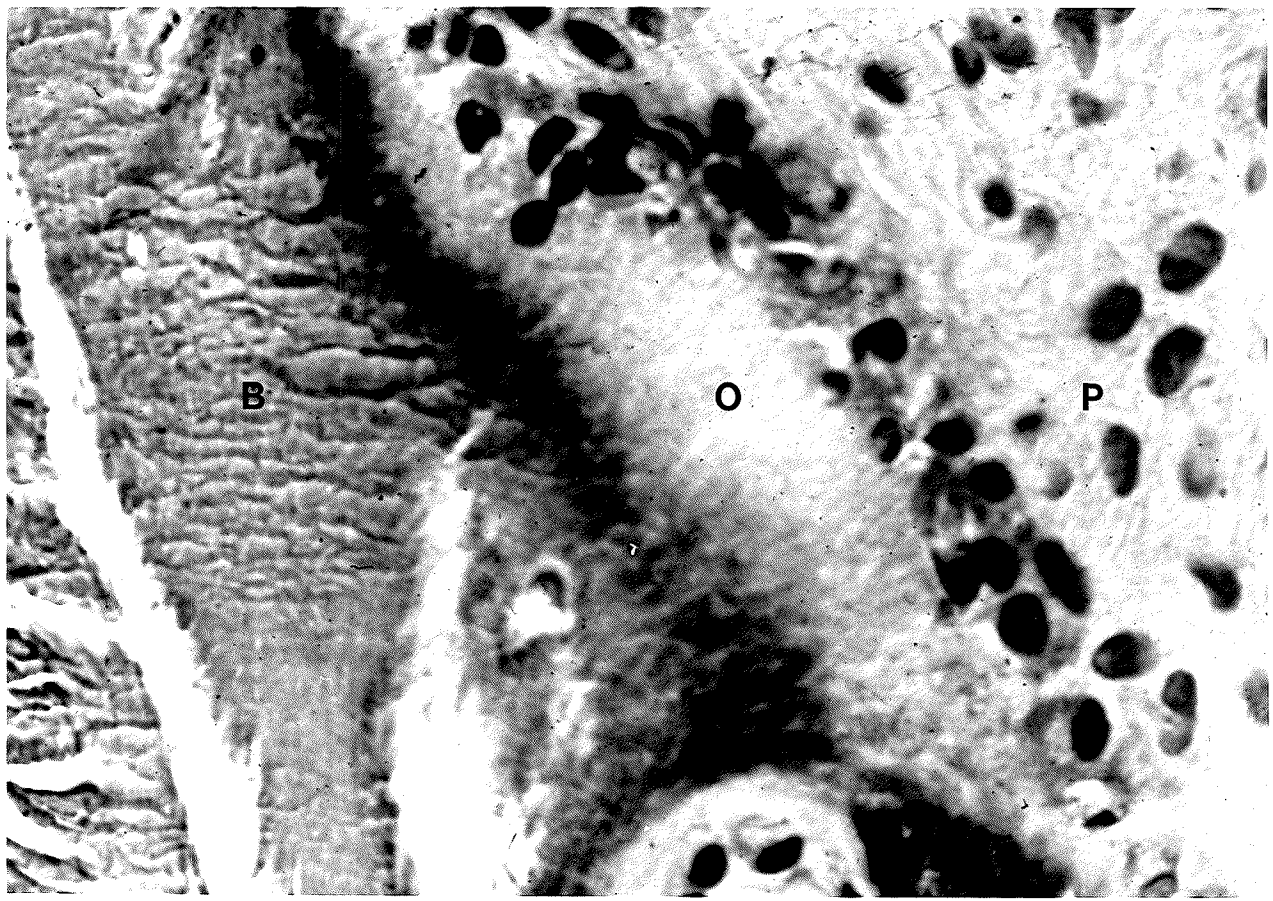


Figure 42: 2-7-Left Graft (x400)

Cortical bone (B), osteoid (O), periosteum (P)

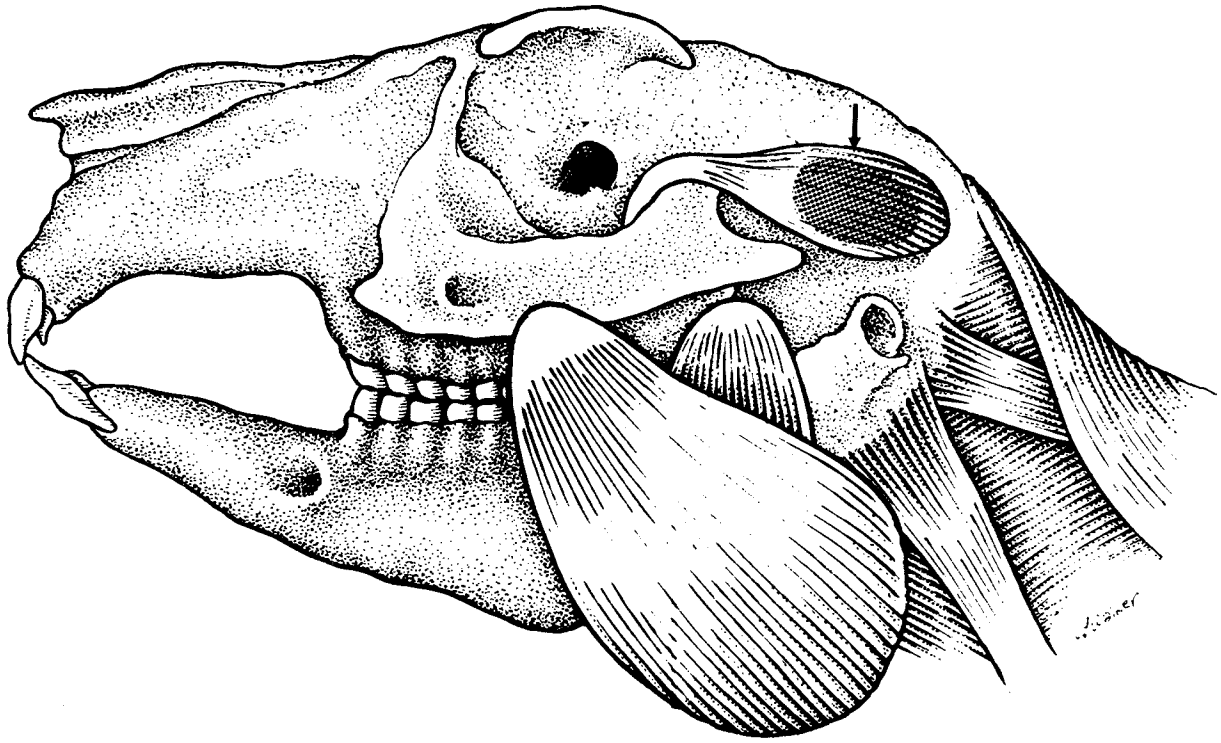


Figure 43: Tibial periosteum grafted on to temporalis muscle belly

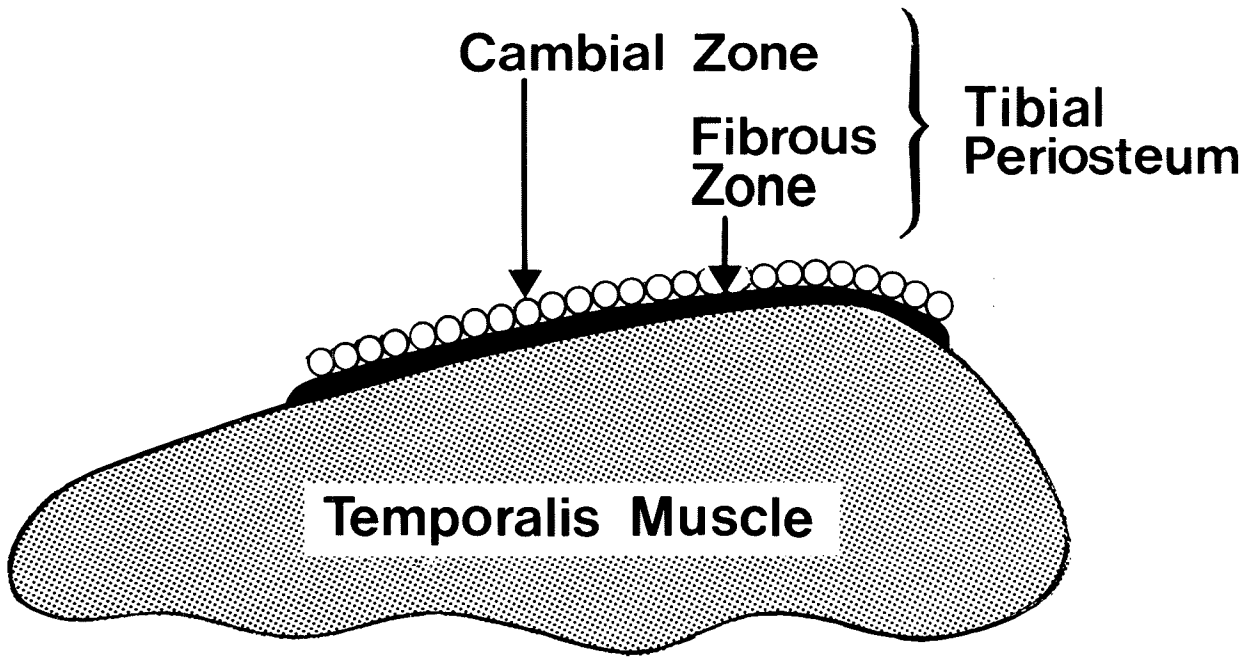


Figure 44: Tibial periosteum on temporalis muscle belly

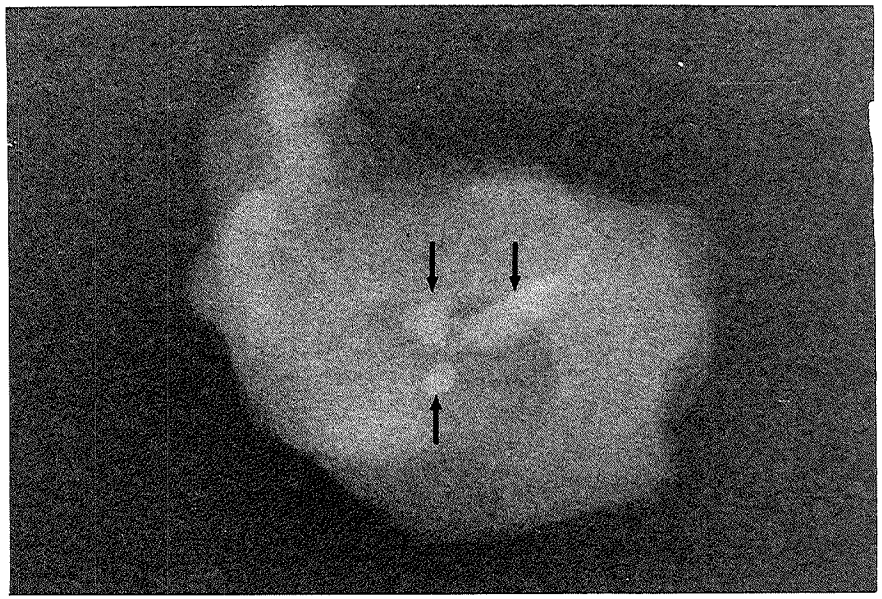


Figure 45: Radiograph of tibial periosteum grafted to temporalis muscle and delayed 2 weeks. Increased radiopacities within graft, suggestive of osteoneogenesis

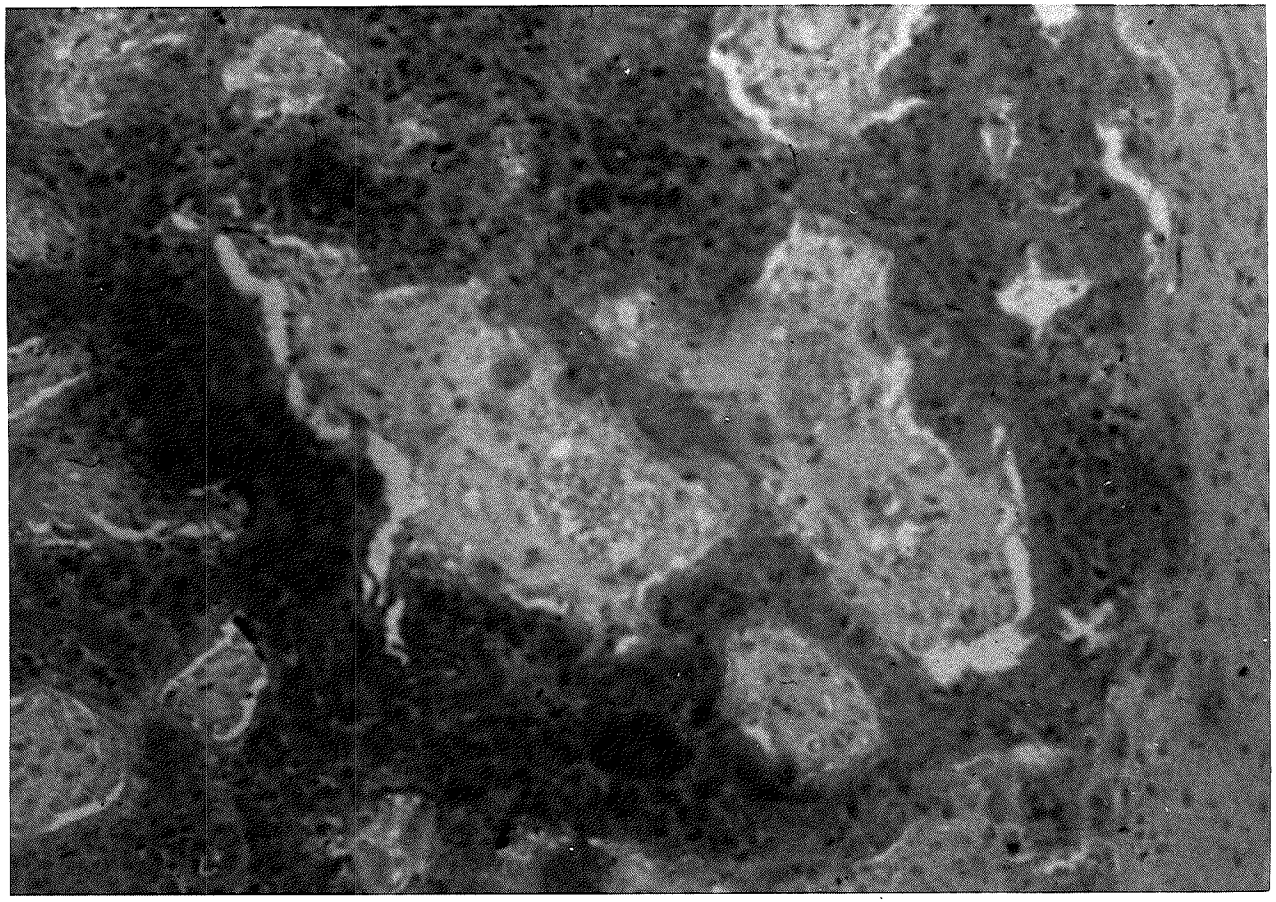


Figure 46: Intramembranous osteoneogenesis within tibial periosteum grafted to temporalis muscle 2 weeks previously (x400)

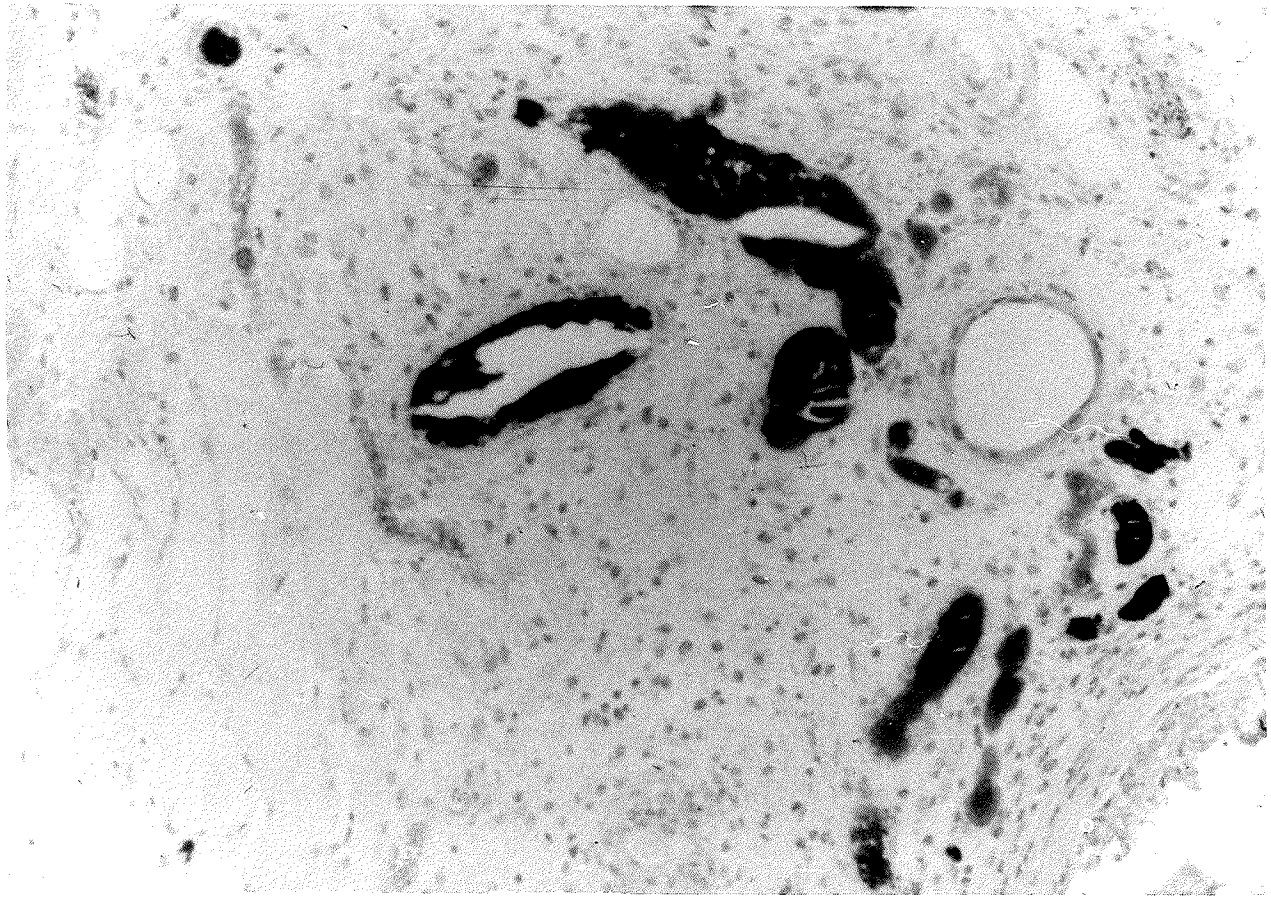


Figure 47: India Ink within tibial periosteum grafted to temporalis muscle 2 weeks previously (x100)

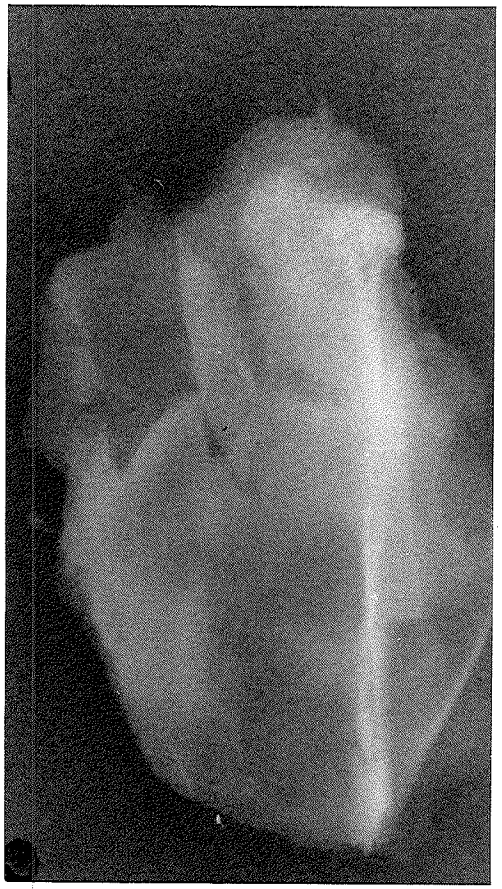


Figure 48: 4-1-Right Flap

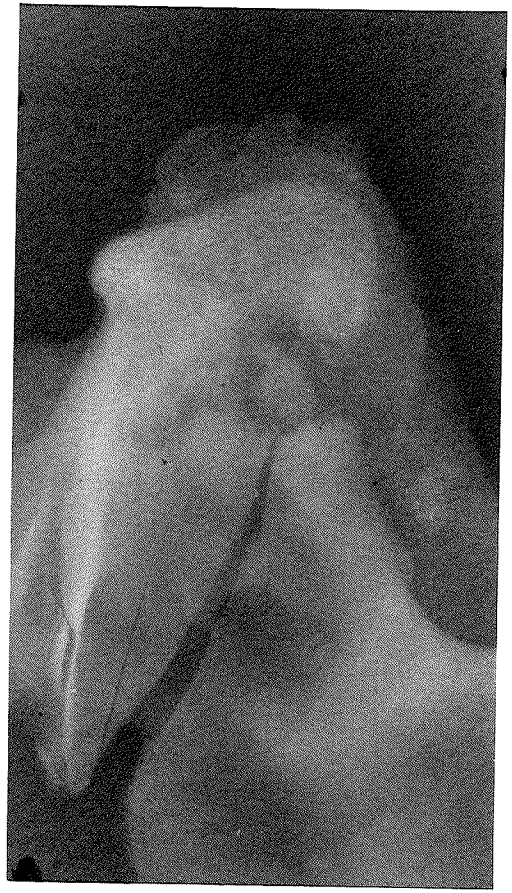


Figure 49: 4-1-Left Graft



Figure 50: 4-2-Right Flap

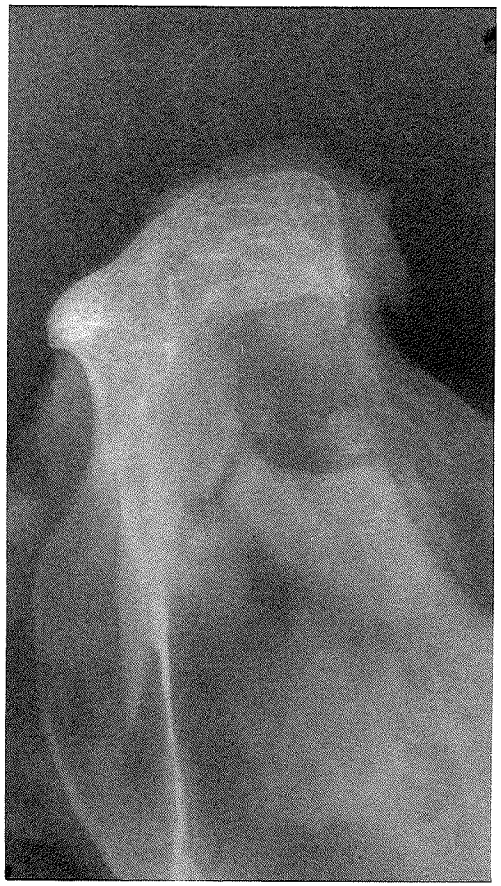


Figure 51: 4-2-Left Graft

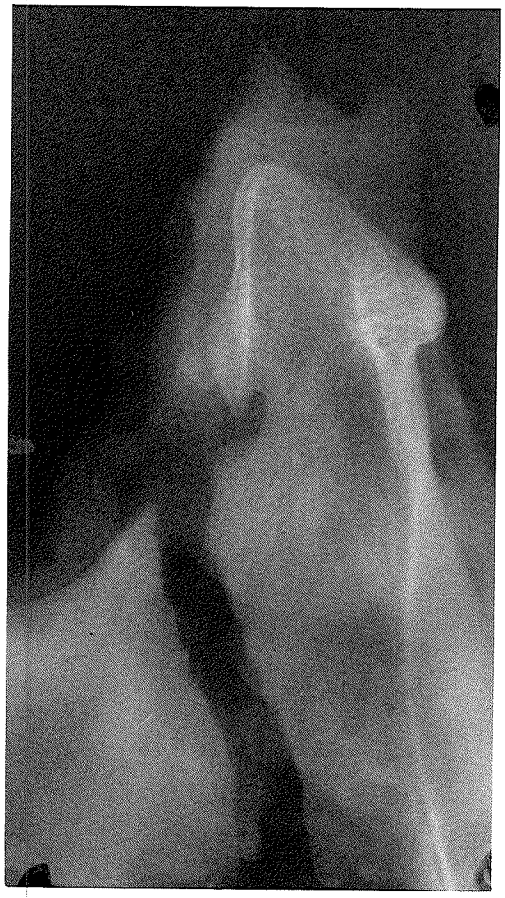


Figure 52: 4-3-Right Graft

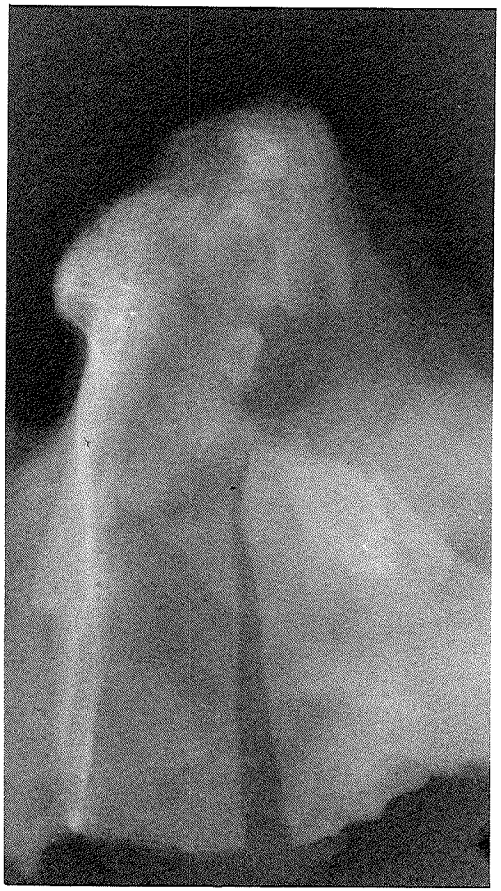


Figure 53: 4-3-Left Flap



Figure 54: 4-4-Right Flap

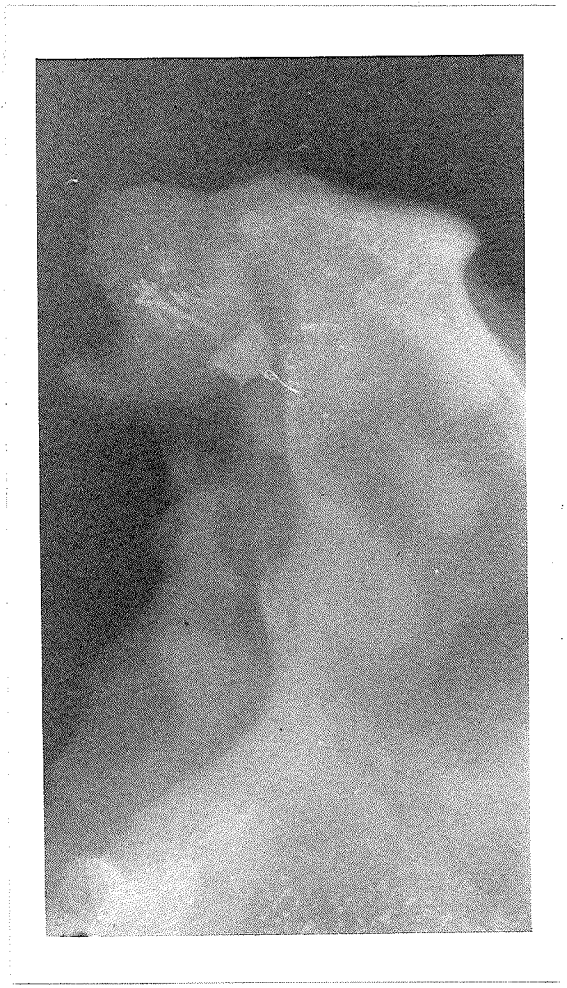


Figure 55: 4-5-Right Flap



Figure 56: 4-5-Left Graft

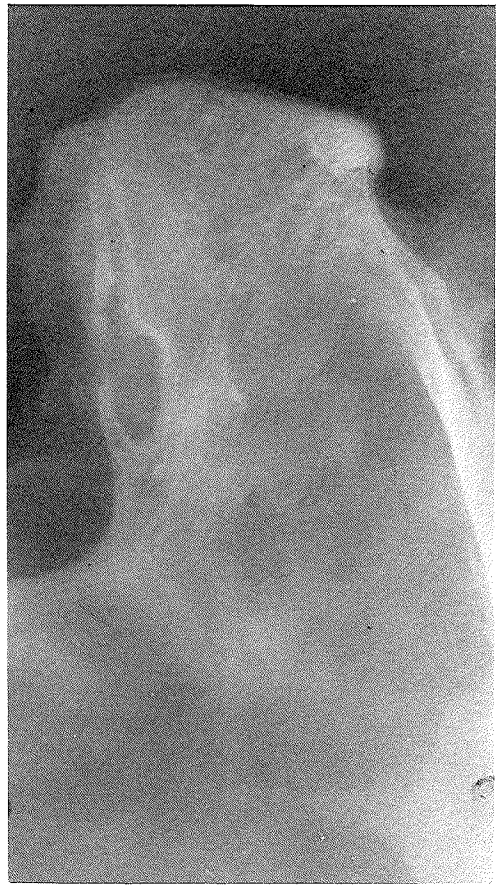


Figure 57: 4-6-Right Flap

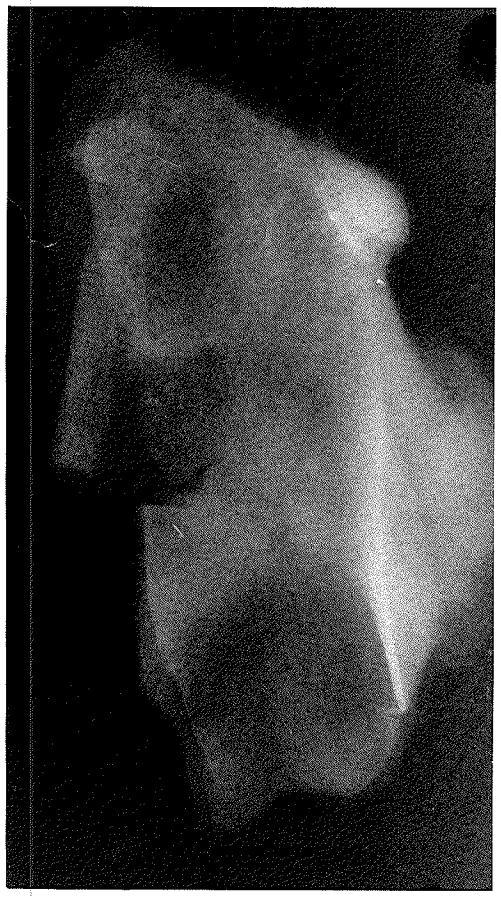


Figure 58: 4-7-Right Flap



Figure 59: 4-7-Left Graft



Figure 60: 4-1-Right Flap (x40)

Cartilage (C), intramembranous bone (B)

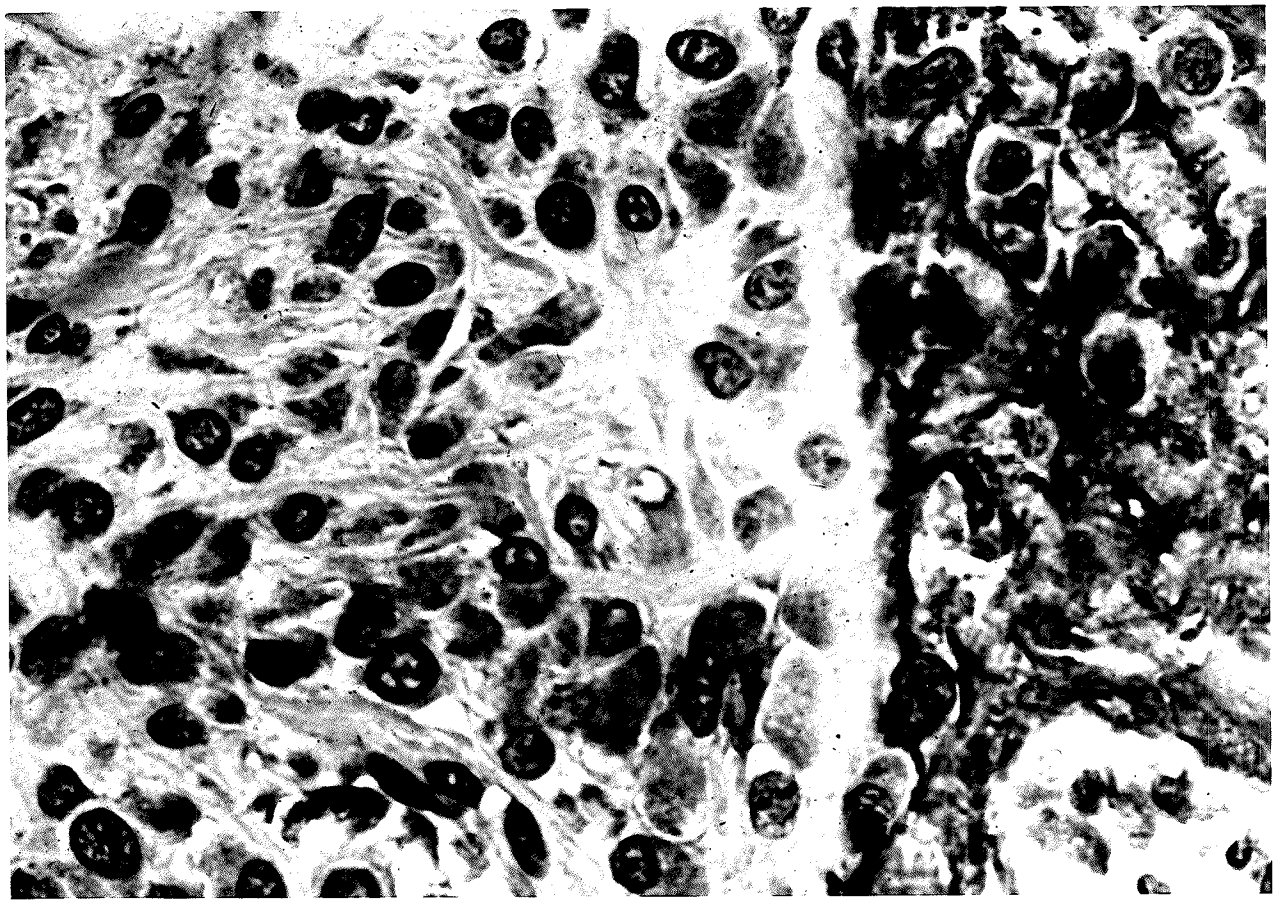


Figure 61: 4-1-Right Flap (x400)

Detail of subperiosteal osteoneogenesis

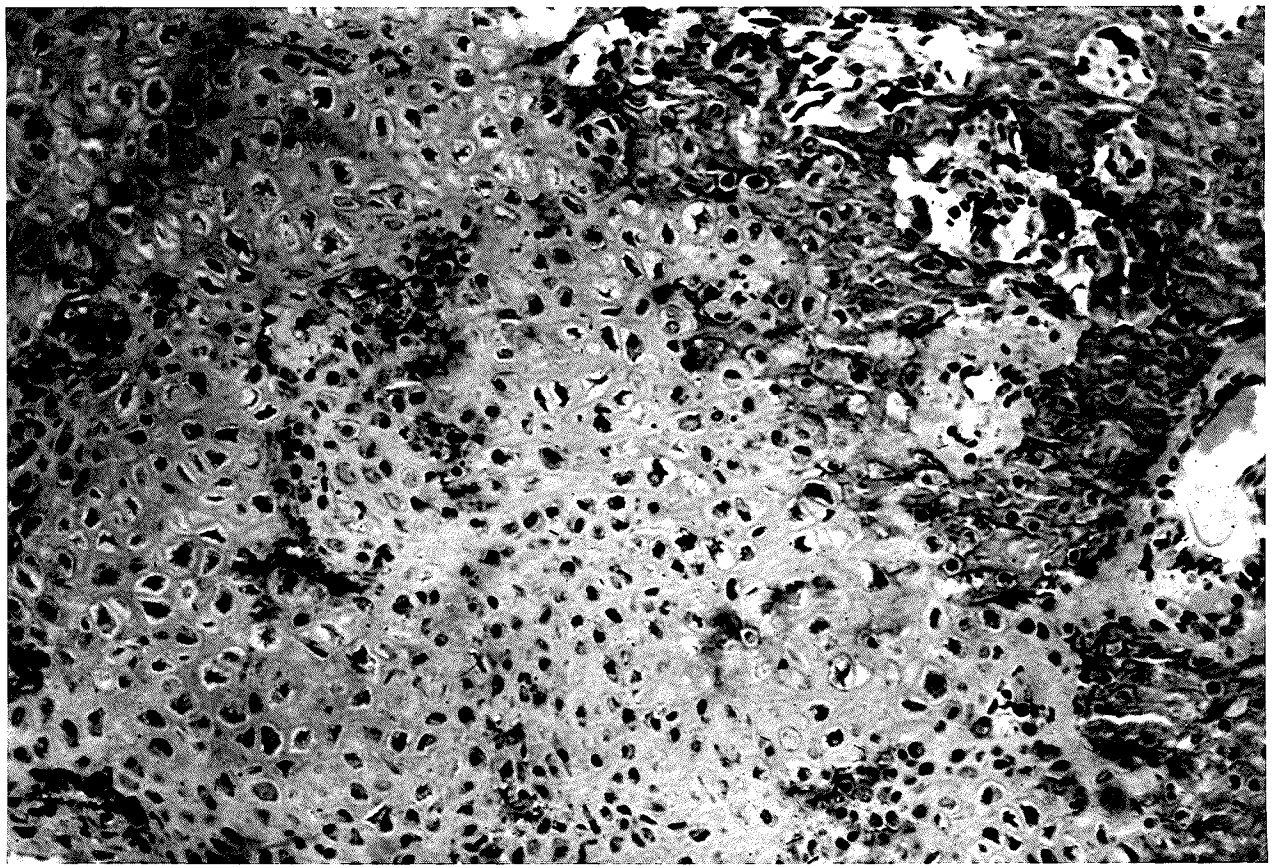


Figure 62: 4-1-Left Graft (x100)

Cartilage and intramembranous bone formation

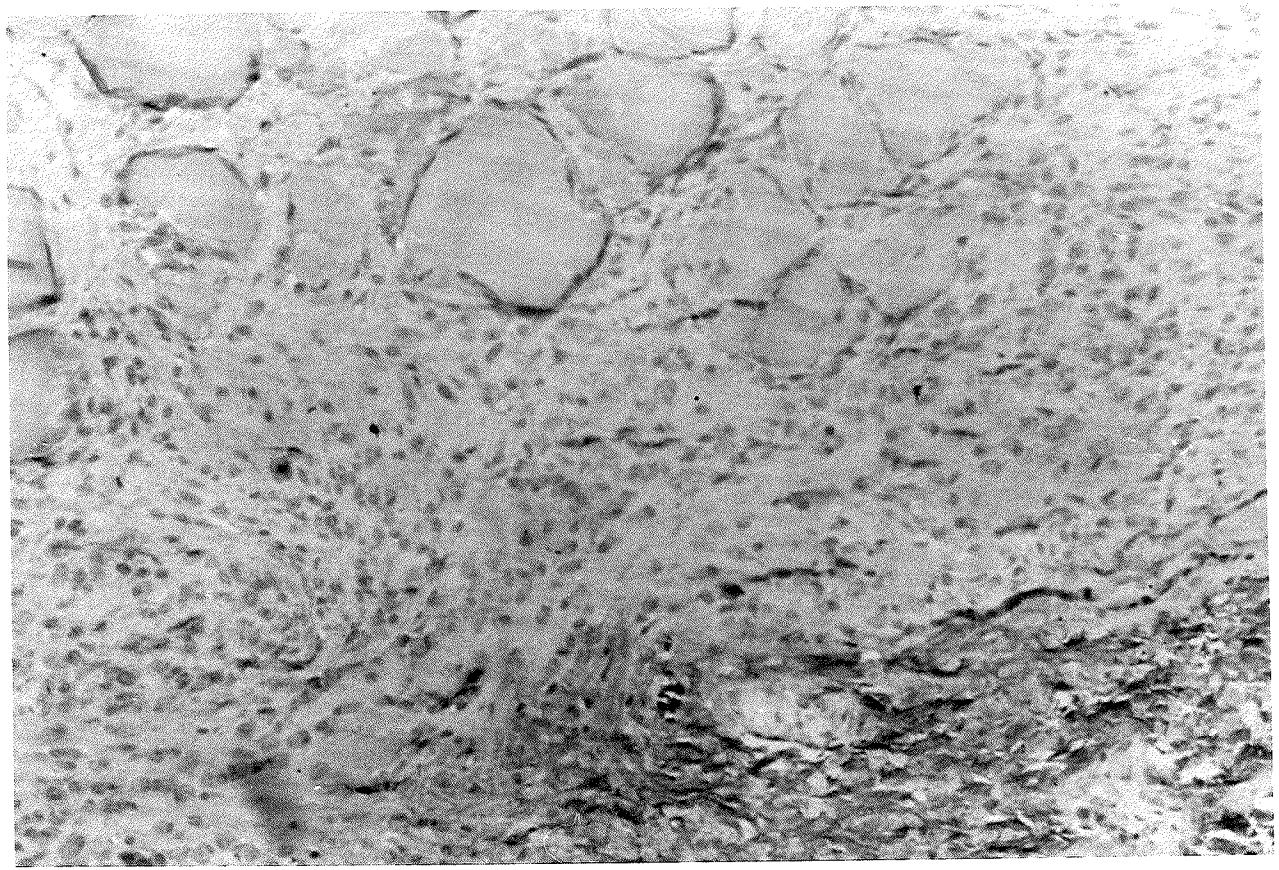


Figure 63: 4-2-Right Flap (x100)

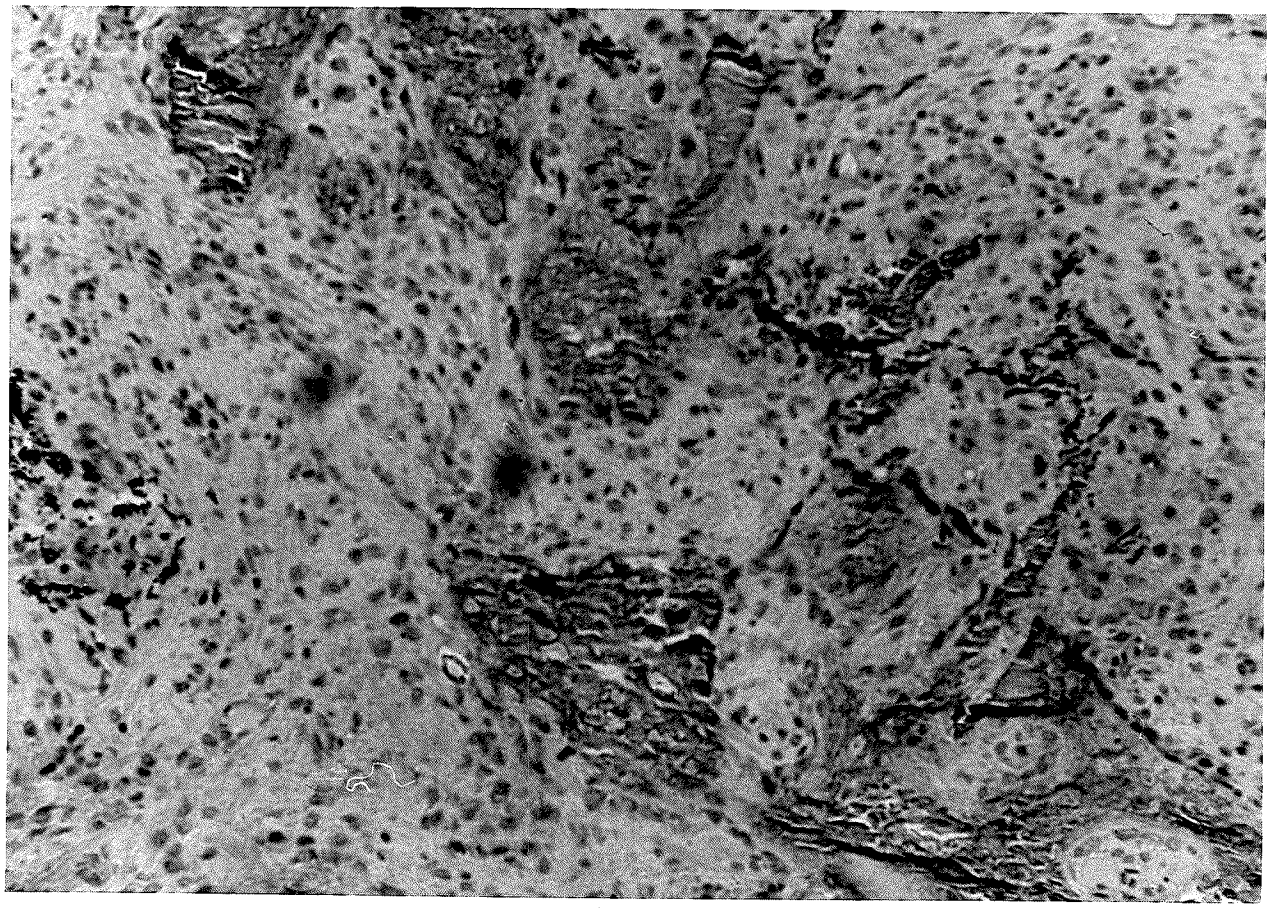


Figure 64: 4-2-Left Graft (x100)

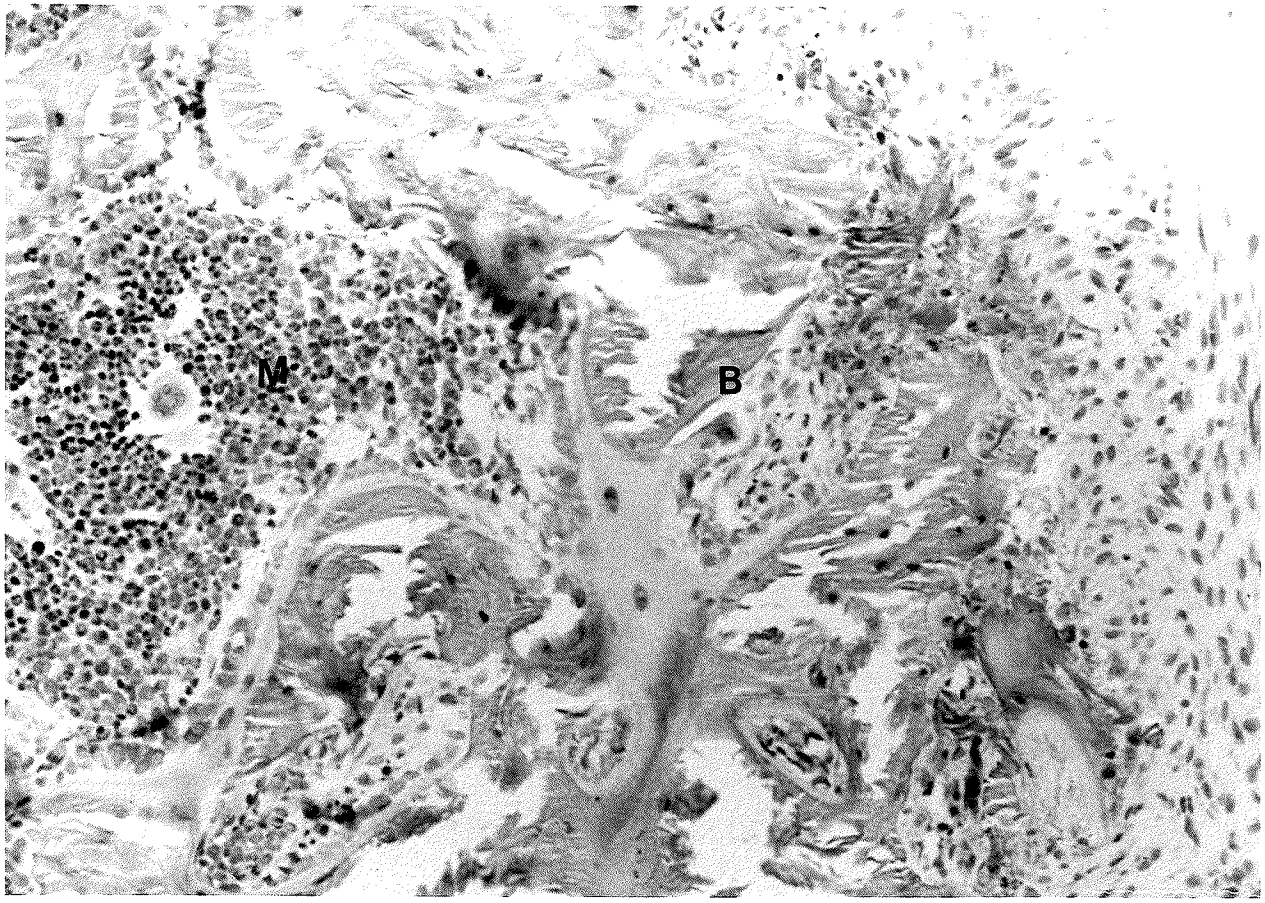


Figure 65: 4-3-Right Graft (x100)

Intramembranous bone formation (B), embyonic marrow (M)

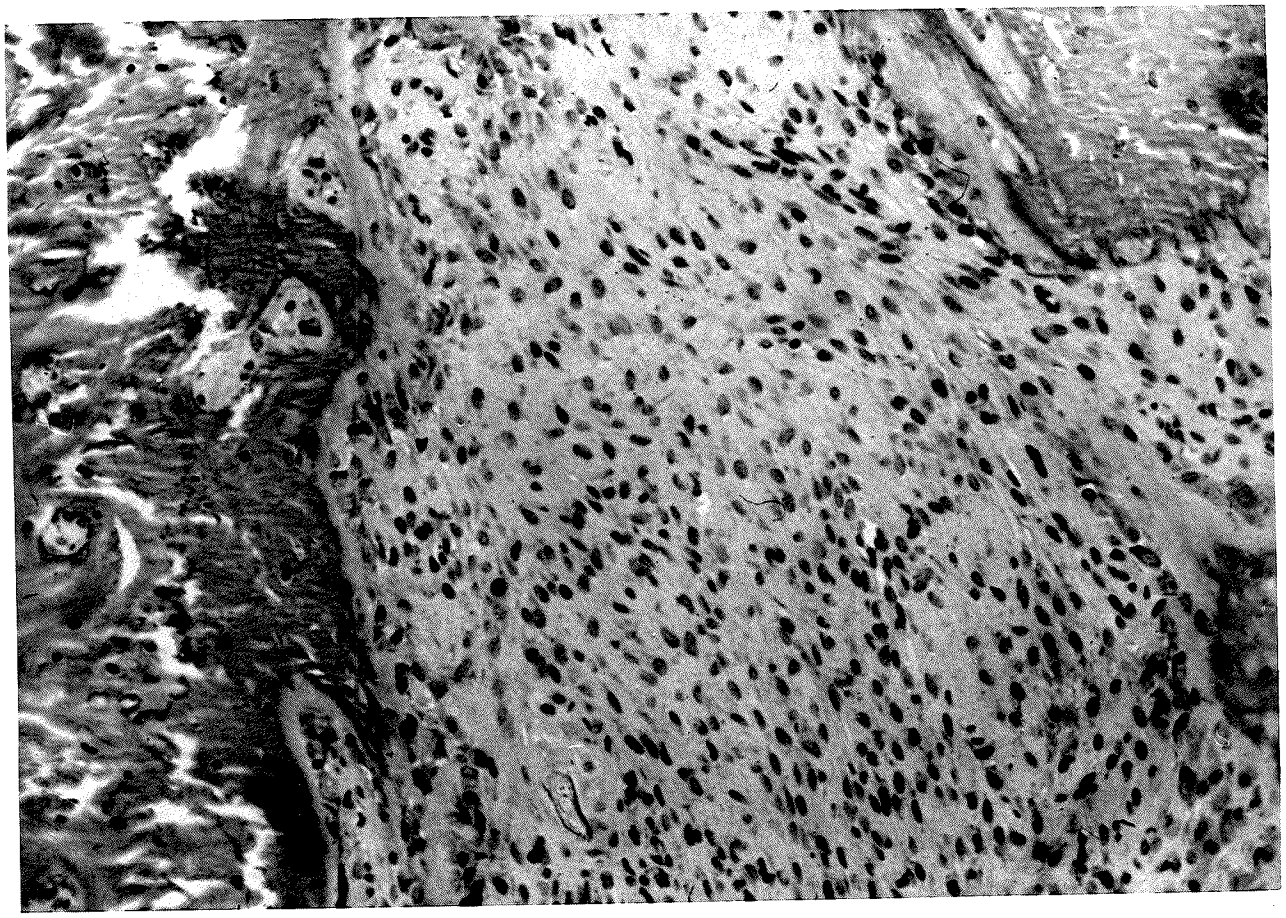


Figure 66: 4-3-Left Flap (x100)



Figure 67: 4-4-Right Flap (x100)



Figure 68: 4-4-Right Flap (x40)

Intramembranous bone (B), embryonic marrow (M)

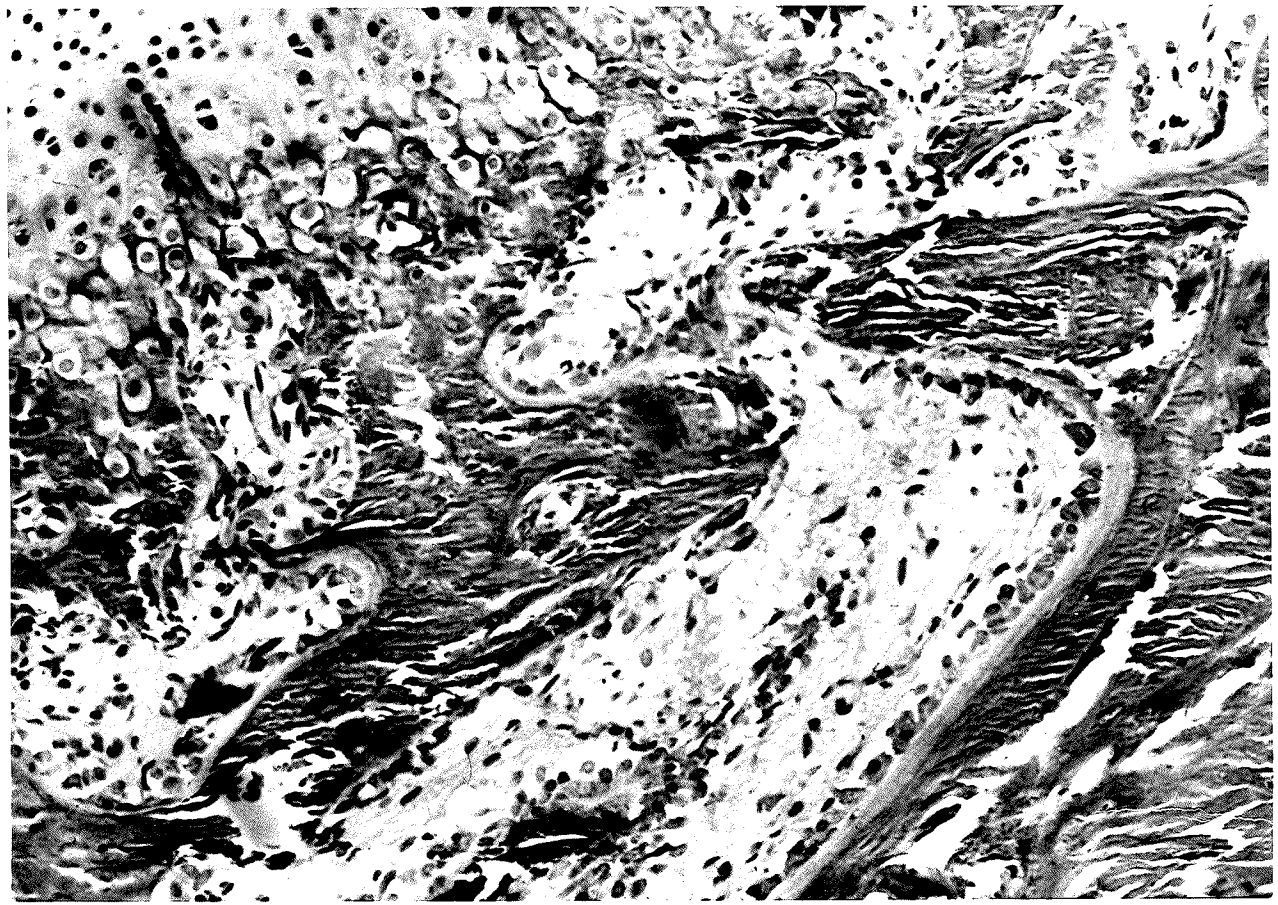


Figure 69: 4-5-Right Flap (x100)

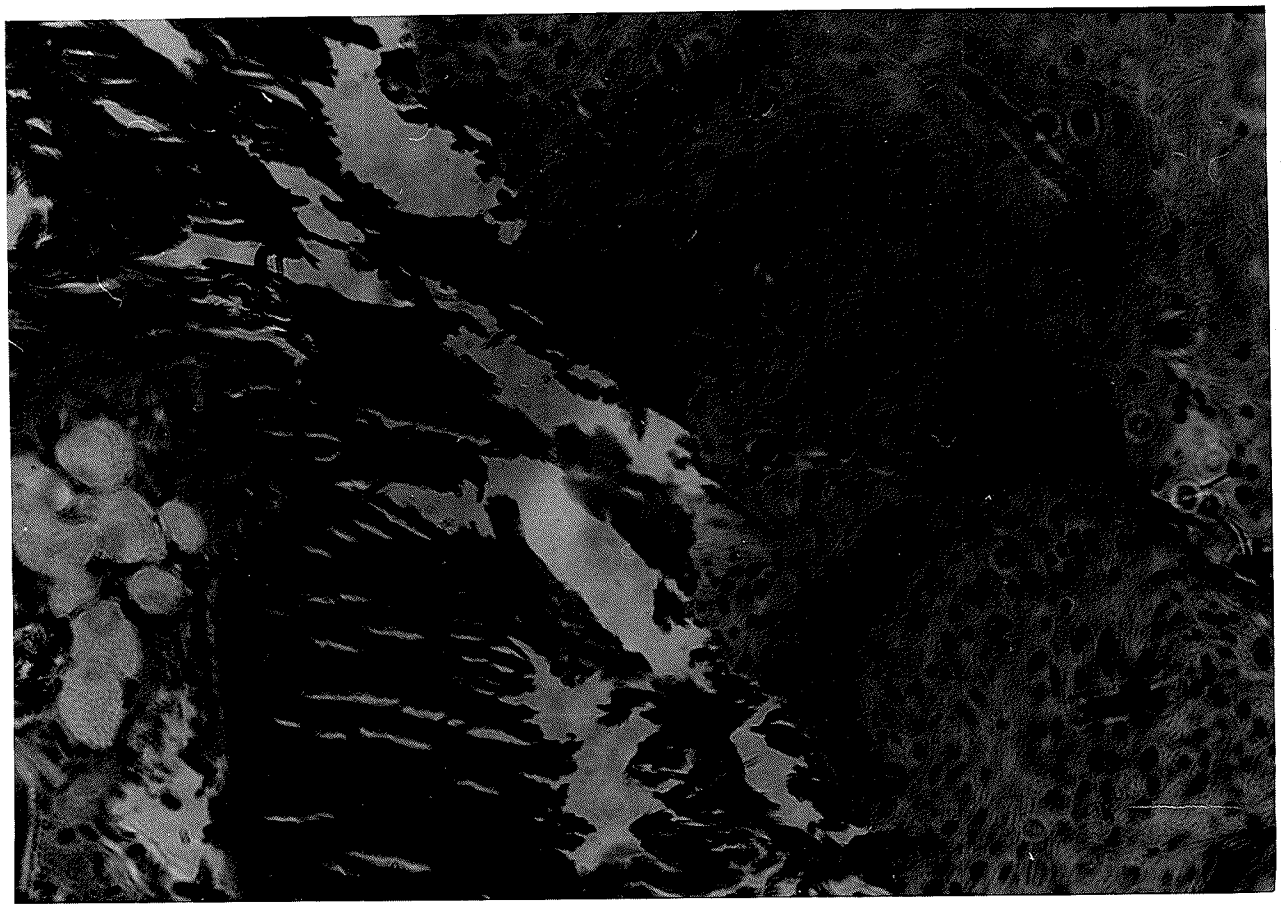


Figure 70: 4-5-Left Flap (x100)

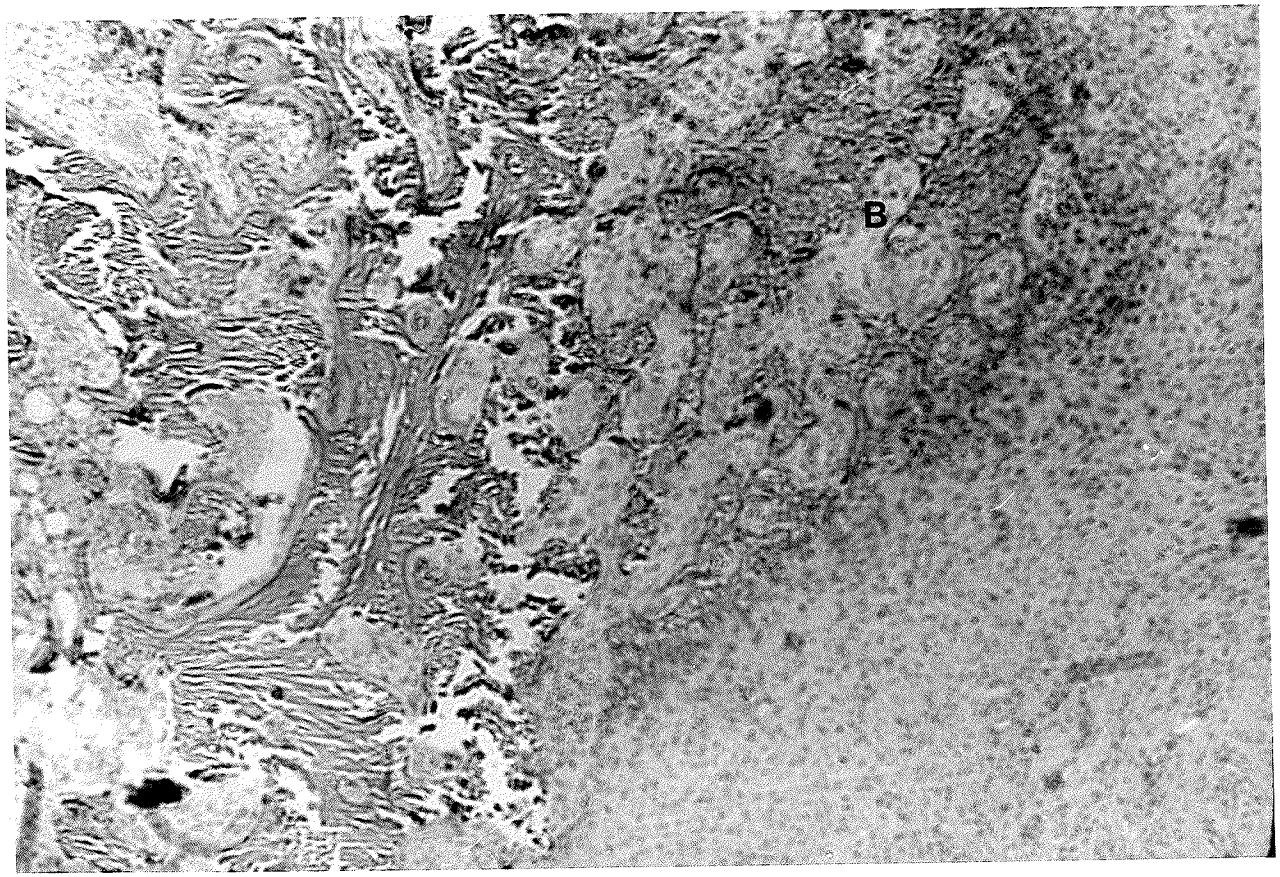


Figure 71: 4-6-Right Flap (x40)

Intramembranous bone formation (B)

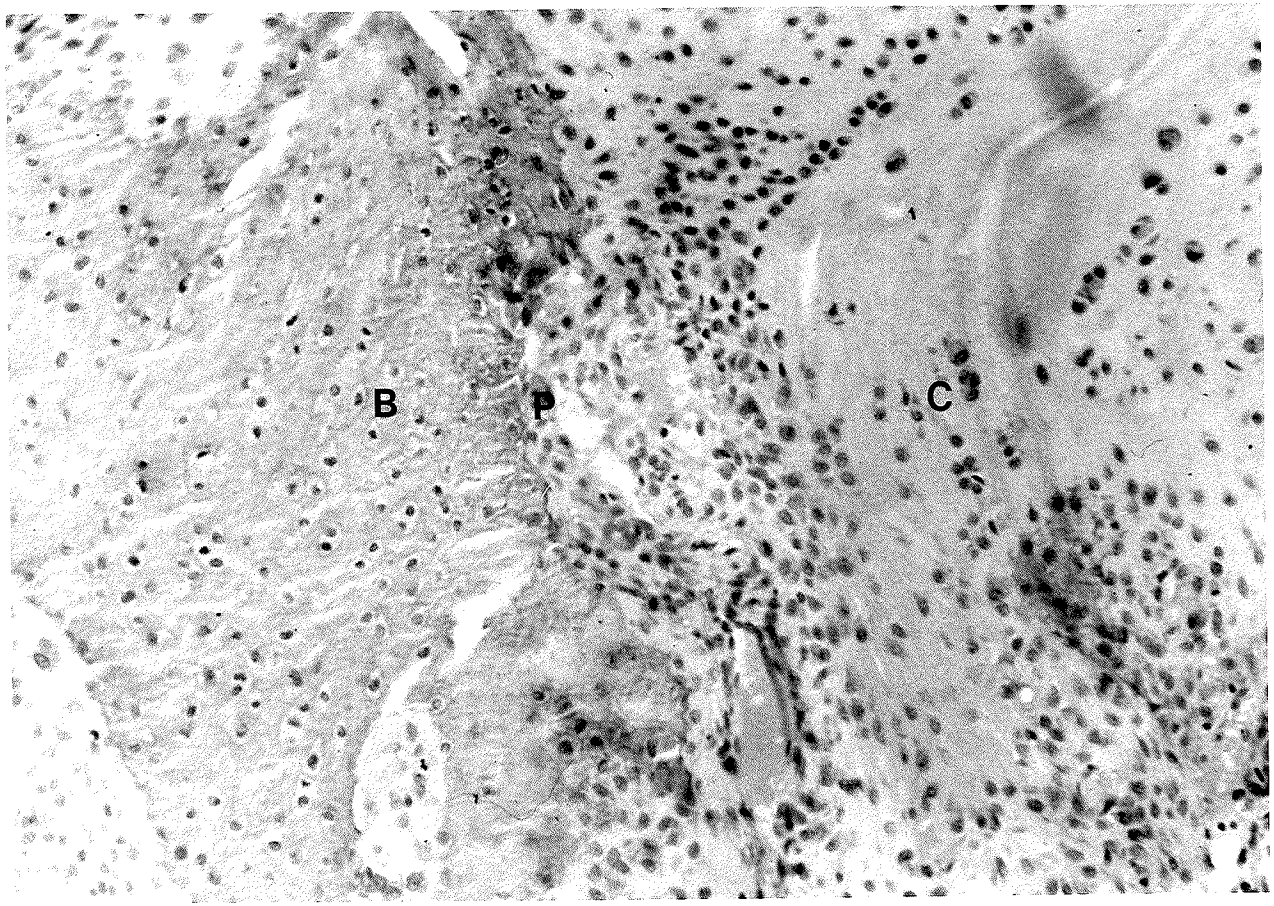


Figure 72: 4-6-Left Graft (x100)

Cartilage (C), periosteum (P), new bone formation (B)

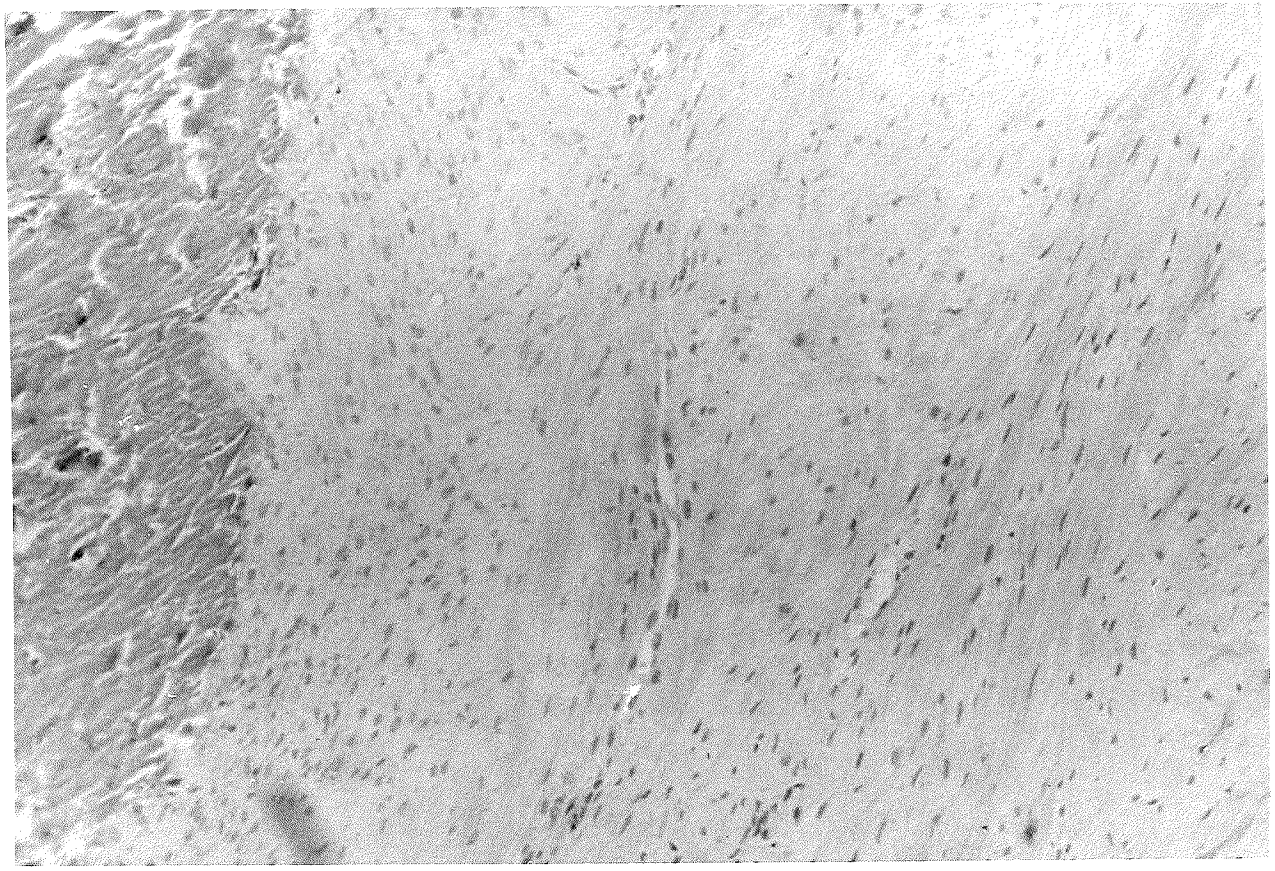


Figure 73: 4-7-Right Flap (x100)

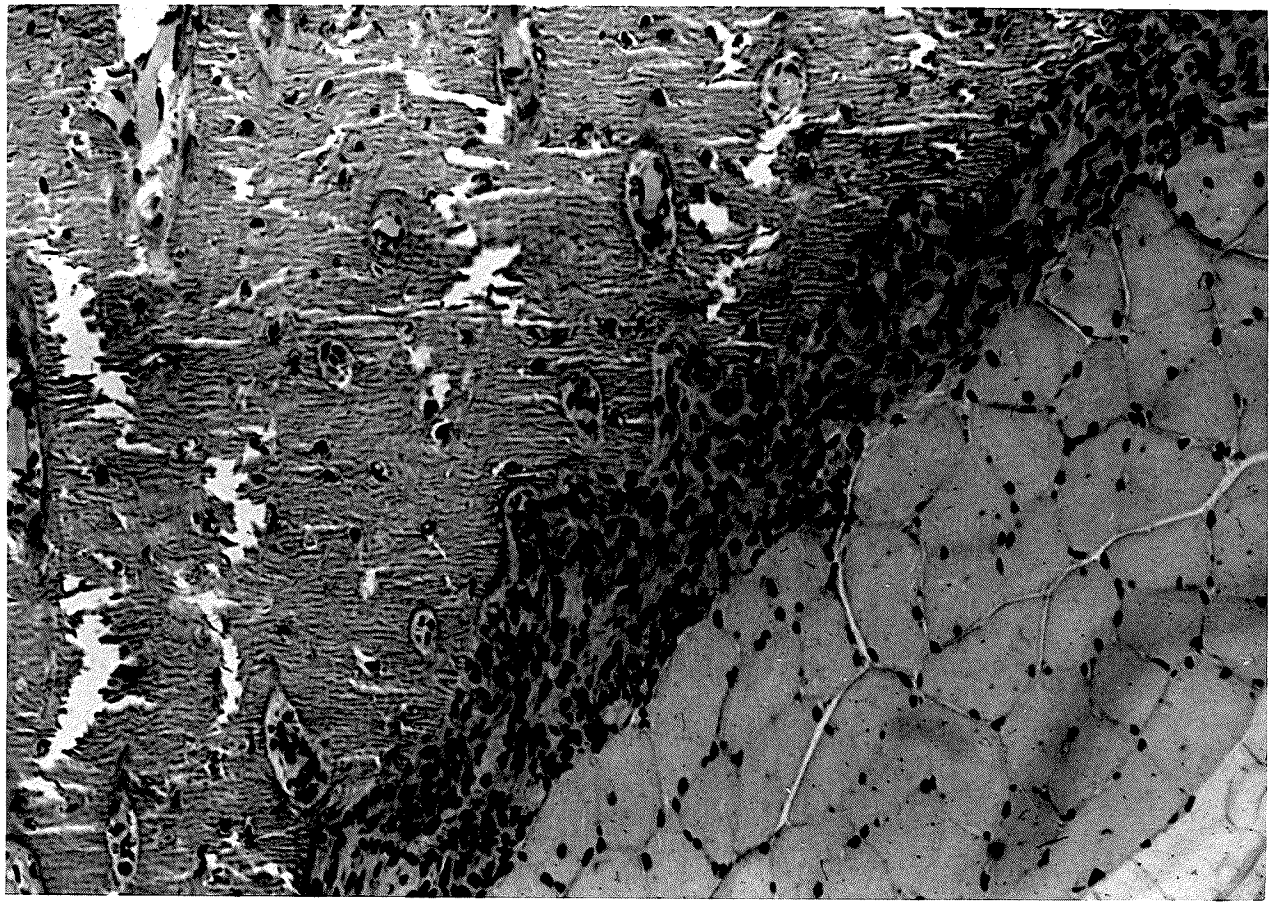


Figure 74: 4-7-Left Graft (x100)

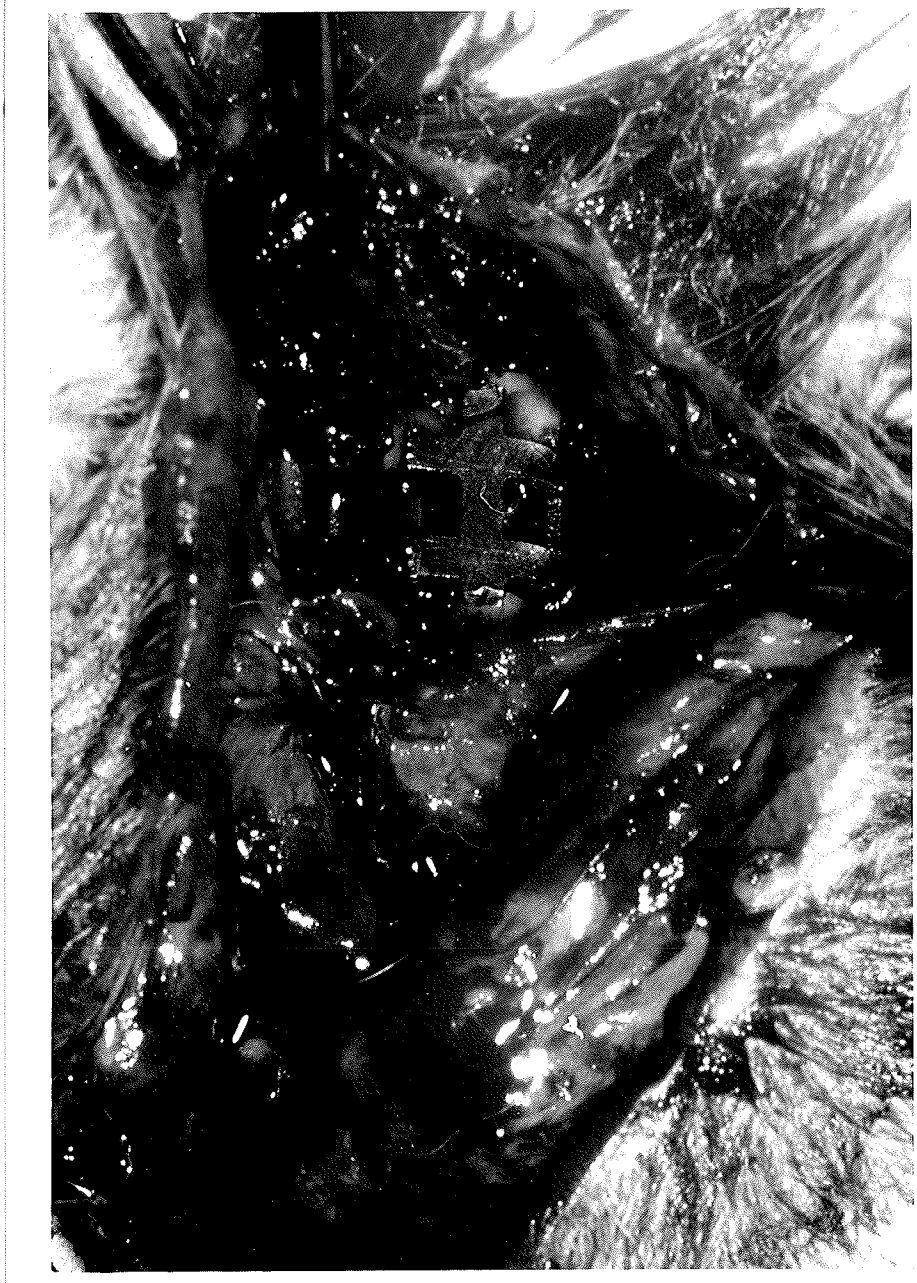


Figure 75: Vitallium (Tm) mesh placed over mandibular defect

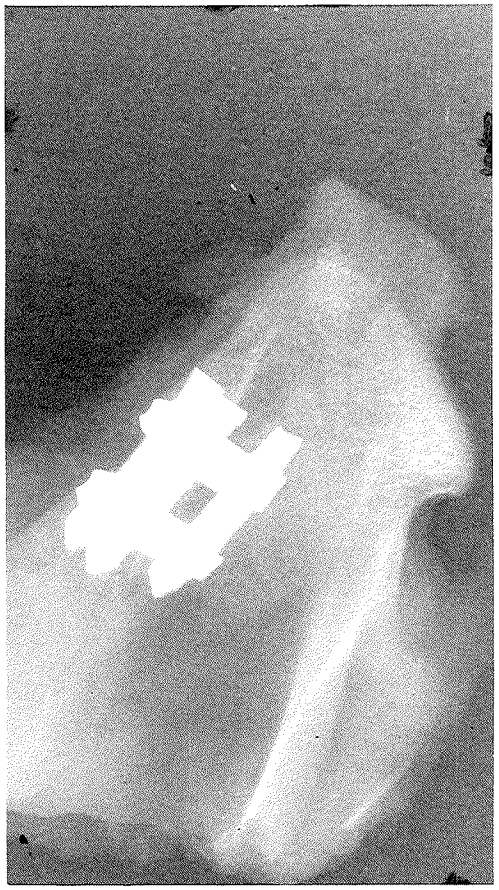


Figure 76: Radiograph of mesh placed over defect

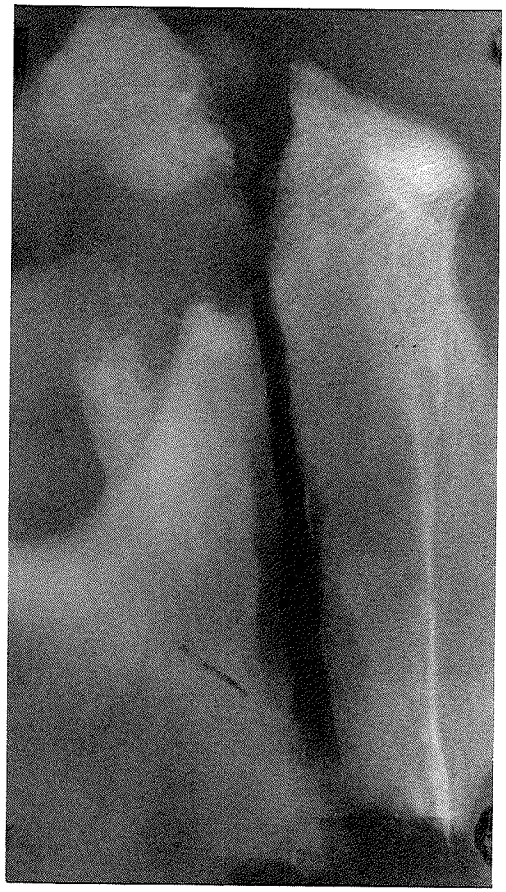


Figure 77: 5-1-Right Graft



Figure 78: 5-2-Right graft

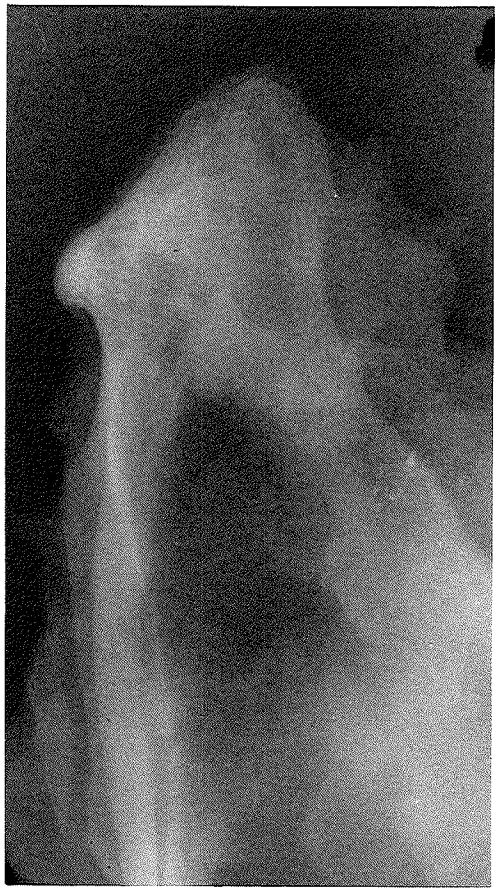


Figure 79: 5-4-Left Graft

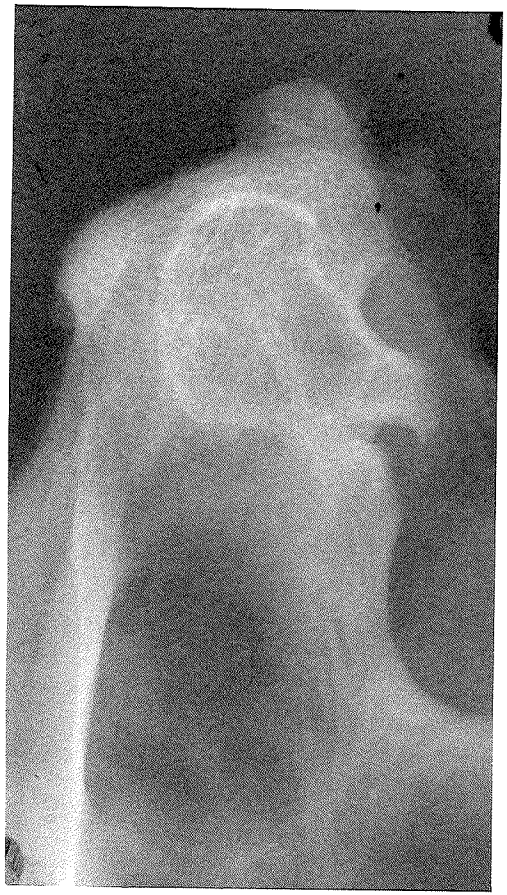


Figure 80: 5-5-Left Graft

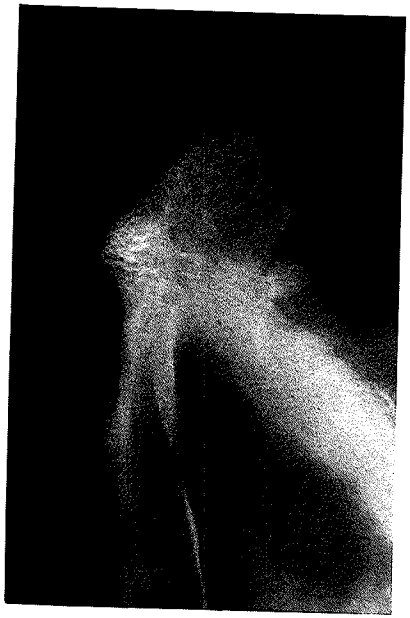


Figure 81: 5-6-Left Graft



Figure 82: 5-8-Left Graft

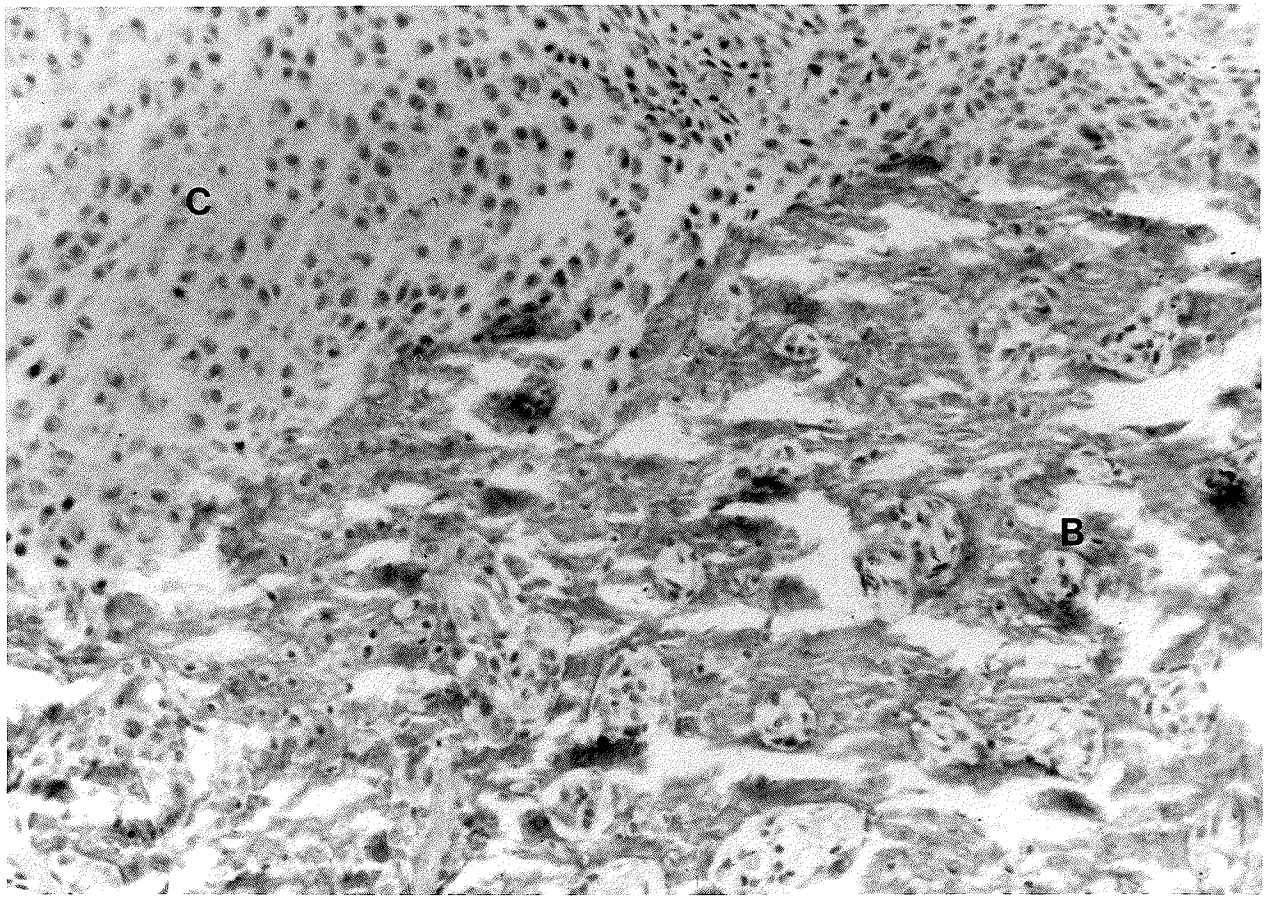


Figure 83: 5-1-Right Graft (x100)

Cartilage (C), intramembranous bone (B)

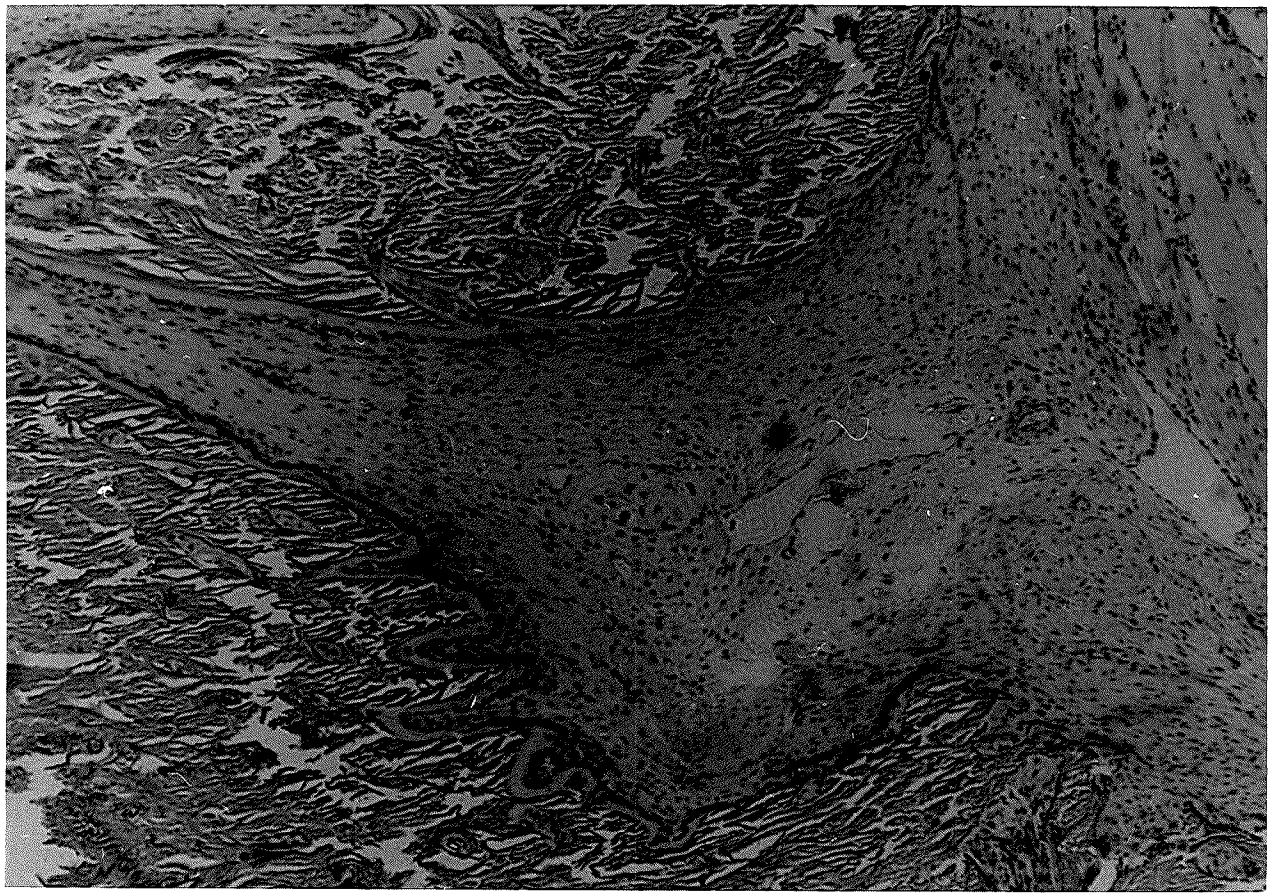


Figure 84: 5-1-Left Flap (x40)

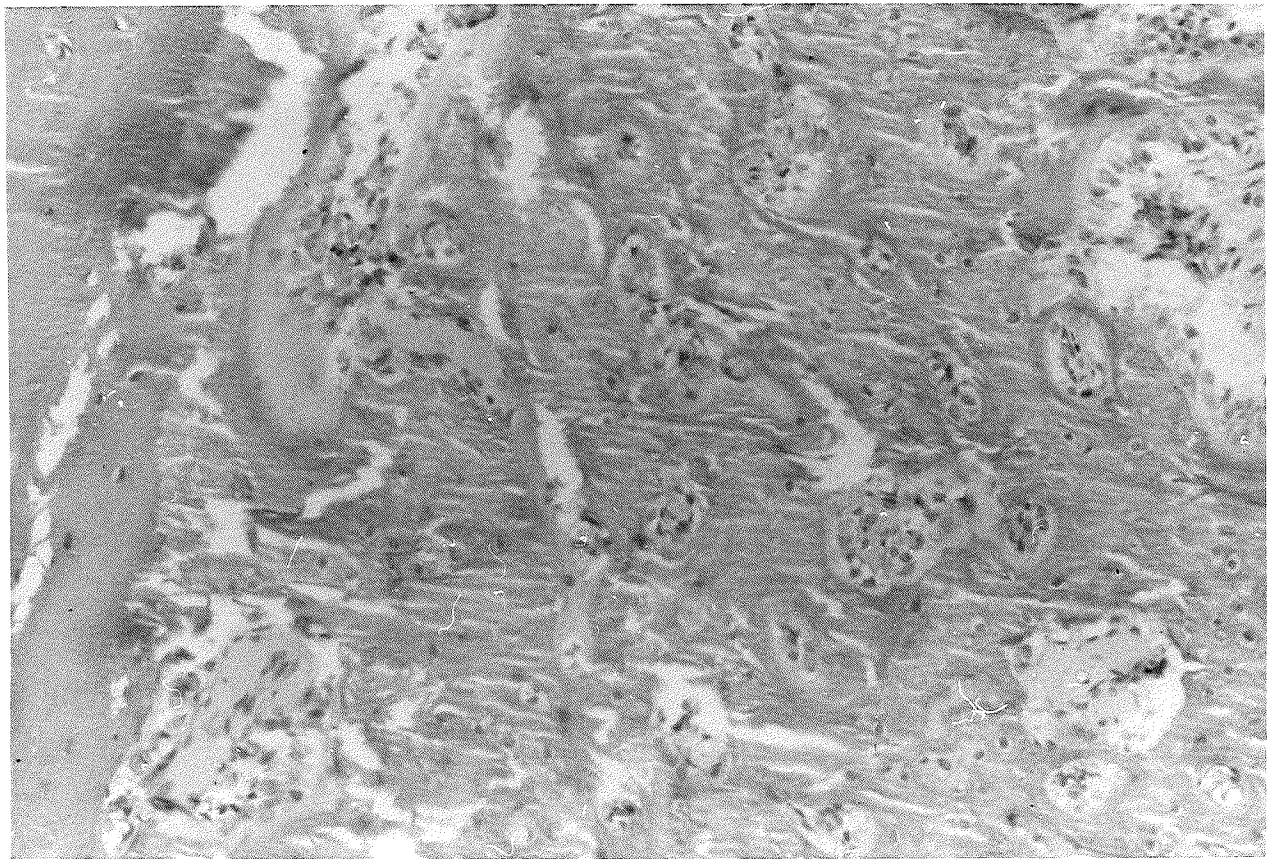


Figure 85: 5-1-Left Flap (x100)

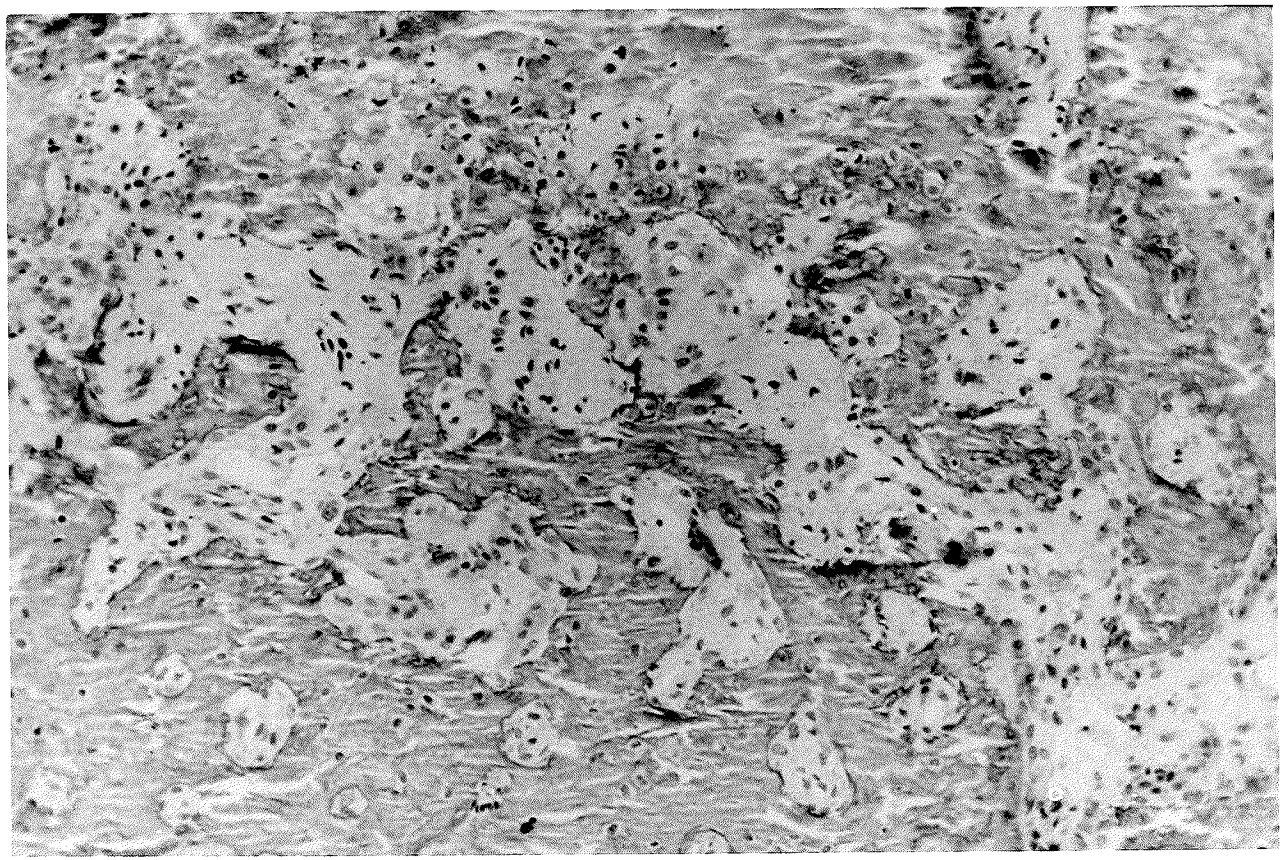


Figure 86: 5-2-Left Flap (x100)



Figure 87: 5-4-Right Flap (x40)

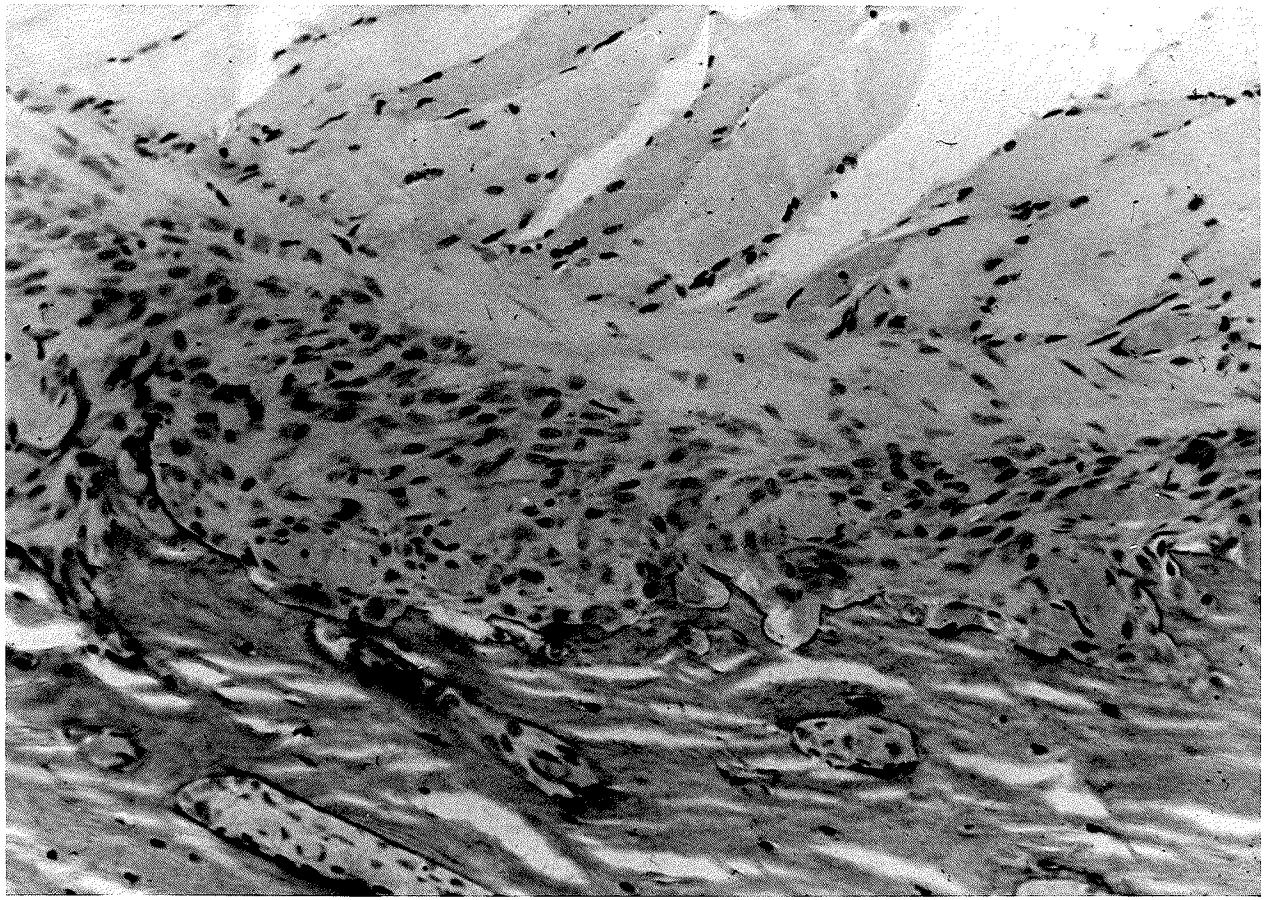


Figure 88: 5-4-Left Graft (x100)

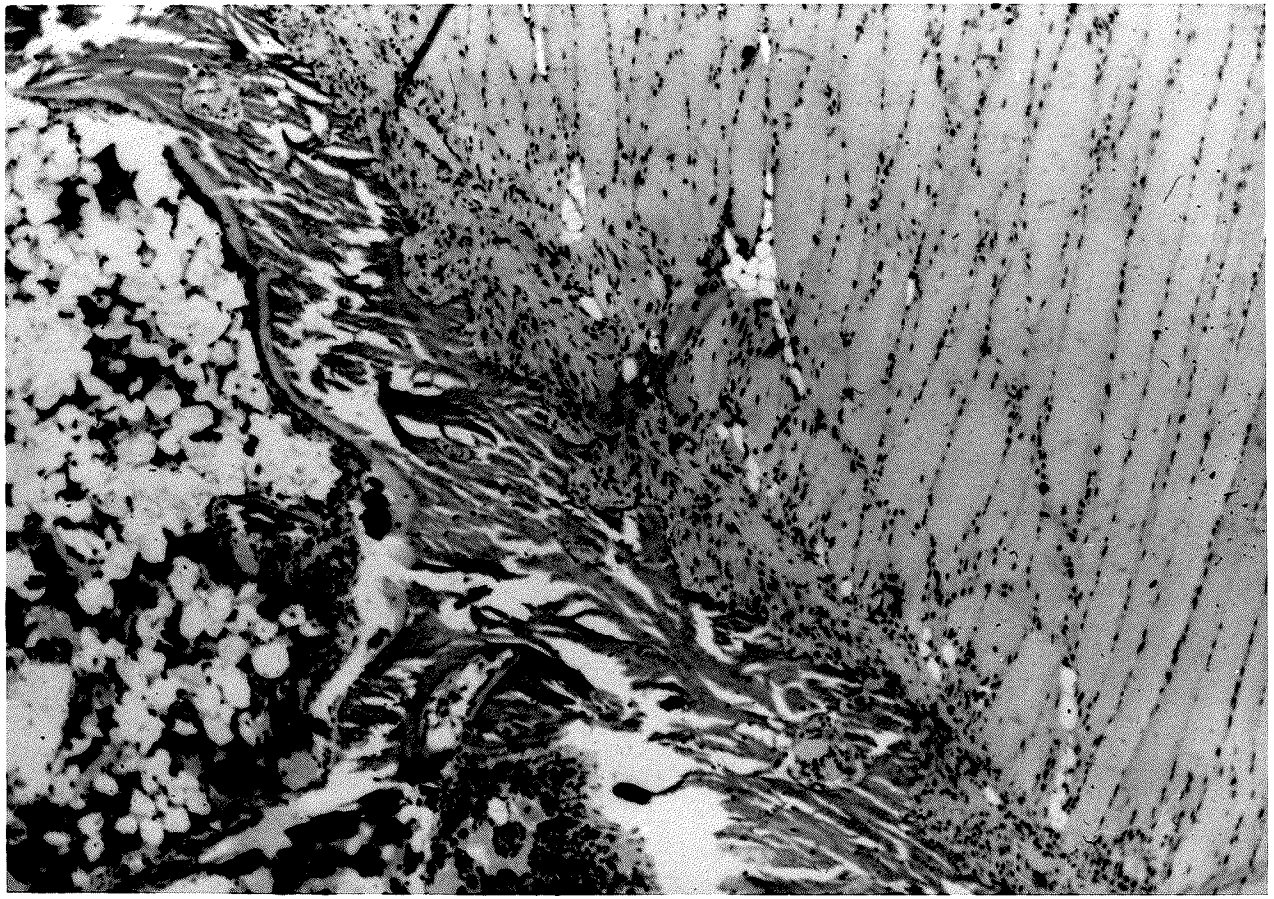


Figure 89: 5-5-Right Flap (x100)

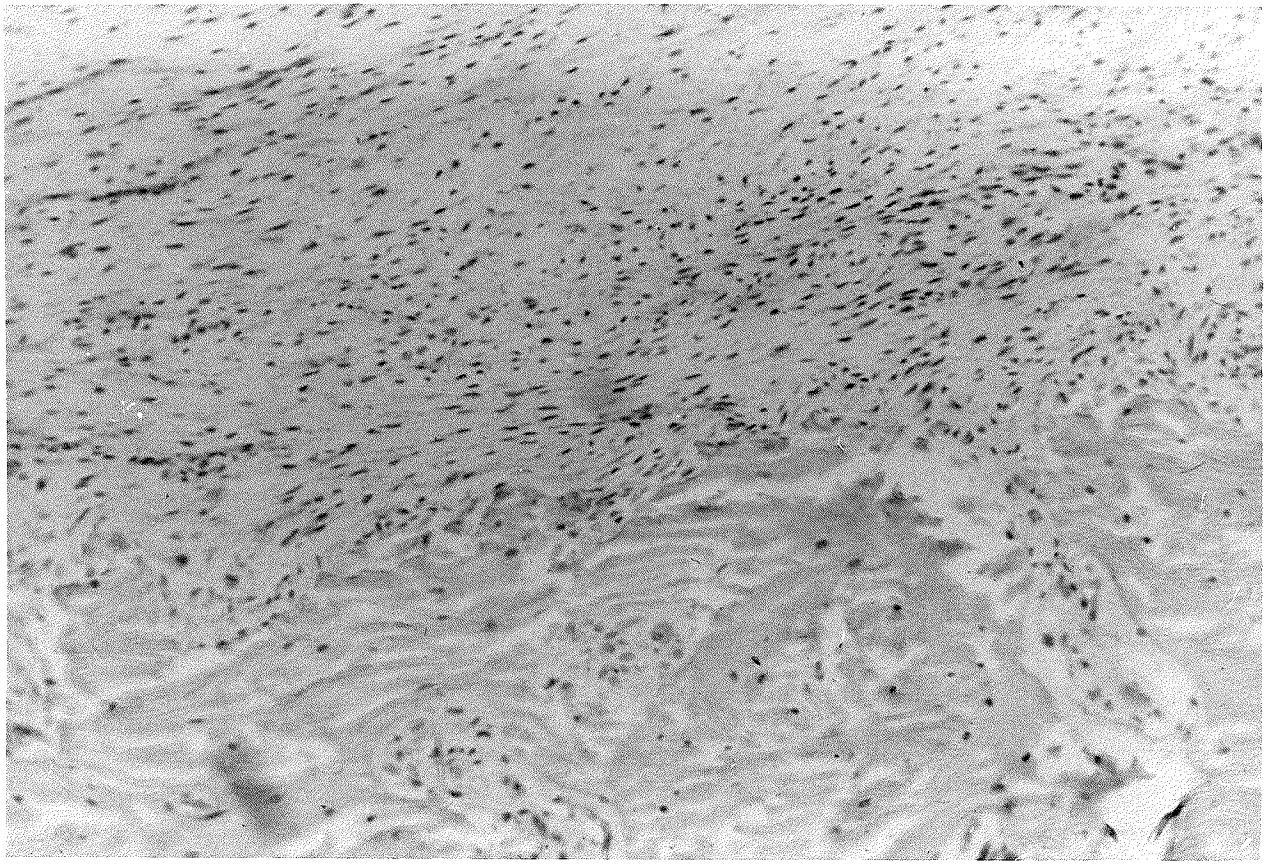


Figure 90: 5-5-Left Graft (x100)

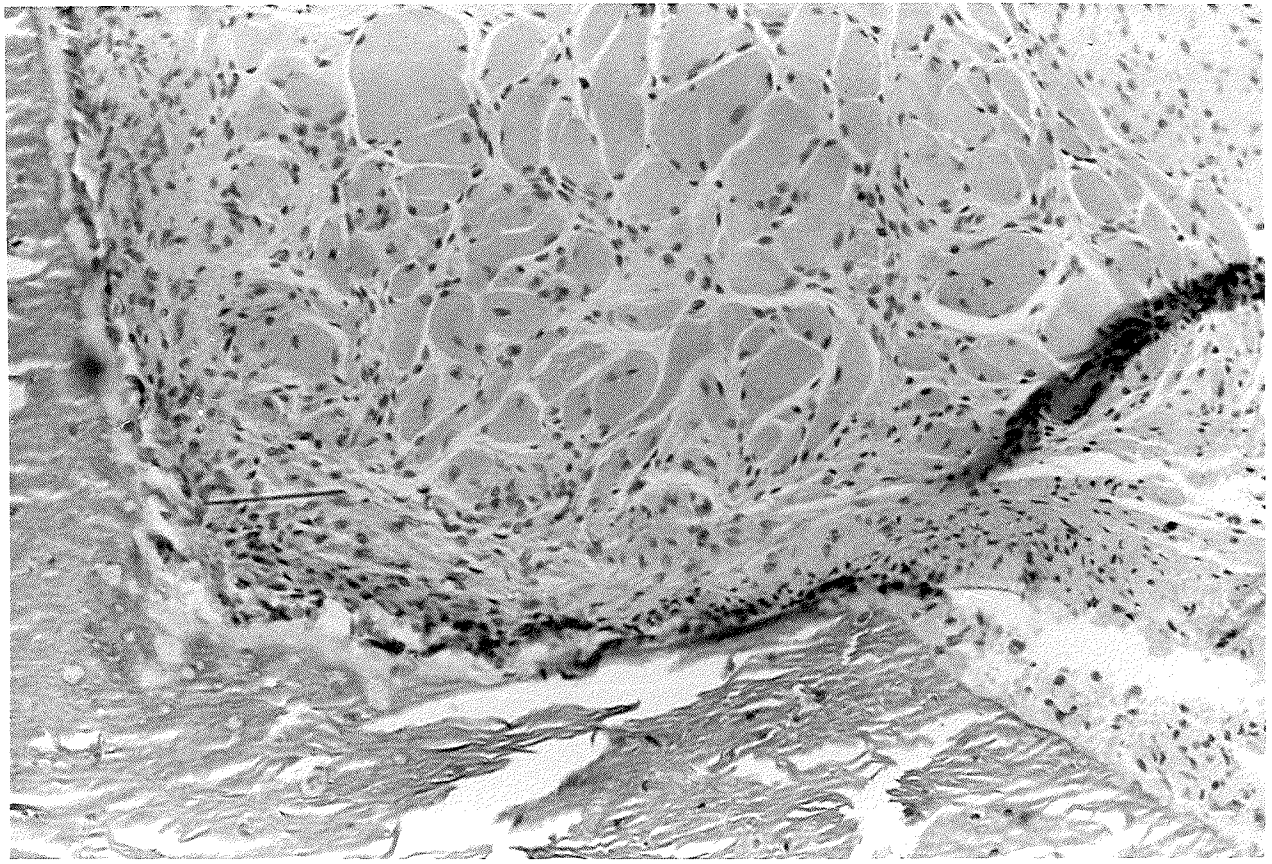


Figure 91: 5-6-Right Flap (x100)

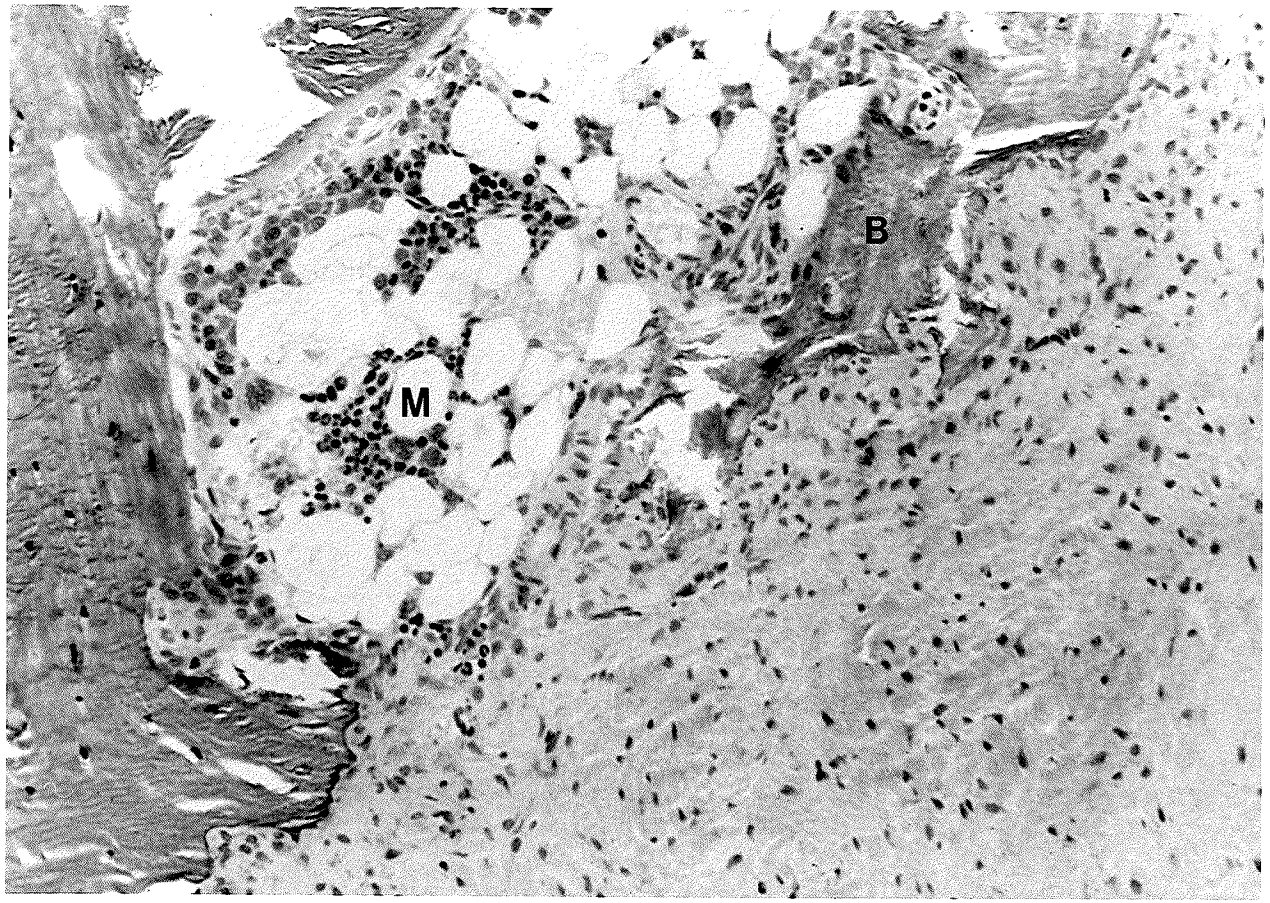


Figure 92: 5-6-Left Graft (x100)

Intramembranous bone (B), marrow (M)

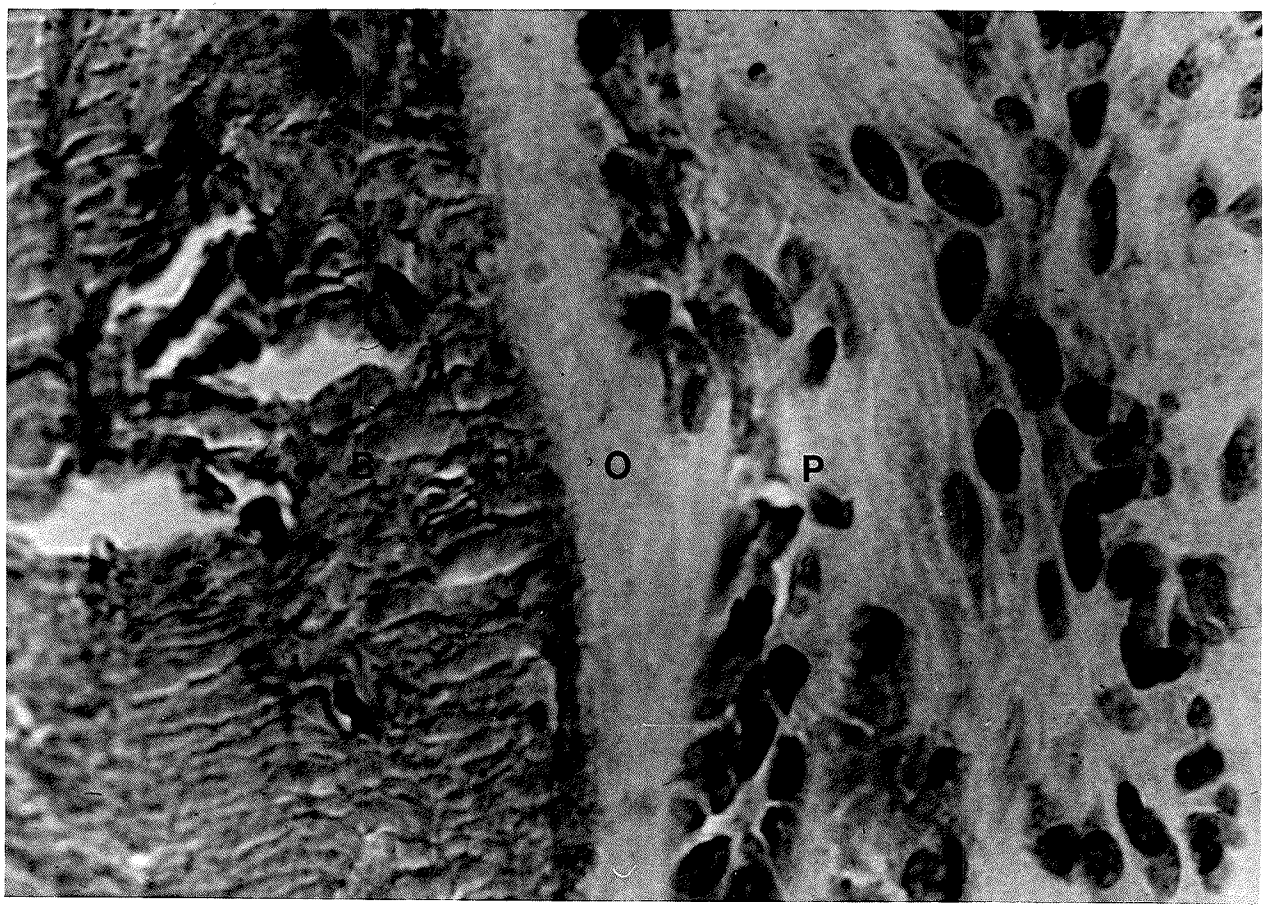


Figure 93: 5-6-Left Graft (x400)

Periosteum (P), osteoid (O), bone (B)

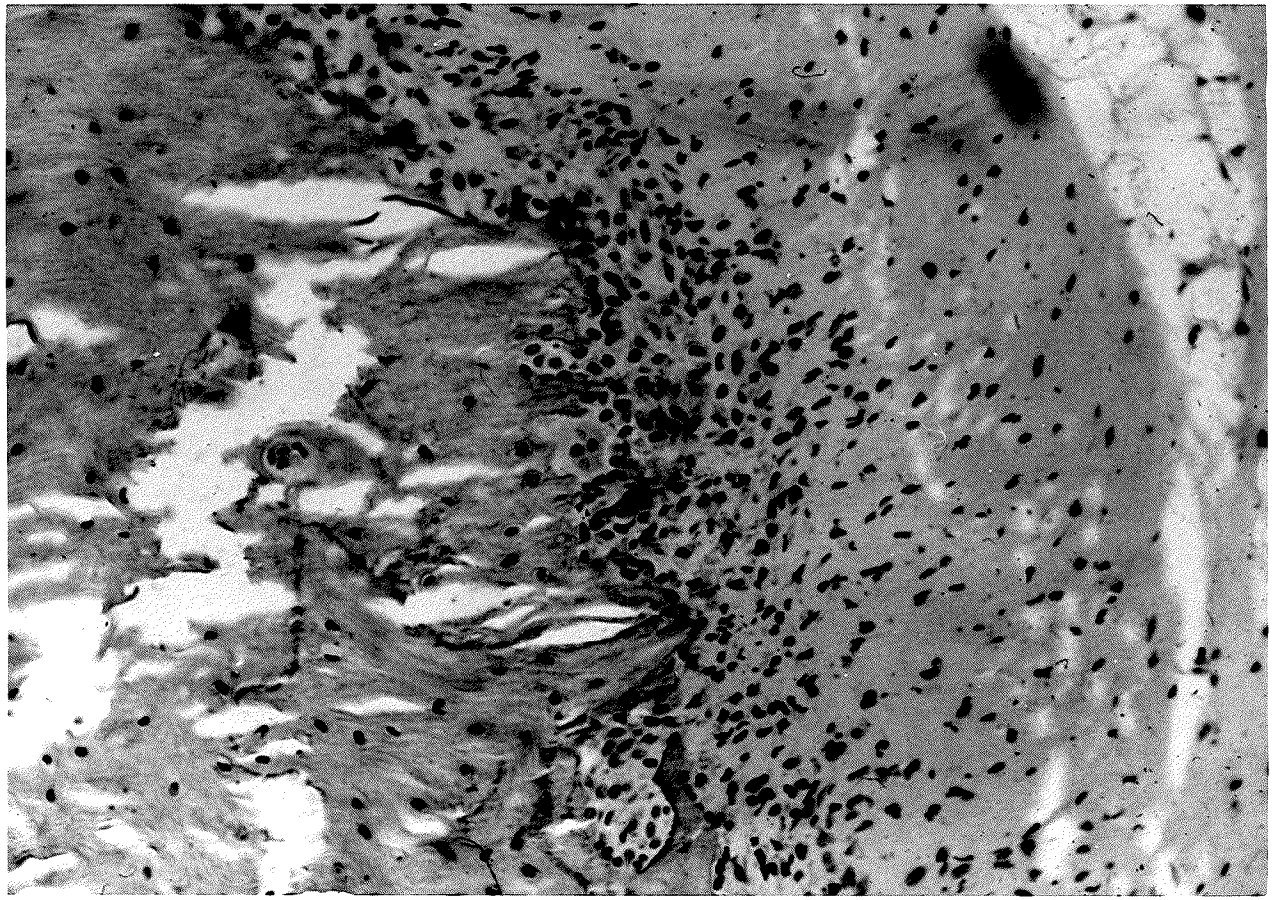


Figure 94: 5-7-Left Flap (x100)



Figure 95: 5-8-Right Flap (x40)

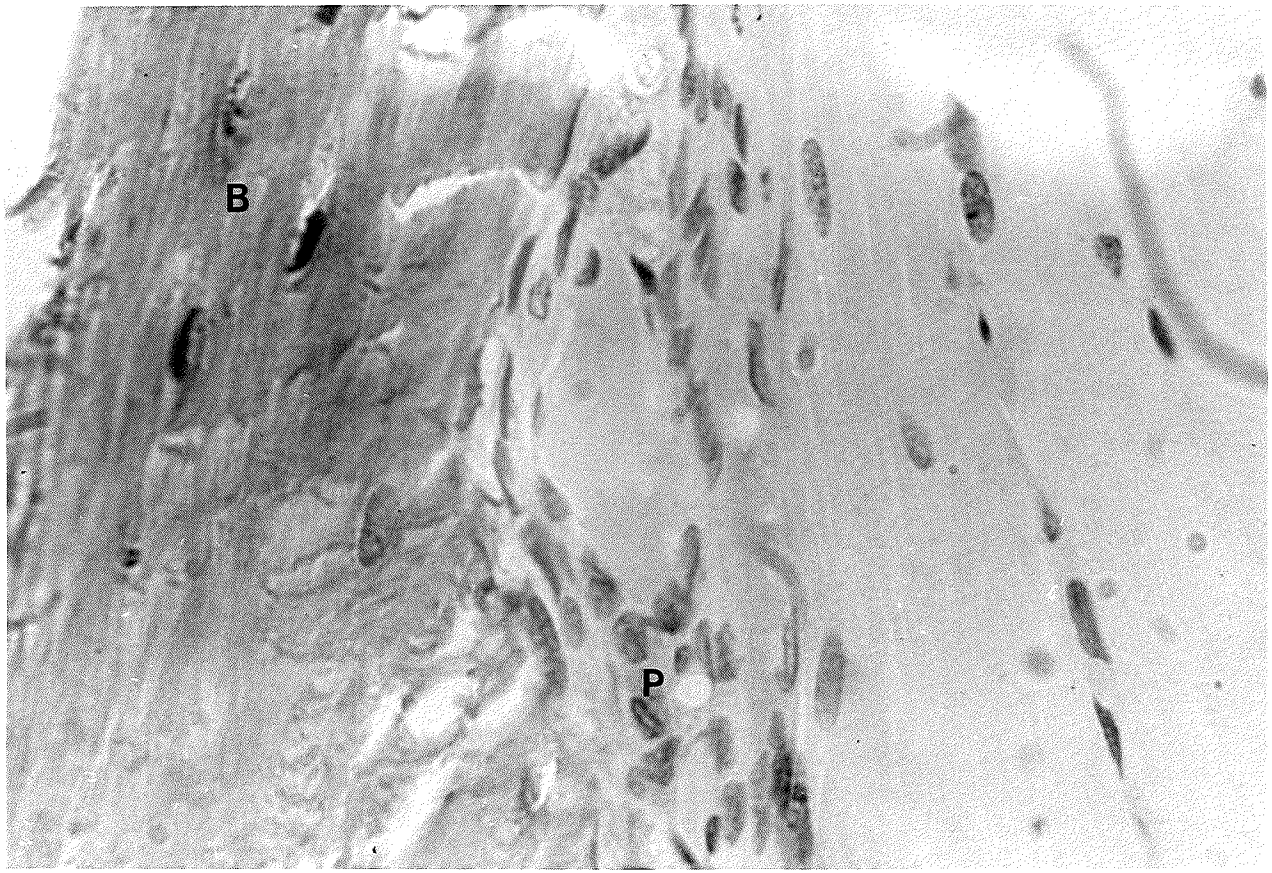


Figure 96: 5-8-Left Graft (x400)

Periosteum (P), Bone (B)

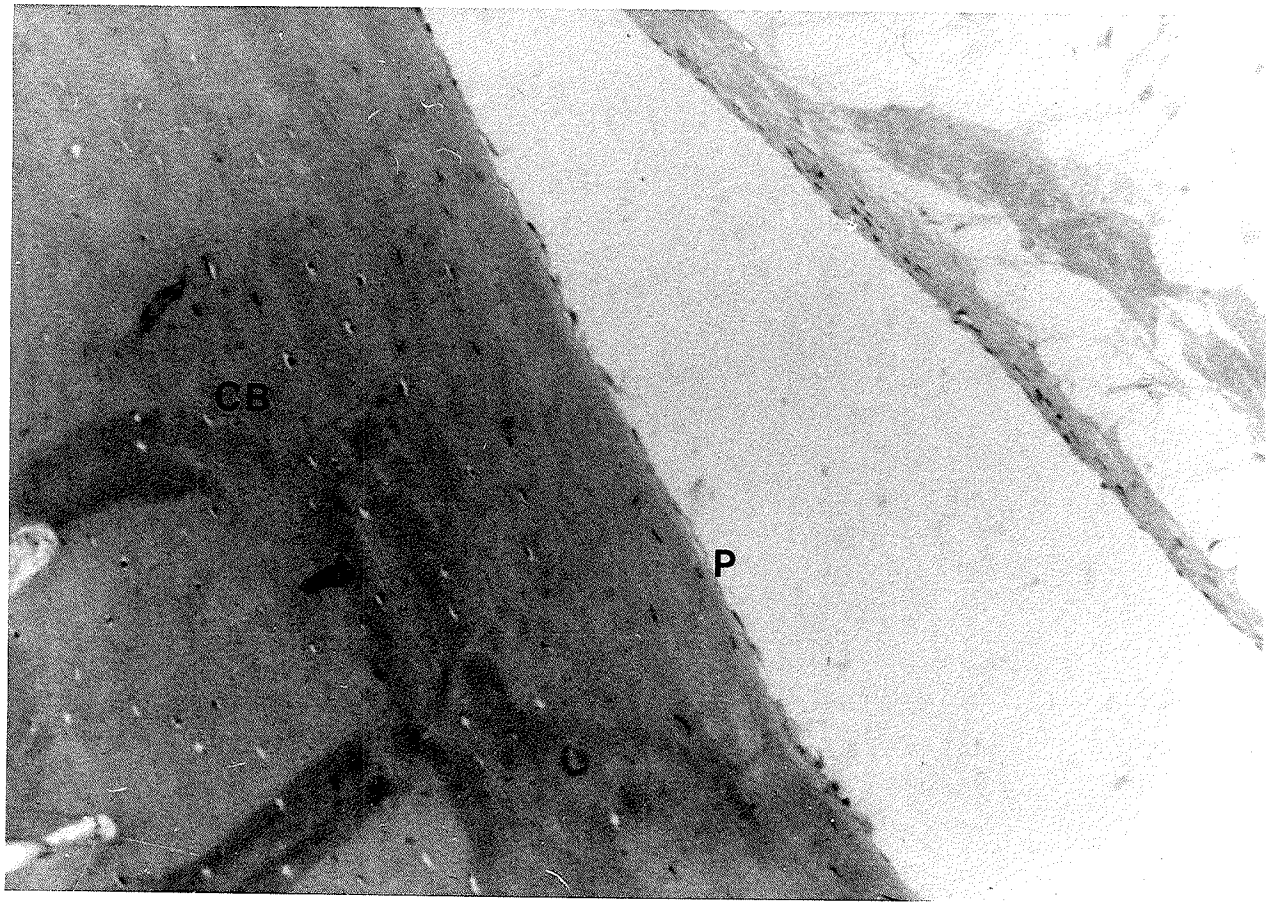


Figure 97: Periosteum stripped quickly from calvarial bone (CB). A substantial part of cambial periosteum (P) remains on the cortical bone